(54) Title: NOVEL THERAPEUTIC AGENTS THAT MODULATE ALPHA-1A ADRENERGIC RECEPTORS

(57) Abstract

Disclosed are novel multi-binding compounds (agents) which bind alpha-1A adrenergic receptors. The compounds of this invention comprise a plurality of ligands each of which can bind to such receptors thereby modulating the biological processes/functions thereof. Each of the ligands is covalently attached to a linker or linkers which may be the same or different to provide for the multi-binding compound. The linker is selected such that the multi-binding compound so constructed demonstrates increased modulation of the biological processes mediated by the alpha-1A adrenergic receptor.
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NOVEL THERAPEUTIC AGENTS THAT MODULATE ALPHA-1A ADRENERGIC RECEPTORS

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to novel therapeutic agents which bind to mammalian receptors and modulate their activity. More particularly, the invention relates to novel therapeutic agents that bind to and modulate the *in vivo* activity of alpha-1A adrenergic receptors in mammals by acting as multi-binding compounds. The therapeutic agents or multi-binding compounds described herein 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 alpha-1A adrenergic receptor. The linking moiety is chosen such that the multi-binding compounds so constructed demonstrate increased biological activity as compared to individual units of the ligand. The invention also relates 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 mammalian conditions that are mediated by the alpha-1A adrenergic receptors targeted by the ligands, such as benign prostatic hyperplasia ("BPH") and
hypertension. Accordingly, this invention also relates to pharmaceutical compositions comprising a pharmaceutically acceptable excipient and an effective amount of a multi-binding compound of this invention.

Additionally, the multi-binding compounds are useful as affinity resins for affinity chromatography. When so employed, 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.

References

The following publications are cited in this application as superscript numbers:


All of the above publications are herein incorporated by reference in their entirety to the same extent as if each individual publication 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 can exist entirely outside the cell.
(extracellular receptors), within the cell membrane (but presenting sections of the receptor to the extracellular milieu and cytosol), or entirely within the cell (intracellular receptors). 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 to function in the transport of molecules and ions into and out of the cell.

A ligand is a binding partner for a specific receptor or family of receptors. 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.

The ligands that bind to cellular receptors 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 surmountable 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 measurable properties that pertain 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; and

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.

Current drugs (ligands) targeting receptors have clinical shortcomings identified by one or more 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. Accordingly, it would be beneficial to develop ligands that have improved affinity, efficacy, selectivity, onset of action and duration of action.

**Affinity of ligand for target receptor**

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

**Efficacy of ligand at a target receptor (functional effect)**

An increased ligand efficacy at a target receptor 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 or antagonist may provide clinical benefit by modulating the biological response.

**Selectivity of ligand compared across receptor subtypes**

An increase in the selectivity of the ligand across receptor subtypes requires that the affinity or efficacy of the ligand at other receptors is reduced relative to the desired receptor. A decrease in the selectivity of the ligand may also be desired.

**Onset of Action**

More rapid onset of action of the ligand to effect a biological response is often preferred.

**Duration of Action**

An increased duration of action of the ligand to effect a biological response may be preferred. For example β₂ adrenergic agonists such as albuterol have a relatively short duration of action of approximately 3-4 hours and an increase in duration of action would simplify the dosing regimen required to administer this drug (ligand).
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 downregulation is generally slower, in 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. Alternatively, receptor desensitization may occur through changes in receptor structure, such as receptor phosphorylation.

Receptor oligomerization also plays a role in receptor function. The family alpha-1 adrenergic receptors (alpha-1A, alpha-1B, alpha-1D and alpha-1L), mediate various actions of the peripheral sympathetic nervous system through the binding of the catecholamines, epinephrine and norepinephrine.

Human adrenergic receptors are integral membrane proteins which have been classified into two broad classes, the alpha and the beta adrenergic receptors. Both types mediate the action of the peripheral sympathetic nervous system upon binding of catecholamines, norepinephrine and epinephrine.

Norepinephrine is produced by adrenergic nerve endings, while epinephrine is produced by the adrenal medulla. The binding affinity of
adrenergic receptors for these compounds forms one basis of the classification. Alpha receptors bind norepinephrine more strongly than epinephrine and much more strongly than the synthetic compound isoproterenol. The binding affinity of these hormones is reversed for the beta receptors. In many tissues, the functional responses, such as smooth muscle contraction, induced by alpha receptor activation are opposed to responses induced by beta receptor binding.\(^4\)

Benign prostatic hyperplasia ("BPH") is a progressive condition which is characterized by a nodular enlargement of prostatic tissue resulting in obstruction of the urethra.\(^6\) This condition occurs in over 50% of the male population above age 60 and leads to a variety of urological symptoms including increased frequency in urination, nocturia, a poor urine stream and hesitancy or delay in starting the urine flow.\(^6\) Further, BPH can lead to lower urinary tract infections and benign prostatic enlargement. Currently, a variety of alpha-1 adrenergic receptor antagonists are used clinically to treat BPH which relax the smooth muscle of the prostate.\(^6\) However, these agents have been shown to cause significant side effects including dizziness, decreased blood pressure, nasal congestion and impotence, presumably as a result of their lack of selectivity for any one of the receptor subtypes. Examples of these antagonists include prazosin, terazosin, doxazosin and alfuzosin.\(^6\) It has been demonstrated that the alpha-1A subtype is the predominant receptor mediating smooth muscle contraction in the human prostate. Thus, an alpha-1A selective antagonist will show better efficacy in the treatment of BPH, with reduced side effects, than nonselective alpha-1 antagonists.

Further, because the alpha-1A subtype mediates smooth vascular muscle contraction, it is useful in the treatment of hypertension and other related cardiovascular disorders.
Accordingly, novel ligands having desired potency and therapeutic effect for the alpha-1A adrenergic receptor would be particularly desirable in order to treat benign prostatic hyperplasia and hypertension in mammalian patients. Such novel ligands would preferably achieve the desired potency and therapeutic effect by modulating one or more of the ligand’s properties as to efficacy, affinity, safety profile, selectivity, duration of action and/or onset of action.

SUMMARY OF THE INVENTION

This invention is directed to general synthetic methods for generating large libraries of diverse multimeric compounds 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 is also directed to libraries of diverse multimeric compounds which multimeric compounds 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.

This invention is directed, in part, to novel multi-binding compounds that bind alpha-1A adrenergic receptors and consequently these compounds can be used
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to treat conditions mediated by alpha-1A adrenergic receptors such as benign prostatic hyperplasia and hypertension.

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, at least one of said ligands comprising a ligand domain capable of binding to an alpha-1A adrenergic receptor. Preferably, at least two and more preferably each of the ligands comprises a ligand domain capable of binding to an alpha-1A adrenergic receptor.

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

\[(L)_p(X)_q\]

wherein each L is independently selected from ligands comprising a ligand domain capable of binding to an alpha-1A adrenergic receptor; X is independently 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 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, at least one of said ligands comprising a ligand domain capable of binding to one or more alpha-1A adrenergic receptors.
Preferably, said ligands comprising a ligand domain capable of binding to one or more alpha-1A adrenergic receptors that modulate benign prostatic hyperplasia in mammals. More preferably, said ligands are selected from the group consisting of terazosin, prazosin, doxazosin, alfuzosin, tamsulosin, RS 100975 (Roche Biosciences), A-131701 (Abbott), L794-191 (Merck), L757464 (Merck), REC 15-2739 (SB216469, Recordati/SKB), KMD-3213 (Kissei Pharmaceuticals), and derivatives thereof.

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:

\[(L)_p(X)_q\]  

wherein each L is independently selected from ligands comprising a ligand domain capable of binding to an alpha-1A adrenergic receptor; 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, and more preferably the ligand is selected from the group consisting of terazosin, prazosin, doxazosin, alfuzosin, tamsulosin, RS 100975 (Roche Biosciences), A-131701 (Abbott), L794-191 (Merck), L757464 (Merck), REC 15-2739 (SB216469, Recordati/SKB), KMD-3213 (Kissei Pharmaceuticals), and derivatives thereof.

In one of its method aspects, this invention is directed to a method for treating benign prostatic hyperplasia and/or hypertension in a mammal mediated by alpha-1A adrenergic receptors which method comprises administering to said mammal 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, at least two of said ligands comprising a ligand domain capable of binding to an alpha-1A adrenergic receptor.

In another of its method aspects, this invention is directed to a method for treating benign prostatic hyperplasia and/or hypertension in a mammal mediated by alpha-1A adrenergic receptors which method comprises administering to said mammal an effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable excipient and a multi-binding compound represented by formula I:

\[(L)_p(X)_q\]  

wherein each \(L\) is independently selected from ligands comprising a ligand domain capable of binding to an alpha-1A adrenergic receptor; \(X\) 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 \(p\), and more preferably, the ligand is selected from the group consisting of terazosin, prazosin, doxasosin, alfuzosin, tamsulosin, RS 100975 (Roche Biosciences), A-131701 (Abbott), L794-191 (Merck), L757464 (Merck), REC 15-2739 (SB216469, Recordati/SKB), KMD-3213 (Kissei Pharmaceuticals), and derivatives thereof.

Accordingly, in one of its method aspects, this invention is directed to a method for identifying multimeric ligand compounds possessing multibinding properties which method comprises:
(a) identifying a ligand or a mixture of ligands wherein each ligand is capable of binding to an alpha-1A adrenergic 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 possessing multibinding properties which method comprises:

(a) identifying a library of ligands wherein each ligand is capable of binding to an alpha-1A adrenergic 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 is preferred when at least a portion of the multimeric compounds
prepared are homomultimeric compounds.

The assay protocols recited in (d) can be conducted on the multimeric
ligand compound library 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 possess multivalent properties which
library is prepared by the method comprising:

(a) identifying a ligand or a mixture of ligands wherein each ligand is
capable of binding to an alpha-1A adrenergic 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; 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 possess multivalent properties which library is prepared by the method comprising:

(a) identifying a library of ligands wherein each ligand is capable of binding to an alpha-1A adrenergic 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 ligand having a ligand binding domain capable of binding to an alpha-1A adrenergic receptor, 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 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 least from 2-50 times, even more preferably from at least 3 to 50 times, and still more preferably at least 5-50 times.

**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 the ligand with the cellular receptor can be used to treat these conditions. Of particular interest are mammalian alpha-1A adrenergic receptors which are known to mediate the contraction and relaxation of the smooth muscles of the prostate gland in mammals. As noted above, this invention is directed, in part, to multi-binding compounds that bind alpha-1A adrenergic receptors.

The "affinity" and "specificity" of the alpha-1A adrenergic receptor and a ligand thereto 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 multi-binding compounds of this invention are capable of acting as multi-binding agents and the surprising activity of these compounds arises at least in part from their ability to bind in a multivalent manner with mammalian alpha-1A adrenergic receptors. Multivalent binding interactions are characterized by the concurrent interaction of multiple ligands with multiple ligand binding sites on one or more alpha-1A adrenergic 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 cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminocarbonyl, aminocarboxyloxy, oxyaminocarbonyl, azido, cyano, halogen, hydroxyl, keto, thiol keto, carboxyl, carboxyalkyl, thioaryloxy, thioheteroaryloxy, thioheterocycloxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocycloxy, hydroxy-amino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-substituted aryl, -SO-aryl, -SO2-alkyl, -SO2-substituted alkyl, -SO2-aryl and -SO2-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 (-CH2-), ethylene
(-CH₂CH₂⁻), the propylene isomers (e.g., -CH₂CH₂CH₂⁻ and -CH(CH₃)CH₂⁻) 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, cycloalkeny1, substituted cycloalkeny1, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxy1alkyl, thiocarboxy1oxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-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, cycloalkeny1, substituted cycloalkeny1, 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-, cycloalkeny1-O-, and alkylnyl-O-, where alkyl, alkenyl, cycloalkyl, cycloalkeny1, and alkylnyl 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, alkylen and substituted alkylene are as defined herein. Preferred alkylalkoxy groups are alkylene-O-alkyl and include, by way of example, methylenemethoxy (-CH₂OCH₃), ethylenemethoxy (-CH₂CH₂OCH₃), n-propylene-iso-propoxy (-CH₂CH₂CH₂OCH(CH₃)₂), methylene-t-butoxy (-CH₂-O-C(CH₃)₃) and the like.

The term "alkylthioalkoxy" refers to the group -alkylene-S-alkyl, alkylen-S-substituted alkyl, substituted alkylene-S-alkyl and substituted alkylene-S-substituted alkyl wherein alkyl, substituted alkyl, alkylen and substituted alkylene are as defined herein. Preferred alkylthioalkoxy groups are alkylene-S-alkyl and include, by way of example, methylenethiomethoxy (-CH₂SCH₃), ethylenethiomethoxy (-CH₂CH₂SCH₃), n-propylene-iso-thiopropoxy (-CH₂CH₂CH₂SCH(CH₃)₂), methylene-t-thiobutoxy (-CH₂SC(CH₃)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=CH₂), n-propenyl (-CH₂CH=CH₂), iso-propenyl (-C(CH₃)=CH₂), 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, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-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 ethylene (-CH=CH-), the propenylene isomers (e.g., -CH₂CH=CH- and -C(CH₃)=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, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-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≡CH₂), propargyl (-CH₂C≡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, thiketo, 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, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-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≡C-), propargylene (-CHC≡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, thiketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocycloxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-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" 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" 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, aminoacetyl, acylamino, alkaryl, aryl, arloxy, azido, carboxyl, carboxylalkyl, cyano, halo, nitro, heteroaryl, heteroaryloxy, heterocyclic, heterocycloxy, 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-naphthylen and the like.

The term “amino” refers to the group -NH₂.

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 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, thioheterocycloxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocycloxy, 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, thioheterocycloxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocycloxy, 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, arylxy, azido, carboxyl, carboxylalkyl, cyano, halo, nitro, heteroaryl, heteroaryloxy, heterocyclic, heterocycloxy, aminoacyloxy, oxyacylmino, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioheteroaryloxy, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl, -SO₂-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., indolizinyld or benzothienyl). Preferred heteroaryl 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-pyridylene, 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, aminocarbonyl, aminocarboxamide, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thiol, thioalkoxy, substituted thiaalkoxy, aryl, arylloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxo, hydroxyaminocarbonyl, alkoxyaminocarbonyl, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-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, phenanthrolinone, isothiazole, phenazine, isoxazole, phenoxazine, phenothiazine, imidazolidine, imidazole, 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 \([-{(\text{CH}_2)}_m\text{Y}^-]\) where \(m\) is \(\geq 2\), and \(Y\) at each separate occurrence can be O, N, S or P. Examples of crown compounds include, by way of example only, \([-{(\text{CH}_2)}_3\text{-NH}^-]_3\), \([-{(\text{CH}_2)}_2\text{-O}]_4\cdot{(\text{CH}_2)}_2\text{-NH}\text{H}_2\] and the like. Typically such crown compounds can have from 4 to 10 heteroatoms and 8 to 40 carbon atoms.

The term “heterocycloxy” refers to the group heterocyclic-O-.

The term “thioheterocycloxy” 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" refers to the group \(-\text{OC(O)}\text{NRR}\) 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 multi-binding compounds of this invention and which are not biologically or otherwise undesirable. In many cases, the multi-binding 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. 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-butoxycarbonyl (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 (CHCl₃), methylene chloride (or dichloromethane or "CH₂Cl₂"), 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.

"Benign prostatic hyperplasia" ("BPH") refers to nonmalignant enlargement of the prostate that is directly attributable to cellular hyperplasia of both glandular and stromal elements of the gland.⁵ It is diagnosed histologically, developing in the periurethral transition zone of the prostate.⁵ BPH progresses frequently to benign prostatic enlargement and causes benign prostatic obstruction and lower urinary tract symptoms, often referred to as symptomatic BPH.⁵ BPH is currently the most prevalent urological disease in men.⁵ Current treatment for BPH include surgery, ultrasound and androgen-suppressing therapy.⁵

The "alpha-1A adrenergic receptor," also known as alpha-1A adrenoreceptor, is a G-protein coupled transmembrane receptor that is responsible for prostate smooth muscle contraction.⁷ The alpha-1A adrenergic receptor is present in the greatest concentration in the human prostate⁷ and smooth vascular muscle tissue. Antagonists of this receptor relax the smooth muscles of the prostate, for example, thereby decreasing bladder outlet resistance and facilitating urinary flow without affecting detrusor smooth muscle contractility.⁷

It should be recognized that the alpha-1A adrenergic 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 "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 “ligand binding site” as used herein denotes the site on the alpha-1A adrenergic 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.

“Ligand” or “alpha-1A adrenergic ligand” as used herein denotes a compound that is a binding partner for the alpha-1A adrenergic receptor and is bound thereto by complementarity. The specific region or regions of the ligand that is (are) recognized by the alpha-1A adrenergic receptor is designated as the “ligand binding 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).

It is further understood that the term "ligand" or "alpha-1A adrenergic ligand" is not intended to be limited to compounds known to be useful as alpha-1A adrenergic receptor binding compounds (e.g., known drugs). It should also 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 the 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 alpha-1A adrenergic 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 multi-valency. 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 include terazosin, prazosin, doxasosin, alfuzosin, tamsulosin, RS 100975 (Roche Biosciences), A-131701 (Abbott), L794-191 (Merck), L757464 (Merck), REC 15-2739 (SB216469, Recordati/SKB), KMD-3213 (Kissei Pharmaceuticals), and derivatives thereof. Each of these ligands are known to be useful in the treatment of BPH and/or hypertension.

A "multimeric compound" refers to a compound that may be capable of multivalency as defined below, and which has 2 to 10 ligands covalently bound to one or more linkers which may be the same or different. The compound may or may not possess multibinding properties. At least one of the ligands comprises a
ligand binding domain capable of binding to an alpha-1A adrenergic receptor. The multi-binding compound provides a biological and/or therapeutic effect greater than the aggregate of unlinked ligands equivalent thereto which may be the same or different which unlinked ligands comprise a ligand domain capable of binding to one or more alpha-1A adrenergic receptors. That is to say that the biological and/or therapeutic effect of the alpha-1A adrenergic binding ligands attached to the multi-binding compound is greater than that achieved by the same amount of unlinked alpha-1A adrenergic 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 uni-valency 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 three ligands versus a monovalent binding interaction is shown below:

![Diagrams showing univalent and trivalent interactions](image)

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 multivalency 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 response 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 "partial agonist" refers to a receptor agonist which cannot fully elicit a maximal response when it binds to the receptor, no matter how high the concentration of the partial agonist. A partial agonist is able to combine with the receptor, but the full effect of the binding is not elicited. This term is well known
in the art and a discussion of it may be found in *Textbook of Receptor Pharmacology*, ch 1.4, J. Foreman and T. Johansen eds., CRC Press, 1996.

The term "treatment" refers to the treatment of benign prostatic hyperplasia (BPH) and/or hypertension, particularly in a human, and includes:

(i) alleviating the symptoms of BPH and/or hypertension;
(ii) inhibiting BPH and/or hypertension by prophylactic treatment; or
(iii) relieving or reducing BPH and/or hypertension, e.g., relieving or reducing the duration, intensity and/or severity of the condition.

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 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 which may be chiral or achiral that permits attachment of multiple copies of a ligand (which may be the same or different) thereto. 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 ligands and linkers which comprise the multibinding agents of the invention and the multibinding compounds themselves may have various stereoisomeric forms, including enantiomers and diastereomers. It is to be understood that the invention contemplates all possible stereoisomeric forms of multibinding compounds, and mixtures thereof.

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.

**Methodology**

The linker, when covalently attached to the ligands, provides a biocompatible, substantially non-immunogenic multi-binding compound of this invention. 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. In general, the linker may be chosen from any organic molecule construct that orients two or more ligands to the receptors to permit multi-valency. 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.
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., polylcine, 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,
-43-
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, palmitoylphosphatidylcholine, lysophosphatidylcholine, lysophosphatidyl-ethanolamine, dipalmitoylphosphatidylcholine, dioleoylphosphatidylcholine, distearoylphosphatidylcholine 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 deprotection 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 multi-binding compounds described herein is also within the scope of this invention.

As explained above, the multi-binding compounds described herein comprise 2-10 ligands for alpha-1A adrenergic attached to a linker that links the ligands in such a manner that they are presented to the alpha-1A adrenergic 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 multi-valency, 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. 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 multi-binding 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. Tetravalent compounds can be represented as, for example, in a linear array:

\[ L-X-L-X-L-X-L \]

or in a tetrahedral array:

\[ \text{Diagram of tetrahedral array} \]

where \( X \) and \( L \) are as defined herein.

The same considerations apply to higher multibinding compounds of this invention containing 5-10 ligands. 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 directly 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:

\[ \text{Diagram of cyclic chain} \]

and variants thereof.
All of the above variations are intended to be within the scope of the invention defined by the formula \((L)^p(X)^q\).

In view of the above description of the linker, it is understood that the term “linker” when used in combination with the term “multibinding compound” includes both a covalently contiguous single linker (e.g., L-X-L) and multiple covalently non-contiguous linkers (L-X-L-X-L) within the multibinding compound.

**Preparation of Multibinding Compounds**

The multibinding compounds of this invention can be prepared from readily available starting materials using the following general methods and procedures. It will be appreciated that where typical or preferred process conditions (i.e., reaction temperatures, times, mole ratios of reactants, solvents, pressures, etc.) are given, other process conditions can also be used unless otherwise stated. Optimum reaction conditions may vary with the particular reactants or solvent used, but such conditions can be determined by one skilled in the art by routine optimization procedures.

Additionally, as will be apparent to those skilled in the art, conventional protecting groups may be necessary to prevent certain functional groups from undergoing undesired reactions. The choice of a suitable protecting group for a particular functional group as well as suitable conditions for protection and deprotection are well known in the art. For example, numerous protecting groups, and their introduction and removal, are described in T. W. Greene and G. M. Wuts, *Protecting Groups in Organic Synthesis*, Second Edition, Wiley, New York, 1991, and references cited therein.

Any compound which acts as a ligand toward the alpha-1A adrenergic receptor can be used as a ligand in this invention. It is desirable that the ligand or
ligands be antagonists or partial agonists in order to moderate the activity of the alpha-1A adrenergic receptor or lessen or alleviate the condition of BPH and/or hypertension.

Ligands useful in the compound of this invention include terazosin, prazosin, doxasosin, alfuzosin, tamsulosin, RS 100975 (Roche Biosciences), A-131701 (Abbott), L794-191 (Merck), L757464 (Merck), REC 15-2739 (SB216469, Recordati/SKB), KMD-3213 (Kissei Pharmaceuticals), and derivatives thereof. These ligands may be combined to form homo- or heteromers as described herein. In particular, a multimer may be formed by joining any ligand selected from the group terazosin, prazosin, doxasosin, alfuzosin, tamsulosin, RS 100975 (Roche Biosciences), A-131701 (Abbott), L794-191 (Merck), L757464 (Merck), REC 15-2739 (SB216469, Recordati/SKB), KMD-3213 (Kissei Pharmaceuticals), and derivatives thereof with any one or more ligands selected from the group terazosin, prazosin, doxasosin, alfuzosin, tamsulosin, RS 100975 (Roche Biosciences), A-131701 (Abbott), L794-191 (Merck), L757464 (Merck), REC 15-2739 (SB216469, Recordati/SKB), KMD-3213 (Kissei Pharmaceuticals), and derivatives thereof through a linker. The ligands of the invention will be referred to herein as L-1 through L-11, wherein each of L-1 to L-11 corresponds to a ligand selected from the group terazosin, prazosin, doxasosin, alfuzosin, tamsulosin, RS 100975 (Roche Biosciences), A-131701 (Abbott), L794-191 (Merck), L757464 (Merck), REC 15-2739 (SB216469, Recordati/SKB), KMD-3213 (Kissei Pharmaceuticals), and derivatives thereof. Further, each designation of L-1 through L-11 is meant to include derivatives and analogs of the respective ligand.

Combinatorial Libraries

Combinatorial approaches for identifying multimeric compounds which possess multibinding properties will now be discussed.
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. 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, ability to increase insulin
sensitivity, ability to lower serum triglyceride, cholesterol and/or fatty acid levels, 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 \textit{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.

\textbf{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. 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 monomeric 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, terazosin and alfuzosin 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 terazosin ligand and elements proximal to the formal alfuzosin ligand binding site and between the alfuzosin ligand and elements proximal to the formal terazosin ligand binding site. Thus, the dimeric compound may be a more potent and selective antagonist of the alpha-1A androgenic receptor and a superior therapy for benign prostatic hyperplasia.

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 innocuous 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, divergent 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, 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 multimeric 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.

5 **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

10 (hydrophobicity, hydrophilicity, amphiphilicity, polarization, 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 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*.

15 **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.

20 **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

25 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:


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 functionalities 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 library 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 are 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, ion channel blockers, and antimicrobial activity are also determined. Pharmacological data, including oral absorption, everted gut penetration, other pharmacokinetic parameters and efficacy data are 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 is incorporated herein by reference in its entirety. Alternatively, the structure of relevant multivalent compounds can also be determined from soluble and untagged libraries 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 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, dipseudohalides, diketones, dihalides, diisocyanates, diamines, diols, diboronates, 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, which is exemplary only:
## COMPLEMENTARY BINDING CHEMISTRIES

<table>
<thead>
<tr>
<th>First Reactive Group</th>
<th>Second Reactive Group</th>
<th>Linkage</th>
</tr>
</thead>
<tbody>
<tr>
<td>hydroxyl</td>
<td>isocyanate</td>
<td>urethane</td>
</tr>
<tr>
<td>amine</td>
<td>epoxide</td>
<td>β-hydroxyamine</td>
</tr>
<tr>
<td>5</td>
<td>amine</td>
<td>sulfonamide</td>
</tr>
<tr>
<td>sulfonyl halide</td>
<td>amine</td>
<td>amide</td>
</tr>
<tr>
<td>carboxyl acid</td>
<td>amine</td>
<td>ether</td>
</tr>
<tr>
<td>hydroxyl</td>
<td>alkyl/aryl halide</td>
<td>amine</td>
</tr>
<tr>
<td>aldehyde</td>
<td>amine/NaCNBH₃</td>
<td>amine</td>
</tr>
<tr>
<td>ketone</td>
<td>amine/NaCNBH₃</td>
<td>urea</td>
</tr>
<tr>
<td>10</td>
<td>amine/NaCNBH₃</td>
<td></td>
</tr>
<tr>
<td>amine</td>
<td>isocyanate</td>
<td></td>
</tr>
</tbody>
</table>

Exemplary linkers include the following linkers identified as X-1 through X-418 as set forth below in Table 1:
<table>
<thead>
<tr>
<th>Dialdehydes</th>
<th></th>
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</thead>
<tbody>
<tr>
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<table>
<thead>
<tr>
<th>Dibalides</th>
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<table>
<thead>
<tr>
<th>Disocyanates</th>
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</tbody>
</table>
Representative ligands for use in this invention include, by way of example, L-1 through L-11 as identified above.

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 through L-11 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-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-121 L-1/X-122 L-1/X-123 L-1/X-124 L-1/X-125 L-1/X-126
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-163 L-1/X-164 L-1/X-165 L-1/X-166 L-1/X-167 L-1/X-168
L-1/X-175 L-1/X-176 L-1/X-177 L-1/X-178
L-1/X-203 L-1/X-204 L-1/X-205 L-1/X-206 L-1/X-207 L-1/X-208
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:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity (mg/capsule)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active Ingredient</td>
<td>30.0</td>
</tr>
<tr>
<td>Starch</td>
<td>305.0</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>5.0</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity (mg/tablet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active Ingredient</td>
<td>25.0</td>
</tr>
<tr>
<td>Cellulose, microcrystalline</td>
<td>200.0</td>
</tr>
<tr>
<td>Colloidal silicon dioxide</td>
<td>10.0</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>5.0</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active Ingredient</td>
<td>5</td>
</tr>
<tr>
<td>Lactose</td>
<td>95</td>
</tr>
</tbody>
</table>
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:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity (mg/tablet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active Ingredient</td>
<td>30.0 mg</td>
</tr>
<tr>
<td>Starch</td>
<td>45.0 mg</td>
</tr>
<tr>
<td>Microcrystalline cellulose</td>
<td>35.0 mg</td>
</tr>
<tr>
<td>Polyvinylpyrrolidone (as 10% solution in sterile water)</td>
<td>4.0 mg</td>
</tr>
<tr>
<td>Sodium carboxymethyl starch</td>
<td>4.5 mg</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>0.5 mg</td>
</tr>
<tr>
<td>Talc</td>
<td>1.0 mg</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>120 mg</strong></td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity (mg/capsule)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active Ingredient</td>
<td>40.0 mg</td>
</tr>
<tr>
<td>Starch</td>
<td>109.0 mg</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>1.0 mg</td>
</tr>
<tr>
<td>Total</td>
<td>150.0 mg</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active Ingredient</td>
<td>25 mg</td>
</tr>
<tr>
<td>Saturated fatty acid glycerides to</td>
<td>2,000 mg</td>
</tr>
</tbody>
</table>

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:
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**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity (mg/capsule)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active Ingredient</td>
<td>15.0 mg</td>
</tr>
<tr>
<td>Starch</td>
<td>407.0 mg</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>3.0 mg</td>
</tr>
<tr>
<td>Total</td>
<td>425.0 mg</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active Ingredient</td>
<td>5.0 mg</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>1.0 mL</td>
</tr>
</tbody>
</table>

**Formulation Example 10**

A topical formulation may be prepared as follows:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active Ingredient</td>
<td>1-10 g</td>
</tr>
<tr>
<td>Emulsifying Wax</td>
<td>30 g</td>
</tr>
<tr>
<td>Liquid Paraffin</td>
<td>20 g</td>
</tr>
<tr>
<td>White Soft Paraffin</td>
<td>to 100 g</td>
</tr>
</tbody>
</table>

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

The following assays are used to evaluate the multi-binding compounds of this invention.

In vitro binding assay

Using the alpha-1-adrenoceptor binding assay described by Ford et al. ⁹ the affinity (pKi) with the alpha-1A adrenergic receptor is determined for the multi-binding compounds of this invention.

Ex vivo model

Using the guinea pig prostate assay described by Haynes et al., ¹¹ prostate contractile response to the multi-binding compounds of this invention is measured.
In vivo models

Using the conscious dog model described by Hancock et al., the effects of the multibinding compounds on prostatic muscle response is tested.

Additionally, the challenge intraurethral pressure (“cIUP”) model can be used to measure of efficacy of the multibinding compounds and the spontaneously hypertensive rat (“SHR”) model can be used to measure the selectivity of the multibinding compounds. Both of these models are described by Meyer et al.

Utility

The compounds of this invention are useful for modulating alpha-1A adrenergic receptor activity and accordingly, may be used for the treatment of benign prostatic hyperplasia and/or hypertension in animals, including humans. More particularly the compounds may be used in the treatment of medical and veterinary conditions in mammals.

The compounds of the invention are particularly useful in treating benign prostatic hyperplasia mediated in one form or another by alpha-1A adrenergic 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 Celsius (unless otherwise indicated) and all percentages are weight percentages (also unless otherwise indicated). Further, X as used in the reactive schemes depicted in the Examples below denotes a halide, preferably bromide or chloride, as designated in the accompanying experimental text.

Examples 1-19 are given as representative examples of methods for preparing compounds of this invention.

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-hydroxybenzotriazole
HPLC = high performance liquid chromatography
Hunig's base = diisopropylethylamine
MFC = minimum fungicidal concentration
mg = milligram
-92-

MIC = minimum inhibitory concentration
min = minute
mL = milliliter
mm = millimeter
mmol = millimol
N = normal
PyBOP = pyridine benzotriazol-1-yloxy-tris(dimethyl-aminophosphonium hexafluorophosphate

10

∗-BOC = tert-butyloxycarbonyl
TBAF = tetrabutyl ammonium fluoride
TFA = trifluoroacetic acid
THF = tetrahydrofuran
µL = microliters

Based on the basic pharmacophore for alpha-1A adrenergic receptor antagonists and the current available materials, several classes of bivalent alpha-1A adrenergic receptor antagonists are designed, and the syntheses are described in the following examples.

**EXAMPLE 1**: Preparation of a Formula I compound wherein p is 2, q is 1, and the ligand, L, is the phenethylamine moiety of Tamsulosin linked via the amino group to the linker, X.

![Chem Pharm Bull. v 40 (6) 1992, 1443-1451](image)

A solution of 2,2'-oxybist(ethylamine) (1 mmol) in methanol (4 mL) is acidified with acetic acid to pH 6.6 (pH meter) under a nitrogen atmosphere. The ketone, 1-(4-methoxy-3-aminosulfonyl)phenyl-2-oxopropane (Chem. Pharm. Bull. 1992, 40, 1443-51; 2 mmol) is added neat followed by sodium cyanoborohydride (2.1 mmol). The course of the reaction is followed by thin layer chromatography. After reaction occurs, the reaction solution is quenched in water
and the pH of the aqueous mixture is adjusted to 9-10 with aqueous Na₂CO₃. The mixture is extracted with ether, the organic extracts are washed with half-saturated saline, dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The desired Formula I compound (where R is
-CH₂CH₂OCH₂CH₂-) is obtained by purification of the crude product by use of HPLC.

**EXAMPLE 2:** Preparation of a Formula I compound wherein p is 2, q is 1 and the ligand, L, is O-desethyl-tamsulosin linked via the aryloxy group to the linker, X.

Tamsulosin Analog

R preferably contains alkyl, alkyl-ether linkages, hydroxyl groups, hydrogen bond acceptors, aryloxy, heterocyclic groups.

**Example 2: Tamsulosin Analogs**


Step 1. A solution of 1-(2-aminoethoxy)-2-benzylxoybenzene (Chem. Pharm. Bull. 1988, 36, 4121-35; 1 mmol) in methanol (4 mL) is acidified with
acetic acid to pH 6.6 (pH meter) under a nitrogen atmosphere. The ketone, 1-(4-
methoxy-3-aminosulfonyl)phenyl-2-oxopropane (Chem. Pharm. Bull. 1992, 40,
1443-51; 1 mmol) is added neat followed by sodium cyanoborohydride (1.1 mmol).
The course of the reaction is followed by thin layer chromatography. After
reaction occurs, the reaction solution is quenched in water and the pH of the
aqueous mixture is adjusted to 9-10 with aqueous Na₂CO₃. The mixture is
extracted with ether, the organic extracts are washed with half-saturated saline,
dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude
product. The desired compound is obtained by purification of the crude product by
use of HPLC.

Step 2. A solution of BOC-anhydride (5 mmol) and triethylamine (0.1 mL)
in CH₂Cl₂ (5 mL) is stirred under an inert atmosphere. To this is added a solution
the product of the preceding reaction (2 mmol) in CH₂Cl₂ (2 mL) and the resulting
solution is stirred. The progress of the reaction is followed by TLC and when
complete, the reaction is quenched by the addition of aqueous Na₂CO₃. The
mixture is extracted with CH₂Cl₂, the organic extracts are washed with half-
saturated saline, dried (Na₂SO₄), filtered and concentrated under reduced pressure
to give the crude product. The desired N-BOC-product is obtained by purification
of the crude product by use of HPLC.

Step 3. A solution of the compound from the preceding reaction in ethyl
alcohol (10 mL) is hydrogenated at atmospheric pressure in the presence of 10% palladium-on-carbon (100 mg) until TLC evidence shows that reaction is complete.
The mixture is filtered through Celite and the filter pad is washed thoroughly with ethanol. The combined filtrates are concentrated under reduced pressure to give
the crude product. The desired compound is obtained by purification of the crude product with the use of HPLC.
Step 4. Diethyl azodicarboxylate (2 mmol) is added dropwise via a syringe to a stirred solution of triphenylphosphine (2 mmol) in THF (5 mL) at room temperature. To this is added a solution of the product from the preceding reaction (2 mmol) and 2,2’-(1,2-phenylenedioxy)diethanol (1 mmol) in THF (3 mL). The resulting solution is stirred at RT and the progress of the reaction is followed by tlc. After reaction occurs, solvent is removed by evaporation under reduced pressure and the residue is purified by HPLC, giving the desired compound.

Step 5. A solution of the product from the preceding reaction and trifluoroacetic acid (3 mL) in CH₂Cl₂ (5 mL) is stirred at room temperature. The progress of the reaction is followed by tlc. After reaction occurs, more CH₂Cl₂ is added and the solution is washed with aqueous Na₂CO₃ and with H₂O. The organic layer is dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The desired Formula I compound (where R is 1,2-phenylenedioxyethylene) is obtained by purification of the crude product with the use of HPLC.
**EXAMPLE 3:** Preparation of a Formula I compound wherein \( p \) is 2, \( q \) is 1, and the ligand, \( L \), is tamsulosin linked via the sulfonamide group to the linker, \( X \).

Example 3: Tamsulosin Analogs

\[
\text{H}_2\text{N} \text{-} \text{SO}_3 \text{-} \text{CH}_3 \text{-} \text{CH}_2 \text{-} \text{NH} \text{-} \text{SO}_3 \text{-} \text{CH}_3 \text{-} \text{R}
\]

1. (BOC)\(_2\)  
2. 1,5-dibromopentane, DMF  
3. TFA

\( R = -(\text{CH}_2)_5- \)

Background Chemistry:

\[
\text{H}_2\text{N} \text{-} \text{SO}_3 \text{-} \text{CH}_3 \text{-} \text{CH}_2 \text{-} \text{NH} \text{-} \text{SO}_3 \text{-} \text{CH}_3 \text{-} \text{H}_2\text{O}
\]

1. \( \text{ClSO}_2\text{H} \)  
2. \( \text{NH}_3 \)


Reductive alkylation can be accomplished with chiral borane ligands:

\[
\text{H}_2\text{N} \text{-} \text{SO}_3 \text{-} \text{CH}_3 \text{-} \text{CH}_2 \text{-} \text{NH} \text{-} \text{SO}_3 \text{-} \text{CH}_3 \text{-} \text{HB}(L^*)
\]

See Hett, Robert; Fang, Qin Kevin; Gao, Yun; Hong, Yaping; Butler, Hal T.; et al Tetrahedron Lett. 38; 7; 1997; 1125-1128, for the use of chiral oxazaborolidines in the enantioselective reductive alkylation of 2-phenyl ketones.

Step 1. A solution of BOC-anhydride (5 mmol) and triethylamine (0.1 mL) in \( \text{CH}_2\text{Cl}_2 \) (5 mL) is stirred under an inert atmosphere. To this is added a solution of tamsulosin (2 mmol) in \( \text{CH}_2\text{Cl}_2 \) (2 mL) and the resulting solution is stirred. The reaction is followed by TLC and when complete, is quenched by the addition of aqueous \( \text{Na}_2\text{CO}_3 \). The mixture is extracted with \( \text{CH}_2\text{Cl}_2 \), the organic extracts are washed with half-saturated saline, dried (\( \text{Na}_2\text{SO}_4 \)), filtered and concentrated under reduced pressure to give the crude product. The desired N-BOC-tamsulosin is obtained by purification of the crude product by use of HPLC.
Step 2. A mixture of NaH (2.1 mmol) and DMF (1 mL) is prepared under an inert atmosphere in a flask equipped with a stirring bar and a drying tube. To this is first added a solution of N-BOC-tamsulosin (2 mmol) in dry DMF (3 mL) followed by the linker molecule, 1,5-dibromopentane (1 mmol), in dry DMF (1 mL). The resulting mixture is stirred and the course of the reaction is followed by thin layer chromatography. After reaction occurs, the reaction is quenched with cold dilute aq. Na$_2$CO$_3$ and extracted with methylene chloride. The organic layer is dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

Step 3. A solution of the product (2 mmol) of the preceding reaction and trifluoroacetic acid (3 mL) in CH$_2$Cl$_2$ (5 mL) is stirred at room temperature. The progress of the reaction is followed by tlc. After reaction occurs, more CH$_2$Cl$_2$ is added and the solution is washed with aqueous Na$_2$CO$_3$ and with H$_2$O. The organic layer is dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure to give the crude product. The desired Formula I compound [where R is -(CH$_2$)$_5$-] is obtained by purification of the crude product with the use of HPLC.
EXAMPLE 4: Preparation of a Formula I compound wherein p is 2, q is 1, and the ligand, L, is (R)-KMD-3213 linked via the amide group to the linker, X.

Example 4: KMD-3231 Analogs

Step 1. A solution of KMD-3213 (E.P. 0 600 675 B1; 4 mmol) in methanol (4 mL) and aqueous 1M KOH (8 mL) is stirred under an inert atmosphere and is warmed until reaction occurs. The progress of the reaction is followed by tlc. When reaction is complete, the pH of the solution is adjusted to between 1 and 2 by the addition of 1 N HCl. The solution is then lyophilized and the crude reaction product is dried and used directly in the next step described below.

Step 2. A solution of BOC-anhydride (3 mmol) and triethylamine (0.15 mL) in CH₂Cl₂ (5 mL) is stirred under an inert atmosphere. To this is added a solution the product of the preceding reaction (3 mmol) in CH₂Cl₂ (2 mL) and the resulting solution is stirred. The progress of the reaction is followed by TLC and when complete, the reaction is quenched by the addition of dilute aqueous HCl. The mixture is extracted with CH₂Cl₂, the organic extracts are washed with half-saturated saline, dried (Na₂SO₄), filtered and concentrated under reduced pressure
to give the crude product. The desired N-BOC-product is obtained by purification of the crude product by use of HPLC.

Step 3. A solution of the product (2 mmol) from the preceding reaction, 1,7-diaminoheptane (1 mmol), and 1-hydroxybenzotriazole (2.5 mmol) in dry DMF (5 mL) is cooled in an ice-water bath and stirred under an inert atmosphere. To the stirred solution is added dicyclohexylcarbodiimide (2.1 mmol). The course of the reaction is followed by tlc. The cooling bath is removed and after reaction occurs, the reaction mixture is partitioned between methylene chloride and saturated aqueous NaHCO₃. The organic layer is washed with water and brine, dried and concentrated under reduced pressure. The desired product is obtained by purification of the crude product by use of HPLC.

Step 4. A solution of the product from the preceding reaction (1 mmol) and trifluoroacetic acid (3 mL) in CH₂Cl₂ (5 mL) is stirred at room temperature. The progress of the reaction is followed by tlc. After reaction occurs, more CH₂Cl₂ is added and the solution is washed with aqueous Na₂CO₃ and with H₂O. The organic layer is dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The desired Formula I compound (where R is 1,7-heptanediyl) is obtained by purification of the crude product with the use of HPLC.

The Formula I compound prepared by this method is obtained as a racemate when racemic KMD-3213 is used in Step 1.
EXAMPLE 5: Preparation of a Formula I compound wherein p is 2, q is 1, and the ligand, L, is (R)-5-[2-[N-BOC-2-[2-(2,2,2-trifluoroethoxy)phenoxy]ethylaminol]propyl]indoline-7-carboxamide [N-des-(3-hydroxypropyl)-KMD-3213] linked via the amino group of the dihydroindole to the linker, X.

Example 5: KMD-3231 Analogs


Preparation of 1,5-dibromo-3-pentanol-O-TBDMS.

*tert*-Butyldimethylsilyl chloride (0.1 mol) is added to a solution of 1,5-dibromo-3-pentanol (0.05 mol) and imidazole (0.05 mol) in dry pyridine (10 mL) and the resulting solution is stirred at RT. The progress of the reaction is followed by tlc. When reaction is complete, water (25 mL) is added to the solution which is then concentrated by evaporation under reduced pressure (>25 mm Hg, 30°C). The residue is dissolved in EtOAc and the solution is extracted with saturated aq. CuSO₄ to remove residual pyridine. The EtOAc solution is washed with water, dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The pure product is obtained by purification of the crude product by flash chromatography over silica gel.
Compound Preparation.

Step 1. A solution of (R)-5-[2-[N-BOC-2-[2-(2,2,2-trifluoroethoxy)phenoxy]ethylamino]propyl]indoline-7-carboxamide (EP 0600675, the (R), (S), and racemic forms of this compound may be found as the first three entries in the table on page 24 of this EP Specification; 2 mmol) and 1,5-dibromo-3-pentanol-O-TBDMS (1 mmol), and diisopropylethylamine (0.2 mL) in DMF (3 mL) is stirred and warmed under an inert atmosphere. The progress of the reaction is followed by tlc and when reaction is complete, the solution is poured into aqueous 5% NaHCO₃ and the aqueous mixture is extracted with methylene chloride. The organic extract solution is dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

Step 2. A solution of the compound prepared by the preceding reaction and trifluoroacetic acid (3 mL) in CH₂Cl₂ (5 mL) is stirred at room temperature. The progress of the reaction is followed by tlc. After reaction occurs, more CH₂Cl₂ is added and the solution is washed with aqueous Na₂CO₃ and with H₂O. The organic layer is dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The desired Formula I compound [where R is -CH₂CH₂CH(OH)CH₂CH₂-] is obtained by purification of the crude product with the use of HPLC.

The Formula I compound prepared by this method is of the (S) configuration in both ligands when (S)-KMD-3213 is used in Step 1. Likewise, the Formula 1 compound of (R) configuration in both ligands is obtained when (R)-KMD-3213 is used in Step 1. The Formula 1 compound is obtained as a racemate when racemic KMD-3213 is used in Step 1.
By replacing the 1,5-dibromo-3-pentanol-O-TBDMS of the preceding example with other linker molecules, other compounds of Formula I are prepared.

**EXAMPLE 6**: Preparation of a Formula I compound wherein \( p \) is 2, \( q \) is 1, and the ligand, \( L \), is \((R)\)-KMD-3213 linked via the hydroxyl group to the linker, \( X \).

**Example 6: KMD-3213 Analogs**

[Chemical structure image]


---

**Step 1.** Trimethylsilyl chloride (4 mmol) is added to a solution of \((R)\)-KMD-3213 (2 mmol) and imidazole (2 mol) in dry pyridine (5 mL) and the resulting solution is stirred at RT. The progress of the reaction is followed by tlc. When reaction is complete, water (5 mL) is added to the solution which is then concentrated by evaporation under reduced pressure (>25 mm Hg, 30°C). The residue is dissolved in EtOAc and the solution is extracted with saturated aq. CuSO\(_4\) to remove residual pyridine. The EtOAc solution is washed with water, dried (Na\(_2\)SO\(_4\)), filtered and concentrated under reduced pressure to give the crude product. The pure product, \((R)\)-KMD-3213-O-TMS, is obtained by purification of the crude product by flash chromatography over silica gel.

**Step 2.** A mixture of \((R)\)-KMD-3213-O-TMS (2 mmol), di-tert-butylcarbonate (2.5 mmol), dioxane (5 mL) and aq. 2 N NaOH (2 mL) is stirred at RT for 24 hr. The dioxane is removed by evaporation under reduced pressure.
Water (50 mL) is added to the aqueous mixture and the mixture is extracted with CH₂Cl₂ (4 x 25 mL). The combined organic layers are dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. Pure (R)-N-BOC-KMD-3213-O-TMS is obtained by purification of the crude product with the use of flash chromatography over silica gel.

Step 3. A solution of (R)-N-BOC-KMD-3213-O-TMS (2 mmol), prepared by the preceding reaction, and Et₃N-(HF)₃ in MeCN (5 mL) is stirred at room temperature. After reaction occurs as detected by tlc, the solution is diluted with EtOAc and then washed with water-brine. The organic layer is dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The desired compound (R)-N-BOC-KMD-3213 is obtained by purification of the crude product with the use of HPLC.

Step 4. A mixture of NaH (2.1 mmol) and dry DMF (1 mL) is prepared under an inert atmosphere in a flask equipped with a stirring bar and a drying tube. To this is added a solution of the compound (2 mmol) prepared by the preceding reaction in dry DMF (3 mL). Then a solution of 1,4-diiodobutane (1 mmol) in dry DMF (2 mL) is added and the resulting mixture is stirred, warmed and monitored for reaction by tlc. After reaction occurs, the reaction solution is quenched water (25 mL) and brine (25 mL). The mixture is extracted with CH₂Cl₂ (4 x 20 mL) and the combined organic extracts are back-washed with water (3x). The organic layer is dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product with the use of HPLC.

Step 5. A solution of the product from the preceding reaction and trifluoroacetic acid (3 mL) in CH₂Cl₂ (5 mL) is stirred at room temperature. The progress of the reaction is followed by tlc. After reaction occurs, more CH₂Cl₂ is
added and the solution is washed with aqueous Na₂CO₃ and with H₂O. The organic layer is dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The desired Formula I compound [where R is -(CH₂)$_4$-] is obtained by purification of the crude product with the use of HPLC.

The Formula I compound prepared by this method is obtained as a racemate when racemic KMD-3213 is used in Step 1. The Formula I compound with ligands of (S) configuration is obtained when (S)-KMD-3213 is used in Step 1.

**EXAMPLE 7:** Preparation of a Formula I compound wherein p is 2, q is 1, and the ligand, L, is the N-(3-Hydroxy)propyl-7-aminocarbonyl-5-(2-amino)propylidihydroindole moiety of KMD-3213 linked via the amino group to the linker, X.

**Example 7: KMD-3213 Analogs**

![Chemical Structure](image)

Step 1. A solution of 1-acetyl-5-(2-azidopropyl)indoline-7-carbonitrile (EP 0 600 675 B1; 2 mmol) in aqueous 5 N NaOH (2 mL) and ethanol (4 mL) is stirred at room temperature. The course of the reaction is followed by tlc and when complete, the reaction solution is made alkaline by the addition of cold 1 N NaOH. The mixture is extracted with ether, the ether extracts are washed with water and
with brine, dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure to give the crude product. The desired 5-(2-azidopropyl)indoline-7-carbonitrile is obtained by purification of the crude product with the use of HPLC.

Step 2. To a solution of 5-(2-azidopropyl)indoline-7-carbonitrile (2 mmol) in DMSO (3 mL) is added 30% hydrogen peroxide (0.5 mL). The resulting mixture is stirred at room temperature for 20 minutes and then is transferred into a solution of aqueous 5 N NaOH (0.5 mL). This mixture is stirred at room temperature and the progress of the reaction is followed by tlc. When complete, the mixture is neutralized by the addition of acetic acid, water is added, and the mixture is extracted with ethyl acetate. The organic layer is washed with dilute aqueous sodium carbonate, with water and then is dried, filtered and concentrated under reduced pressure. The desired 5-(2-azidopropyl)indoline-7-carboxamide is obtained by purification of the crude product with the use of HPLC.

Step 3. A solution of 5-(2-azidopropyl)indoline-7-carboxamide (2 mmol) together with 3-bromopropan-1-ol (2 mmol) in dioxane is heated and stirred with potassium carbonate. The progress of the reaction is followed by tlc and when complete, the solvent is removed by evaporation under reduced pressure. The residue is partitioned between dilute aq. sodium bicarbonate and ethyl acetate. The organic extract layer is washed with water and with brine, is dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure to give the crude product. The desired 1-(3-hydroxypropyl)-5-(2-azidopropyl)indoline-7-carboxamide is obtained by purification of the crude product with the use of HPLC.

Step 4. Trimethylsilyl chloride (4 mmol) is added to a solution of 1-(3-hydroxypropyl)-5-(2-azidopropyl)indoline-7-carboxamide (2 mmol) and imidazole (2 mol) in dry pyridine (5 mL) and the resulting solution is stirred at RT. The progress of the reaction is followed by tlc. When reaction is complete, water (5
mL) is added to the solution which is then concentrated by evaporation under reduced pressure (> 25 mm Hg, 30°C). The residue is dissolved in EtOAc and the solution is extracted with saturated aq. CuSO₄ to remove residual pyridine. The EtOAc solution is washed with water, dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The pure 1-(3-hydroxypropyl)-5-(2-azidopropyl)indoline-7-carboxamide-O-TMS is obtained by purification of the crude product by flash chromatography over silica gel.

Step 5. A solution of 1-(3-hydroxypropyl)-5-(2-azidopropyl)indoline-7-carboxamide-O-TMS in ethyl acetate (10 mL) is hydrogenated at atmospheric pressure in the presence of 10% palladium-on-carbon (100 mg) until tlc evidence shows that reaction is complete. The mixture is filtered through Celite and the filter pad is washed thoroughly with ethyl acetate. The combined filtrates are concentrated under reduced pressure to give the crude product. The desired 1-(3-hydroxypropyl)-5-(2-aminopropyl)indoline-7-carboxamide-O-TMS is obtained by purification of the crude product with the use of HPLC.

Step 6. A solution 1-(3-hydroxypropyl)-5-(2-aminopropyl)indoline-7-carboxamide-O-TMS (2 mmol) and 1,2-bis(2-bromoethoxy)benzene (Aldrich; 1 mmol), and diisopropylethylamine (0.2 mL) in DMF (3 mL) is stirred and warmed under an inert atmosphere. The progress of the reaction is followed by tlc and when reaction is complete, the solution is poured into aqueous 5% NaHCO₃ and the aqueous mixture is extracted with methylene chloride. The organic extract solution is dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

Step 7. A solution of the product of the preceding reaction in HOAc-H₂O (4:1) (5 mL) is stirred under an inert atmosphere at room temperature. The
reaction is followed by tlc and when complete, is diluted with EtOAc and washed several times with water and dilute aq. Na$_2$CO$_3$. The organic layer is dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure to give the crude product. The desired Formula I compound is obtained by purification of the crude product by use of HPLC.

**EXAMPLE 8:** Preparation of a Formula I compound wherein p is 2, q is 1, and the ligand, L, is the N-(3-Hydroxy)propyl-7-aminocarbonyl-5-(2-amino)propyldihydroindole moiety of KMD-3213 linked via the amino group to a bis catechol linker, X.

**Example 8: KMD-3213 Analogs**

![Chemical Structure](image)

**Preparation of 1,5-Bis[o-(2-bromoethoxy)phenoxy]pentane.**

Step A. Diethyl azodicarboxylate (2 mmol) is added dropwise via a syringe to a stirred solution of triphenylphosphine (2 mmol) in THF (5 mL) at room temperature. To this is added a solution of o-benzylxyphenol (2 mmol) and 2-bromoethanol (2 mmol) in THF (1 mL). The resulting solution is stirred at RT and the progress of the reaction is followed by tlc. After reaction occurs, solvent is removed by evaporation under reduced pressure and the residue is purified by HPLC, giving the desired 1-benzylxy-2-(2-bromoethoxy)benzene.
Step B. A solution of 1-benzyloxy-2-(2-bromoethoxy)benzene in ethyl acetate (10 mL) is hydrogenated at atmospheric pressure in the presence of 10% palladium-on-carbon (100 mg) until tlc evidence shows that reaction is complete. The mixture is filtered through Celite and the filter pad is washed thoroughly with ethyl acetate. The combined filtrates are concentrated under reduced pressure to give the crude product. The desired o-(2-bromo)ethoxyphenol is obtained by purification of the crude product with the use of HPLC.

Step C. Diethyl azodicarboxylate (2 mmol) is added dropwise via a syringe to a stirred solution of triphenylphosphine (2 mmol) in THF (5 mL) at room temperature. To this is added a solution of o-(2-bromoethoxy)phenol (2 mmol) and pentan-1,5-diol (1 mmol) in THF (1 mL). The resulting solution is stirred at RT and the progress of the reaction is followed by tlc. After reaction occurs, solvent is removed by evaporation under reduced pressure and the residue is purified by HPLC, giving the desired 1,5-bis[o-(2-bromoethoxy)phenoxy]pentane.

Compound Preparation

Step 1. A solution 1-(3-hydroxypropyl)-5-(2-aminopropyl)indoline-7-carboxamide-O-TMS (2 mmol), 1,5-bis[o-(2-bromoethoxy)phenoxy]pentane (1 mmol), and diisopropylethylamine (0.2 mL) in DMF (3 mL) is stirred and warmed under an inert atmosphere. The progress of the reaction is followed by tlc and when reaction is complete, the solution is poured into aqueous 5% NaHCO₃ and the aqueous mixture is extracted with methylene chloride. The organic extract solution is dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

Step 2. A solution of the product of the preceding reaction in HOAc-H₂O (4:1) (5 mL) is stirred under an inert atmosphere at room temperature. The
reaction is followed by tlc and when complete, is diluted with EtOAc and washed several times with water and dilute aq. Na₂CO₃. The organic layer is dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The desired Formula I compound is obtained by purification of the crude product by use of HPLC.

**EXAMPLE 9**: Preparation of a Formula I compound wherein p is 2, q is 1, and the ligand, L, is 4-amino-2-(N-piperazinyl)-6,7-dimethoxy-2-quinazoline linked through the piperazine amino group via an amide to the linker, X.

![Chemical structure](image)


A solution of 4-amino-2-(N-piperazinyl)-6,7-dimethoxy-2-quinazoline (2 mmol), 2,3-dimethoxybenzene-1,4-dicarboxylic acid (1 mmol), and 4-dimethylaminopyridine (10 mg) in CH₂Cl₂ (5 mL) is prepared under argon in a flask equipped with magnetic stirrer and a drying tube. To this solution is added dicyclohexylcarbodiimide (solid, 2.2 mmol). The progress of the reaction is followed by tlc and after reaction occurs, the reaction solution is quenched in water, aqueous sodium bicarbonate is added and the aqueous mixture is extracted with methylene chloride. The organic layer is washed with aqueous Na₂CO₃ and H₂O, dried (Na₂SO₄), filtered and concentrated under reduced pressure to
give the crude product. The desired Formula I compound is obtained by purification of the crude product with the use of HPLC.

**EXAMPLE 10**: Preparation of a Formula I compound wherein \( p \) is 2, \( q \) is 1, and the ligand, \( L \), is 4-amino-2-(N-piperazinyl)-6,7-dimethoxy-2-quinazoline linked through the piperazine amino group via a carbamate to the linker, \( X \).

A solution of di(ethylene glycol) (2 mmol) and pyridine (2.5 mmol) in dry THF (4 mL) is cooled (ice-water bath) and stirred under an inert atmosphere. To this a solution of 4-nitrophenylchloroformate (2.1 mmol) in dry THF (1 mL) is added dropwise. After the addition is completed, the ice-water cooling bath is removed and the solution is stirred and allowed to reach ambient temperature. The reaction is followed by tlc and after reaction is complete, THF is removed under reduced pressure. The residue is protected from atmospheric moisture and is dissolved in dry DMF (2 mL). To this solution are added 4-dimethylaminopyridine (1 mg), 1-hydroxybenzotriazole (3 mmol), and a solution of 4-amino-2-(N-piperazinyl)-6,7-dimethoxy-2-quinazoline (4 mmol) in dry DMF (2 mL). The resulting solution is stirred at room temperature and the progress of the reaction is followed by tlc. When complete, the reaction solution is quenched by addition of cold aq. Na\(_2\)CO\(_3\) and brine. The mixture is extracted with methylene chloride, the organic layers are combined, washed with water and with brine, are dried (Na\(_2\)SO\(_4\)), filtered and concentrated under reduced pressure to give the crude
product. The desired Formula I compound (where R is -CH₂CH₂OCH₂CH₂-) is obtained by purification of the crude product by use of HPLC.

EXAMPLE 11: Preparation of a Formula I compound wherein ligand, L₁, the phenethylamine moiety of Tamsulosin is linked via the amino group to ligand, L₂, the phenylbutoxyhexyl moiety of Salmeterol.

\[
\text{Example 11: Salmeterol analogs}
\]

Step 1. A solution of benzylamine (2 mmol) in methanol (4 mL) is acidified with acetic acid to pH 6.6 (pH meter) under a nitrogen atmosphere. The ketone, 1-(4-methoxy-3-aminosulfonyl)phenyl-2-oxopropane (Chem. Pharm. Bull. 1992, 40, 1443-51; 2 mmol) is added neat followed by sodium cyanoborohydride (2.1 mmol). The course of the reaction is followed by thin layer chromatography. After reaction occurs, the reaction solution is quenched in water and the pH of the aqueous mixture is adjusted to 9-10 with aqueous Na₂CO₃. The mixture is extracted with ether, the organic extracts are washed with half-saturated saline, dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

Step 2. A solution of the product of the preceding reaction and n-(4-phenyl)butyl-n-(6-bromo)hexyl ether (or 1-bromo-11-phenyl-7-oxaundecane) (2 mmol), and diisopropylethylamine (0.2 mL) in DMF (3 mL) is stirred and warmed under an inert atmosphere. The progress of the reaction is followed by tlc and
when reaction is complete, the solution is poured into aqueous 5% NaHCO₃ and the aqueous mixture is extracted with methylene chloride. The organic extract solution is dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

Step 3. A solution of the compound from the preceding reaction in ethanol (5 mL) is stirred under hydrogen at atmospheric pressure in the presence of 10% palladium-on-carbon (100 mg) until tlc evidence shows that reaction is complete. The mixture is filtered through Celite and the filter pad is washed thoroughly with ethyl acetate. The combined filtrates are concentrated under reduced pressure to give the crude product. The desired Formula I compound is obtained by purification of the crude product with the use of HPLC.

**EXAMPLE 12:** Preparation of a Formula I compound wherein ligand, L₁, the dihydroindole moiety of KMD-3213 is linked via the amino group to ligand, L₂, the phenylbutoxyhexyl moiety of Salmeterol.

**Example 12: Salmeterol analogs**

![Diagram of molecular structures]


Step 1. A solution of 1-(3-hydroxypropyl)-5-(2-aminopropyl)indoline-7-carboxamide-O-TMS (2 mmol) in methanol (4 mL) is acidified with acetic acid to pH 6.6 (pH meter) under a nitrogen atmosphere. Benzaldehyde (2.2 mmol) is
added neat followed by sodium cyanoborohydride (2.1 mmol). The course of the reaction is followed by thin layer chromatography. After reaction occurs, the reaction solution is quenched in water and the pH of the aqueous mixture is adjusted to 9-10 with aqueous Na₂CO₃. The mixture is extracted with ether, the organic extracts are washed with half-saturated saline, dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

Step 2. A solution of the product of the preceding reaction and n-(4-phenyl)butyl-n-(6-bromo)hexyl ether (or 1-bromo-11-phenyl-7-oxaundecane) (2 mmol), and diisopropylethylamine (0.2 mL) in DMF (3 mL) is stirred and warmed under an inert atmosphere. The progress of the reaction is followed by tlc and when reaction is complete, the solution is poured into aqueous 5% NaHCO₃ and the aqueous mixture is extracted with methylene chloride. The organic extract solution is dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

Step 3. A solution of the compound from the preceding reaction in ethanol (5 mL) is stirred under hydrogen at atmospheric pressure in the presence of 10% palladium-on-carbon (100 mg) until tlc evidence shows that reaction is complete. The mixture is filtered through Celite and the filter pad is washed thoroughly with ethyl acetate. The combined filtrates are concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product with the use of HPLC.

Step 4. A solution of the product of the preceding reaction in HOAc-H₂O (4:1) (5 mL) is stirred under an inert atmosphere at room temperature. The reaction is followed by tlc and when complete, is diluted with EtOAc and washed
several times with water and dilute aq. Na₂CO₃. The organic layer is dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The desired Formula I compound is obtained by purification of the crude product by use of HPLC.

**EXAMPLE 13:** Preparation of a Formula I compound wherein ligand, L₁, the dihydroindole moiety of KMD-3213 is linked via the amino group to ligand, L₂, the phenethylamine moiety of Tamsulosin. (See Fig. above.)

Step 1. A solution of 1-(3-hydroxypropyl)-5-(2-aminopropyl)indoline-7-carboxamide-O-TMS (2 mmol) in methanol (4 mL) is acidified with acetic acid to pH 6.6 (pH meter) under a nitrogen atmosphere. 1-(4-methoxy-3-aminosulfonyl)phenyl-2-oxopropane (2 mmol) is added neat followed by sodium cyanoborohydride (2.1 mmol). The course of the reaction is followed by thin layer
chromatography. After reaction occurs, the reaction solution is quenched in water and the pH of the aqueous mixture is adjusted to 9-10 with aqueous Na$_2$CO$_3$. The mixture is extracted with ether, the organic extracts are washed with half-saturated saline, dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

Step 2. A solution of the product of the preceding reaction in HOAc-H$_2$O (4:1) (5 mL) is stirred under an inert atmosphere at room temperature. The reaction is followed by tlc and when complete, is diluted with EtOAc and washed several times with water and dilute aq. Na$_2$CO$_3$. The organic layer is dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure to give the crude product. The desired Formula I compound is obtained by purification of the crude product by use of HPLC.

**EXAMPLE 14**: Preparation of a Formula I compound wherein ligand, L$_1$, the dihydroindole moiety of KMD-3213 is linked via the amino group to ligand, L$_2$, the desmethyl-phenethylamine moiety of Tamsulosin. (See Fig. above.)

Step 1. A solution of 1-(3-hydroxypropyl)-5-(2-aminopropyl)indoline-7-carboxamide-O-TMS (2 mmol) in methanol (4 mL) is acidified with acetic acid to pH 6.6 (pH meter) under a nitrogen atmosphere. 1-(4-methoxy-3-aminosulfonyl)phenylacetaldehyde (2 mmol) is added neat followed by sodium cyanoborohydride (2.1 mmol). The course of the reaction is followed by thin layer chromatography. After reaction occurs, the reaction solution is quenched in water and the pH of the aqueous mixture is adjusted to 9-10 with aqueous Na$_2$CO$_3$. The mixture is extracted with ether, the organic extracts are washed with half-saturated saline, dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure to give
the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

Step 2. A solution of the product of the preceding reaction in HOAc-H₂O (4:1) (5 mL) is stirred under an inert atmosphere at room temperature. The reaction is followed by tlc and when complete, is diluted with EtOAc and washed several times with water and dilute aq. Na₂CO₃. The organic layer is dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The desired Formula I compound is obtained by purification of the crude product by use of HPLC.

EXAMPLE 15: Preparation of a Formula I compound wherein p is 2, q is 1, and the ligand, L, is the compound prepared in Example 13 linked through the chain amino group to the linker, X.

Step 1. A solution of the product from Example 13, Step 1 (2 mmol) and 2,2’-(1,2-phenylenedioxy)diethylbromide (1 mmol), and diisopropylethylamine (0.2 mL) in DMF (3 mL) is stirred and warmed under an inert atmosphere. The progress of the reaction is followed by tlc and when reaction is complete, the solution is poured into aqueous 5% NaHCO₃ and the aqueous mixture is extracted with methylene chloride. The organic extract solution is dried (Na₂SO₄), filtered
and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

Step 2. A solution of the product of the preceding reaction in HOAc-H₂O (4:1) (5 mL) is stirred under an inert atmosphere at room temperature. The reaction is followed by tlc and when complete, is diluted with EtOAc and washed several times with water and dilute aq. Na₂CO₃. The organic layer is dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The desired Formula I compound is obtained by purification of the crude product by use of HPLC.

**EXAMPLE 16**: Preparation of a Formula I compound wherein p is 2, q is 1, and the ligand, L, is the compound prepared in Example 14 linked through the chain amino group to the linker, X.

Step 1. A solution of the product from Example 14, Step 1 (2 mmol) and 2-bromoethyl ether (1 mmol), and diisopropylethylamine (0.2 mL) in DMF (3 mL) is stirred and warmed under an inert atmosphere. The progress of the reaction is followed by tlc and when reaction is complete, the solution is poured into aqueous
5% NaHCO₃ and the aqueous mixture is extracted with methylene chloride. The organic extract solution is dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

Step 2. A solution of the product of the preceding reaction in HOAc-H₂O (4:1) (5 mL) is stirred under an inert atmosphere at room temperature. The reaction is followed by tlc and when complete, is diluted with EtOAc and washed several times with water and dilute aq. Na₂CO₃. The organic layer is dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The desired Formula I compound is obtained by purification of the crude product by use of HPLC.

**EXAMPLE 17**: Preparation of a Formula I compound wherein p is 2, q is 1, and wherein one ligand L₁, is the dihydroindole moiety of KMD-3213 linked via the chain amino group to the linker, X, and the second ligand, L₂, is the phenethylamine moiety of Tamsulosin linked to X via the amino group.
Step 1. A solution of 4-amino-1-butanol (2 mmol) in methanol (4 mL) is acidified with acetic acid to pH 6.6 (pH meter) under a nitrogen atmosphere. 1-(4-methoxy-3-aminosulfonyl)phenyl-2-oxopropane (2 mmol) is added neat followed by sodium cyanoborohydride (2.1 mmol). The course of the reaction is followed by thin layer chromatography. After reaction occurs, the reaction solution is quenched in water and the pH of the aqueous mixture is adjusted to 9-10 with aqueous Na$_2$CO$_3$. The mixture is extracted with ether, the organic extracts are washed with half-saturated saline, dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

Step 2. A solution of the product of the preceding reaction (2 mmol) in pyridine (5 mL) containing 4-dimethylaminopyridine (2-10 mg) is cooled in an ice bath and benzyl chloroformate (0.5 mL) is added. The cooling bath is removed and the reaction solution is stirred at room temperature. Progress of the reaction is followed by tlc and when complete, the reaction is diluted with ethyl acetate, washed with 5% aq. sodium bisulfate, dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

Step 3. A solution of the product of the preceding reaction (2 mmol) and 4-methylmorpholine N-oxide (NMO, 3 mmol) in MeCN (10 mL) and methylene chloride (5 mL) is treated with 4-A molecular sieves (50 mg) and stirred at ambient temperature for 10 min. Tetrapropylammonium perruthenate (TPAP, 0.05 mmol) is added and the reaction mixture is stirred at room temperature. The progress of the reaction is followed by tlc and when complete, the reaction is diluted with methylene chloride and the mixture is filtered through silica gel. The filtrate is concentrated, giving the crude product.
Step 4. A solution of the product from Example 12, Step 1 (2 mmol) in methanol (4 mL) is acidified with acetic acid to pH 6.6 (pH meter) under a nitrogen atmosphere. To this is added a solution of the aldehyde (2 mmol) prepared by the preceding step in methanol (1 mL) followed by sodium cyanoborohydride (3 mmol). The course of the reaction is followed by thin layer chromatography. After reaction occurs, the reaction solution is quenched in water and the pH of the aqueous mixture is adjusted to 9-10 with aqueous Na₂CO₃. The mixture is extracted with ether, the organic extracts are washed with half-saturated saline, dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

Step 5. A solution of the product of the preceding reaction in HOAc-H₂O (4:1) (5 mL) is stirred under an inert atmosphere at room temperature. The reaction is followed by tlc and when complete, is diluted with EtOAc and washed several times with water and dilute aq. Na₂CO₃. The organic layer is dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

Step 6. A solution of the compound from the preceding reaction in ethyl acetate (10 mL) is hydrogenated at atmospheric pressure in the presence of 10% palladium-on-carbon (100 mg) until tlc evidence shows that reaction is complete. The mixture is filtered through Celite and the filter pad is washed thoroughly with ethyl acetate. The combined filtrates are concentrated under reduced pressure to give the crude product. The desired Formula I compound is obtained by purification of the crude product with the use of HPLC.
EXAMPLE 18: Preparation of a Formula I compound wherein p is 2, q is 1, and wherein one ligand L₁, is the dihydroindole moiety of KMD-3213 linked via the chain amino group to the linker, X, and the second ligand, L₂, is the (4-amino-6,7-dimethoxy-2-quinazolinyl)piperazine moiety of Cardura linked to X via the piperazine amino group. (See Fig. above.)

Step 1. A mixture of 4-amino-2-(N-piperazinyl)-6,7-dimethoxy-2-quinazoline (2 mmol), dicyclohexylcarbodiimide (2.2 mmol), and 4-dimethylaminopyridine (10 mg) in CH₂Cl₂ (5 mL) is prepared under nitrogen in a flask equipped with magnetic stirrer and a drying tube. To the stirred mixture is added a solution of 6-bromohexanoic acid (1 mmol) in CH₂Cl₂ (3 mL). The
mixture is stirred at room temperature and the progress of the reaction is followed by tlc. After reaction occurs, the reaction solution is quenched in water, aqueous sodium bicarbonate is added and the aqueous mixture is extracted with methylene chloride. The organic layer is washed with aqueous Na₂CO₃ and with H₂O, dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product with the use of HPLC.

Step 2. A solution of the product of the preceding reaction (1 mmol) and the dihydroindole derivative prepared in Example 12, Step 1 (1 mmol), and diisopropylethylamine (0.2 mL) in DMF (3 mL) is stirred and warmed under an inert atmosphere. The progress of the reaction is followed by tlc and when reaction is complete, the solution is poured into aqueous 5% NaHCO₃ and the aqueous mixture is extracted with methylene chloride. The organic extract solution is dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

Step 3. A solution of the compound of the preceding reaction in ethyl acetate (10 mL) is hydrogenated at atmospheric pressure in the presence of 10% palladium-on-carbon (100 mg) until tlc evidence shows that reaction is complete. The mixture is filtered through Celite and the filter pad is washed thoroughly with ethyl acetate. The combined filtrates are concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product with the use of HPLC.

Step 4. A solution of the product of the preceding reaction in HOAc-H₂O (4:1) (5 mL) is stirred under an inert atmosphere at room temperature. The reaction is followed by tlc and when complete, is diluted with EtOAc and washed.
several times with water and dilute aq. Na$_2$CO$_3$. The organic layer is dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure to give the crude product. The desired Formula I compound, where R is -(CH$_2$)$_4$-, is obtained by purification of the crude product by use of HPLC.

**EXAMPLE 19:** Preparation of a Formula I compound wherein p is 2, q is 1, and wherein one ligand L$_1$, is the phenethyamine moiety of Tamsulosin linked via the chain amino group to the linker, X, and the second ligand, L$_2$, is the (4-amino-6,7-dimethoxy-2-quinazoliny1)piperazine moiety of Cardura linked to X via the piperazine amino group. (See Fig. above.)

Step 1. A solution of the product prepared by the method of Example 17, Step 1 (1 mmol) and 1-(4-methoxy-3-aminosulfonyl)phenyl-2-benzylaminopropane (prepared as in Example 11, Step 1; 1 mmol), and diisopropylethylamine (0.2 mL) in DMF (3 mL) is stirred and warmed under an inert atmosphere. The progress of the reaction is followed by tlc and when reaction is complete, the solution is poured into aqueous 5% NaHCO$_3$ and the aqueous mixture is extracted with methylene chloride. The organic extract solution is dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

Step 2. A solution of the compound of the preceding reaction in ethyl acetate (10 mL) is hydrogenated at atmospheric pressure in the presence of 10% palladium-on-carbon (100 mg) until tlc evidence shows that reaction is complete. The mixture is filtered through Celite and the filter pad is washed thoroughly with ethyl acetate. The combined filtrates are concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product with the use of HPLC.
Step 3. A solution of the product of the preceding reaction in HOAc-H$_2$O (4:1) (5 mL) is stirred under an inert atmosphere at room temperature. The reaction is followed by tlc and when complete, is diluted with EtOAc and washed several times with water and dilute aq. Na$_2$CO$_3$. The organic layer is dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure to give the crude product. The desired Formula I compound [where R is -(CH$_2$)$_4$-] is obtained by purification of the crude product by use of HPLC.

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 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 or different, at least one of said ligands comprising a ligand binding domain capable of binding to an alpha-1A adrenergic receptor.

2. The multi-binding compound according to Claim 1 wherein at least two of the ligands comprises a ligand binding domain capable of binding to an alpha-1A adrenergic receptor.

3. A multibinding compound represented by Formula I:

\[(L)_p(X)_q\]  

(I)

and pharmaceutically acceptable salts thereof;

wherein:

- each L is a ligand that may be the same or different at each occurrence;
- each X is a linker that may be the same or different at each occurrence;
- p is an integer of from 2 to 10; and
- q is an integer of from 1 to 20;

wherein each of said ligands comprises a ligand domain capable of binding to an alpha-1A adrenergic receptor, and where q is less than p.

4. The multibinding compound of claim 3, wherein each of said ligands is capable of modulating the activity of the alpha-1A adrenergic receptor.

5. The multibinding compound of claim 4, wherein each ligand capable of binding to the alpha-1A adrenergic receptor is independently selected from the group consisting of terazosin, prazosin, doxasosin, alfuzosin, tamsulosin, RS
6. The multibinding compound of claim 4, wherein each divalent linker X is independently selected from a structure of Table 1.

7. The multibinding compound of claim 6, wherein p is an integer of from 2 to 4, and q is less than p.

8. 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, at least one of said ligands comprising a ligand domain capable of binding to one or more alpha-1A adrenergic receptors.

9. The pharmaceutical composition according to Claim 8 wherein said ligands comprising a ligand domain capable of binding to one or more alpha-1A adrenergic receptors modulate benign prostatic hyperplasia and/or hypertension in mammals.

10. The pharmaceutical composition according to Claim 9 wherein said ligands are selected from the group consisting of terazosin, prazosin, doxazosin, alfuzosin, tamsulosin, RS 100975, A-131701, L794-191, L757464, REC 15-2739 and KMD-3213, and derivatives thereof.

11. A pharmaceutical composition comprising a pharmaceutically acceptable excipient and a therapeutically effective amount of one or more multibinding compounds represented by Formula I,

\[(L)_p(X)_q\]  

(I)
and pharmaceutically acceptable salts thereof;
wherein:

- each L is a ligand that may be the same or different at each occurrence;
- each X is a linker that may be the same or different at each occurrence;

\[ p \] is an integer of from 2 to 10; and
\[ q \] is an integer of from 1 to 20;

wherein each of said ligands comprises a ligand domain capable of binding to an alpha-1A adrenergic receptor, and where \( q \) is less than \( p \).

12. The pharmaceutical composition of claim 11, wherein said multibinding compound is capable of modulating activity of the alpha-1A adrenergic receptor.

13. The pharmaceutical composition of claim 12, wherein each ligand is independently selected from the group consisting of terazosin, prazosin, doxazosin, alfuzosin, tamsulosin, RS 100975, A-131701, L794-191, L757464, REC 15-2739, KMD-3213 and derivatives thereof.

14. The pharmaceutical composition of claim 12, wherein each linker X is independently selected from a structure of Table 1.

15. The pharmaceutical composition of claim 14, wherein \( p \) is an integer of from 2 to 4, and \( q \) is less than \( p \).

16. A method of preparing a multibinding compound represented by formula I:

\[
(L)_p(X)_q
\]

wherein each L is a ligand that may be the same or different at each occurrence;
\( X \) is a linker that may be the same or different at each occurrence;
\( p \) is an integer of from 2 to 10; and
\( q \) is an integer of from 1 to 20;
wherein each of said ligands comprises a ligand domain capable of binding to an alpha-1A adrenergic receptor, and where \( q \) is less than \( p \),
(a) providing at least \( p \) equivalents of a ligand \( L \) or precursors thereof and at least \( q \) equivalents of linker or linkers \( X \); and
(b) covalently attaching said ligands to said linkers to produce a multibinding compound; or
(b') covalently attaching said ligand precursors to said linkers and completing the synthesis of said ligands thereupon, thereby to produce a multibinding compound.

17. The method of claim 16, wherein \( p \) is an integer of from 2 to 4, and \( q \) is less than \( p \).

18. The method of claim 16, wherein each ligand is independently selected from the group consisting of terazosin, prazosin, doxasosin, alfuzosin, tamsulosin, RS 100975, A-131701, L794-191, L757464, REC 15-2739, KMD-3213 and derivatives thereof.

19. A method for treating benign prostatic hyperplasia and/or hypertension in a mammal which method comprises administering to said mammal an effective amount of 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, at least one of said ligands comprising a ligand domain capable of binding to one or more an alpha-1A adrenergic receptors

20. A method for modulating or alleviating benign prostatic hyperplasia and/or hypertension in a mammal, which method comprises administering to a mammal in
need of such treatment a therapeutically effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable excipient and one or more multibinding compounds represented by formula I,

\[(L)_p(X)_q\] (I)

and pharmaceutically acceptable salts thereof,

wherein

each L is a ligand that may be the same or different at each occurrence;

X is a linker that may be the same or different at each occurrence;

p is an integer of from 2 to 10; and

q is an integer of from 1 to 20;

wherein each of said ligands comprises a ligand domain capable of binding to an alpha-1A adrenergic receptor, and where q is less than p.

21. The method of claim 20, wherein p is an integer of from 2 to 4 and q is less than p.

22. The method according to Claim 20 wherein the ligand is selected from the group consisting of terazosin, prazosin, doxasosin, alfuzosin, tamsulosin, RS 100975, A-131701, L794-191, L757464, REC 15-2739, KMD-3213 and derivatives thereof.

23. A method for identifying multimeric ligand compounds possessing multibinding properties which method comprises:

(a) identifying a ligand or a mixture of ligands wherein each ligand contains at least one reactive functionality and has a ligand binding domain capable of binding to an alpha-1A adrenergic receptor;

(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.

24. A method for identifying multimeric ligand compounds possessing multibinding properties which method comprises:

(a) identifying a library of ligands wherein each ligand contains at least one reactive functionality and has a ligand binding domain capable of binding to an alpha-1A adrenergic receptor;

(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.

25. The method according to Claim 23 or 24 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).

26. The method according to Claim 25 wherein the multimeric ligand compounds comprising the multimeric ligand compound library are dimeric.

27. The method according to Claim 26 wherein the dimeric ligand compounds comprising the dimeric ligand compound library are heterodimeric.

28. The method according to Claim 27 wherein the heterodimeric ligand compound library is prepared by sequential addition of a first and second ligand.

29. The method according to Claim 23 or 24 wherein, prior to procedure (d), each member of the multimeric ligand compound library is isolated from the library.

30. The method according to Claim 29 wherein each member of the library is isolated by preparative liquid chromatography mass spectrometry (LCMS).

31. The method according to Claim 23 or Claim 24 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.

32. The method according to Claim 31 wherein the linkers comprise linkers of different chain length and/or having different complementary reactive groups.
33. The method according to Claim 32 wherein the linkers are selected to have different linker lengths ranging from about 2 to 100Å.

34. The method according to Claim 23 or 24 wherein the ligand or mixture of ligands is selected to have reactive functionality at different sites on said ligands.

35. The method according to Claim 34 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.

36. The method according to Claim 23 or Claim 24 wherein the multimeric ligand compound library comprises homomeric ligand compounds.

37. The method according to Claim 23 or Claim 24 wherein the multimeric ligand compound library comprises heteromeric ligand compounds.

38. A library of multimeric ligand compounds which may possess multivalent properties which library is prepared by the method comprising:
   (a) identifying a ligand or a mixture of ligands wherein each ligand contains at least one reactive functionality and has a ligand binding domain capable of binding to an alpha-1A adrenergic receptor;
   (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.

39. A library of multimeric ligand compounds which may possess multivalent properties which library is prepared by the method comprising:
   (a) identifying a library of ligands wherein each ligand contains at least one reactive functionality and has a ligand binding domain capable of binding to an alpha-1A adrenergic receptor;
   (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.

40. The library according to Claim 38 or Claim 39 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.

41. The library according to Claim 40 wherein the linkers comprise linkers of different chain length and/or having different complementary reactive groups.
42. The library according to Claim 41 wherein the linkers are selected to have different linker lengths ranging from about 2 to 100 Å.

43. The library according to Claim 38 or 39 wherein the ligand or mixture of ligands is selected to have reactive functionality at different sites on said ligands.

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44. The library according to Claim 43 wherein said reactive functionality is selected from the group consisting of carboxylic acids, carboxylic acid halides, carboxyl esters, amines, halides, pseudo-halides, 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.

45. The library according to Claim 38 or Claim 39 wherein the multimeric ligand compound library comprises homomeric ligand compounds.

46. The library according to Claim 38 or Claim 39 wherein the multimeric ligand compound library comprises heteromeric ligand compounds.

47. An iterative 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 an alpha-1A adrenergic receptor with a linker or mixture of linkers wherein said ligand or mixture of ligands comprises at least one reactive functionality and has a ligand binding domain capable of binding to an alpha-1A adrenergic receptor, 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.

48. The method according to Claim 47 wherein steps (e) and (f) are repeated from 2-50 times.

49. The method according to Claim 47 wherein steps (e) and (f) are repeated from 5-50 times.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.
US CL : Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/1.11, 9.1, 178.1, 193.1; 435/7.1, 7.2; 436/501, 518; 530/345, 389.1, 402, 807; 549/449

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tr>
<td>X</td>
<td>US 4,304,721 A (FAHRENHOLTZ et al) 08 December 1981 (08/12/81), see entire document, especially Abstract and column 23 line 36 through column 24 line 38.</td>
<td>1, 8</td>
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</tbody>
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[X] Further documents are listed in the continuation of Box C.  See patent family annex.

Date of the actual completion of the international search 24 OCTOBER 1999

Date of mailing of the international search report 18 NOV 1999

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<table>
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<td>Y</td>
<td>US 4,587,046 A (GOODMAN et al) 06 May 1986 (05/06/86), see entire document, especially Abstract and columns 4-7.</td>
<td>1-49</td>
</tr>
<tr>
<td>Y</td>
<td>SHUKER et al. Discovering High-Affinity Ligands for Proteins: SAR by NMR. Science. 29 November 1996, Vol. 274, pages 1531-1534, see entire article, especially Figure 1.</td>
<td>23-4923-4</td>
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</table>
A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):
A61K 38/00, 39/00, 39/44, 39/395, 51/00; C07D 317/10; C07K 2/00, 4/00; G01N 33/53, 33/543, 33/566

A. CLASSIFICATION OF SUBJECT MATTER:
US CL:
424/1.11, 9.1, 178.1, 193.1; 435/7.1, 7.2; 436/501, 518; 530/345, 389.1, 402, 807; 549/449

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):
WEST, STN (CAPLUS, BIOSIS, MEDLINE, SCISEARCH)
Search terms: alpha adrenergic receptor, multibinding, polyvalent, multimeric, ligand, link?, bind?, combinatorial, library