METHODS OF MAKING PHARMACOKINETICALLY IMPROVED COMPOUNDS COMPRISING FUNCTIONAL RESIDUES OR GROUPS AND PHARMACEUTICAL COMPOSITIONS COMPRISING SAID COMPOUNDS

Background of the Invention

[0001] The physiological and clinical effects of inhibitors of cyclic guanosine 3',5'-monophosphate specific phosphodiesterase (cGMP-specific PDE) suggest that such inhibitors have utility in a variety of disease states in which modulation of smooth muscle, renal, hemostatic, inflammatory, and/or endocrine function is desired. Type 5 cGMP-specific phosphodiesterase (PDE5) is the major cGMP hydrolyzing enzyme in vascular smooth muscle. Thus, an inhibitor of PDE5 may be indicated in the treatment of cardivascular disorders, including but not limited to hypertension, cerebrovascular disorders, and disorders of the urogenital system, particularly erectile dysfunction.

[0002] Pharmaceutical products that provide selective inhibition of PDE5 are currently available. Vardenafil, marketed under the tradename Levitra® is a potent and selective inhibitor of PDE5 and is currently indicated for the treatment of erectile dysfunction. There is a present need to improve the pharmacokinetic properties of PDE5 inhibitors.

[0003] The development of a new pharmaceutical agent requires careful optimization of the chemical and biological properties of a lead compound. For example, a successful drug candidate must be safe and effective for its intended use. Further, the compound must possess desired pharmacokinetic and pharmacodynamic profiles. This arduous development process usually requires extensive experimentation. In many cases, the process for determining the optimal compound can often require preparation of thousands of structurally similar compounds.

[0004] Among the properties that can limit the utility of a potential pharmaceutical agent is the degree to which the compound is complexed to proteins *in vivo*. If a high percentage of the compound present *in vivo* is non-specifically bound, for example by components of blood and blood plasma, this leaves only a very small amount of free compound available to tissue to perform its therapeutic function. Thus, binding of the

compound to various proteins and other plasma components may require an unacceptably large dosage of compound to achieve the desired therapeutic effect.

[0005] Traditional approaches have sought to alter pharmacokinetic properties.

[0006] Pegylation, the process of the conjugating or linking of biomolecules and drug delivery systems, e.g. liposomes, proteins, enzymes, drugs, nanoparticles, with polyethylene glycol, is a known method for altering pharmacokinetics by improving the circulating half-life of protein and liposomal pharmaceuticals. (See, Bhadra et al. Pharmazie 2002 Jan; 5791):5-29) Pegylated drugs have a large molecular weight polyethylene glycol (PEG) shell around the drug which protects the drug from enzymatic degradation, and allows the drug to cross the gut, i.e. provides oral availability and also acts as a shield to prevent recognition of the pegylated drug by cells of the immune system and protects the drug from renal clearance. (See, Molineux, Cancer Treat Rev. 2002 Apr, 28 Suppl A:13-16) As a result, pegylated proteins, for example, have improved pharmacokinetics due to decreased hydrolysis and a longer circulating half-life. Anticancer agents have a suboptimal pharmacokinetic profile that requires prolonged or repetitive administration of the drug. Pegylated anticancer agents, e.g. pegfilgrastim, a pegylated filgrastim, have been shown to maintain drug efficacy and patient tolerability that are at least equivalent to those of unmodified filgrastim with only one administration per chemotherapy cycle. (See, Crawford, Cancer Treat Rev. 2002 Apr; 28 Suppl A:7-11) Pegylated liposomal doxorubicin, another chemotherapeutic agent, has been found to be more effective and less cardiotoxic than the unpegylated or liposome-encapsulated doxorubicin. (See, Crawford, 2002). In addition to improved pharmacokinetics, pegylated drugs permit reduced dosing schedules, e.g. a fixed dose rather than a weight based dose. (See, Yowell and Blackwell, Cancer Treat Rev. 2002 Apr; 28 Suppl A:3-6) Since the PEG size, its geometry and attachment site of the pegylated therapeutic agent, e.g. proteins, determine the drug pharmacokinetics, therapeutic agents must be designed on a protein-by-protein basis. (See, Harris et al. Clin. Pharmacokinet. 2001, 40(7):539-551) A shortcoming of pegylated agents is potential reduced drug activity at the target site due to steric hindrance of the large PEG molecule. PEG molecule size is more of a concern in small molecules than with proteins.

[0007] The present invention relates to improvement of the compound by designing it such that it incorporates at least one functional unit or group in the compound, thereby improving its pharmacokinetic properties. In an embodiment, the present invention relates to improvement of the pharmacokinetic profile of a compound by attaching at least one functional unit or group to the compound, thereby improving its non-specific binding characteristics and pharmacokinetic properties. In one embodiment of the invention, at least one sarcosine residue or sarcosine derivative may be attached to the compound. The sarcosine unit serves to decrease protein binding, thereby increasing the amount of free form of the compound. The functional residues which are attached to a compound differ in their chemical structure from the groups used in PEG techniques, e.g. the functional residue may be an ethylene glycol derivative, the functional residues are of a significantly smaller molecular weight, e.g. approximately MW of 100 daltons compared to 5000 daltons or more used in standard pegylation. Accordingly, the chemical or biological activity of compounds comprising the functional residues of the present invention is not altered due to less steric hindrance and greater drug accessibility to the target site(s) of the compound.

Summary of the Invention

[0008] The present invention relates to compounds comprising at least one functional unit or group, wherein the functional residue is a hydrophobic group, an ether, an oligo(ethylene glycol) group or a derivative thereof, an amine, an ammonium salt, a simple amide, an amide based on amino acids, a crown ether, a sugar, or a nitrile. In a further embodiment of the present invention, a compound comprising a sarcosine residue or oligomer is provided. In addition, the present invention relates to pharmaceutical compositions of compounds comprising at least one functional unit or group. In an example embodiment of the present invention, a composition comprising a sarcosine residue or oligomer is provided.

[0009] In another aspect, the present invention relates generally to a method of modulating the pharmacokinetic and/or pharmacodynamic profile of a compound by attaching at least one functional unit or group to the compound, thereby improving its non-specific binding and/or pharmacokinetic properties. Attachment of the functional unit or group to the compound generates an active agent with improved biological and chemical properties, preferably including but not limited to increased oral absorption of

the compound, improved metabolism for increased bio availability, decreased protein binding, improved ability to cross the blood brain barrier or combinations thereof, without concomitant increased toxicity compared to the toxicity prior to such modification. In preferred embodiments, the active agent has improved solubility, lower IC_{50}, and/or is substantially less protein bound in comparison to the original compound.

[00010] In general, functional groups are characterized by minimized H-bond donors, a large number of H-bond acceptors, and may have an overall neutral charge. Compounds of the invention result from substitution or replacement of such functional groups into known drugs or design of novel drugs by incorporating such functional groups. Such compounds tend to conform to the Lipinski rule of five. However, because such compounds incorporate functional groups, they are also resistant to non-specific interactions with other biological molecules, particularly plasma proteins. In example embodiments of the present invention, the functional residue attached to the compound may be a hydrophobic group, an ether, an oligo(ethylene glycol) group or a derivative thereof, an amine, an ammonium salt, a simple amide, an amide based on amino acids, a crown ether, a sugar, or a nitrile. In further example embodiments of the present invention, the functional group attached to the compound may be a trimethyl-ethylenediamine, a methyl group, an acetyl group, an oxalamide, a sarcosine residue, a sarcosine oligomer, or a sarcosine derivative.

DESCRIPTION OF THE DRAWINGS

[00011] Figure 1 depicts a scaffold or backbone of an active compound (Compound B), a PDE5 inhibitor, which may be substituted with an functional residue or group as the R group to produce new compounds of the present invention.

[0010] Figure 2 shows Compound 1 of the invention.

[0011] Figure 3 shows various compounds that represent embodiment of the present invention.

[0012] Figure 4 shows Compound 2 of the invention.

[0013] Figure 5 shows various compounds that represent embodiment of the present invention.

[0014] Figure 6 shows Compound 3 of the invention.

[0015] Figure 7 shows permutations of Compound 3.

[0016] Figure 8 shows a known active compound, known as vardenafil.

[0017] Figure 9 shows metabolites of Compound 1.

[0018] Figures 10A-10B depicts some functional groups that may be used to prepare pharmacokinetically improved compounds of the invention. (From Ostuni et al. Langmuir 2001, 17:5605-5620). These functional groups are effective in rendering surfaces protein resistant.

DETAILED DESCRIPTION

[0019] One aspect of the present invention relates generally to a method of modulating the pharmacokinetic and/or pharmacodynamic profile of a compound by attaching at least one functional unit or group to the compound to generate an active agent, wherein the active agent has improved pharmacokinetic properties compared to the unmodified, i.e. parent compound. In an example embodiment of the present invention, a hydrophobic group, an ether, an oligo(ethylene glycol) group or a derivative thereof, an amine, an ammonium salt, a simple amide, an amide based on amino acids, a crown ether, a sugar, or a nitrile, an amine group, an oxalamide, a sarcosine residue, a sarcosine oligomer, or a sarcosine derivative may be attached to the compound. In a preferred embodiment, the sarcosine derivative is attached to the compound by a covalent bond to

the N-terminal nitrogen atom of the sarcosine residue or oligomer. In another preferred embodiment, the sarcosine is attached to a compound by a covalent bond to the carboxy terminus of the sarcosine residue or oligomer. In certain embodiments, the active agent has improved physicochemical properties, pharmacokinetics, metabolism, or toxicity profile. In a preferred embodiment, the active agent has superior solubility, lower IC_{50}, and/or is substantially less protein bound *in vivo* compared to the compound lacking the at least one functional residue.

[0020] It is believed that the group having the formula C modulates the pharmacokinetic and/or pharmacodynamic profile of the compound and may result in improved pharmacokinetic properties compared to the unmodified, i.e. parent compound. In certain embodiments, R4 is an active agent having improved physicochemical properties, pharmacokinetics, metabolism, or toxicity profile. In a preferred embodiment, the active agent has superior solubility, lower IC50, and/or is substantially less protein bound in vivo compared to the compound lacking the at least one functional residue.

[0021] Another aspect of the present invention relates to compounds comprising at least one functional unit or group. In example embodiments, compounds comprising at least one attached functional unit or group include but are not limited to chemical compounds, e.g. active drug agents and bioactive substances, and proteins. Chemical compounds include but are not limited to inhibitors and activators of proteins and enzymes (*e.g.*, phosphodiesterases such as PDE5, PDE1, PDE4 and PDE6, kinases, growth factor receptors, and proteases) and chemotherapeutic compounds (e.g. antineoplastic and antitumor substances). In an example embodiment of the present invention, a compound comprising at least one functional residue which is a hydrophobic group, an ether, an oligo(ethylene glycol) group or a derivative thereof, an amine, an ammonium salt, a simple amide, an amide based on amino acids, a crown ether, a sugar, or a nitrile is provided. In another example embodiment of the present invention, a compound comprising at least one functional residue which is a trimethyl-ethylenediamine, an oxalamide, a sarcosine residue, a sarcosine oligomer, a sarcosine metabolite, a sarcosine-ethylenediamine, or a branched analog thereof is provided. In a preferred embodiment, the sarcosine appendage is a sarcosine monomer or dimer. In a

preferred embodiment, the at least one functional unit or group is attached to a compound by a covalent bond to the N-terminal nitrogen atom or by a covalent bond to the carboxy terminus of the functional unit or group. In an example embodiment, sarcosine is attached to a compound by a covalent bond to the N-terminal nitrogen atom of the sarcosine residue or oligomer. In another preferred embodiment, the sarcosine is attached to a compound by a covalent bond to the carboxy terminus of the sarcosine residue or oligomer. In certain embodiments, the at least one functional unit or group is attached to the compound to form an amide, sulfonamide, or amine functional group. In an example embodiment sarcosine is attached to the compound to form an amide, sulfonamide, or amine functional group.

[0022] In addition, the present invention relates to pharmaceutical compositions of compounds that comprise at least one functional unit or group. In a preferred example embodiment of the present invention, a pharmaceutical composition comprising at least one functional residue which is a trimethyl-ethylenediamine, a methyl group, an acetyl group, an oxalamide, a sarcosine residue, a sarcosine oligomer, a sarcosine metabolite, a sarcosine-ethylenediamine, a branched analog thereof, or a sarcosine derivative is provided.

[0023] The basic approach to identifying these chemical groups is as follows: 1) A set of chemical groups under evaluation are immobilized on a solid surface (planar or 3D) via an appropriate linkage group. The groups are spatially discrete so that each can be measured individually, i.e., they are either on a single chip in an array; or on individual chips; or in separate wells of a microtitre plate. 2) A test or assay is then done on the set of chemical groups to determine whether they have a property that might improve the PK properties of a small molecule once that chemical group is synthetically added to the molecule. The assay performed at this stage on the immobilized chemical groups approximates the assay that is performed on the small molecule therapeutics that measures the PK property being optimized. The assay usually takes the form of the addition of a suitable reagent, followed by an incubation period, followed by a detection or measurement event to detect any modification. Examples of properties and assays are given below. 3) Once a chemical group or a set of chemical groups are identified as having a potential positive benefit on the pharmacokinetic property of interest using the surface assay system then the target small molecule is synthesized with this group or

groups appended in the appropriate position determined by SAR. These compounds are then tested in the corresponding, standard assay that is used to measure the pharmacokinetic property of interest.

[0024] An alternative approach to planar substrates is to immobilize the chemical groups onto beads where each bead provides spatial isolation for each group during an assay.

[0025] According to the invention, individual compounds may be designed and synthesized, for example, based on knowledge of existing pharmacophores. The invention further includes synthesis and/or screening of libraries of compounds. Usually, a library of compounds comprises a one or more related core moieties or pharmacophores, each combined with one or more functional groups. The compounds are spatially addressed or bound to a solid support.

[0026] Accordingly, the invention also includes methods of using the indexed compounds, for example in surface-based arrays, to identify functional groups that modify pharmacophores and affect their pharmacokinetic properties in desirable ways.

[0027] In general, indexed arrays of modified pharmacophores are used in a method that comprises: a) preparing a universal library of drug functional group modifier compounds, b) contacting or combining drug functional group modifier compounds from the universal library with pharmaceutical drugs of interest; and c) determining the strength of interaction/function of the modified drug compounds. The determination in step (c) involves evaluation of one or more physical and biochemical properties. Physical properties include, for example, lipophilicity, solubility, polar surface area, and the like. Biochemical properties include, for example, potency, target specificity, stability, bioavailability, and minimized non-specific interaction with host molecules, particularly molecules such as serum components, that would otherwise sequester the compounds and prevent them from reaching their targets.

Definitions

[0028] For convenience, certain terms employed in the specification, examples, and appended claims are collected here.

[0029] The term "functional unit or group" as used herein means a residue which is attached to a compound or protein. A residue may be a structural component of a molecule comprising from one atom of an element up to a complex molecule, wherein the complex molecule is a functional moiety or is comprised of a plurality of functional moieties. In a preferred embodiment, the functional residue to be attached as substitutents or as replacement residues of known active compounds may have a minimum number of hydrogen donors, a large number of hydrogen bond acceptors and a may have a neutral electrical charge. Not altering the inherent functional properties of the compound, as used herein means that the modification of the compound or protein resulting from the attachment of the residue thereto does not either decrease the desired chemical or biological activity of the compound or increase any of its deleterious side effects, e.g. toxicity. Such an functional unit or group may be attached to a compound or protein to replace an existing functional unit or group or may be attached as a residue to an existing functional unit or group. The functional unit or group may be attached to a compound or protein by covalent bonds. The functional unit or group may be inert to protein binding.

[0030] The term "heteroatom" as used herein means an atom of any element other than carbon or hydrogen. Preferred heteroatoms are boron, nitrogen, oxygen, phosphorus, sulfur and selenium.

[0031] The term "alkyl" refers to the radical of saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. In preferred embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (e.g., C1-C30 for straight chain, C3-C30 for branched chain), and more preferably 20 or fewer. Likewise, preferred cycloalkyls have from 3-10 carbon atoms in their ring structure, and more preferably have 5, 6 or 7 carbons in the ring structure.

[0032] Unless the number of carbons is otherwise specified, "lower alkyl" as used herein means an alkyl group, as defined above, but having from one to ten carbons, more preferably from one to six carbon atoms in its backbone structure. Likewise, "lower

alkenyl" and "lower alkynyl" have similar chain lengths. Preferred alkyl groups are lower alkyls. In preferred embodiments, a substituent designated herein as alkyl is a lower alkyl.

[0033] The term "aralkyl", as used herein, refers to an alkyl group substituted with an aryl group (e.g., an aromatic or heteroaromatic group).

[0034] The terms "alkenyl" and "alkynyl" refer to unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively.

[0035] The term "aryl" as used herein includes 5-, 6- and 7-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, anthracene, naphthalene, pyrene, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycles" or "heteroaromatics." The aromatic ring can be substituted at one or more ring positions with such substituents as described above, for example, halogen, azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, alkoxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, sulfonamido, ketone, aldehyde, ester, heterocyclyl, aromatic or heteroaromatic moieties, $-CF_3$, -CN, or the like. The term "aryl" also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings (the rings are "fused rings") wherein at least one of the rings is aromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls.

[0036] The terms ortho, meta and para apply to 1,2-, 1,3- and 1,4-disubstituted benzenes, respectively. For example, the names 1,2-dimethylbenzene and ortho-dimethylbenzene are synonymous.

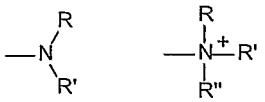
[0037] The terms "heterocyclyl" or "heterocyclic group" refer to 3- to 10-membered ring structures, more preferably 3- to 7-membered rings, whose ring structures include

one to four heteroatoms. Heterocycles can also be polycycles. Heterocyclyl groups include, for example, thiophene, thianthrene, furan, pyran, isobenzofuran, chromene, xanthene, phenoxathiin, pyrrole, imidazole, pyrazole, isothiazole, isoxazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, pyrimidine, phenanthroline, phenazine, phenarsazine, phenothiazine, furazan, phenoxazine, pyrrolidine, oxolane, thiolane, oxazole, piperidine, piperazine, morpholine, lactones, lactams such as azetidinones and pyrrolidinones, sultams, sultones, and the like. The heterocyclic ring can be substituted at one or more positions with such substituents as described above, as for example, halogen, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, ketone, aldehyde, ester, a heterocyclyl, an aromatic or heteroaromatic moiety, $-CF_3$, -CN, or the like.

[0038] The terms "polycyclyl" or "polycyclic group" refer to two or more rings (e.g., cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls) in which two or more carbons are common to two adjoining rings, e.g., the rings are "fused rings". Rings that are joined through non-adjacent atoms are termed "bridged" rings. Each of the rings of the polycycle can be substituted with such substituents as described above, as for example, halogen, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, ketone, aldehyde, ester, a heterocyclyl, an aromatic or heteroaromatic moiety, -CF3, -CN, or the like.

[0039] As used herein, the term "nitro" means -NO2; the term "halogen" designates -F, -Cl, -Br or -I; the term "sulfhydryl" means -SH; the term "hydroxyl" means -OH; and the term "sulfonyl" means -SO2-.

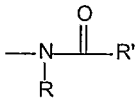
[0040] The terms “amine” and “amino” are art-recognized and refer to both unsubstituted and substituted amines, e.g., a moiety that can be represented by the general formula:



or

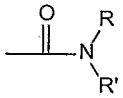
wherein R, R' and R" each independently represent a group permitted by the rules of valence, preferably H, alkyl, alkenyl, alkynyl, aralkyl, aryl, and heterocyclic group.

[0041] The term "acylamino" is art-recognized and refers to a moiety that can be represented by the general formula:



wherein R and R' are as defined above.

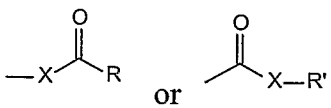
[0042] The term "amido" is art recognized as an amino-substituted carbonyl and includes a moiety that can be represented by the general formula:



wherein R and R' are as defined above. Preferred embodiments of the amide will not include imides which may be unstable.

[0043] The term "alkylthio" refers to an alkyl group, as defined above, having a sulfur radical attached thereto. In preferred embodiments, the "alkylthio" moiety is represented by one of -S-alkyl, -S-alkenyl, -S-alkynyl, and -S-$(CH_2)_m$-R_8, wherein m and R_8 are defined above. Representative alkylthio groups include methylthio, ethyl thio, and the like.

[0044] The term "carbonyl" is art recognized and includes such moieties as can be represented by the general formula:

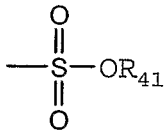


or

wherein X is a bond or represents an oxygen or a sulfur, and R and R' are as defined above. Where X is an oxygen and R or R' is not hydrogen, the formula represents an "ester". Where X is an oxygen, and R is as defined above, the moiety is referred to herein as a carboxyl group, and particularly when R is a hydrogen, the formula represents a "carboxylic acid". Where X is an oxygen, and R' is hydrogen, the formula represents a "formate". In general, where the oxygen atom of the above formula is replaced by sulfur, the formula represents a "thiolcarbonyl" group. Where X is a sulfur and R or R' is not hydrogen, the formula represents a "thiolester." Where X is a sulfur and R is hydrogen, the formula represents a "thiolcarboxylic acid." Where X is a sulfur and R' is hydrogen, the formula represents a "thiolformate." On the other hand, where X is a bond, and R is not hydrogen, the above formula represents a "ketone" group. Where X is a bond, and R is hydrogen, the above formula represents an "aldehyde" group.

[0045] The terms "alkoxyl" or "alkoxy" as used herein refers to an alkyl group, as defined above, having an oxygen radical attached thereto. Representative alkoxyl groups include methoxy, ethoxy, propyloxy, tert-butoxy and the like. An "ether" is two hydrocarbons covalently linked by an oxygen.

[0046] The term "sulfonate" is art recognized and includes a moiety that can be represented by the general formula:

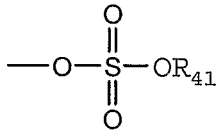


in which R_{41} is an electron pair, hydrogen, alkyl, cycloalkyl, or aryl.

[0047] The terms triflyl, tosyl, mesyl, and nonaflyl are art-recognized and refer to trifluoromethanesulfonyl, p-toluenesulfonyl, methanesulfonyl, and nonafluorobutanesulfonyl groups, respectively. The terms triflate, tosylate, mesylate, and nonaflate are art-recognized and refer to trifluoromethanesulfonate ester, p-toluenesulfonate ester, methanesulfonate ester, and nonafluorobutanesulfonate ester functional groups and molecules that contain said groups, respectively.

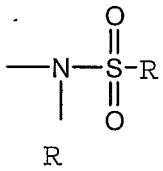
[0048] The abbreviations Me, Et, Ph, Tf, Nf, Ts, Ms represent methyl, ethyl, phenyl, trifluoromethanesulfonyl, nonafluorobutanesulfonyl, p-toluenesulfonyl and methanesulfonyl, respectively. A more comprehensive list of the abbreviations utilized by organic chemists of ordinary skill in the art appears in the first issue of each volume of the Journal of Organic Chemistry; this list is typically presented in a table entitled Standard List of Abbreviations. The abbreviations contained in said list, and all abbreviations utilized by organic chemists of ordinary skill in the art are hereby incorporated by reference.

[0049] The term "sulfate" is art recognized and includes a moiety that can be represented by the general formula:

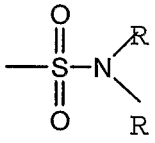


in which R_{41} is as defined above.

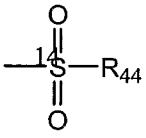
[0050] The term "sulfonylamino" is art recognized and includes a moiety that can be represented by the general formula:



[0051] The term "sulfamoyl" is art-recognized and includes a moiety that can be represented by the general formula:

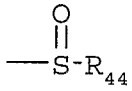


[0052] The term "sulfonyl", as used herein, refers to a moiety that can be represented by the general formula:



in which R_{44} is selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, aryl, or heteroaryl.

[0053] The term "sulfoxido" as used herein, refers to a moiety that can be represented by the general formula:



in which R_{44} is selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, aralkyl, or aryl.

[0054] A "selenoalkyl" refers to an alkyl group having a substituted seleno group attached thereto. Exemplary "selenoethers" which may be substituted on the alkyl are selected from one of -Se-alkyl, -Se-alkenyl, -Se-alkynyl, and -Se-$(CH_2)_m$-R_7, m and R_7 being defined above.

[0055] Analogous substitutions can be made to alkenyl and alkynyl groups to produce, for example, aminoalkenyls, aminoalkynyls, amidoalkenyls, amidoalkynyls, iminoalkenyls, iminoalkynyls, thioalkenyls, thioalkynyls, carbonyl-substituted alkenyls or alkynyls.

[0056] As used herein, the definition of each expression, e.g. alkyl, m, n, etc., when it occurs more than once in any structure, is intended to be independent of its definition elsewhere in the same structure.

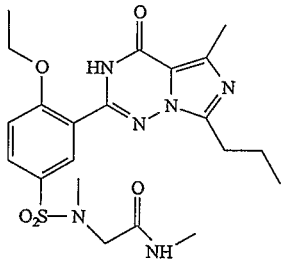
[0057] It will be understood that "substitution" or "substituted with" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc.

[0058] As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic substituents of organic compounds. Illustrative substituents include, for example, those described herein above. The permissible substituents can be one or more and the same or different for appropriate organic compounds. For purposes of this invention, the heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. This invention is not intended to be limited in any manner by the permissible substituents of organic compounds.

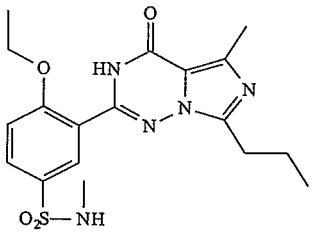
[0059] The phrase "protecting group" as used herein means temporary substituents which protect a potentially reactive functional group from undesired chemical transformations. Examples of such protecting groups include esters of carboxylic acids, silyl ethers of alcohols, and acetals and ketals of aldehydes and ketones, respectively. The field of protecting group chemistry has been reviewed (Greene, T.W.; Wuts, P.G.M. *Protective Groups in Organic Synthesis*, 2nd ed.; Wiley: New York, 1991).

[0060] As used herein, the term "metabolite" refers to a compound that has been metabolized in the body. For example, an embodiment of the invention, Compound 1 (Figure 2) is metabolized in the body to give the metabolites shown in Figure 9.

[0061] The present invention also includes metabolites of any of the above compounds. Preferred metabolites include compounds having the formula:



or



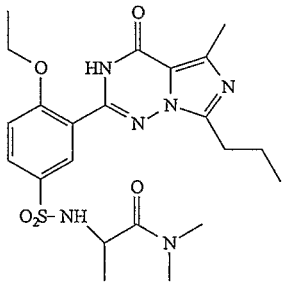
.

[0062] Table 1-3 summarizes certain biological and pharmacological properties of the above-described modified compounds of A. Table 3 includes selectivity index against

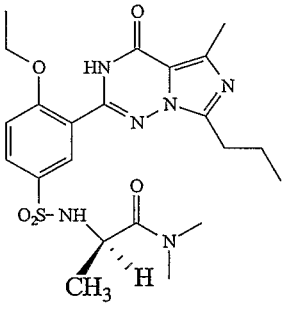
several PDEs. The protein binding, permeability, and solubility of the above-described compounds are set forth in Table 2.

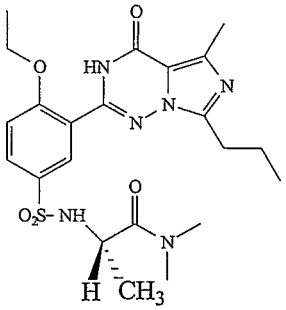
Compounds of the Invention

[0063] Certain compounds of the present invention may exist in particular geometric or stereoisomeric forms. The present invention contemplates all such compounds, including *cis*- and *trans*-isomers, *R*- and *S*-enantiomers, diastereomers, (D)-isomers, (L)-isomers, the racemic mixtures thereof, and other mixtures thereof, as falling within the scope of the invention. Additional asymmetric carbon atoms may be present in a substituent such as an alkyl group. All such isomers, as well as mixtures thereof, are intended to be included in this invention. For example, in one embodiment, the compound or pharmaceutical composition is a permutation of Compound 2 having the structure:



[0064] The following isomers of this compound are included in this invention:





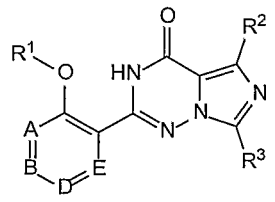
[0065] In addition, if, for instance, a particular enantiomer of a compound of the present invention is desired, it may be prepared by asymmetric synthesis, or by derivation with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomers. Alternatively, where the molecule contains a basic functional group, such as amino, or an acidic functional group, such as carboxyl, diastereomeric salts are formed with an appropriate optically-active acid or base, followed by resolution of the diastereomers thus formed by fractional

crystallization or chromatographic means well known in the art, and subsequent recovery of the pure enantiomers.

[0066] Contemplated equivalents of the compounds described above include compounds which otherwise correspond thereto, and which have the same general properties thereof (e.g., functioning as analgesics), wherein one or more simple variations of substituents are made which do not adversely affect the efficacy of the compound in binding to sigma receptors. In general, the compounds of the present invention may be prepared by the methods illustrated in the general reaction schemes as, for example, described below, or by modifications thereof, using readily available starting materials, reagents and conventional synthesis procedures. In these reactions, it is also possible to make use of variants which are in themselves known, but are not mentioned here.

[0067] For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 67th Ed., 1986-87, inside cover.

[0068] In one embodiment, the present invention provides a compound of the formula A^1:



(A)

or pharmaceutically acceptable salt, stereoisomer, or hydrate thereof, wherein

R^1 is lower alkyl;

R^2 and R^3 are independently selected from lower alkyl, and lower alkenyl and lower alkynyl, wherein the lower alkyl, lower alkenyl, and lower alkynyl may be optionally substituted with one or more halogen, lower alkoxy, hydroxy, CN, NO_2, amino, acylamino, amido, carbonyl, and alkylthio;

A is N or C-H;

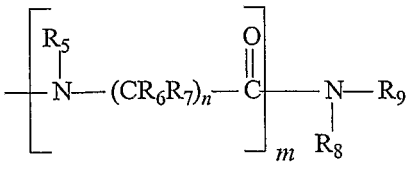
B is N, C-H, C-(SO_2-R^4), or C-CO-R^4;

D is N, C-H, C-(SO_2-R^4) or C-CO-R^4;

E is N or C-H;

wherein only one of A, B or E may be N, and one of B or D is C-(SO_2-R^4) or C-CO-R^4;

R^4 is a group having the formula:



(C)

in which each R^5, R^6, R^7 and R^8 are independently selected from H and lower alkyl, wherein the lower alkyl may be optionally substituted with one or more halogen, lower alkoxy, hydroxy, CN, NO_2, amino, acylamino, amido, carbonyl, and alkylthio; and additionally or alternatively R^6 and R^5 together form a 5- or 6-membered ring, or R^6 and R^7 together form a 3 to 6 membered ring; R^9 is independently selected from H and lower alkyl, wherein the lower alkyl may be optionally substituted with one or more halogen, lower alkoxy, hydroxy, CN, NO_2, amino, acylamino, amido, carbonyl, and alkylthio; alternatively R^8 and R^9 together with the nitrogen to which they are attached form a 5- or 6-membered ring; *n* is 1 to 4; and *m* is 1 to 6. In a preferred embodiment, R^2 and R^3 are independently selected from lower alkyl.

[0069] It is believed that the group having the formula C modulates the pharmacokinetic and/or pharmacodynamic profile of the compound and may result in improved pharmacokinetic properties compared to the unmodified, i.e. parent compound. In certain embodiments, R^4 is an active agent having improved physicochemical properties, pharmacokinetics, metabolism, or toxicity profile. In a preferred embodiment, the active agent has superior solubility, lower IC_{50}, and/or is substantially less protein bound *in vivo* compared to the compound lacking the at least one functional residue.

[0070] The compounds of the present invention which have been modified by the attachment thereto of at least one residue of the formula C provide modified pharmacokinetic properties, including modified nonspecific *in vivo* protein binding. Such optimal pharmacokinetic properties do not compromise either the selectivity or the potency of the modified compound.

[0071] Compounds of the invention may be designed to incorporate a functional unit or group or an existing compound(s) may be modified by the attachment of at least one functional unit or group, i.e. residue, thereto; such latter compounds include substances which are chemically or biologically active molecules. Such compounds also include therapeutic drugs or proteins, whose desired activity is known by one of skill in the art. Potential compounds are identified by the skilled artisan by the presence of functional groups which provide the desired *in vivo* characteristics of drug potency, solubility, and suitable protein binding required for therapeutic efficacy at the minimal therapeutically effective amount of the compound. Additional compound properties to be considered include the oral absorption, metabolism, ability to cross the blood brain barrier and toxicity of the compound. Potential compounds for modification by the attachment of at least one functional residue are interchangeably referred to herein as "parent" compounds.

[0072] Parent compounds comprise an essential skeleton, scaffold, or backbone, i.e. the pharmacophore to which are attached substituents, typically residues that are not required for the chemical or biological activity of the compound, but which may affect properties including but not limited to solubility and/or *in vivo* protein binding. For example, a potential parent compound may be selected for modification with residues according to the present invention by the presence of a substituent, e.g. a piperazine group or a morpholine group. The substituent group may itself be substituted with a residue or alternatively, be replaced by a residue, to make the compounds of the present invention.

[0073] Further, compounds of the invention may be designed *de novo*. For example, backbones that incorporate functional groups that confer resistance to protein binding can be chosen as the starting point for drug design. Alternatively such backbones may be the basis for combinatorial libraries to be screened for compounds of interest.

[0074] The compounds of the present invention which have been modified by the attachment thereto of at least one residue provide modified pharmacokinetic properties, including modified nonspecific *in vivo* protein binding. Such optimal pharmacokinetic properties do not compromise either the selectivity or the potency of the modified compound. The pharmacokinetically improved compounds of the invention preferably allow a minimum effective amount of the compound to be administered to achieve the desired therapeutic effect of the unbound compound, thereby reducing the dosage amount

(and may improve patient compliance). The compounds provide improved pharmacokinetic properties over the parent compound by modifying the non-specific *in vivo* protein binding of the compound. The attachment of the at least one residue may decrease non-specific protein binding for some compounds. Other improved pharmacokinetic properties of the compounds of the present invention include solubility, oral availability, metabolism, ability to cross the blood-brain barrier and providing the desired tissue distribution, i.e. at target tissue(s).

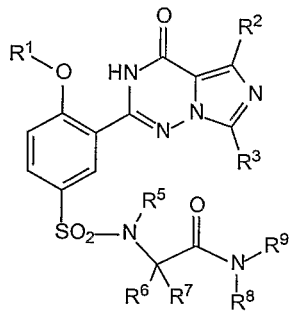
[0075] The modification of protein binding is based on surface technology, i.e. the preparation and screening of surfaces for their ability to resist adsorption of proteins from solution. Surfaces which are resistant to adsorption of proteins from solution are known to one of skill in the art as "protein resistant" surfaces. Functional groups may be screened to identify the group(s) present in protein resistant surfaces, as described in e.g., Chapman et al. Surveying for Surfaces that Resist the Adsorption of Proteins, J. Am. Chem. Soc. 2000, 122:8303-8304; Ostuni et al. A Survey of Structure-Property Relationships of Surfaces that Resist the Adsorption of Protein, Langmuir 2001, 17:5605-5620; Holmlin, et al. Zwitterionic SAMs that Resist Nonspecific Adsorption of Protein from Aqueous Buffer, Langmuir 2001, 17:2841-2850; and Ostuni et al. Self-Assembled Monolayers that Resist the Adsorption of Proteins and the Adhesion of Bacterial and Mammalian Cells, Langmuir 2001, 17:6336-6343.

[0076] In preferred example embodiments of the present invention, functional residues include but are not limited to a hydrophobic group, an ether, an oligo(ethylene glycol) group or a derivative thereof, an amine, an ammonium salt, a simple amide, an amide based on amino acids, a crown ether, a sugar, or a nitrile, and other suitable functional groups shown below, as described by Ostuni et al. Langmuir 2001, 17:5605-5620, the contents of which are hereby incorporated in their entirety, specifically the functional groups that were particularly effective in rendering surfaces protein non-adsorbing, i.e. protein resistant. Figures 10A-10B illustrate some of the various functional groups which may be attached to active compound scaffolds or backbones to produce the compounds of the present invention.

[0077] The chemical compounds of the present invention comprise a variable essential scaffold or backbone structure of a known active compound, i.e. the parent

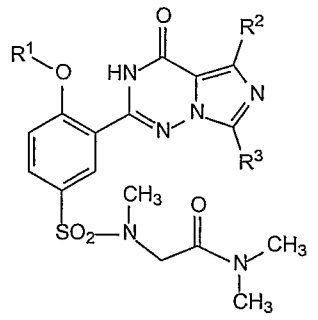
compound, to which modifications may be made by attachment of at least one substituent which is at least one functional group by replacement and/or subtitution of existing non-essential substituent group(s), as discussed above. The variable essential scaffold or backbone structure is referred to herein as "A" or as a radical of an active compound which scaffold or radical may form covalent bonds with the at least one functional residue. The backbone or scaffold of chemically or biologically active molecules, including known therapeutic drugs or proteins, having a desired activity, include but are not limited to the backbone structures of cyclic guanosine 3',5'-monophosphate phosphodiesterases, cGMP PDEs, e.g. cGMP PDE5, as described in e.g., Published PCT applications WO 00/24745 (Bunnage et al.), WO 01/27112 (Allerton et al.), WO 02/074312 (Allerton et al.) and U. S. Patent Nos. 6,440,982 B1(Maw et al.) and 6,583,147 B1 (Yoo et al.).

[0078] In one embodiment, the compound has the following formula:



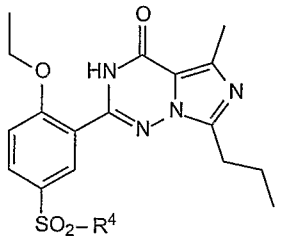
in which each R^5, R^6, R^7 and R^8 are independently selected from H and lower alkyl, wherein the lower alkyl may be optionally substituted with one or more halogen, lower alkoxy, hydroxy, CN, NO_2, amino, acylamino, amido, carbonyl, and alkylthio; and additionally or alternatively R^6 and R^5 together form a 5- or 6-membered ring, or R^6 and R^7 together form a 3 to 6 membered ring; R^9 is independently selected from H and lower alkyl, wherein the lower alkyl may be optionally substituted with one or more halogen, lower alkoxy, hydroxy, CN, NO_2, amino, acylamino, amido, carbonyl, and alkylthio; alternatively R^8 and R^9 together with the nitrogen to which they are attached form a 5- or 6-membered ring.

[0079] Preferably, the functional group attached to the sulfonyl group is a sarcosine derivative or oligomer. A preferred embodiment has the formula:



in which R^1, R^2 and R^3 are independently selected from H and lower alkyl, wherein the lower alkyl may be optionally substituted with one or more halogen, lower alkoxy, hydroxy, CN, NO_2, amino, acylamino, amido, carbonyl, and alkylthio.

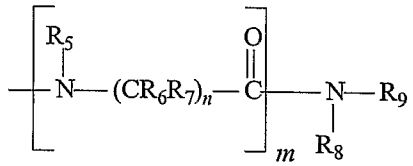
[0080] In a most preferred embodiment, the scaffold or backbone may be a PDE5 inhibitor having the formula (B):



(B)

wherein R represents at least one functional residue which replaces the original piperazine by attachment to the sulfur of the parent compound by covalent bonding.

[0081] In compound A or B, R^4 may be a compound having the formula C:

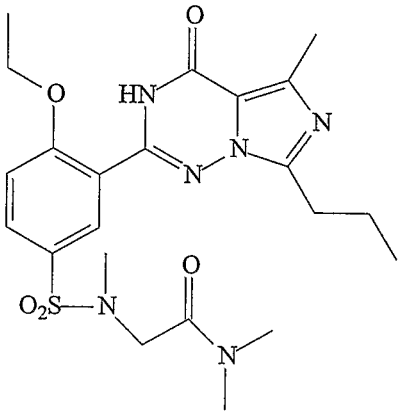


(C)

wherein each R^5, R^6, R^7 and R^8 are independently selected from H and lower alkyl, wherein the lower alkyl may be optionally substituted with one or more halogen, lower alkoxy, hydroxy, CN, NO_2, amino, acylamino, amido, carbonyl, and alkylthio; and additionally or alternatively R^6 and R^5 together form a 5- or 6-membered ring, or R^6 and R^7 together form a 3 to 6 membered ring; R^9 is independently selected from H and lower alkyl, wherein the lower alkyl may be optionally substituted with one or more halogen, lower alkoxy, hydroxy, CN, NO_2, amino, acylamino, amido, carbonyl, and alkylthio;

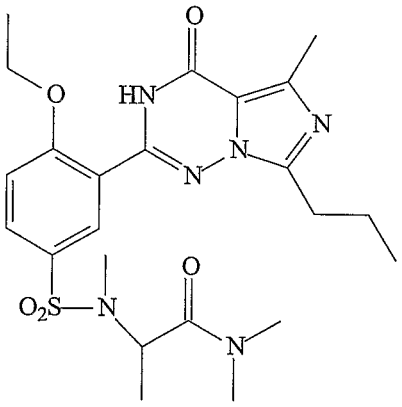
alternatively R^8 and R^9 together with the nitrogen to which they are attached form a 5- or 6-membered ring; n is 1 to 4; and m is 1 to 6.

[0082] In a preferred embodiment, R^4 is methyl-amino-dimethylacetamide, to give a compound having the formula I:



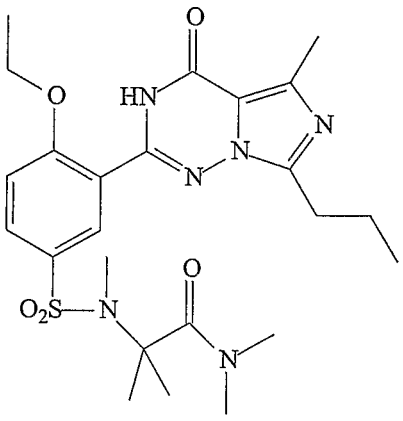
(I)

[0083] In another embodiment, R^4 is methyl-alanine-dimethylamide, to give a compound having the formula II:



(II)

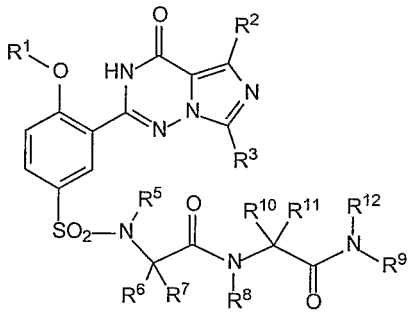
[0084] In another embodiment, R^4 is 2-methylamino-2-trimethyl-propionamide, to give a compound having the formula III:



(III)

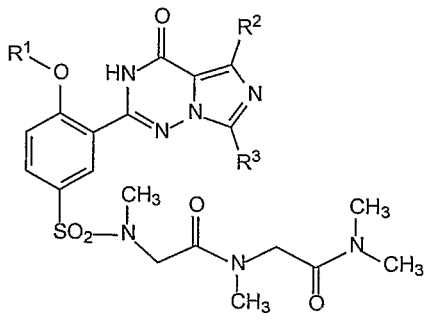
[0085] In other preferred embodiments, m is 1 or 2 for R^4 (Formula C), which is attached to Compound A. Preferably, n is 1.

[0086] In a further embodiment, the compound has the following formula:



in which R^1, R^2, R^3 R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11} and R^{12} are independently selected from H and lower alkyl, wherein the lower alkyl may be optionally substituted with one or more halogen, lower alkoxy, hydroxy, CN, NO_2, amino, acylamino, amido, carbonyl, and alkylthio; and additionally or alternatively R^6 and R^5, or R^8 and R^{10} together form a 5- or 6-membered ring, or R^6 and R^7, or R^{10} and R^{11} together form a 3 to 6 membered ring; and R^9 and R^{12} together with the nitrogen to which they are attached form a 5- or 6-membered ring.

[0087] For the above embodiment, preferably, R^6, R^7, R^{10}, and R^{11} are each hydrogen. More preferably, a sarcosine dimer is attached to the sulfonyl group, and the compound has the formula:



in which R^1, R^2 and R^3 are independently selected from H and lower alkyl, wherein the lower alkyl may be optionally substituted with one or more halogen, lower alkoxy, hydroxy, CN, NO_2, amino, acylamino, amido, carbonyl, and alkylthio. In a most preferred embodiment, R^1 is ethyl, R^2 is methyl, and R^3 is propyl.

[0088] Table 1 summarizes the certain properties of the above-described modified compounds of B, including IC50 and CLogP.

[0089] Table 2 summarizes certain properties of the modified compounds, including protein binding, solubility and non-specific binding. The selectivity index against several PDEs of the above-described compounds are set forth in Table 3.

[0090] The present invention relates to a method of modulating the pharmacokinetic and/or pharmacodynamic properties of a compound, comprising:
attaching at least one functional residue, wherein the at least one functional residue reduces non-specific protein binding, to a known active compound by (a) replacing a nonessential residue, i.e. a variant scaffold, of the compound with the at least one functional residue or (b) substituting a nonessential residue of the compound with the at least one functional residue, thereby improving the pharmacokinetic properties of the compound.

[0091] In a preferred embodiment of the above-described method, the functional residue to be attached as a substitutent or as a replacement residue comprises a minimum number of hydrogen donors, a large number of hydrogen bond acceptors and a neutral electrical charge.

[0092] In the above-described method, the at least one functional residue which is attached to the known active compound may be a hydrophobic group, an ether, an oligo(ethylene glycol) group or a derivative thereof, an amine, an ammonium salt, a simple amide, an amide based on amino acids, a crown ether, a sugar, or a nitrile, an amine group, an oxalamide, a sarcosine residue, a sarcosine derivative or a sarcosine oligomer. In a preferred embodiment, the pharmacokinetic property is nonspecific protein binding.

[0093] The present invention relates to a method of modulating the pharmacokinetic and/or pharmacodynamic properties of a compound, comprising: attaching a residue from the group consisting of a hydrophobic group, an ether, an oligo (ethylene glycol) group or a derivative thereof, an amine, an ammonium salt, a simple amide, an amide based on amino acids, a crown ether, a sugar, or a nitrile, an amine group, an oxalamide, a sarcosine residue, a sarcosine derivative or a sarcosine oligomer to a known active compound to generate a second compound.

[0094] In general, protein binding is assessed by measuring the capacity of molecules of the invention to bind to one or more human serum components or mimics thereof. In one embodiment, suitable functional residues may be identified by screening of surfaces comprising such residues for their ability to resist adsorption of serum components, including, but not limited to serum proteins, and preferably human serum proteins. Candidate residues can be screened directly by attaching them to a solid support and testing the support for protein resistance. Alternatively, candidate residues are incorporated into, or linked to molecules of pharmaceutical interest. Such compounds may be synthesized on a solid support, or bound to a solid support after synthesis. In a non-limiting example of a direct binding assay, immobilized candidate functional residues or molecules incorporating such residues are tested for their ability to bind serum components. The serum components can be labeled with a signaling moiety for detection, or a labeled secondary reagent that binds to such serum components can be used.

[0095] Surfaces which are resistant to adsorption of proteins from solution are known as "protein resistant" surfaces. Functional groups may be screened to identify the group(s) present in protein resistant surfaces, as described in e.g., Chapman et al. Surveying for

Surfaces that Resist the Adsorption of Proteins, J. Am. Chem. Soc. 2000, 122:8303-8304; Ostuni et al. A Survey of Structure-Property Relationships of Surfaces that Resist the Adsorption of Protein, Langmuir 2001, 17:5605-5620; Holmlin, et al. Zwitterionic SAMs that Resist Nonspecific Adsorption of Protein from Aqueous Buffer, Langmuir 2001, 17:2841-2850; and Ostuni et al. Self-Assembled Monolayers that Resist the Adsorption of Proteins and the Adhesion of Bacterial and Mammalian Cells, Langmuir 2001, 17:6336-6343.

[0096] Upon identification of a functional residue which provides such protein resistance, one of skill in the art will readily determine a suitable chemical skeleton or backbone of a known biologically or chemically active compound to which the functional residue may be attached by either substitution of functional group of the active compound or by replacement of a nonessential functional group of the active compound. For example, as discussed above, the presence of a piperazine group on a compound will indicate that such group may be either replaced or substituted with an functional residue. One of skill in the art, e.g. a medicinal chemist, will recognize other suitable groups on known active compounds which may be replaced or substituted with at least one functional residue. Accordingly, a combinatorial library of compounds, may be generated as described infra, wherein the compounds are modified compounds comprising a conjugate of an active site of the compound (an essential backbone of a compound having a particular desired activity), e.g. compound A, and at least one functional residue attached thereto, wherein each conjugate has a different functional residue attached thereto, e.g. residues having formula C, wherein the R group is selected from the various groups described herein, or the various functional groups shown in Figs. 10A-10B. Accordingly, a library may be used to screen a plurality of different functional residues for improved pharmacokinetic and/or pharmacodynamic properties including non-specific protein binding of the modified compound.

[0097] In preferred embodiments, the solid support itself is chosen or modified to minimize its interaction with the serum components. Examples of such supports and assay systems are described in International Application WO 02/48676, WO 03/12392, WO 03/18854, WO 03/54515, herein incorporated by reference. Alternatively, the molecules of the invention may be mixed with one or more serum components in liquid phase, and the amount of unbound molecules determined.

[0098] A direct binding analysis can also be preformed in liquid phase. For example, test compounds can be mixed with one or more serum components in liquid phase, and the unbound molecules determined.

[0099] In an example of a preferred embodiment, molecules having reduced protein binding are identified as follows: a self-assembled monolayer of thiol molecules terminated with anhydride groups is formed at a gold surface. A set of small molecules with amine groups at one end, and groups that are designed to resist binding to albumin, for example, at the other end are then attached to the surface via reaction between the amine and anhydride. The set of molecules are spotted onto spatially distinct regions on the gold surface to create an array of molecules that might resist protein binding. This array is then exposed to a solution containing albumin that is fluorescently labeled. After a suitable incubation period, the gold surface is washed and scanned on a fluorescent scanner. The immobilized chemical groups that bound to albumin will be identified by the presence of a fluorescent signal; groups that resist albumin binding will have low fluorescence in that part of the array. If a fluorescent protein is not available then antibodies against the protein of interest in combination with fluorescent secondary antibodies can be used to detect protein binding to the chemical groups. If an antibody is not available then a labeless detection method such as surface plasmon resonance (SPR) or MALDI mass spectrometry can be used to identify the presence of the protein at individual elements in the array. SPR also has the advantage of providing kinetic information on the binding of protein to the chemical groups.

[00100] The use of this system is not limited to albumin; any protein of pharmacokinetic interest can be tested for binding potential. For example, blood proteins that bind small molecules, such as α-acid glycoprotein (AAG, AGP) and lipoproteins, could be exposed to the array and protein binding detected.

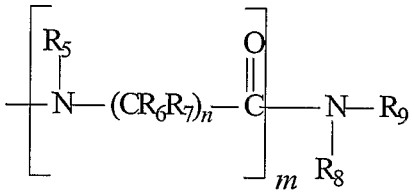
[00101] In an embodiment of the invention, chemical groups can be identified that resist binding to P-glycoprotein (PGP) and therefore have the potential to reduce efflux when appended to a small molecule therapeutic. This is particularly important for development of anti-cancer drugs provide effective treatment where multiple drug resistance (MDR) has developed.

[00102] The method could also be used to identify chemical groups that resist binding to proteins such as thrombin, anti-thrombin, and Factor Xa and therefore have the potential to control coagulation.

[00103] This method would also be useful for identifying groups that improve therapeutics that are designed as supplemental or replacement therapies where protein binding and PK properties are very important, e.g., hormones and their binding proteins, and steroids and their binding proteins such as testosterone and sex hormone binding globulin (SHBG).

[00104] In another aspect, the present invention relates to a method of modulating the pharmacokinetic and/or pharmacodynamic property of a compound, comprising the step of:

attaching a first compound selected from the group consisting of any of the aforementioned known skeletons or backbone of active compounds, including but not limited to active drug agents, bioactive substances, proteins and chemical to a residue represented by formula **C** to generate a second compound,



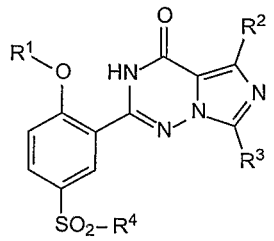
C

wherein R^5, R^6, R^7, R^8 and R^9 may be H and lower alkyl, wherein the lower alkyl may be substituted with halogen, lower alkoxy, hydroxy, CN, NO_2, amino, acylamino, amido, carbonyl, and alkylthio; and R^6 and R^5 together form a 5- or 6-membered ring, or R^6 and R^7 together form a 3 to 6 membered ring; R^8 and R^9 together may with the nitrogen to which they are attached form a 5- or 6-membered ring; *n* is 1 to 4; and *m* is 1 to 6.

[00105] In certain embodiments, the present invention relates to the aforementioned method, wherein *m* is 1 or 2.

[00106] In certain embodiments, the present invention relates to the aforementioned method, wherein *n* is 1.

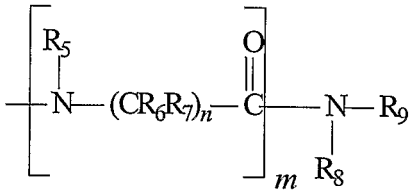
[00107] In certain embodiments, the present invention relates to a method of modulating the pharmacokinetic and/or pharmacodynamic property of a compound, whereby the compound has the formula A^1:



(A)

or pharmaceutically acceptable salt, stereoisomer, or hydrate thereof, wherein, R^1, R^2 and R^3 are independently selected from H, lower alkyl, lower alkenyl, and lower alkynyl, wherein the lower alkyl, lower alkenyl, and lower alkynyl may be optionally substituted with one or more halogen, lower alkoxy, hydroxy, CN, NO_2, amino, acylamino, amido, carbonyl, and alkylthio, comprising:

attaching R^4 having the formula C:

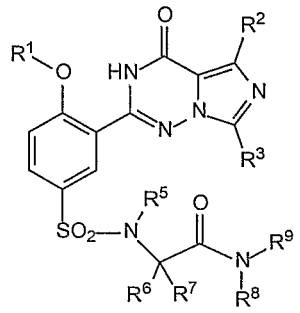


C

wherein R^5, R^6, R^7, R^8 and R^9 may be H and lower alkyl, wherein the lower alkyl may be substituted with halogen, lower alkoxy, hydroxy, CN, NO_2, amino, acylamino, amido, carbonyl, and alkylthio; and R^6 and R^5 together form a 5- or 6-membered ring, or R^6 and R^7 together form a 3 to 6 membered ring; R^8 and R^9 together may with the nitrogen to

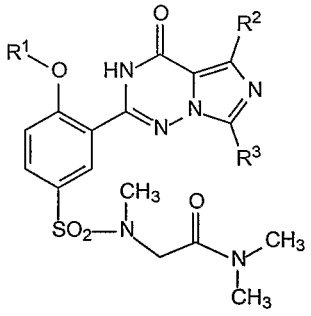
which they are attached form a 5- or 6-membered ring; *n* is 1 to 4; and *m* is 1 to 6, to generate a second compound.

[00108] In certain embodiments, the present invention relates to the aforementioned method, wherein the second compound has the formula:



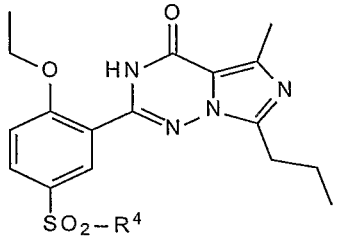
wherein,R^1, R^2 and R^3 are independently selected from H, lower alkyl, lower alkenyl, and lower alkynyl, wherein the lower alkyl, lower alkenyl, and lower alkynyl may be optionally substituted with one or more halogen, lower alkoxy, hydroxy, CN, NO_2, amino, acylamino, amido, carbonyl, and alkylthio.

[00109] In certain embodiments, the present invention relates to the aforementioned method, wherein the second compound has the formula:



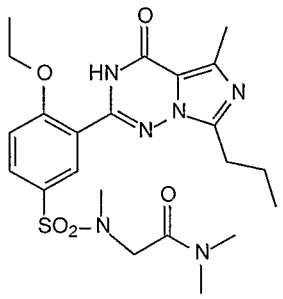
wherein, R^1, R^2 and R^3 are independently selected from H and lower alkyl, wherein the lower alkyl may be optionally substituted with one or more halogen, lower alkoxy, hydroxy, CN, NO_2, amino, acylamino, amido, carbonyl, and alkylthio.

[00110] In certain embodiments, the present invention relates to the aforementioned method, wherein the compound has the formula B:



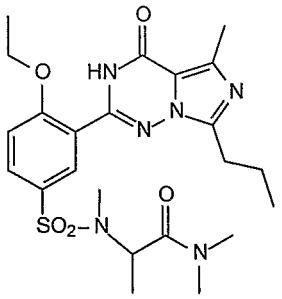
(B).

[00111] In certain embodiments, the present invention relates to the aforementioned method, wherein R^4 is methyl-amino-dimethylacetamide, to give a compound having the formula I:



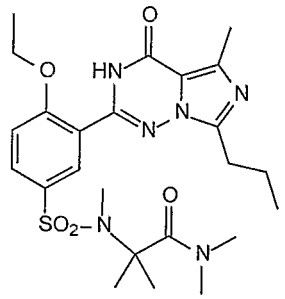
(I).

[00112] In certain embodiments, the present invention relates to the aforementioned method, wherein R^4 is methyl-alanine-dimethylacetamide, to give a compound having the formula II:



(II).

[00113] In certain embodiments, the present invention relates to the aforementioned method, wherein R^4 is 2-methylamino-2-trimethyl-propionamide, to give a compound having the formula III:

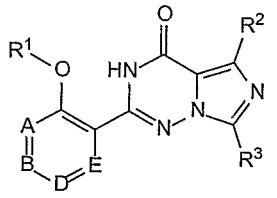


(III).

[00114] In certain embodiments, the present invention relates to the aforementioned methods, wherein *m* is 1 or 2 for R^4 (Formula C), which is attached to Compound A. Preferably, *n* is 1.

[00115] In certain embodiments, the present invention relates to the aforementioned method, wherein said first compound is a known skeletons or backbone of active compounds, including but not limited to active drug agents, bioactive substances, proteins and chemical compounds. For example, the compound may be a compound having formula A or B.

[00116] The present invention also relates to the aforementioned methods that includes metabolites of any of the above compounds. In one embodiment of the invention, the metabolite may have a structure represented by the formula D:



(D)

wherein

R^1 is lower alkyl;

R^2 and R^3 are independently selected from lower alkyl, and lower alkenyl and lower alkynyl, wherein the lower alkyl, lower alkenyl, and lower alkynyl may be optionally substituted with one or more halogen, lower alkoxy, hydroxy, CN, NO_2, amino, acylamino, amido, carbonyl, and alkylthio;

A is N or C-H;

B is N, C-H, C-(SO_2-NH-R^{13}), or C-CO-NH-R^{13};

D is N, C-H, C-(SO_2-NH-R^{13}) or C-CO-NH-R^{13};

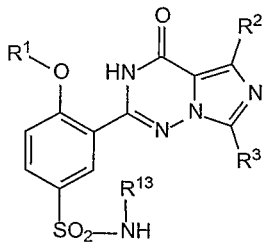
E is N or C-H;

wherein only one of A, B or E may be N, and one of B or D is C-(SO_2-NH-R^{13}) or C-CO-NH-R^{13};

R^{13} is lower alkyl.

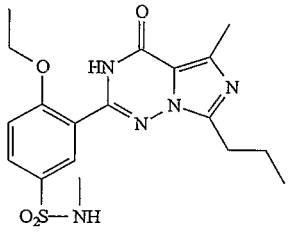
[00117] In a preferred embodiment, R^2 and R^3 are independently selected from lower alkyl. In a further preferred embodiment, R^{13} is methyl.

[00118] Preferred metabolites include compounds having the formula D_1:



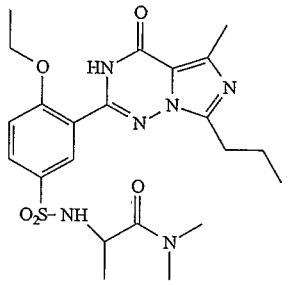
(D_1)

wherein R^1, R^2 and R^3 are as defined above for Compound A, and R^{13} is selected from lower alkyl, preferably methyl. Preferred embodiments include the compound of the formula

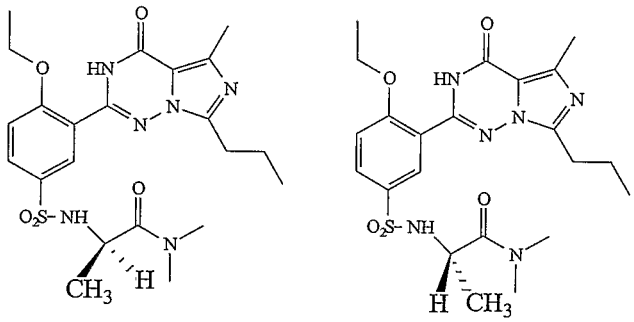


[00119] Compounds of the Formula D and D_1, or pharmaceutically acceptable salts, stereoisomers, hydrates or prodrugs thereof, may be administered directly to a patient. Alternatively, compounds of the Formula D and D_1 may be formed in the body of the patient after the administration of a parent compound or prodrug. Thus in one embodiment, the invention is directed to methods for inhibiting PDEs, particularly PDE5, by administering to a human subject compounds of the formula D or D_1. A further embodiment of the invention is directed to methods for inhibiting PDEs, particularly PDE5, by administering to a human subject, a prodrug forms a compound of the formula D or D_1 *in vivo*, for example, as a result of being metabolized.

[00120] Certain compounds derived by the aforementioned methods of the present invention may exist in particular geometric or stereoisomeric forms. The present invention contemplates all such compounds, including *cis*- and *trans*-isomers, *R*- and *S*-enantiomers, diastereomers, (D)-isomers, (L)-isomers, the racemic mixtures thereof, and other mixtures thereof, as falling within the scope of the invention. Additional asymmetric carbon atoms may be present in a substituent such as an alkyl group. All such isomers, as well as mixtures thereof, are included in this invention. For example, in one embodiment, the compound or pharmaceutical composition is a permutation of Compound 2 having the structure:



[00121] The following isomers of this compound are included in this invention:



[00122] In addition, if, for instance, a particular enantiomer of a compound of the present invention is desired, it may be prepared by asymmetric synthesis, or by derivation with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomers. Alternatively, where the molecule contains a basic functional group, such as amino, or an acidic functional group, such as carboxyl, diastereomeric salts are formed with an appropriate optically-active acid or base, followed by resolution of the diastereomers thus formed by fractional crystallization or chromatographic means well known in the art, and subsequent recovery of the pure enantiomers.

[00123] Synthetic small molecules often have low solubility that limits their ability to dissolve in the gut, be absorbed by the body, and hit the therapeutic target. Conversely, some molecules are so hydrophilic that they cannot pass through the hydrophobic membrane that surrounds cells and therefore are not able to hit their therapeutic target. Chemical groups that allow synthetic chemists to either increase solubility or reduce the hydrophilicity of small molecules are therefore desirable.

[00124] The following describes a surface-based method for identifying groups that can improve the solubility of small molecules. A self-assembled monolayer of thiol molecules terminated with maleimide groups is formed at a gold surface. A set of small molecules with thiol groups at one end, and groups that are hydrophilic at the other end are then attached to the surface via reaction between the thiol and maleimide. The set of molecules are spotted onto spatially distinct regions on the gold surface to create an array of molecules that might increase the solubility of a small molecule. Droplets of both polar (e.g., water) and hydrophobic (e.g., octanol) liquids are then placed onto each element of the array. The contact angles of the two liquids on each element are then measured at each element of the array using a goniometer. Alternatively, the wettability of a particular liquid at a surface presenting a chemical group can be determined by measuring the area of the surface covered by a droplet when viewed from above (high contact angle will yield droplets of small area; low contact angles cover greater areas). The contact angle of a liquid on a surface presenting a chemical group is inversely proportional to the miscibility of that chemical group with that liquid (solvent). For example, a chemical group for which water has a high contact angle when it is presented at the surface, such as methyl (CH3), has low miscibility with water, i.e., it will tend to reduce the solubility of a small molecule. Conversely, a chemical group for which water has a low contact angle when it is presented at the surface, such as carboxyl (COOH), has high miscibility with water, i.e., it will tend to increase the solubility of a small molecule. Sets of chemical groups can therefore be screened rapidly using contact angles on surfaces to identify groups that improve solubility or reduce hydrophilicity. This approach can be used to evaluate the effect on solubility of chemical groups used according to the invention.

[00125] A common parameter for the ability of a small molecule to cross the lipid membrane of a cell is logP where P is the partition coefficient of the compound between

octanol and water. The relative contact angle of a surface presenting chemical groups for octanol and water therefore offers a rapid, empirical method for ranking large sets of chemical groups for their potential effect on the logP of a compound.

[00126] The pH dependence of the solubility of small molecules can be addressed in this method by measuring the contact angles of solutions at different pHs. The parameter equivalent to logP in this case is logD, where D is the distribution coefficient, defined as the ratio of the sum of the concentrations of all species of the compound in octanol to the sum of the concentrations of all species of the compound in water at various pHs. Contact angles measured at different pHs therefore offer the possibility of an equivalent measure to logD.

[00127] It will also be useful to screen candidate compounds for their capacity to be actively transported across cell membranes and cells, or for their resistance to such transport. For example, it is well known that pharmaceutically useful anti-cancer molecules may be limited in their effectiveness due to active transport out of target tumor cells. Similarly, monolayers of brain capillary endothelial cells have been observed to unidirectionally transport vincristine from basal side to apical side, effectively preventing the anti-cancer agent from entering the central nervous system. In some instances, chemical groups of value will, in addition to reducing non-specific protein binding, improve pharmcokinetics by enhancing passive or active transport towards their site of action, and/or inhibiting transport from the site of action.

[00128] The brain is one of the most difficult tissues for small molecules to penetrate. The neurovascular junctions are tight and contain very few active transporters that are mostly responsible for clearing small molecules out of the brain. The paracellular route (between cell junctions) is not available to small molecules, but only the transcellular route is (through cell membranes). Classically, molecules to target the brain, such as benzodiazepines, are hydrophobic to allow them to penetrate cell membranes. The instant invention is compatible with the search for chemical groups that confer protein resistant and alleviate the common problem of excessive protein binding associated with molecules such as the benzodiazepines; this requires high dosing to account for the large percentage of binding to serum proteins. The approaches described earlier for the

identification of binders of PGP will be of help to optimize molecules for improved residence time in the brain.

[00129] Several model systems are available, employing monolayers of various cell types, for evaluation of active transport of pharmaceutically active substances. For example, monolayers of Caco-2 intestinal epithelial cells can be used to evaluate active transport of substances between the intestine and the bloodstream. When plated on a surface which allows the flow of material from apical to basolateral and vice versa, such cells form a biological membrane which can be used to simulate physiological absorption and bio-availability. In another example, mouse brain capillary endothelial cell (MBEC) lines have been established to evaluate active transport in and out of the central nervous system. Another example of such cells is HT29 human colon carcinoma cells. Further, monolayers expressing particular transporter proteins can be established using transfected cells. For example, Sasaki et al (2002) J. Biol. Chem. 8:6497 used a double-transfected Madin-Darby canine kidney cell monolayer to study transport of organic anions.

[00130] Alternatives to cell monolayers may of course be utilized to examine permeability. Alternatives typically comprise a biological structure capable of active transport and include, but are not limited to, organs of the digestive tract obtained from lab animals and reconstituted organs or membranes created in vitro from cells seeded in an artificial matrix.

[00131] Many small molecules that are potential therapeutics are not effective in the body because the body's enzymes, especially in the liver and intestines, metabolize them. For example, CYP450 enzymes cause the Phase I oxidation of small molecules thereby altering their physical and biological properties that may have a positive or negative result on its therapeutic impact. Chemical groups that resist oxidation by the CYP450 enzymes can be useful in modulating the metabolism of small molecules without compromising their efficacy against the therapeutic target. Alternatively, it might be desirable to identify chemical groups that act as sacrificial targets for oxidative enzymes thereby diverting the metabolic pathway away from the active pharmacophore. This approach might lead to new pro-drugs and/or metabolic protecting groups for metabolically susceptible pharmacophores.

[00132] One way to identify chemical groups that modulate the metabolic profile of small molecules is as follows: a self-assembled monolayer of thiol molecules terminated with anhydride groups is formed at a gold surface. A set of small molecules with amine groups at one end, and groups that are designed to resist oxidation by CYP450, for example, at the other end are then attached to the surface via reaction between the amine and anhydride. This set of molecules is spotted onto spatially distinct regions of the gold surface to create an array of molecules that might resist enzymatic oxidation. This array is then exposed to a solution containing the CYP450 enzyme, for example, a microsomal preparation. Such microsomes are available for all of the major isozymes in the CYP450 class. Once the array has been exposed to the enzyme for a suitable incubation period the gold surface is washed and placed in a MALDI mass spectrometer (MS). The chemical compositions of the set of chemical groups immobilized at the surface are then determined by performing a mass spectrometric analysis at each element in the array on gold (see previous disclosures). If the chemical groups were modified by the enzyme then this will be revealed by changes to their mass spectra. If the chemical groups were not modified by the CYP450 then their mass spectrum will not have changed from before exposure to enzyme. Groups that resist modification are good candidates for replacing groups on small molecules that are susceptible to oxidation by CYP450.

Metabolic stability or profile.

[00133] This approach is not limited to microsomes containing CYP450 enzymes of human origin. This approach can be used with natural microsomal preparations from many species in order to screen en masse to predict (and account for) different metabolic pathways for different species. These data would help in choosing the most relevant animal species to look at preclinically.

[00134] Similarly, groups that resist modification by other Phase I enzymes that cause the reduction and hydrolysis of small molecules could be identified by exposing these arrays to these enzymes and reading the array out on a MALDI-MS. Groups that resist conjugation and synthesis by Phase II enzymes could be identified by exposing the array to the enzyme and co-factor and reading out the array on a MALDI-MS.

[00135] Drug-Drug interactions. In a similar fashion, the surface array approach could be used to identify dangerous drug-drug interactions that might increase the toxicity of

small molecule therapeutics. In this method, a set of common "metabolism" motifs found on existing drugs (e.g., aspirin and anti-histamines) is immobilized on a surface. The surface is then exposed to a CYP450 microsome mixed with a potential therapeutic, and the metabolism of the immobilized motifs are then analyzed using MALDI-MS. If the test compound interfered with the metabolism of the immobilized groups by CYP450 then this result could be indicative of potential drug-drug interactions. While this example does not fall into the category of identifying chemical groups to improve PK performance it does show the wide utility of the surface array approach.

Toxicity

[00136] We could also use this format to identify what CYP450s are involved in the metabolism of a particular chemical group. This information could lead to designing drugs for particular sub-populations with certain mutations in their CYP450.

[00137] Small molecules often fail as therapeutics because they are toxic to cells. Groups that reduce toxicity would therefore be beneficial in improving such molecules. The following describes a method for screening the effect on toxicity of a set of chemical groups. Solutions of the chemical groups are spotted and dried in arrays onto a planar substrate. The cell type of interest is then deposited as a monolayer onto the array. The set of molecules are then electroporated through the cell monolayer and into the cells. After a period of incubation, the array is scanned for the viability of the cells. Any areas that contain dead cells would indicate that the electroporated chemical group is toxic.

Pharmaceutical Compositions

[00138] In another aspect, the present invention provides pharmaceutically acceptable compositions which comprise a therapeutically-effective amount of one or more of the compounds of the present invention, including but not limited to the compounds described above and those shown in the Figures, formulated together with one or more pharmaceutically acceptable carriers (additives) and/or diluents. As described in detail below, the pharmaceutical compositions of the present invention may be specially formulated for administration in solid or liquid form, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, e.g., those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; (2) parenteral

administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; (3) topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin; (4) intravaginally or intrarectally, for example, as a pessary, cream or foam; (5) sublingually; (6) ocularly; (7) transdermally; or (8) nasally.

[00139] The phrase "therapeutically-effective amount" as used herein means that amount of a compound, material, or composition comprising a compound of the present invention which is effective for producing some desired therapeutic effect in at least a sub-population of cells in an animal at a reasonable benefit/risk ratio applicable to any medical treatment, e.g. reasonable side effects applicable to any medical treatment.

[00140] The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals with toxicity, irritation, allergic response, or other problems or complications, commensurate with a reasonable benefit/risk ratio.

[00141] The phrase "pharmaceutically-acceptable carrier" as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, manufacturing aid (e.g., lubricant, talc magnesium, calcium or zinc stearate, or steric acid), or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14)

buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) pH buffered solutions; (21) polyesters, polycarbonates and/or polyanhydrides; and (22) other non-toxic compatible substances employed in pharmaceutical formulations.

[00142] As set out above, certain embodiments of the present compounds may contain a basic functional group, such as amino or alkylamino, and are, thus, capable of forming pharmaceutically-acceptable salts with pharmaceutically-acceptable acids. The term "pharmaceutically-acceptable salts" in this respect, refers to the relatively non-toxic, inorganic and organic acid addition salts of compounds of the present invention. These salts can be prepared *in situ* in the administration vehicle or the dosage form manufacturing process, or by separately reacting a purified compound of the invention in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed during subsequent purification. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, napthylate, mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts and the like. (See, for example, Berge et al. (1977) "Pharmaceutical Salts", *J. Pharm. Sci.* 66:1-19)

[00143] The pharmaceutically acceptable salts of the subject compounds include the conventional nontoxic salts or quaternary ammonium salts of the compounds, e.g., from non-toxic organic or inorganic acids. For example, such conventional nontoxic salts include those derived from inorganic acids such as hydrochloride, hydrobromic, sulfuric, sulfamic, phosphoric, nitric, and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, palmitic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicyclic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isothionic, and the like.

[00144] In other cases, the compounds of the present invention may contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically-acceptable salts with pharmaceutically-acceptable bases. The term "pharmaceutically-

acceptable salts" in these instances refers to the relatively non-toxic, inorganic and organic base addition salts of compounds of the present invention. These salts can likewise be prepared *in situ* in the administration vehicle or the dosage form manufacturing process, or by separately reacting the purified compound in its free acid form with a suitable base, such as the hydroxide, carbonate or bicarbonate of a pharmaceutically-acceptable metal cation, with ammonia, or with a pharmaceutically-acceptable organic primary, secondary or tertiary amine. Representative alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts and the like. Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like. (See, for example, Berge et al., *supra*)

[00145] Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

[00146] Examples of pharmaceutically-acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

[00147] Formulations of the present invention include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will

range from about 0.1 per cent to about ninety-nine percent of active ingredient, preferably from about 5 per cent to about 70 per cent, most preferably from about 10 per cent to about 30 per cent.

[00148] In certain embodiments, a formulation of the present invention comprises an excipient selected from the group consisting of cyclodextrins, celluloses, liposomes, micelle forming agents, e.g., bile acids, and polymeric carriers, e.g., polyesters and polyanhydrides; and a compound of the present invention. In certain embodiments, an aforementioned formulation renders orally bioavailable a compound of the present invention.

[00149] Methods of preparing these formulations or compositions include the step of bringing into association a compound of the present invention with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a compound of the present invention with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

[00150] Formulations of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a compound of the present invention as an active ingredient. A compound of the present invention may also be administered as a bolus, electuary or paste.

[00151] In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules, trouches and the like), the active ingredient is mixed with one or more pharmaceutically-acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-

agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds and surfactants, such as poloxamer and sodium lauryl sulfate; (7) wetting agents, such as, for example, cetyl alcohol, glycerol monostearate, and non-ionic surfactants; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, zinc stearate, sodium stearate, stearic acid, and mixtures thereof; (10) coloring agents; and (11) controlled release agents such as crospovidone or ethyl cellulose. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-shelled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

[00152] A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

[00153] The tablets, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be formulated for rapid release, e.g., freeze-dried. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active

ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

[00154] Liquid dosage forms for oral administration of the compounds of the invention include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

[00155] Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

[00156] Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

[00157] Formulations of the pharmaceutical compositions of the invention for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing one or more compounds of the invention with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active compound.

[00158] Formulations of the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

[00159] Dosage forms for the topical or transdermal administration of a compound of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with a pharmaceutically-acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

[00160] The ointments, pastes, creams and gels may contain, in addition to an active compound of this invention, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

[00161] Powders and sprays can contain, in addition to a compound of this invention, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

[00162] Transdermal patches have the added advantage of providing controlled delivery of a compound of the present invention to the body. Such dosage forms can be made by dissolving or dispersing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the compound in a polymer matrix or gel.

[00163] Ophthalmic formulations, eye ointments, powders, solutions and the like, are also contemplated as being within the scope of this invention.

[00164] Pharmaceutical compositions of this invention suitable for parenteral administration comprise one or more compounds of the invention in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or nonaqueous

solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain sugars, alcohols, antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

[00165] Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

[00166] These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms upon the subject compounds may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

[00167] In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

[00168] Injectable depot forms are made by forming microencapsule matrices of the subject compounds in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer

employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue.

[00169] When the compounds of the present invention are administered as pharmaceuticals, to humans and animals, they can be given per se or as a pharmaceutical composition containing, for example, 0.1 to 99% (more preferably, 10 to 30%) of active ingredient in combination with a pharmaceutically acceptable carrier.

[00170] The preparations of the present invention may be given orally, parenterally, topically, or rectally. They are of course given in forms suitable for each administration route. For example, they are administered in tablets or capsule form, by injection, inhalation, eye lotion, ointment, suppository, etc. administration by injection, infusion or inhalation; topical by lotion or ointment; and rectal by suppositories. Oral administrations are preferred.

[00171] The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticulare, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

[00172] The phrases "systemic administration," "administered systemically," "peripheral administration" and "administered peripherally" as used herein mean the administration of a compound, drug or other material other than directly into the central nervous system, such that it enters the patient's system and, thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

[00173] These compounds may be administered to humans and other animals for therapy by any suitable route of administration, including orally, nasally, as by, for example, a spray, rectally, intravaginally, parenterally, intracisternally and topically, as by powders, ointments or drops, including buccally and sublingually.

[00174] Regardless of the route of administration selected, the compounds of the present invention, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically-acceptable dosage forms by conventional methods known to those of skill in the art.

[00175] Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

[00176] The selected dosage level will depend upon a variety of factors including the activity of the particular compound of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion or metabolism of the particular compound being employed, the rate and extent of absorption, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compound employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

[00177] A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

[00178] In general, a suitable daily dose of a compound of the invention will be that amount of the compound which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above. Generally, oral, intravenous, intracerebroventricular and subcutaneous doses of the compounds of this invention for a patient, when used for the indicated analgesic effects, will range from about 0.0001 to about 100 mg per kilogram of body weight per day.

[00179] If desired, the effective daily dose of the active compound may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms. Preferred dosing is one administration per day.

[00180] While it is possible for a compound of the present invention to be administered alone, it is preferable to administer the compound as a pharmaceutical formulation (composition).

[00181] The compounds according to the invention may be formulated for administration in any convenient way for use in human or veterinary medicine, by analogy with other pharmaceuticals.

[00182] In another aspect, the present invention provides pharmaceutically acceptable compositions which comprise a therapeutically-effective amount of one or more of the subject compounds, as described above, formulated together with one or more pharmaceutically acceptable carriers (additives) and/or diluents. As described in detail below, the pharmaceutical compositions of the present invention may be specially formulated for administration in solid or liquid form, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, boluses, powders, granules, pastes for application to the tongue; (2) parenteral administration, for example, by subcutaneous, intramuscular or intravenous injection as, for example, a sterile solution or suspension; (3) topical application, for example, as a cream, ointment or spray applied to the skin, lungs, or mucous membranes; or (4) intravaginally or intrarectally, for example, as a pessary, cream or foam; (5) sublingually or buccally; (6) ocularly; (7) transdermally; or (8) nasally.

[00183] The term "treatment" is intended to encompass also prophylaxis, therapy and cure.

[00184] The patient receiving this treatment is any animal in need, including primates, in particular humans, and other mammals such as equines, cattle, swine and sheep; and poultry and pets in general.

[00185] The compound of the invention can be administered as such or in admixtures with pharmaceutically acceptable carriers and can also be administered in conjunction with antimicrobial agents such as penicillins, cephalosporins, aminoglycosides and glycopeptides. Conjunctive therapy, thus includes sequential, simultaneous and separate administration of the active compound in a way that the therapeutical effects of the first administered one is not entirely disappeared when the subsequent is administered.

[00186] The addition of the active compound of the invention to animal feed is preferably accomplished by preparing an appropriate feed premix containing the active compound in an effective amount and incorporating the premix into the complete ration.

[00187] Alternatively, an intermediate concentrate or feed supplement containing the active ingredient can be blended into the feed. The way in which such feed premixes and complete rations can be prepared and administered are described in reference books (such as "Applied Animal Nutrition", W.H. Freedman and CO., San Francisco, U.S.A., 1969 or "Livestock Feeds and Feeding" O and B books, Corvallis, Ore., U.S.A., 1977).

Micelles

[00188] Recently, the pharmaceutical industry introduced microemulsification technology to improve bioavailability of some lipophilic (water insoluble) pharmaceutical agents. Examples include Trimetrine (Dordunoo, S. K., et al., Drug Development and Industrial Pharmacy, 17(12), 1685-1713, 1991 and REV 5901 (Sheen, P. C., et al., J Pharm Sci 80(7), 712-714, 1991). Among other things, microemulsification provides enhanced bioavailability by preferentially directing absorption to the lymphatic system instead of the circulatory system, which thereby bypasses the liver, and prevents destruction of the compounds in the hepatobiliary circulation.

[00189] In one aspect of invention, the formulations contain micelles formed from a compound of the present invention and at least one amphiphilic carrier, in which the micelles have an average diameter of less than about 100 nm. More preferred embodiments provide micelles having an average diameter less than about 50 nm, and even more preferred embodiments provide micelles having an average diameter less than about 30 nm, or even less than about 20 nm.

[00190] While all suitable amphiphilic carriers are contemplated, the presently preferred carriers are generally those that have Generally-Recognized-as-Safe (GRAS) status, and that can both solubilize the compound of the present invention and microemulsify it at a later stage when the solution comes into a contact with a complex water phase (such as one found in human gastro-intestinal tract). Usually, amphiphilic ingredients that satisfy these requirements have HLB (hydrophilic to lipophilic balance) values of 2-20, and their structures contain straight chain aliphatic radicals in the range of C-6 to C-20. Examples are polyethylene-glycolized fatty glycerides and polyethylene glycols.

[00191] Particularly preferred amphiphilic carriers are saturated and monounsaturated polyethyleneglycolyzed fatty acid glycerides, such as those obtained from fully or partially hydrogenated various vegetable oils. Such oils may advantageously consist of tri-. di- and mono-fatty acid glycerides and di- and mono-polyethyleneglycol esters of the corresponding fatty acids, with a particularly preferred fatty acid composition including capric acid 4-10, capric acid 3-9, lauric acid 40-50, myristic acid 14-24, palmitic acid 4-14 and stearic acid 5-15%. Another useful class of amphiphilic carriers includes partially esterified sorbitan and/or sorbitol, with saturated or mono-unsaturated fatty acids (SPAN-series) or corresponding ethoxylated analogs (TWEEN-series).

[00192] Commercially available amphiphilic carriers are particularly contemplated, including Gelucire-series, Labrafil, Labrasol, or Lauroglycol (all manufactured and distributed by Gattefosse Corporation, Saint Priest, France), PEG-mono-oleate, PEG-di-oleate, PEG-mono-laurate and di-laurate, Lecithin, Polysorbate 80, etc (produced and distributed by a number of companies in USA and worldwide).

Polymers

[00193] Hydrophilic polymers suitable for use in the present invention are those which are readily water-soluble, can be covalently attached to a vesicle-forming lipid, and which are tolerated in vivo without toxic effects (i.e., are biocompatible). Suitable polymers include polyethylene glycol (PEG), polylactic (also termed polylactide), polyglycolic acid (also termed polyglycolide), a polylactic-polyglycolic acid copolymer, and polyvinyl alcohol. Preferred polymers are those having a molecular weight of from about 100 or

120 daltons up to about 5,000 or 10,000 daltons, and more preferably from about 300 daltons to about 5,000 daltons. In a particularly preferred embodiment, the polymer is polyethyleneglycol having a molecular weight of from about 100 to about 5,000 daltons, and more preferably having a molecular weight of from about 300 to about 5,000 daltons. In a particularly preferred embodiment, the polymer is polyethyleneglycol of 750 daltons (PEG(750)). The polymers used in the present invention have a significantly smaller molecular weight, approximately 100 daltons, compared to the large MW of 5000 daltons or greater that used in standard pegylation techniques. Polymers may also be defined by the number of monomers therein; a preferred embodiment of the present invention utilizes polymers of at least about three monomers, such PEG polymers consisting of three monomers (approximately 150 daltons).

[00194] Other hydrophilic polymers which may be suitable for use in the present invention include polyvinylpyrrolidone, polymethoxazoline, polyethyloxazoline, polyhydroxypropyl methacrylamide, polymethacrylamide, polydimethylacrylamide, and derivatized celluloses such as hydroxymethylcellulose or hydroxyethylcellulose.

[00195] In certain embodiments, a formulation of the present invention comprises a biocompatible polymer selected from the group consisting of polyamides, polycarbonates, polyalkylenes, polymers of acrylic and methacrylic esters, polyvinyl polymers, polyglycolides, polysiloxanes, polyurethanes and co-polymers thereof, celluloses, polypropylene, polyethylenes, polystyrene, polymers of lactic acid and glycolic acid, polyanhydrides, poly(ortho)esters, poly(butic acid), poly(valeric acid), poly(lactide-co-caprolactone), polysaccharides, proteins, polyhyaluronic acids, polycyanoacrylates, and blends, mixtures, or copolymers thereof.

Cyclodextrins

[00196] Cyclodextrins are cyclic oligosaccharides, consisting of 6, 7 or 8 glucose units, designated by the Greek letter .alpha., .beta. or .gamma., respectively. Cyclodextrins with fewer than six glucose units are not known to exist. The glucose units are linked by alpha-1,4-glucosidic bonds. As a consequence of the chair conformation of the sugar units, all secondary hydroxyl groups (at C-2, C-3) are located on one side of the ring, while all the primary hydroxyl groups at C-6 are situated on the other side. As a result, the external faces are hydrophilic, making the cyclodextrins water-soluble. In contrast, the

cavities of the cyclodextrins are hydrophobic, since they are lined by the hydrogen of atoms C-3 and C-5, and by ether-like oxygens. These matrices allow complexation with a variety of relatively hydrophobic compounds, including, for instance, steroid compounds such as 17.beta.-estradiol (see, e.g., van Uden et al. Plant Cell Tiss. Org. Cult. 38:1-3-113 (1994)). The complexation takes place by Van der Waals interactions and by hydrogen bond formation. For a general review of the chemistry of cyclodextrins, see, Wenz, Agnew. Chem. Int. Ed. Engl., 33:803-822 (1994).

[00197] The physico-chemical properties of the cyclodextrin derivatives depend strongly on the kind and the degree of substitution. For example, their solubility in water ranges from insoluble (e.g., triacetyl-beta-cyclodextrin) to 147% soluble (w/v) (G-2-beta-cyclodextrin). In addition, they are soluble in many organic solvents. The properties of the cyclodextrins enable the control over solubility of various formulation components by increasing or decreasing their solubility.

[00198] Numerous cyclodextrins and methods for their preparation have been described. For example, Parmeter (I), et al. (U.S. Pat. No. 3,453,259) and Gramera, et al. (U.S. Pat. No. 3,459,731) described electroneutral cyclodextrins. Other derivatives include cyclodextrins with cationic properties [Parmeter (II), U.S. Pat. No. 3,453,257], insoluble crosslinked cyclodextrins (Solms, U.S. Pat. No. 3,420,788), and cyclodextrins with anionic properties [Parmeter (III), U.S. Pat. No. 3,426,011]. Among the cyclodextrin derivatives with anionic properties, carboxylic acids, phosphorous acids, phosphinous acids, phosphonic acids, phosphoric acids, thiophosphonic acids, thiosulphinic acids, and sulfonic acids have been appended to the parent cyclodextrin [see, Parmeter (III), supra]. Furthermore, sulfoalkyl ether cyclodextrin derivatives have been described by Stella, et al. (U.S. Pat. No. 5,134,127).

Liposomes

[00199] Liposomes consist of at least one lipid bilayer membrane enclosing an aqueous internal compartment. Liposomes may be characterized by membrane type and by size. Small unilamellar vesicles (SUVs) have a single membrane and typically range between 0.02 and 0.05 μm in diameter; large unilamellar vesicles (LUVS) are typically larger than 0.05 μm Oligolamellar large vesicles and multilamellar vesicles have multiple,

usually concentric, membrane layers and are typically larger than 0.1 μm. Liposomes with several nonconcentric membranes, i.e., several smaller vesicles contained within a larger vesicle, are termed multivesicular vesicles.

[00200] One aspect of the present invention relates to formulations comprising liposomes containing a compound of the present invention, where the liposome membrane is formulated to provide a liposome with increased carrying capacity. Alternatively or in addition, the compound of the present invention may be contained within, or adsorbed onto, the liposome bilayer of the liposome. The compound of the present invention may be aggregated with a lipid surfactant and carried within the liposome's internal space; in these cases, the liposome membrane is formulated to resist the disruptive effects of the active agent-surfactant aggregate.

[00201] According to one embodiment of the present invention, the lipid bilayer of a liposome contains lipids derivatized with polyethylene glycol (PEG), such that the PEG chains extend from the inner surface of the lipid bilayer into the interior space encapsulated by the liposome, and extend from the exterior of the lipid bilayer into the surrounding environment.

[00202] Active agents contained within liposomes of the present invention are in solubilized form. Aggregates of surfactant and active agent (such as emulsions or micelles containing the active agent of interest) may be entrapped within the interior space of liposomes according to the present invention. A surfactant acts to disperse and solubilize the active agent, and may be selected from any suitable aliphatic, cycloaliphatic or aromatic surfactant, including but not limited to biocompatible lysophosphatidylcholines (LPCs) of varying chain lengths (for example, from about C.sub.14 to about C.sub.20). Polymer-derivatized lipids such as PEG-lipids may also be utilized for micelle formation as they will act to inhibit micelle/membrane fusion, and as the addition of a polymer to surfactant molecules decreases the CMC of the surfactant and aids in micelle formation. Preferred are surfactants with CMCs in the micromolar range; higher CMC surfactants may be utilized to prepare micelles entrapped within liposomes of the present invention, however, micelle surfactant monomers could affect liposome bilayer stability and would be a factor in designing a liposome of a desired stability.

[00203] Liposomes according to the present invention may be prepared by any of a variety of techniques that are known in the art. See, e.g., U.S. Pat. No. 4,235,871; Published PCT applications WO 96/14057; New RRC, Liposomes: A practical approach, IRL Press, Oxford (1990), pages 33-104; Lasic DD, Liposomes from physics to applications, Elsevier Science Publishers BV, Amsterdam, 1993.

[00204] For example, liposomes of the present invention may be prepared by diffusing a lipid derivatized with a hydrophilic polymer into preformed liposomes, such as by exposing preformed liposomes to micelles composed of lipid-grafted polymers, at lipid concentrations corresponding to the final mole percent of derivatized lipid which is desired in the liposome. Liposomes containing a hydrophilic polymer can also be formed by homogenization, lipid-field hydration, or extrusion techniques, as are known in the art.

[00205] In another exemplary formulation procedure, the active agent is first dispersed by sonication in a lysophosphatidylcholine or other low CMC surfactant (including polymer grafted lipids) that readily solubilizes hydrophobic molecules. The resulting micellar suspension of active agent is then used to rehydrate a dried lipid sample that contains a suitable mole percent of polymer-grafted lipid, or cholesterol. The lipid and active agent suspension is then formed into liposomes using extrusion techniques as are known in the art, and the resulting liposomes separated from the unencapsulated solution by standard column separation.

[00206] In one aspect of the present invention, the liposomes are prepared to have substantially homogeneous sizes in a selected size range. One effective sizing method involves extruding an aqueous suspension of the liposomes through a series of polycarbonate membranes having a selected uniform pore size; the pore size of the membrane will correspond roughly with the largest sizes of liposomes produced by extrusion through that membrane. See e.g., U.S. Pat. No. 4,737,323 (Apr. 12, 1988).

Release Modifiers

[00207] The release characteristics of a formulation of the present invention depend on the encapsulating material, the concentration of encapsulated drug, and the presence of release modifiers. For example, release can be manipulated to be pH dependent, for

example, using a pH sensitive coating that releases only at a low pH, as in the stomach, or a higher pH, as in the intestine. An enteric coating can be used to prevent release from occurring until after passage through the stomach. Multiple coatings or mixtures of cyanamide encapsulated in different materials can be used to obtain an initial release in the stomach, followed by later release in the intestine. Release can also be manipulated by inclusion of salts or pore forming agents, which can increase water uptake or release of drug by diffusion from the capsule. Excipients which modify the solubility of the drug can also be used to control the release rate. Agents which enhance degradation of the matrix or release from the matrix can also be incorporated. They can be added to the drug, added as a separate phase (i.e., as particulates), or can be co-dissolved in the polymer phase depending on the compound. In all cases the amount should be between 0.1 and thirty percent (w/w polymer). Types of degradation enhancers include inorganic salts such as ammonium sulfate and ammonium chloride, organic acids such as citric acid, benzoic acid, and ascorbic acid, inorganic bases such as sodium carbonate, potassium carbonate, calcium carbonate, zinc carbonate, and zinc hydroxide, and organic bases such as protamine sulfate, spermine, choline, ethanolamine, diethanolamine, and triethanolamine and surfactants such as Tween.RTM. and Pluronic.RTM.. Pore forming agents which add microstructure to the matrices (i.e., water soluble compounds such as inorganic salts and sugars) are added as particulates. The range should be between one and thirty percent (w/w polymer).

[00208] Uptake can also be manipulated by altering residence time of the particles in the gut. This can be achieved, for example, by coating the particle with, or selecting as the encapsulating material, a mucosal adhesive polymer. Examples include most polymers with free carboxyl groups, such as chitosan, celluloses, and especially polyacrylates (as used herein, polyacrylates refers to polymers including acrylate groups and modified acrylate groups such as cyanoacrylates and methacrylates).

Combinatorial Libraries

[00209] The subject compounds may be synthesized using the methods of combinatorial synthesis described in this section. Combinatorial libraries of the compounds may be used for the screening of pharmaceutical, agrochemical or other biological or medically-related activity or material-related qualities. A combinatorial library for the purposes of the present invention is a mixture of chemically related

compounds which may be screened together for a desired property; said libraries may be in solution or covalently linked to a solid support. The preparation of many related compounds in a single reaction greatly reduces and simplifies the number of screening processes which need to be carried out. Screening for the appropriate biological, pharmaceutical, agrochemical or physical property may be done by conventional methods.

[00210] Diversity in a library can be created at a variety of different levels. For instance, the substrate aryl groups used in a combinatorial approach can be diverse in terms of the core aryl moiety, e.g., a variegation in terms of the ring structure, and/or can be varied with respect to the other substituents.

[00211] A variety of techniques are available in the art for generating combinatorial libraries of small organic molecules. See, for example, Blondelle et al. (1995) Trends Anal. Chem. 14:83; the Affymax U.S. Patents 5,359,115 and 5,362,899: the Ellman U.S. Patent 5,288,514: the Still et al. PCT publication WO 94/08051; Chen et al. (1994) JACS 116:2661: Kerr et al. (1993) JACS 115:252; PCT publications WO92/10092, WO93/09668 and WO91/07087; and the Lerner et al. PCT publication WO93/20242). Accordingly, a variety of libraries on the order of about 16 to 1,000,000 or more diversomers can be synthesized and screened for a particular activity or property.

[00212] In an exemplary embodiment, a library of substituted diversomers can be synthesized using the subject reactions adapted to the techniques described in the Still et al. PCT publication WO 94/08051, e.g., being linked to a polymer bead by a hydrolyzable or photolyzable group, e.g., located at one of the positions of substrate. According to the Still et al. technique, the library is synthesized on a set of beads, each bead including a set of tags identifying the particular diversomer on that bead. In one embodiment, which is particularly suitable for discovering enzyme inhibitors, the beads can be dispersed on the surface of a permeable membrane, and the diversomers released from the beads by lysis of the bead linker. The diversomer from each bead will diffuse across the membrane to an assay zone, where it will interact with an enzyme assay. Detailed descriptions of a number of combinatorial methodologies are provided below.

Direct Characterization

[00213] A growing trend in the field of combinatorial chemistry is to exploit the sensitivity of techniques such as mass spectrometry (MS), e.g., which can be used to characterize sub-femtomolar amounts of a compound, and to directly determine the chemical constitution of a compound selected from a combinatorial library. For instance, where the library is provided on an insoluble support matrix, discrete populations of compounds can be first released from the support and characterized by MS. In other embodiments, as part of the MS sample preparation technique, such MS techniques as MALDI can be used to release a compound from the matrix, particularly where a labile bond is used originally to tether the compound to the matrix. For instance, a bead selected from a library can be irradiated in a MALDI step in order to release the diversomer from the matrix, and ionize the diversomer for MS analysis.

Multipin Synthesis

[00214] The libraries of the subject method can take the multipin library format. Briefly, Geysen and co-workers (Geysen et al. (1984) PNAS 81:3998-4002) introduced a method for generating compound libraries by a parallel synthesis on polyacrylic acid-grated polyethylene pins arrayed in the microtitre plate format. The Geysen technique can be used to synthesize and screen thousands of compounds per week using the multipin method, and the tethered compounds may be reused in many assays. Appropriate linker moieties can also been appended to the pins so that the compounds may be cleaved from the supports after synthesis for assessment of purity and further evaluation (c.f., Bray et al. (1990) Tetrahedron Lett 31:5811-5814; Valerio et al. (1991) Anal Biochem 197:168-177; Bray et al. (1991) Tetrahedron Lett 32:6163-6166).

Divide-Couple-Recombine

[00215] In yet another embodiment, a variegated library of compounds can be provided on a set of beads utilizing the strategy of divide-couple-recombine (see, e.g., Houghten (1985) PNAS 82:5131-5135; and U.S. Patents 4,631,211; 5,440,016; 5,480,971). Briefly, as the name implies, at each synthesis step where degeneracy is introduced into the library, the beads are divided into separate groups equal to the number of different substituents to be added at a particular position in the library, the different substituents coupled in separate reactions, and the beads recombined into one pool for the next iteration.

[00216] In one embodiment, the divide-couple-recombine strategy can be carried out using an analogous approach to the so-called "tea bag" method first developed by Houghten, where compound synthesis occurs on resin sealed inside porous polypropylene bags (Houghten et al. (1986) PNAS 82:5131-5135). Substituents are coupled to the compound-bearing resins by placing the bags in appropriate reaction solutions, while all common steps such as resin washing and deprotection are performed simultaneously in one reaction vessel. At the end of the synthesis, each bag contains a single compound.

Combinatorial Libraries by Light-Directed, Spatially Addressable Parallel Chemical Synthesis

[00217] A scheme of combinatorial synthesis in which the identity of a compound is given by its locations on a synthesis substrate is termed a spatially-addressable synthesis. In one embodiment, the combinatorial process is carried out by controlling the addition of a chemical reagent to specific locations on a solid support (Dower et al. (1991) Annu Rep Med Chem 26:271-280; Fodor, S.P.A. (1991) Science 251:767; Pirrung et al. (1992) U.S. Patent No. 5,143,854; Jacobs et al. (1994) Trends Biotechnol 12:19-26). The spatial resolution of photolithography affords miniaturization. This technique can be carried out through the use protection/deprotection reactions with photolabile protecting groups.

[00218] The key points of this technology are illustrated in Gallop et al. (1994) J Med Chem 37:1233-1251. A synthesis substrate is prepared for coupling through the covalent attachment of photolabile nitroveratryloxycarbonyl (NVOC) protected amino linkers or other photolabile linkers. Light is used to selectively activate a specified region of the synthesis support for coupling. Removal of the photolabile protecting groups by light (deprotection) results in activation of selected areas. After activation, the first of a set of amino acid analogs, each bearing a photolabile protecting group on the amino terminus, is exposed to the entire surface. Coupling only occurs in regions that were addressed by light in the preceding step. The reaction is stopped, the plates washed, and the substrate is again illuminated through a second mask, activating a different region for reaction with a second protected building block. The pattern of masks and the sequence of reactants define the products and their locations. Since this process utilizes photolithography techniques, the number of compounds that can be synthesized is limited only by the

number of synthesis sites that can be addressed with appropriate resolution. The position of each compound is precisely known; hence, its interactions with other molecules can be directly assessed.

[00219] In a light-directed chemical synthesis, the products depend on the pattern of illumination and on the order of addition of reactants. By varying the lithographic patterns, many different sets of test compounds can be synthesized simultaneously; this characteristic leads to the generation of many different masking strategies.

Encoded Combinatorial Libraries

[00220] In yet another embodiment, the subject method utilizes a compound library provided with an encoded tagging system. A recent improvement in the identification of active compounds from combinatorial libraries employs chemical indexing systems using tags that uniquely encode the reaction steps a given bead has undergone and, by inference, the structure it carries. Conceptually, this approach mimics phage display libraries, where activity derives from expressed peptides, but the structures of the active peptides are deduced from the corresponding genomic DNA sequence. The first encoding of synthetic combinatorial libraries employed DNA as the code. A variety of other forms of encoding have been reported, including encoding with sequenceable bio-oligomers (e.g., oligonucleotides and peptides), and binary encoding with additional non-sequenceable tags.

[00221] The principle of using oligonucleotides to encode combinatorial synthetic libraries was described in 1992 (Brenner et al. (1992) PNAS 89:5381-5383), and an example of such a library appeared the following year (Needles et al. (1993) PNAS 90:10700-10704). A combinatorial library of nominally 7^7 (= 823,543) peptides composed of all combinations of Arg, Gln, Phe, Lys, Val, D-Val and Thr (three-letter amino acid code), each of which was encoded by a specific dinucleotide (TA, TC, CT, AT, TT, CA and AC, respectively), was prepared by a series of alternating rounds of peptide and oligonucleotide synthesis on solid support. In this work, the amine linking functionality on the bead was specifically differentiated toward peptide or oligonucleotide synthesis by simultaneously preincubating the beads with reagents that generate protected OH groups for oligonucleotide synthesis and protected NH_2 groups for peptide synthesis

(here, in a ratio of 1:20). When complete, the tags each consisted of 69-mers, 14 units of which carried the code. The bead-bound library was incubated with a fluorescently labeled antibody, and beads containing bound antibody that fluoresced strongly were harvested by fluorescence-activated cell sorting (FACS). The DNA tags were amplified by PCR and sequenced, and the predicted peptides were synthesized. Following such techniques, compound libraries can be derived for use in the subject method, where the oligonucleotide sequence of the tag identifies the sequential combinatorial reactions that a particular bead underwent, and therefore provides the identity of the compound on the bead.

Tagging with sequenceable bio-oligomers

[00222] The use of oligonucleotide tags permits exquisitely sensitive tag analysis. Even so, the method requires careful choice of orthogonal sets of protecting groups required for alternating co-synthesis of the tag and the library member. Furthermore, the chemical lability of the tag, particularly the phosphate and sugar anomeric linkages, may limit the choice of reagents and conditions that can be employed for the synthesis of non-oligomeric libraries. In preferred embodiments, the libraries employ linkers permitting selective detachment of the test compound library member for assay.

[00223] Peptides have also been employed as tagging molecules for combinatorial libraries. Two exemplary approaches are described in the art, both of which employ branched linkers to solid phase upon which coding and ligand strands are alternately elaborated. In the first approach (Kerr JM et al. (1993) J Am Chem Soc 115:2529-2531), orthogonality in synthesis is achieved by employing acid-labile protection for the coding strand and base-labile protection for the compound strand.

[00224] In an alternative approach (Nikolaiev et al. (1993) Pept Res 6:161-170), branched linkers are employed so that the coding unit and the test compound can both be attached to the same functional group on the resin. In one embodiment, a cleavable linker can be placed between the branch point and the bead so that cleavage releases a molecule containing both code and the compound (Ptek et al. (1991) Tetrahedron Lett 32:3891-3894). In another embodiment, the cleavable linker can be placed so that the test compound can be selectively separated from the bead, leaving the code behind. This last construct is particularly valuable because it permits screening of the test compound

without potential interference of the coding groups. Examples in the art of independent cleavage and sequencing of peptide library members and their corresponding tags has confirmed that the tags can accurately predict the peptide structure.

Non-sequenceable Tagging: Binary Encoding

[00225] An alternative form of encoding the test compound library employs a set of non-sequencable electrophoric tagging molecules that are used as a binary code (Ohlmeyer et al. (1993) PNAS 90:10922-10926). Exemplary tags are haloaromatic alkyl ethers that are detectable as their trimethylsilyl ethers at less than femtomolar levels by electron capture gas chromatography (ECGC). Variations in the length of the alkyl chain, as well as the nature and position of the aromatic halide substituents, permit the synthesis of at least 40 such tags, which in principle can encode 2^{40} (e.g., upwards of 10^{12}) different molecules. In the original report (Ohlmeyer et al., supra) the tags were bound to about 1% of the available amine groups of a peptide library via a photocleavable o-nitrobenzyl linker. This approach is convenient when preparing combinatorial libraries of peptide-like or other amine-containing molecules. A more versatile system has, however, been developed that permits encoding of essentially any combinatorial library. Here, the compound would be attached to the solid support via the photocleavable linker and the tag is attached through a catechol ether linker via carbene insertion into the bead matrix (Nestler et al. (1994) J Org Chem 59:4723-4724). This orthogonal attachment strategy permits the selective detachment of library members for assay in solution and subsequent decoding by ECGC after oxidative detachment of the tag sets.

[00226] Although several amide-linked libraries in the art employ binary encoding with the electrophoric tags attached to amine groups, attaching these tags directly to the bead matrix provides far greater versatility in the structures that can be prepared in encoded combinatorial libraries. Attached in this way, the tags and their linker are nearly as unreactive as the bead matrix itself. Two binary-encoded combinatorial libraries have been reported where the electrophoric tags are attached directly to the solid phase (Ohlmeyer et al. (1995) PNAS 92:6027-6031) and provide guidance for generating the subject compound library. Both libraries were constructed using an orthogonal attachment strategy in which the library member was linked to the solid support by a photolabile linker and the tags were attached through a linker cleavable only by vigorous

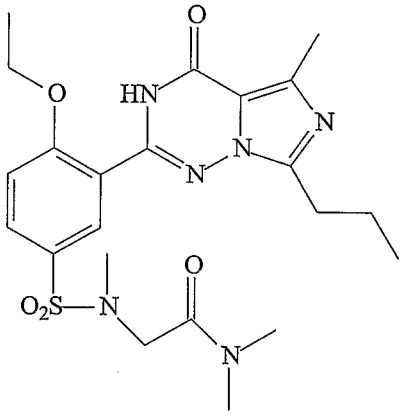
oxidation. Because the library members can be repetitively partially photoeluted from the solid support, library members can be utilized in multiple assays. Successive photoelution also permits a very high throughput iterative screening strategy: first, multiple beads are placed in 96-well microtiter plates; second, compounds are partially detached and transferred to assay plates; third, a metal binding assay identifies the active wells; fourth, the corresponding beads are rearrayed singly into new microtiter plates; fifth, single active compounds are identified; and sixth, the structures are decoded.

EXAMPLES

[00227] The invention now being generally described will be more readily understood by reference to the following examples, which are included merely for purposes of illustration of certain aspects and embodiments of the present invention, and are not intended to limit the invention.

Example 1

[00228] Preparation of Compound 1

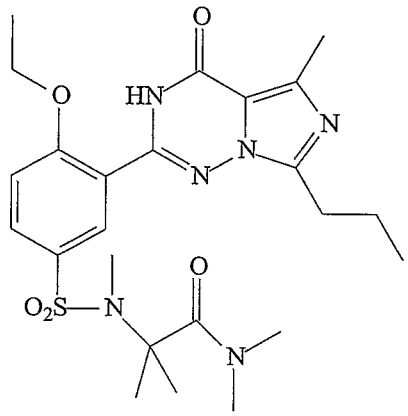


Compound 1

A solution of sarcosine dimethyl amide (0.22 mmol) and triethylamine (0.6 mmol) in methanol/methylene chloride (1:9, 1.2 mL) was treated with sulfonyl chloride (0.122 mmol) and stirred for 4 hours at 23°C. The clear solution was diluted with methylene chloride (10 mL) and washed with 10% aqueous citric acid. The separated aqueous layer was extracted with dichloromethane (10 mL), and the combined organic layers were washed with brine and dried over sodium sulfate. The clear oil from concentration in vacuo was purified by silica gel chromatography (0.6% water/1.2% methanol in ethyl acetate as eluent) to yield a white solid in 87% yield.

Example 2

[00229] Preparation of Compound 3.



Compound 3

A solution of N-methyl-aminoisobutyl dimethylamide (0.22 mmol) and sulfonyl chloride (0.122 mmol) were placed in a flask and concentrated three times from dry 1,2-dichloroethane (10 mL). The residue was restored in dry dichloromethane and triethylamine (0.6 mmol), and 4-(dimethylamino)pyridine (2.5 mg) was added. The mixture was stirred for 8 hours at 23°C. The clear solution was diluted with methylene chloride (10 mL) and washed with 10% aqueous citric acid. The separated aqueous layer was extracted with dichloromethane (10 mL), and the combined organic layers were washed with brine and dried over sodium sulfate. The clear oil from concentration in vacuo was purified by silica gel chromatography (0.6% water/1.2% methanol in ethyl acetate as eluent) to yield a white solid in 87% yield.

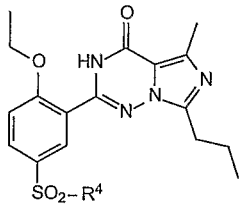
Example 3

[00230] Vardenafil (Levitra®) is a selective inhibitor of PDE5. The structures of vardenafil and analogs known in the art were compared with regard to activity and pharmacokinetic properties. The design of the compounds of the present invention involved modifying molecules using functional groups to provide new compounds that exhibit improved pharmacokinetic properties. The functional groups were placed so as to affect pharmacokinetic properties, rather than activity. The set of compounds that are designed and synthesized included elements that are predicted to be metabolic products of other elements of the set.

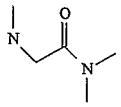
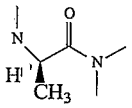
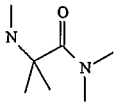
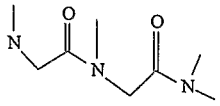
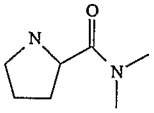
[00231] Among the substitutions tested, sarconine derivatives methyl-amino-dimethylacetamide, resulted in reduction of protein binding, while maintaining potency and solubility.

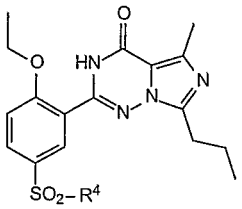
[00232] Phosphodiesterase inhibition was determined by methods known to one of skill in the art. Most of the compounds tested had activity comparable to vardenafil. Selectivity of most of the compounds for human PDE-5 relative to PDE-1, PDE-3 and PDE-6 was also comparable.

[00233] **Table 1. Properties of Compounds Modified by Attachment of Functional Residues**

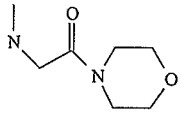
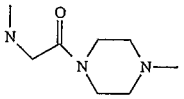
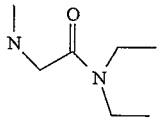
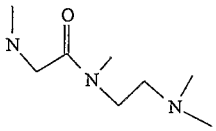
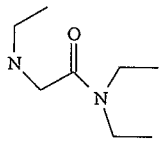
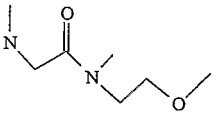
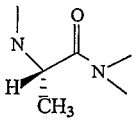
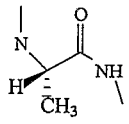
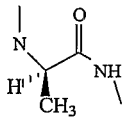


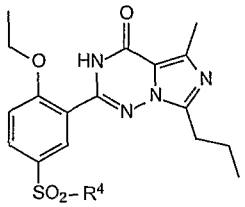
COMPOUND B

Compound No.	R^4	Molecular Weight	Molecular Formula	CLogP	IC_{50}
1		490.576	$C_{22}H_{30}N_6O_5S$	0.98	0.12 nM
2		504.602	$C_{23}H_{32}N_6O_5S$	1.289	0.10 nM
3		518.629	$C_{24}H_{34}N_6O_5S$	1.598	0.14 nM
4		561.654	$C_{24}H_{35}N_7O_6S$	1	0.084 nM
5		516.613	$C_{24}H_{32}N_6O_5S$	1.792	1.6 nM

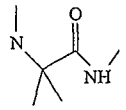
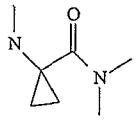
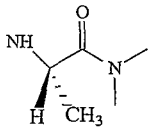
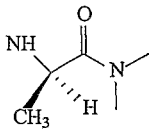
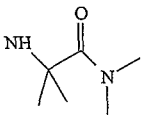
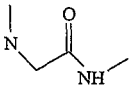


COMPOUND B

Compound No.	R^4	Molecular Weight	Molecular Formula	CLogP	IC_{50}
6		532.612	$C_{24}H_{32}N_6O_6S$	1.1	0.68 nM
7		545.654	$C_{25}H_{35}N_7O_5S$	1.54	0.54 nM
8		518.629	$C_{24}H_{34}N_6O_5S$	2.04	
9		547.67	$C_{25}H_{37}N_7O_5S$	1.75	1.5 nM
10		532.656	$C_{25}H_{37}N_7O_5S$	2.57	0.32 nM
11		534.628	$C_{24}H_{34}N_6O_6S$	1.54	0.15 nM
12		504.602	$C_{24}H_{34}N_6O_6S$	1.2889	0.3 nM
13		490.576	$C_{22}H_{30}N_6O_5S$	0.859	0.17 nM
14		490.576	$C_{22}H_{30}N_6O_5S$	0.859	0.35 nM



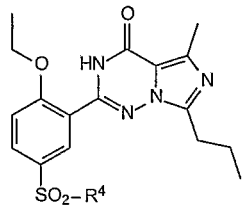
COMPOUND B

Compound No.	R^4	Molecular Weight	Molecular Formula	CLogP	IC_{50}
15		504.602	$C_{23}H_{32}N_6O_5S$	1.168	0.26 nM
16		516.613	$C_{24}H_{32}N_6O_5S$	1.234	0.42 nM
17		490.576	$C_{22}H_{30}N_6O_5S$	0.91	0.66 nM
18		490.576	$C_{22}H_{30}N_6O_5S$	0.91	0.36 nM
19		504.602	$C_{23}H_{32}N_6O_5S$	1.219	0.19 nM
20		405.471	$C_{18}H_{23}N_5O_5S$	1.045	0.24 nM
21		476.549	$C_{21}H_{28}N_6O_5S$	0.55	0.25 nM

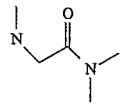
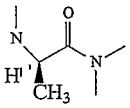
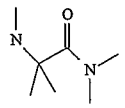
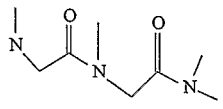
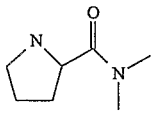
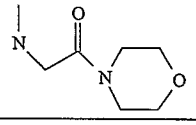
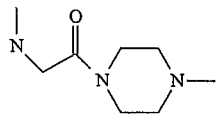
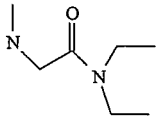
[00234] Table 2. Physiochemical and Pharmacokinetic data for compounds formed by substitution of compound A

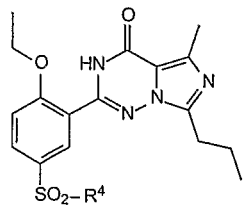
Compound No.	Caco-2 (10^{-6} cm/s)		PAMPA (10^{-6} cm/s)		Solubility (ug/mL)		RLM $T_{1/2}$ (min)	Non-Spec Binding	Protein Binding
	AtoB	BtoA	Hex	Lec	pH 7.4	pH 2.7			
1	17.46	48.88	5.49	0.128	96.12	> 100	22	11.07 %	91.4 %
2	33.33	61.33	18.2		> 100	> 100	32.6	20.7 %	96.8 %
3	28.85	58.45	9.11		> 100	> 100	72.7	14.3 %	92.4 %
4	2.97	25.95	0.47	0.056	109	112	64	7 %	82 %
5	19.4	60.2	13.18		93	100	30.1		
6	11.16	55.15	0.78		> 100	> 100		7.3 %	91.6 %
7	4.35	35.58	0.18		> 100	> 100		2.6 %	84.5 %
8	42.03	77.41	21.81		> 100	> 100	15	19 %	97.6 %
9	2.33	11.31	0.03		> 100	> 100		9.8 %	75.4 %
10			67.04		78.47	77.87			
11	11.4	63.3	1.45		70.87	66.7		0 %	95.8 %
12	33.39	50.43	13.93		100	96	32.23	30.7 %	94.2 %
13			0.42		74.6	69.4			
14			0.47		63.95	62.65			
15	6.8	34.9	0.25						
16			3.41		82.5	74.7			
17			0.26		78.4	70.05			
18			0.18		75.75	70.3			
19	2.43	33	0.17		7.1	> 100			
20	24.27	53.02	0.37	0.00384	> 100	> 100	60.23	16.23 %	96.03 %
21	5.15	31.45	0.24		> 100	> 100			

[00235] Table 3. PDE-5 Selectivity Data. Comparison of selectivity for PDE-5, PDE-1, PDE-3 and PDE-6 for compounds formed by substitution of compound A

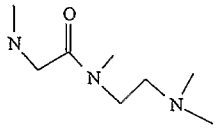
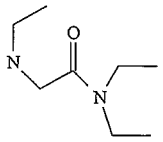
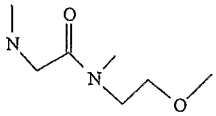
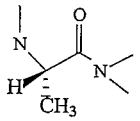
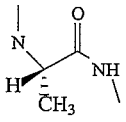
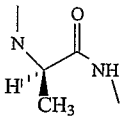
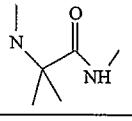
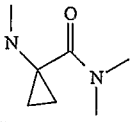


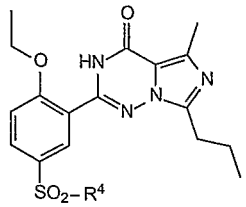
COMPOUND B

Comp. No.	R^4	PDE-1	PDE-3	PDE-5 bovine	PDE-5 human	PDE-6	Cytotox LC50 (mM)		Cytotox GC50 (uM)
							Chronic	Acute	
1		25 nM	986 nM	0.122 nM	0.097 nM	3.50 nM	.075	0.10	76.7
2		13 nM	507 nM	0.101 nM	0.0395 nM	0.605 nM	0.10	0.10	94.6
3		10 nM	412 nM	0.138 nM	0.16 nM	1.70 nM	0.10	0.10	100
4		25 nM		.084 nM	0.11 nM				
5		253 nM		1.6 nM	2.3 nM	7.4 nM			
6		19 nM	1900 nM		0.68 nM	4.8 nM			
7		33.5 nM	1200 nM		0.535 nM	2.7 nM			
8		34.5 nM	1250 nM		0.78 nM	3.0 nM			

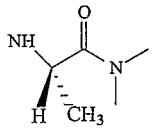
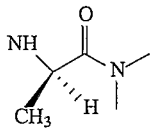
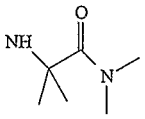
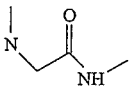


COMPOUND B

Comp. No.	R^4	PDE-1	PDE-3	PDE-5 bovine	PDE-5 human	PDE-6	Cytotox LC50 (mM)		Cytotox GC50 (uM)
							Chronic	Acute	
9		96 nM	2500 nM		1.5 nM	9.6 nM			
10		8.30 nM	1200 nM		0.32 nM	3.6 nM			
11		34.0 nM	2300 nM		0.15 nM	6.0 nM			
12		14.8 nM	712 nM		0.298 nM	3.11 nM	0.10	0.10	99.3
13		40.0 nM	995 nM		0.17 nM	7.0	0.10		94.0
14		25.0 nM	700 nM		0.35 nM	3.7 nM	0.10		88.0
15		17.6 nM	523 nM		0.256 nM	2.93 nM	0.10		95.0
16		13.0 nM	400 nM		0.42 nM	3.80 nM	0.10		100



COMPOUND B

Comp. No.	R^4	PDE-1	PDE-3	PDE-5 bovine	PDE-5 human	PDE-6	Cytotox LC50 (mM)		Cytotox GC50 (uM)
							Chronic	Acute	
17		35.0 nM	1100 nM		0.660 nM	2.90 nM	0.10		100
18		20.0 nM	1700 nM		0.36 nM	3.00 nM	0.10		100
19		17.0 nM	1300 nM		0.193 nM	2.86 nM	0.10		100
20		28.0 nM	1010 nM		0.243 nM	4.18	0.10	0.10	100
21		16.0 nM	443 nM		0.25 nM	1.90 nM			

[00236] All of the patents and publications cited herein are hereby incorporated by reference in their entireties.

Equivalents

[00237] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

We claim:

1. A method of modulating the pharmacokinetic and/or pharmacodynamic property of a compound, comprising the step of:

attaching at least one functional group to a known active compound by (a) replacing a nonessential residue; or (b) substituting a nonessential residue of the compound with the at least one function group, thereby improving the pharmacokinetic properties of the compound.

2. The method of claim 1, wherein said at least one functional group is selected from a hydrophobic group, an ether, an oligo(ethylene glycol) group or a derivative thereof, an amine, an ammonium salt, a simple amide, an amide based on amino acids, a crown ether, a sugar, or a nitrile, an amine group, an oxalamide, a sarcosine residue, a sarcosine derivative or a sarcosine oligomer.

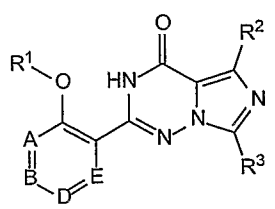
3. The method of claim 2, wherein said functional group is a sarcosine residue or sarcosine derivative.

4. The method of claim 1, wherein said pharmacokinetic property is reduced non-specific protein binding.

5. A method of modulating the pharmacokinetic and/or pharmacodynamic properties of a compound, comprising: attaching a residue from the group consisting of a hydrophobic group, an ether, an oligo (ethylene glycol) group or a derivative thereof, an amine, an ammonium salt, a simple amide, an amide based on amino acids, a crown ether, a sugar, or a nitrile, an amine group, an oxalamide, a sarcosine residue, a sarcosine derivative or a sarcosine oligomer to a known active compound to generate a second compound.

6. The method of any one of claims 1 to 5, wherein said active compound is Vardenafil.

7. The method of any one of claims 1 to 5, wherein said modulated compound has the following formula:



(A)

or pharmaceutically acceptable salt, stereoisomer, or hydrate thereof, wherein

R^1 is lower alkyl;

R^2 and R^3 are independently selected from lower alkyl, and lower alkenyl and lower alkynyl, wherein the lower alkyl, lower alkenyl, and lower alkynyl may be optionally substituted with one or more halogen, lower alkoxy, hydroxy, CN, NO_2, amino, acylamino, amido, carbonyl, and alkylthio;

A is N or C-H;

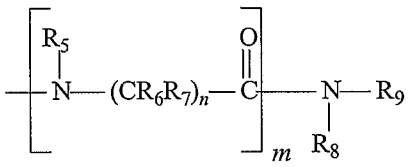
B is N, C-H, C-(SO_2-R^4), or C-CO-R^4;

D is N, C-H, C-(SO_2-R^4) or C-CO-R^4;

E is N or C-H;

wherein only one of A, B or E may be N, and one of B or D is C-(SO_2-R^4) or C-CO-R^4;

R^4 is a group having the formula:



(C)

in which each R^5, R^6, R^7 and R^8 are independently selected from H and lower alkyl, wherein the lower alkyl may be optionally substituted with one or more halogen, lower alkoxy, hydroxy, CN, NO_2, amino, acylamino, amido, carbonyl, and alkylthio; and additionally or alternatively R^6 and R^5 together form a 5- or 6-membered ring, or R^6 and R^7 together form a 3 to 6 membered ring; R^9 is independently selected from H and lower alkyl, wherein the lower alkyl may be optionally substituted with one or more halogen, lower alkoxy, hydroxy, CN, NO_2, amino, acylamino, amido, carbonyl, and alkylthio; alternatively R^8 and R^9 together with the nitrogen to which they are attached form a 5- or 6-membered ring; *n* is 1 to 4; and *m* is 1 to 6.

8. The method of claim 7, wherein

A is C-H;

B is C-H;

D is C-(SO_2-R^4); and

E is C-H.

9. The method of claim 8, wherein *m* is 1 or 2.

10. The method of claim 9, wherein *n* is 1.

11. The method of any one of claims 1 to 5, wherein:

R^1 is ethyl

R^2 is methyl

R^3 is propyl

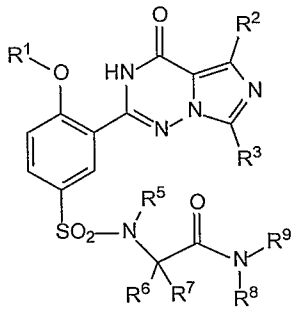
A is C-H;

B is C-H;

D is C-(SO_2-R^4); and

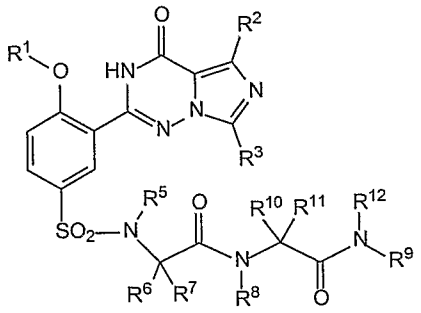
E is C-H.

12. The method of any one of claims 1 to 5, wherein said modulated compound has the following formula:



in which each R^5, R^6, R^7 and R^8 are independently selected from H and lower alkyl, wherein the lower alkyl may be optionally substituted with one or more halogen, lower alkoxy, hydroxy, CN, NO_2, amino, acylamino, amido, carbonyl, and alkylthio; and additionally or alternatively R^6 and R^5 together form a 5- or 6-membered ring, or R^6 and R^7 together form a 3 to 6 membered ring; R^9 is independently selected from H and lower alkyl, wherein the lower alkyl may be optionally substituted with one or more halogen, lower alkoxy, hydroxy, CN, NO_2, amino, acylamino, amido, carbonyl, and alkylthio; alternatively R^8 and R^9 together with the nitrogen to which they are attached form a 5- or 6-membered ring.

13. The method of claims 1 to 5, wherein said modulated compound has the following formula:



wherein,

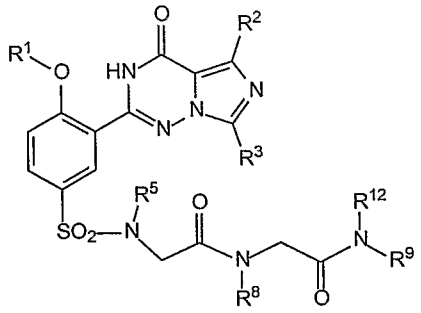
R^1 is lower alkyl;

R^2 and R^3 are independently selected from lower alkyl, and lower alkenyl and lower alkynyl, wherein the lower alkyl, lower alkenyl, and lower alkynyl may be optionally substituted with one or more halogen, lower alkoxy, hydroxy, CN, NO_2, amino, acylamino, amido, carbonyl, and alkylthio;

R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11} and R^{12} are independently selected from H and lower alkyl, wherein the lower alkyl may be optionally substituted with one or more halogen, lower alkoxy, hydroxy, CN, NO_2, amino, acylamino, amido, carbonyl, and alkylthio;

and additionally or alternatively R^6 and R^5, or R^8 and R^{10} together form a 5- or 6-membered ring, or R^6 and R^7, or R^{10} and R^{11} together form a 3 to 6 membered ring; and R^9 and R^{12} together with the nitrogen to which they are attached form a 5- or 6-membered ring.

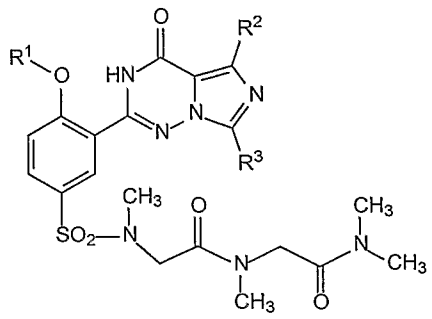
15. The method of claim 14, wherein said modulated compound has the following formula:



wherein,

R^1, R^2, R^3 R^5, R^8, R^9 and R^{12} are as defined in claim 14.

16. The method of claim 15, wherein said modulated compound has the following formula:

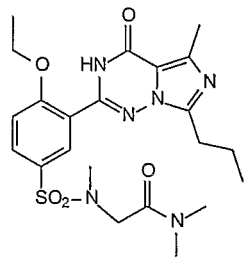
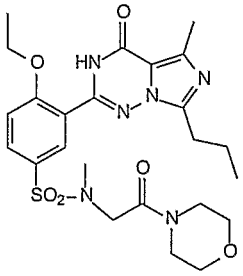
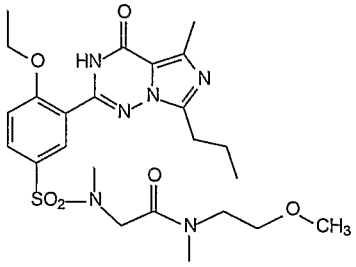
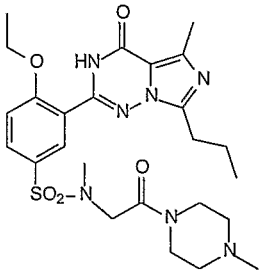
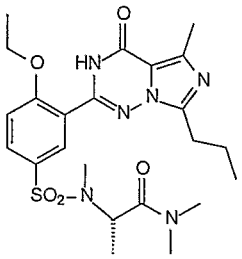
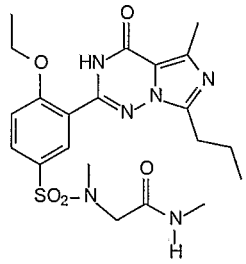
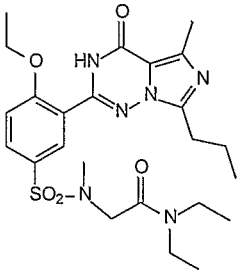
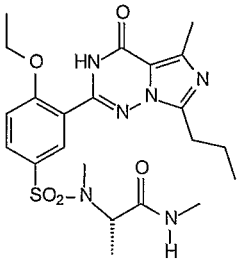
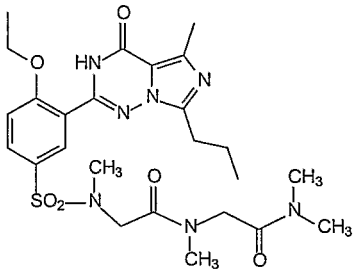
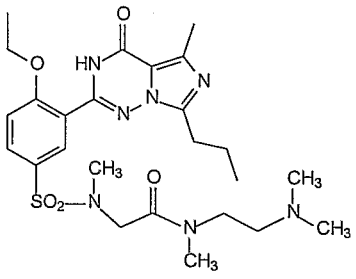
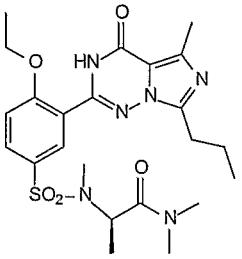
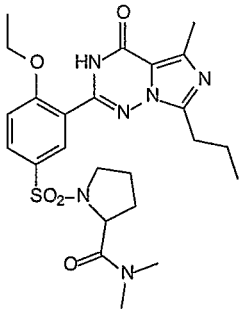
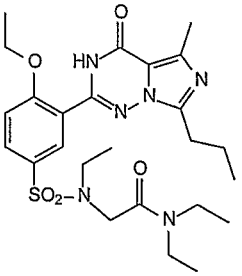
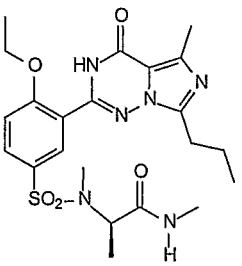


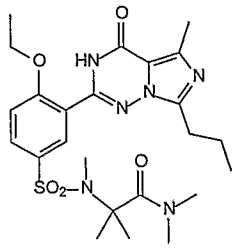
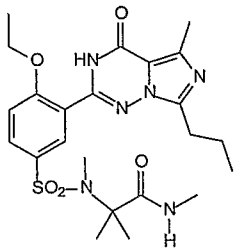
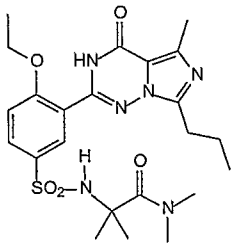
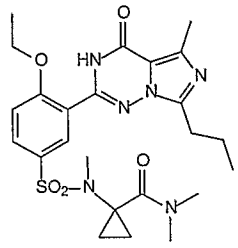
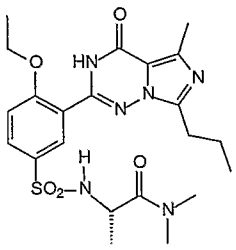
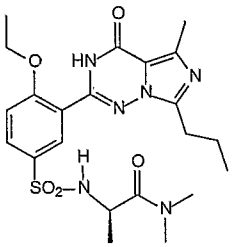
wherein,

R^1 is lower alkyl; and

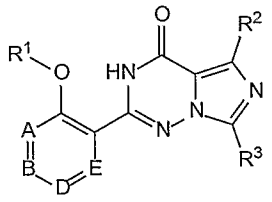
R^2 and R^3 are independently selected from lower alkyl, and lower alkenyl and lower alkynyl, wherein the lower alkyl, lower alkenyl, and lower alkynyl may be optionally substituted with one or more halogen, lower alkoxy, hydroxy, CN, NO_2, amino, acylamino, amido, carbonyl, and alkylthio.

17. The method of claims 1 to 5, wherein said modulated compound is selected from

18. The method of any one of claims 1-5, wherein said modulated compound has the following formula D:



(D)

or pharmaceutically acceptable salt, stereoisomer, or hydrate thereof, wherein:

R^1 is lower alkyl;

R^2 and R^3 are independently selected from lower alkyl, and lower alkenyl and lower alkynyl, wherein the lower alkyl, lower alkenyl, and lower alkynyl may be optionally substituted with one or more halogen, lower alkoxy, hydroxy, CN, NO_2, amino, acylamino, amido, carbonyl, and alkylthio;

A is N or C-H;

B is N, C-H, C-(SO_2-NH-R^{13}), or C-CO-NH-R^{13};

D is N, C-H, C-(SO_2-NH-R^{13}) or C-CO-NH-R^{13};

E is N or C-H;

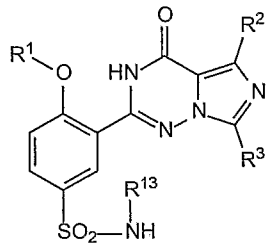
wherein only one of A, B or E may be N, and one of B or D is C-(SO_2-NH-R^{13}) or C-CO-NH-R^{13};

R^{13} is lower alkyl.

19. The method of claim 18, wherein R^{13} is methyl.

20. The method of claim 18, wherein R^2 and R^3 are independently selected from lower alkyl.

21. The method of claim 18, wherein the compound has the formula D_1:



(D_1)

or pharmaceutically acceptable salt, stereoisomer, or hydrate thereof, wherein

R^1 is lower alkyl;

R^2 and R^3 are independently selected from lower alkyl, and lower alkenyl and lower alkynyl, wherein the lower alkyl, lower alkenyl, and lower alkynyl may be optionally substituted with one or more halogen, lower alkoxy, hydroxy, CN, NO_2, amino, acylamino, amido, carbonyl, and alkylthio; and

R^{13} is selected from lower alkyl.

22. The method of claim 21, wherein R^{13} is methyl.

23. The method of claim 21, wherein R^2 and R^3 are independently selected from lower alkyl.

24. The method of claim 21, wherein the compound has the formula

