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(72) Inventor; and
(71) Applicant : **LU, Xiandan (sharon)** [US/US]; 15 Meadow Lane, Boxborough, MA 01719 (US).

(74) Agent: **ZHANG, Yin, Philip**; Milstein Zhang & Wu LLC, 2000 Commonwealth Avenue Suite 400, Newton, MA 02466 (US).

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(54) Title: METHODS AND COMPOSITIONS FOR IMPROVING ADMET PROPERTIES

(57) Abstract: The invention generally relates to methods and compositions that improve one or more ADMET properties of a therapeutic agent. More particularly, the invention relates to methods and compositions thereof, for improving one or more ADMET properties, thus leading to superior drug with one or more efficacy and/or safety-related properties improved over the parent compound.

METHODS AND COMPOSITIONS FOR IMPROVING ADMET PROPERTIES

Technical Fields of the Invention

[0001] The invention generally relates to methods and compositions that improve one or more ADMET properties of a therapeutic agent. More particularly, the invention relates to methods and compositions thereof, for improving one or more ADMET properties, thus leading to superior drug with one or more efficacy and/or safety-related properties improved over the parent compound.

Background of the Invention

[0002] Absorption, distribution, metabolism, excretion, and toxicity (or ADMET) are important properties for any therapeutics. Sometimes, the ADMET properties of a drug severely limit its clinical efficacy and/or safety. Some marketed drugs and drug candidates require high dose and/or more frequent dose to see efficacy, resulting in a reduced therapeutic window and placing patients under heightened risk for severe side effects. Some drugs have absorption issues, metabolic stability issues, solubility issues, delivery issues, which can lead to low or diminished efficacy and high systemic toxicity. These issues can be found in all the therapeutic agents which are marketed drugs, off labels uses, on-going clinical trials.

[0003] The pharmaceutical industry faces the challenge of high R&D costs with diminishing returns. It costs over a billion dollars to delivery one drug to the market. With the heavily involvement of ADME function in the early discovery phase, the attrition due to ADME is greatly reduced to about 9% while toxicity accounts for over 40%, followed by efficacy-based attrition of about 30%. According to the most recent published data, efficacy has climbed to be the number one reason (with an average of over 40%) for attrition (Roberts **2003** *Curr Opin Drug Discov Devel.* 6:66-80; Kola, *et al.* **2004** *Nat Rev Drug Discov.* 3:711-5; *Impact Report*, Tufts Center for the Study of Drug Development, Tufts University, **2010**, Vol. 12, No. 5). Especially, for the first-in-class strategy, the high risk of failure is due to the lack of predictability of animal models, particular in some therapeutic area such as oncology and neurosciences, and low predictive power (only about 10%) in the traditional toxicology tests (Littman, *et al.* **2005** *Nat Rev Drug Discov* 4, 631; Sankar **2005** *The Scientist* 19, 32). Therefore, an urgent need continues to exist to complement the current drug discovery strategies.

[0004] In order to balance the risk and return, different companies implemented different portfolio strategies with the combination of first-in-class, best-in-class, fast follower, and generics. There are companies focused on repositioning of marked and/or failed candidate compounds for different therapeutic utility other than original intended disease (Louis A. Tartaglia *et al.*, **2010** *Drug Discovery World*, on-line Journal Fall Issue). This strategy presents

itself as an additional attractive possibility for enhancing productivity. However, this strategy still has the same limitation as the current drug discovery which may not work on the right science.

[0005] In 90's, ADME accounted for over 40% attrition (Roberts SA, **2003** *Curr Opin Drug Discov Devel.* 6:66-80; Kola I *et al.*, **2004** *Nat Rev Drug Discov.* 3:711-5). For the marked drugs coming out before and/or around 90's, there are a lot of ADME related issues. This offers us a niche or shortcut for drug development. What we propose here is through the pro-drug approach to improve ADME properties, thus toxicity and efficacy profile through knowledge based design and/or target positioning of the therapy to the designated tissue and/or organ. The released therapy has already been clinically proved for both efficacy and safety. Meanwhile, the target positioning will greatly decrease the systemic toxicity, thus increase the safety margin. One might view this approach as a distinct path to enhance the overall productivity, reduce costs and clinical attrition. After being proved in animal models, the therapies can be directly translated into clinical.

[0006] The above concept can be demonstrated through both traditional prodrug design and formulation strategies (Florence, *et al.* **2000** *J of Controlled Release* 65, 253-259; Dufes, *et al.* **2005** *Cancer Res* 65, 8079-84; Ke, *et al.* **2008** *J of Pharm Sciences* Vol. 97, No. 6, 2208-2216; Yang, *et al.* **2009** *Expert Opin. Drug Deliv.* 6, 851-864; Wu, *et al.* **2006** *Mol Cancer Ther* 5, 52-59; Soto-Castro, *et al.* **2010** *Molecules* 15, 8082-8092; Kannan, *et al.* **2004** *J. Biomater. Sci. Polymer Edn.* Vol. 15, No. 3, 311-330). For example, nanotechnologies have been employed to overcome low/no efficacy and systemic toxicity, including liposomes, micelles, polymeric nanoparticles, and dendrimers (Devalapally, *et al.* **2007** *J of Pharm Sciences* Vol 96, NO. 10, 2547-2565; Peer, *et al.* **2007** *Nature Nanotechnology* Vol. 2, 751-760).

[0007] One approach is to use a carrier to mask and replace the unfavorable biopharmaceutical properties of the drug molecule, and form a nano-delivery system through complex or encapsulation with drug molecules.

[0008] Another approach involves the covalent conjugation of the molecules with carrier and targeting moieties that override the drug's poor biopharmaceutical properties, improve pharmacokinetics, biodistribution, thus toxicity and efficacy, through enhanced permeation and retention and target delivery (Maeda, *et al.* **2009** *Eur J of Pharm. and Biopharm.* 71, 409-419; Fox, *et al.* **2009** *Mol. Pharm.* Vol. 6, No. 5, 1562-1572; Lo, *et al.* **2010** *Mol. Pharm.*Vol. 7, No. 4, 993-1006; Lee, *et al.* **2005** *Bioconj Chem.* 16, 535-541; Cheng, et al. **2008** *Eur J of Med Chem.* 43, 2291-2297).

[0009] To this point, only liposomal systems have been approved for general clinical use. Dendrimers have recently been explored as alternative systems for improved targeting compared

to liposomal drug formulations (Szoka *et al.***2006** *Proc. Natl. Acad. Sci. U.S.A.* 103, 16649-16654; Minko *et al.* **2008** *J Controlled Release* 130:107-114). Normally, Dendrimer is globular shape with multivalency. The monodisperse of dendrimers leads to batch-to-batch consistency, and reproducible pharmacodynamics and pharmacokinetics. Covalent attachment is favored over noncovalent association due to higher drug payload with more controlled drug release via enzymatic or pH dependent cleavage.

[0010] PAMAM (Kannan RM, *et al.* **2004** *J. Biomater. Sci Polymer Edn.*15, 311-330; Ghandehari H. *et al.***2010** *Bioconjug Chem.* 21, 1804-10; Ghandehari H. *et al.* **2011** *J Control Release* 150, 318-25) with functional groups of –OH, -COOH, and –NH-, triazine dendrimer (Eric E. Simanek *et al.***2009** *Bioconjug Chem.* 20, 2154-2161; Eric E. Simanek *et al.***2010** *Molecular Pharm.* 7, 993-1006), PEGylated Poly(L-lysine), PEGylated poly-ester dendrimer (Reul R *et al.* **2011** *Int J Pharm*, 407, 190-6), poly(ester) dendronized linear poly(4-hydroxystyrene) (Szoka FC *et al.* **2005** *Bioconjug Chem.* 16, 535-541), active targeting dendrimer such as folate-PEG-PAMAM (Diwan PV, *et al.* **2007** *J Biomed Mater Res A.* 82, 92-103; Jain NK, *et al.***2008** *Bioconjug. Chem.*19, 2239-2252), non-toxic poly propyl ether imine (PETIM) dendrimer (Khanduja KL, **2010** *Eur J Med Chem.* 45, 4997-5005), hyperbranched polymer such as polyol (Kannan RM *et al.* **2009** *Bioconjug Chem.* 20, 842-846; Kannan RM, *et al.* **2004** *J. Biomater. Sci Polymer Edn.*15, 311-330), and biodegradable star HPMA-PAMAM copolymer/dendrimer (Ulbrich K. *et al.* **2011** Eur J Pharm Sci. 42(5):527-39) have been used to improve systemic circulation with oral bioavailability (Ghandehari H. *et al.* **2011** *J Control Release* 150, 318-25), permeability, solubility (Eric E. Simanek *et al.***2010** *Molecular Pharm.* 7, 993-1006; Delia Soto-Castro *et al.*, **2010** *Molecules* 15, 8082-8092; Sujatha Kannan *et al.*, **2004** *J. Biomater. Sci. Polymer Edn.* Vol. 15, No. 3, 311-330). Furthermore, forming micelle and polymer conjugation may diminish uptake by reticuloendothelial system or macrophage or efflux transporter (Maeda, *et al.* **2009** *Eur J of Pharm and Biopharm*, 71, 409-419; Hu, *et al.* **2009** *Current Drug Metabolism* 10, 836-841; Mohammad, *et al.* **2007** *Bioconj Chem.* 18, 937-946).

[0011] Here, we report a systemic prodrug and formulation excipient conjugates through linkers and spacers to improve ADMET and efficacy through change of physical chemical properties and target delivery.

Summary of the Invention

[0012] The invention is based, in part, on the discovery of methods and related compositions that help improve one or more ADMET properties of a drug or a drug candidate. The present invention addresses the shortcomings of the previous methods in that the methods of the invention provide a systematic approach to addressing ADMET challenges of drugs.

[0013] Methods of the invention apply knowledge of ADMET combined with current advanced platforms and technologies, and cutting-edge technology of nanomedicine to generate prodrugs, drug (therapeutic agents)-formulation excipient conjugates, to expand the efficacy with higher safety margin through improvements in PK profile (with longer systemic circulation), target delivery to specific organs, and bioavailability, for examples anti-cancer drug Velcade for solid tumors with increased dose limit and reduction in the toxicity of myelosuppression, gastrointestinal disturbance, and peripheral neuropathy; Lipitor with better bioavailability, efficacy and lower muscular toxicity; metformin, with consistent bioavailability during the dose range, higher systemic exposure of once daily therapy, and lower GI toxicity.

[0014] Prodrugs and/or drug conjugates of formulation excipients can also be used in combo with other therapeutic agents within and/or outside of this patent claims to further improve efficacy.

[0015] Prodrugs and/or drug conjugates of formulation excipients can be applied to different indications/diseases based on the new bio-distribution of the compounds, which can also be used in combination with other therapeutic agents within and/or outside of the present disclosure to further improve efficacy. (Tartaglia, *et al.* **2010** *Drug Discovery World*, on-line Journal Fall Issue).

[0016] In one aspect, the method comprises chemically coupling the compound (D), through one or more of -CO_2H, -OH, -SO_2NH-, -SO_2NH_2, -SO_3H, -NH_2, =N-NH2, -CONH-, -$CONH_2$, -B(OH)-, -CHO, -CO-, -S-, –SH, F, Cl, Br, and I moieties, with one or more pendant groups comprising a cyclic moiety having 4-8 membered rings or aliphatic or fused ring structures (R), whereby the coupling resulting on one or more bones of –N-O-, -N-S-, -C-N-, -C-O-, -N-N-, -B-O-, -B-S-, -B-N-, -C-S-, -O-O-, and –S-S-.

[0017] The modifying reagent, L-R can be amino acids, fatty acids (saturated or unsaturated with carbon atoms between 4 and 28), peptides, small molecule formulation excipients, denoted as E.

[0018] The modifying reagent, L-R, has the formula $R(CH_2)_nL$ (n can be 0,1, and 2).

[0019] L can be any of the moiety such as -CO_2H, -OH, -SO_2NH-, -SO_2NH_2, -SO_3H, -NH_2, =N-NH2, -CONH-, -$CONH_2$, -B(OH)-, -CHO, -CO-, -S-, –SH, F, Cl, Br, and I.

[0020] The modified agent can be the dimer or trimer of the original compound.

[0021] In another aspect, polymer/copolymer (P) is used to conjugate with D with or without spacer (A), among which dendrimer and tubular shaped dendronized linear polymer/copolymer are preferred.

[0022] The selected dendrimers' scaffolds include tubular shape, star Shape, spherical and tear shape with functional groups of either –NH2 or –OH or –COOH or -SO3H or guanidine or

phosphonate or PEGylated, and combinations of the above. The dendrimers can be cationic, anionic and neutral in physiological or tumor's acidic surrounding environment, among which neutral state is preferred. The construct should be non-toxic, non-immunogenic and biodegradable, for example ester and/or amide, which can be cleaved in the body, and non-cytotoxic with the surface functional groups of hydroxyl and/or carboxyl.

[0023] Covalent attachment over noncovalent association is preferred due to higher drug payload with more controlled drug release via enzymatic or pH dependent cleavage of different linkers with or without A.

[0024] Linkers with or without A will affect therapeutic agent's bio-distribution, accumulation, for example into solid tumor, and efficacy. The proposed the linkers and/or bonds include –N-O-, -N-S-, -C-N-, -C-O-, -N-N-, -B-O-, -B-S-, -B-N-, -C-S-, -O-O-, and -S-S- under the name, for example ether hydrazone, peptide, ester, amide and etc. The selection criteria are that the linker with or without spacer will offer long systemic circulation with high accumulation of D at targets.

[0025] Spacer, A, is constructed as formula $X_1(CR'_m)_nY_1$, which can directly link to D and polymer/copolymer/dendrimer (P) or connect after activation of A and/or D and/or P. X_1 and Y_1 can be any of the following groups: OH, SH, NH_2, N-NH2, COOH, SO_3H, CONH2, CONH-NH_2 SO2NH2, CHO, F, Cl, Br, I, -O-, -S-, CONH-, -SO_2-, SONH-, and -NH-; R' can be H, CH_3, CN; m=0 to 2; n = 0 to 30; R', m and n are individually independent, can be in any combination with each other and within; C-C bond within $(CR'_m)_n$ can be single or double or triple; Either single or double bond are between X_1 and $(CR'_m)_n$, and $(CR'_m)_n$ and Y_1.

[0026] In another construct for A, $X_2(CR'_m)_n R_s(CR'_m)_nY_2$, X_2 and Y_2 can be any moiety from –H, -CO_2H, -OH, -SO_2NH-, -SO_2NH_2, -SO_3H, -NH_2, =N-NH2, -CONH-, -$CONH_2$, -B(OH)-, -CHO, -CO-, -S-, –SH, F, Cl, Br, and I; R' can be H, CH_3, CN; m=0 to 2; n = 0 to 10; R', m and n are individually independent, can be in any combination with each other and within; C-C bond within $(CR'_m)_n$ can be single or double or triple; Either single or double bond are between X_2 and $(CR'_m)_n$, and $(CR'_m)_n$ and Y_2; R_s comprises a cyclic moiety of 4-8 membered rings or fused rings or polycyclic structures with one or more of N, O, and S, aromatic and non-aromatic, saturated and unsaturated, with substitute groups.

Definitions

[0027] Definitions of specific functional groups and chemical terms are described in more detail below. General principles of organic chemistry, as well as specific functional moieties and reactivity, are described in "Organic Chemistry", Thomas Sorrell, University Science Books, Sausalito: **1999**.

[0028] 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.

[0029] Isomeric mixtures containing any of a variety of isomer ratios may be utilized in accordance with the present invention. For example, where only two isomers are combined, mixtures containing 50:50, 60:40, 70:30, 80:20, 90:10, 95:5, 96:4, 97:3, 98:2, 99:1, or 100:0 isomer ratios are contemplated by the present invention. Those of ordinary skill in the art will readily appreciate that analogous ratios are contemplated for more complex isomer mixtures.

[0030] 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 methods well known in the art, and subsequent recovery of the pure enantiomers.

[0031] Given the benefit of this disclosure, one of ordinary skill in the art will appreciate that synthetic methods, as described herein, may utilize a variety of protecting groups. By the term "protecting group", as used herein, it is meant that a particular functional moiety, e.g., O, S, N, or B is temporarily blocked so that a reaction can be carried out selectively at another reactive site in a multifunctional compound. In preferred embodiments, a protecting group reacts selectively in good yield to give a protected substrate that is stable to the projected reactions; the protecting group should be selectively removable in good yield by preferably readily available, non-toxic reagents that do not attack the other functional groups; the protecting group forms an easily separable derivative (more preferably without the generation of new stereogenic centers); and the protecting group has a minimum of additional functionality to avoid further sites of reaction. Oxygen, sulfur, nitrogen, and carbon protecting groups may be utilized. Examples of a variety of protecting groups can be found in Protective Groups in Organic Synthesis, Third Ed. Greene, T.W. and Wuts, P.G., Eds., John Wiley & Sons, New York: **1999**.

[0032] It will be appreciated that the compounds, as described herein, may be substituted with any number of substituents or functional moieties.

[0033] As used herein, (C_x-C_y) refers in general to groups that have from x to y (inclusive) carbon atoms. Therefore, for example, C_1-C_6 refers to groups that have 1, 2, 3, 4, 5, or 6 carbon atoms, which encompass C_1-C_2, C_1-C_3, C_1-C_4, C_1-C_5, C_2-C_3, C_2-C_4, C_2-C_5, C_2-C_6, and all like combinations. (C_1-C_{20}) and the likes similarly encompass the various combinations between 1 and 20 (inclusive) carbon atoms, such as (C_1-C_6), (C_1-C_{12}) and (C_3-C_{12}).

[0034] As used herein, the term "(C_x-C_y)alkyl" refers to a saturated linear or branched free radical consisting essentially of x to y carbon atoms, wherein x is an integer from 1 to about 10 and y is an integer from about 2 to about 40. Exemplary (C_x-C_y)alkyl groups include "(C_1-C_{40})alkyl," which refers to a saturated linear or branched free radical consisting essentially of 1 to 40 carbon atoms and a corresponding number of hydrogen atoms. Exemplary (C_1-C_{40})alkyl groups include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, dodecanyl, etc. Of course, other (C_1-C_{40})alkyl groups will be readily apparent to those of skill in the art given the benefit of the present disclosure.

[0035] As used herein, the term "halogen" refers to fluorine, chlorine, bromine, or iodine.

[0036] As used herein, the term "amino" refers to a free radical having a nitrogen atom (i) covalently bonded to two hydrogen atoms, or alternatively (ii) covalently bonded to one hydrogen atom and one carbon radical. As such, the term amino generally refers to primary and secondary amines. In embodiments where the free radical is covalently bonded to a carbon atom, the term "amino" also includes tertiary amines. Those of skill in the art given the benefit of the present disclosure will readily be able to identify when the term "amino" is interchangeably used to refer to primary, secondary, and tertiary amines.

Detailed Description of the Invention

[0037] The invention is based on novel methods and compositions that improve ADMET properties of therapeutic compounds. More particularly, the invention relates to novel methods and compositions thereof, for improving one or more ADMET properties, thus leading to improved one or more efficacy and/or safety-related properties. For instance, the methods of the invention include prodrug and/or drug-formulation excipient conjugate strategies using pH labile and/or bio-labile linkers such as –N-O-, -N-S-, -C-N-, -C-O-, -N-N-, -B-O-, -B-S-, -B-N-, -C-S-, -O-O-, and –S-S-, capable of releasing original therapeutic agent which is pharmacologically active.

[0038] Therapeutic agent represented as D, comprises one or more of -CO_2H, -OH, -SO_2NH-, -SO_2NH_2, -SO_3H, -SO_3-, -NH_2, -NH-, =N-NH2, -CONH-, -$CONH_2$, -B(OH)-, -CHO, -CO-, -S-, –SH, F, Cl, Br, and I moieties.

[0039] Examples of D include, for examples, essential medicines from World Health Organization, Atropine, Acetylcysteine, Naloxone, Cytarabine, Doxorubicin, Carbidopa, L-DOPA, Epinephrine, Methyldopa, Furosemide, Metformin, and Aciclovir.

[0040] Drugs on the top 10 sales list, Atorvastatin, Quetiapine, active thio metabolite of Clopidogrel Hydrogen Sulfate, Valsartan, and Fluticasone.

[0041] Other examples includes methotrexate, leucovorin, irinotecan, mitoxantrone, torisel, 2-methoxyestradiol, prinomastat, carboxyamidotriazole, captopril, Eflornithine, cilengitide, raloxifene, tretinoin, estramustine, nimustine, zorubicin, idarubicin, daunorubicin, mitoxantrone, valrubicin, amrubicin, Velcade, paclitaxel, vindesine, topotecan, pentostatin, trimetrexate, fludarabine, capecitabine, cytarabine, raltitrexed, paltitrexid, tiazofurin, decitabine, pemetrexed, troxacitabine, alanosine, swainsonine, dexrazoxane, lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, Ortho-hydroxylated atorvastatin, para-hydroxylated atorvastatin, celecoxib, corticosteroids, squalamine, mineralocorticoids, dexamethasone, prednisone, prednisolone, methylpred, betamethasone, genistein, combretastatin A-4, flavopiridol, CELEBREX, combretastatin, troglitazone, gemfibrozil, baclofen, fluphenazine, dronabinol, sprycel, floxuridine, ganciclovir, acyclovir, 6-thioguanine, MEXATE, dacarbazine, chlorambucil, camptothecin, 10-hydroxycamptothecin, teniposide, VP-16, docetaxel, vincristine, vinorelbine, thioguanine, testosterone, melphalan propionate, taxanes, procarbazine, Megestrol, leucovorin, hydroxyurea, gemcitabine, etoposide, estramustine, doxorubici glucuronide, epirubicin glucuronide, SN-38, cladribine, cytarabine, and azacytidine.

[0042] Prodrugs and/or therapeutic agent conjugates of formulation excipients, formed based on the claims above, can be hydrolyzed through pH based hydrolysis or from enzymatic reactions by a variety of hydrolytic enzymes, namely, carboxylesterases, cholinesterases, paraoxonase, organophosphatases, peptidases, and enzymes in blood, cytosol and lysosome (Andrew Parkinson, *Casarett & Doull's Toxicology The Basic Science of Poisons, Chapter 6 "Biotransformation of Xenobiotics"*, **2001**; Yin H et al, **1993** *Chem Res Toxicol.*6, 630-4; Satoh T and Hosokawa M., **1998**, *Annu Rev Pharmacol Toxicol.* 38, 257-88). The hydrolysis of carboxylic acid esters (labile bond of –C-O-), amides (labile bond of –C-N-), and thioesters (labile bond of –C-S-) is largely catalyzed by carboxylesterases, which are located in various tissues and serum. Arylesterase is the enzyme for the hydrolysis of aromatic carboxylic acid esters. Both aryl and alkyl (both cyclic and non-cyclic) esters with diversified substitute groups can be hydrolyzed by pseudocholinesterase and true acetylcholinesterase in blood. Aldehyde dehydrogenase has esterase activity (Beretta, *et al.* **2010** *J Biol Chem.*, 285: 943-52). Arylacetamide deacetylase (AADAC) could efficiently catalyze deacetylation reaction (Nakjima, *et al.* **2011** *Biochem Pharmacol.*, Aug 12). Proteins such as human serum albumin (HAS) may

play a role in in vivo conversion of sulfonamide prodrugs (with labile bond of -N-S-) (Ni-Addae KW, *et al.* **2011** *J Pharm Sci.* 100: 3023-7). Disulfide bonds can be cleaved by glutathione or other intracellular thiols (EI-Sayed, *et al.* **2005** *J. Controlled Release* 104: 417-427). Peptide linkers can be enzymatically cleaved (Satchi R., *et al.* **2009** *Br. J. Cancer* 85: 1070-1076).

[0043] The conjugates of therapeutic agents and formulation excipients can be hydrolyzed enzymatically and pH based to release original therapeutic agents (Ghandehari H. *er al.* **2010** *Bioconjug Chem.* 21(10):1804-10; Ronald Gust *et al.* **2010** *Bioconjug Chem.* 21:1727-43; Kannan RM. *et al.***2009** *Bioconjug Chem.* 20(5):842-6; Gillies ER *et al.* **2011** *Adv Drug Deliv Rev.* Sep 29).

[0044] In general, enzymatic amide hydrolysis is much slower than that of esters. However, the amide bond can be weakened through the presence of electron-withdrawing substituents. Thus, the rate of hydrolysis can be modulated through different labile bonds and/or the presence of substitute groups.

[0045] In one aspect, D is chemically coupled with one or more pendant groups comprising a cyclic moiety having 4-8 membered rings or aliphatic or fused ring structures, or classes of compounds of fatty acids, amino acids and peptides, whereby the coupling resulting on one or more bones of –N-O-, -N-S-, -C-N-, -C-O-, -N-N-, -B-O-, -B-S-, -B-N-, -C-S-, -O-O-, and –S-S-.

[0046] L-R introduces the modification to D, which forms prodrug through chemically modifying the functional groups in D at one or multiple locations, for example, through loss of water. The conjugation may result from the direct reaction of D with modifying agents L-R, or the activation of D and/or L-R.

[0047] L-R can be amino acids, fatty acids (saturated or unsaturated with carbon atoms between 4 and 28), peptides, nucleotides and small molecule formulation excipients, denoted as E.

[0048] E can be acetic acid, acetylmethylamine, Anecortave acid form, benzoic acid, butylated hydroxytolune, Capryol 90 ($C_{11}H_{22}O_3$), carboxymethylcellulose (CMC, $C_8H_{16}O_8$), cetyl alcohol ($C_{16}H_{34}O_1$), citric acid, dextrose ($C_6H_{12}O_6$), EDTA, ethanol, glucose, glycerol, isopropyl alcohol, lactose, ascorbic acid, maltol, mannitol, methane sulfonic acid, 2-methoxy-6-methyl-phenol, propylene glycol, succinate, tartaric acid, transcutol, and xylitol;

[0049] E can also be surfactants for dispersing or wetting or solubilizing, such as condensation products of amino acids and fatty acids, for example, N-Lauroyl-L-alanine; alkyl naphthalene sulfonates, condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethylene-oxycetanol, Brij 35; alkyl sulfates, for example ammonium lauryl sulfate, sodium lauryl ether sulfate, and sodium myreth sulfate; sulfonates, for example dioctyl sodium sulfosuccinate, perfluorooctane sulfonate, and perfluorobutanesulfonate;

carboxylates, for example sodium stearate, sodium lauroyl sarcosinate, perfluorononanoate, and perfluorooctanoate; zwitterionic for example CHAPS; non-ionic, for example sorbitan monostearate;

[0050] E can also be inclusion excipients, for example cyclodextrin (α, β, and γ), 2-hydroxypropyl-beta-cyclodextrin, Captisol, and cyclodextrin derivatives;

[0051] Other E can be lipid (single component), which includes fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolopids, and polyketides.

[0052] L is independently selected from -CO_2H, -OH, -SO_2NH-, -SO_2NH_2, -SO_3H, -NH_2, =N-NH2, -CONH-, -$CONH_2$, -B(OH)-, -CHO, -CO-, -S-, -SH, -F, -Cl, -Br, and -I.

[0053] R, preferably, are metabolic stable to make sure that the modified agents can release original therapeutic agents through the liable bonds such as –N-O-, -N-S-, -C-N-, -C-O-, -N-N-, -B-O-, -B-S-, -B-N-, -C-S-, -O-O-, and –S-S-.

[0054] R, the leaving groups, preferably, should not generate structure alerts with potential toxicity (Kalgutkar, *et al.* **2005** *Current Drug Metabolism* 6, 161-225) includes, for example Michael receptors quinones, aniline, quinone-imine (if using "non-oxidizable" groups, such as –F, to block the oxidizable positions/substitute groups, the toxicity potential can be reduced); hydrazine, such as phenylhydrazine (forms alkylation with proteins, liver toxicity); nitroarene analogs; fused azaheterocycles, such as clozapine (leads to frequent agranulo cytosis and liver toxicity); benzylamine with caution of forming reactive hydroxylamine, arene oxide and Ar-CH_2-NH-(CO)-X. However, with the substitution on the benzylic carbon, using "non-oxidizable" group –F, -Cl, and –I, the bioactivation through α-carbon oxidation will be hindered; Imides (can form free radicals, such as phenytoin, mephenytoin, nirvanol, and trimethadione).

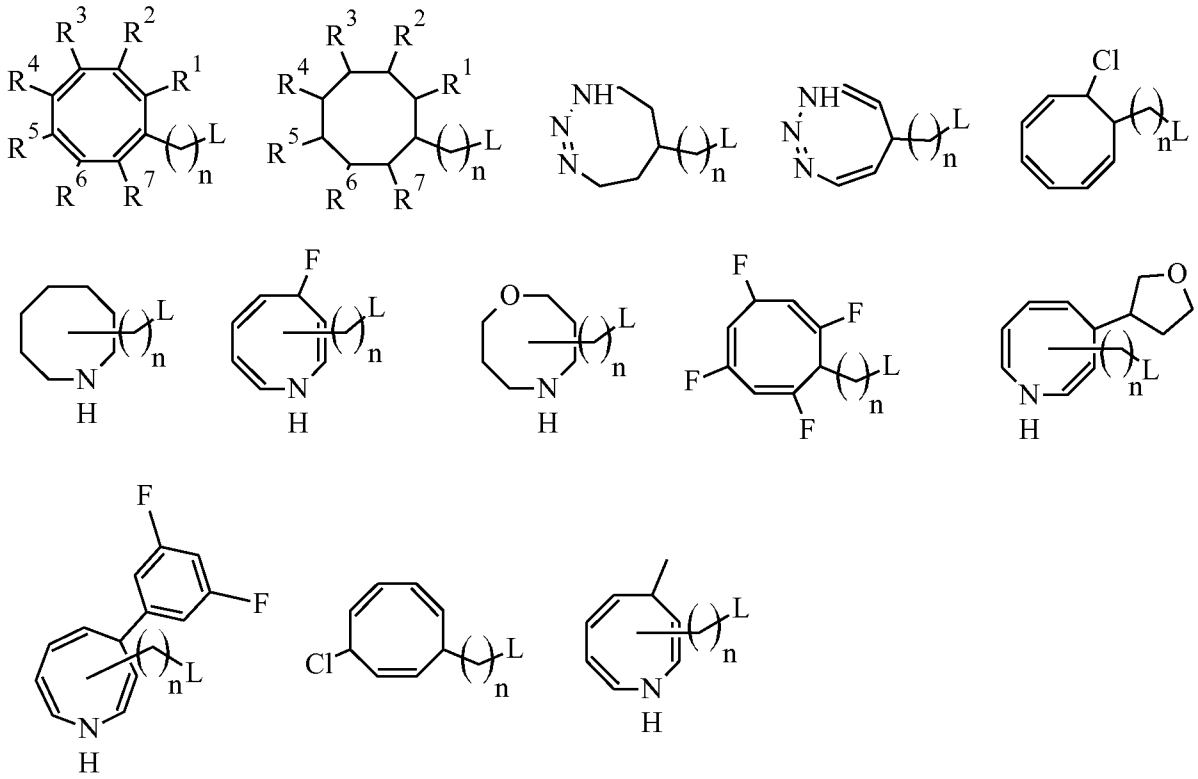
[0055] Experimental approaches which can help to control the selection of R groups: (a) microsomes incubation with prodrug of D, with LC/MS/MS to look for reactive species and/or modification sites of proteins; (b) GSH and/or cyanide trapping experiment with microsome incubation to look for conjugate moieties of GSH or –CN; (c) cell-based MTT assay to screen L-R.

[0056] R, the leaving groups, preferably, should not be a potent agonist or antagonist for any therapeutic targets.

[0057] In some embodiments, R comprises an aromatic or non-aromatic 8-member ring, which may have one or more of N, O, and S in the backbone of the ring, with linker L to have formula $R(CH_2)_nL$ (n can be 0,1, and 2), and the ring may be substituted by any of the following groups (R^n, n=1 to 7): H, F, Cl, I, OH, SH, NH_2, COOH, SO_3H, NO_2, $CONH_2$, amino acids, fatty acids, nucleotides and C_1-C_{40} of alkyl, alkenyl, alkynyl, phenyl, benzyl, haloalkyl, haloformyl, carbonyl, aldehyde, carbonate ester, carboxyl, carboxylate, carboxamide, primary amine,

secondary amine, tertiary amine, primary ketimine, secondary ketimine, primary aldimine, secondary aldimine, imide, azide, azo, cyanate, isocyanide, isothiocyanate, nitrate, nitrile, nitrosooxy, nitro, nitroso, pyridyl, phosphate, phosphono, sulfonyl, sulfo, sulfinyl, sulfhydryl, thiocyanate, disulfide, primary hydroxyl, secondary hydroxyl, tertiary hydroxyl, pyridinyl, pyridazinyl, pyrimidyl, pyrazyl, triazinyl, pyrrolyl, pyrazolyl, imidazolyl, (1,2,3)- and (1,2,4)-triazolyl, pyrazinyl, pyrimidinyl-, tetrazolyl, furyl, thienyl, isoxazolyl, thiazolyl, phenyl, isoxazolyl, oxazolyl, and peptides.

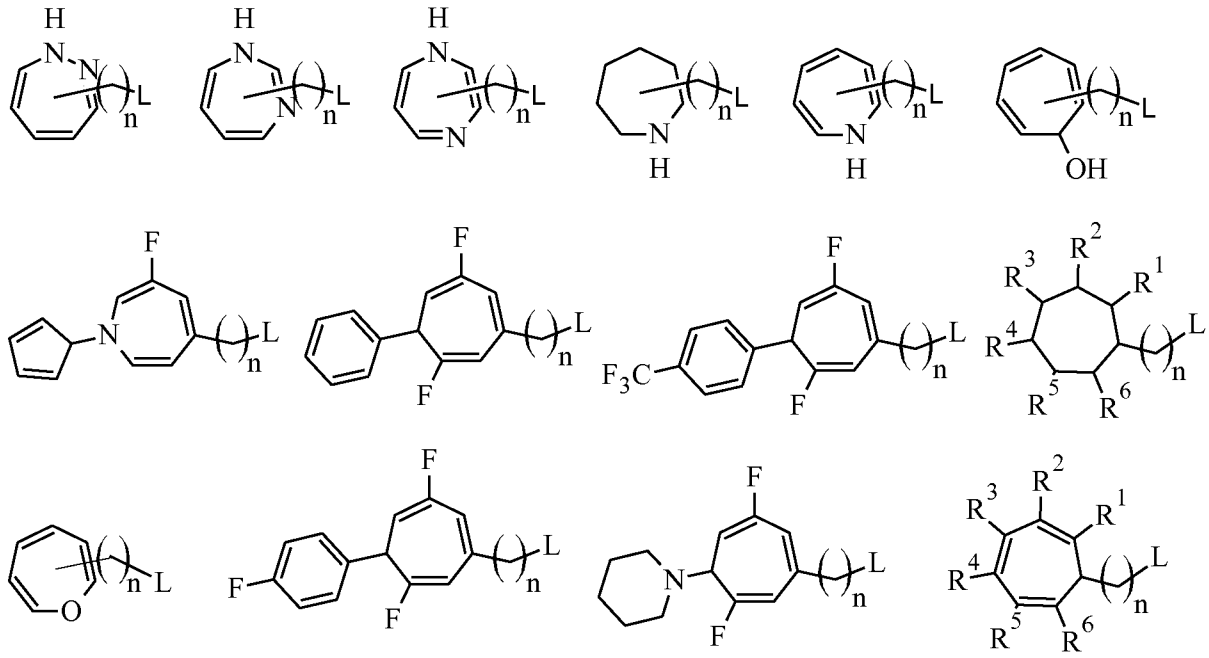
[0058] Examples of R-L include (n=0-2):



[0059] In some embodiments, R comprises an aromatic or non-aromatic 7-member ring, which may have one or more of N, O, and S in the backbone of the ring, with linker L to have formula $R(CH_2)_nL$ (n can be 0,1, and 2), and the ring may be substituted by any of the following groups (R^n, n=1 to 6): H, F, Cl, I, OH, SH, NH_2, COOH, SO_3H, NO_2, amino acids, fatty acids, nucleotides and C_1-C_{40} of alkyl, alkenyl, alkynyl, phenyl, Benzyl, haloalkyl, haloformyl, carbonyl, aldehyde, carbonate ester, carboxyl, carboxylate, carboxamide, primary amine, secondary amine, tertiary amine, primary ketimine, secondary ketimine, primary aldimine, secondary aldimine, imide, azide, azo, cyanate, isocyanide, isothiocyanate, nitrate, nitrile, nitrosooxy, nitro, nitroso, pyridyl, phosphate, phosphono, sulfonyl, sulfo, sulfinyl, sulfhydryl, thiocyanate, disulfide, primary hydroxyl, secondary hydroxyl, tertiary hydroxyl, pyridinyl, pyridazinyl, pyrimidyl, pyrazyl, triazinyl, pyrrolyl, pyrazolyl, imidazolyl, (1,2,3)- and (1,2,4)-

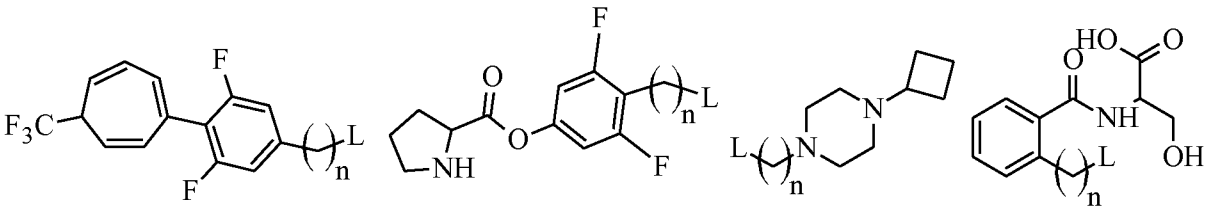
triazolyl, pyrazinyl, pyrimidinyl, tetrazolyl, furyl, thienyl, isoxazolyl, thiazolyl, phenyl, isoxazolyl, oxazolyl and peptides.

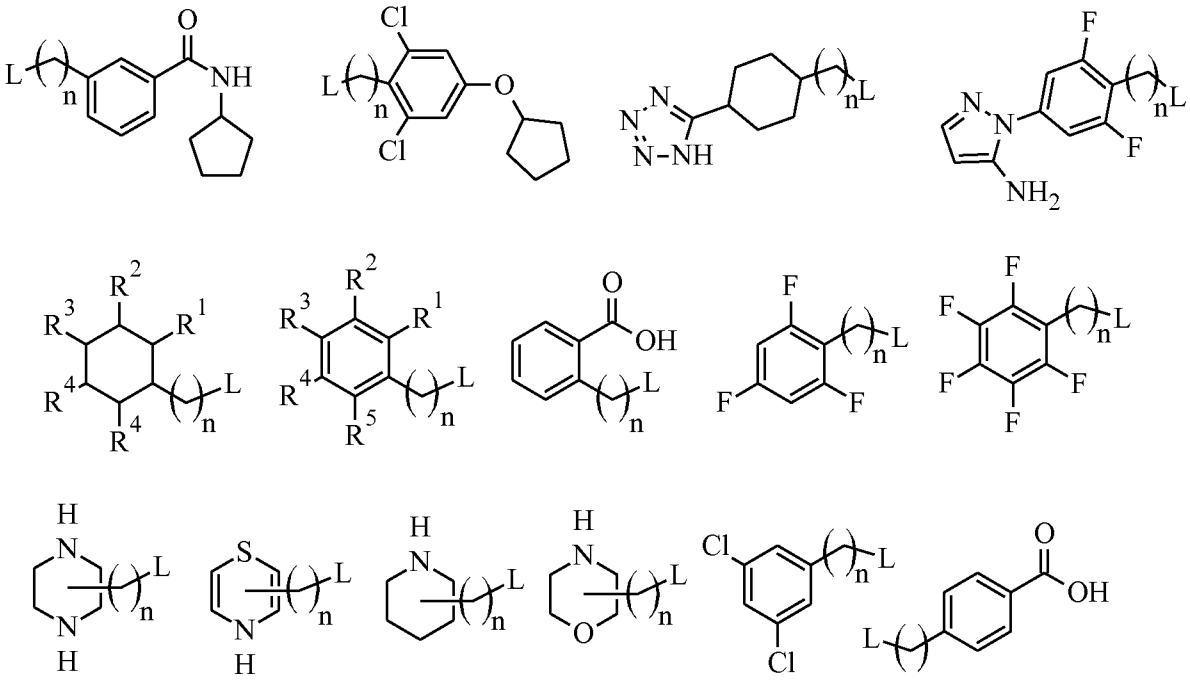
[0060] Examples of R-L (n=0-2):



[0061] In some embodiments, R comprises an aromatic or non-aromatic 6-member ring, which may have one or more of N, O, and S in the backbone of the ring, with linker L to have formula $R(CH_2)_nL$ (n can be 0,1, and 2), and the ring may be substituted by any of the following groups (R^n, n=1 to 5): H, F, Cl, I, OH, SH, NH_2, COOH, SO_3H, NO_2, amino acids, fatty acids, nucleotides and C_1-C_{40} of alkyl, alkenyl, alkynyl, phenyl, Benzyl, haloalkyl, haloformyl, carbonyl, aldehyde, carbonate ester, carboxyl, carboxylate, carboxamide, primary amine, secondary amine, tertiary amine, primary ketimine, secondary ketimine, primary aldimine, secondary aldimine, imide, azide, azo, cyanate, isocyanide, isothiocyanate, nitrate, nitrile, nitrosooxy, nitro, nitroso, pyridyl, phosphate, phosphono, sulfonyl, sulfo, sulfinyl, sulfhydryl, thiocyanate, disulfide, primary hydroxyl, secondary hydroxyl, tertiary hydroxyl, pyridinyl, pyridazinyl, pyrimidyl, pyrazyl, triazinyl, pyrrolyl, pyrazolyl, imidazolyl, (1,2,3)- and (1,2,4)-triazolyl, pyrazinyl, pyrimidinyl, tetrazolyl, furyl, thienyl, isoxazolyl, thiazolyl, phenyl, isoxazolyl, oxazolyl and peptides.

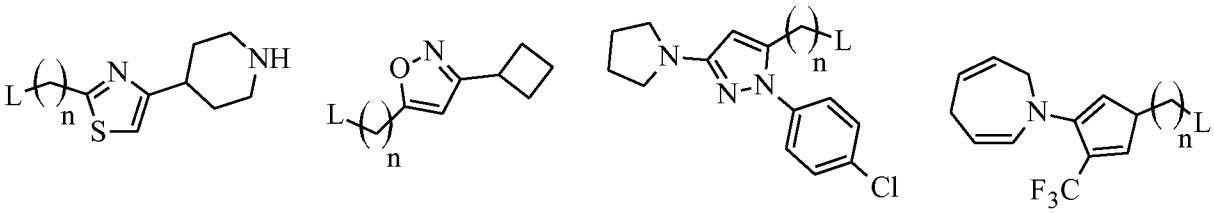
[0062] Examples of R-L (n=0-2):

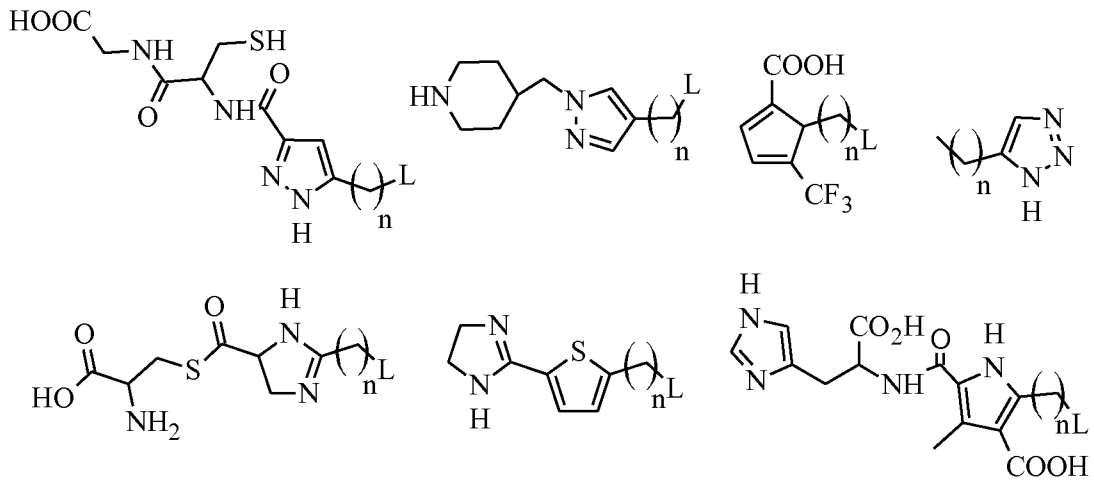




[0063] In some embodiments, R comprises an aromatic or non-aromatic 5-member ring, which may have one or more of N, O, and S in the backbone of the ring, with linker L to have formula $R(CH_2)_nL$ (n can be 0,1, and 2), and the ring may be substituted by any of the following groups (R^n, n=1 to 4): H, F, Cl, I, OH, SH, NH_2, COOH, SO_3H, NO_2, amino acids, fatty acids, nucleotides and C_1-C_{40} of alkyl, alkenyl, alkynyl, phenyl, Benzyl, haloalkyl, haloformyl, carbonyl, aldehyde, carbonate ester, carboxyl, carboxylate, carboxamide, primary amine, secondary amine, tertiary amine, primary ketimine, secondary ketimine, primary aldimine, secondary aldimine, imide, azide, azo, cyanate, isocyanide, isothiocyanate, nitrate, nitrile, nitrosooxy, nitro, nitroso, pyridyl, phosphate, phosphono, sulfonyl, sulfo, sulfinyl, sulfhydryl, thiocyanate, disulfide, primary hydroxyl, secondary hydroxyl, tertiary hydroxyl, pyridinyl, pyridazinyl, pyrimidyl, pyrazyl, triazinyl, pyrrolyl, pyrazolyl, imidazolyl, (1,2,3)- and (1,2,4)-triazolyl, pyrazinyl, pyrimidinyl, tetrazolyl, furyl, thienyl, isoxazolyl, thiazolyl, phenyl, isoxazolyl, oxazolyl and peptides.

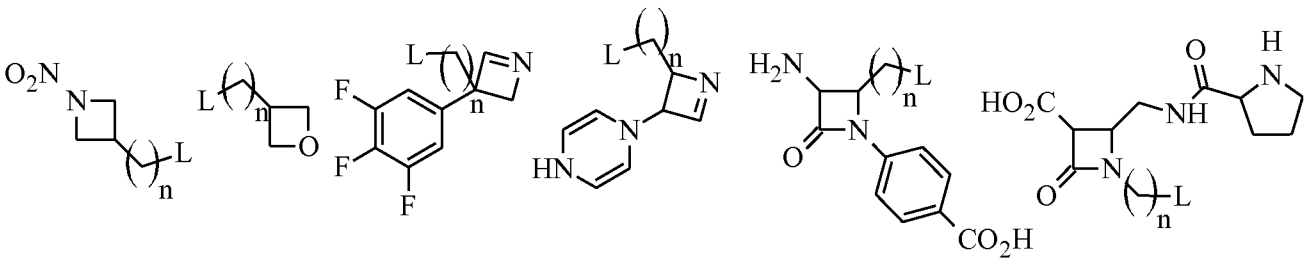
[0064] Examples of R-L (n=0-2):





[0065] In some embodiments, R comprises an aromatic or non-aromatic 4-member ring, which may have one or more of N, O, and S in the backbone of the ring, with linker L to have formula $R(CH_2)_nL$ (n can be 0,1, and 2), and the ring may be substituted by any of the following groups (R^n, n=1 to 3): H, F, Cl, I, OH, SH, NH_2, COOH, SO_3H, NO_2, amino acids, fatty acids, nucleotides and C_1-C_{40} of alkyl, alkenyl, alkynyl, phenyl, Benzyl, haloalkyl, haloformyl, carbonyl, aldehyde, carbonate ester, carboxyl, carboxylate, carboxamide, primary amine, secondary amine, tertiary amine, primary ketimine, secondary ketimine, primary aldimine, secondary aldimine, imide, azide, azo, cyanate, isocyanide, isothiocyanate, nitrate, nitrile, nitrosooxy, nitro, nitroso, pyridyl, phosphate, phosphono, sulfonyl, sulfo, sulfinyl, sulfhydryl, thiocyanate, disulfide, primary hydroxyl, secondary hydroxyl, tertiary hydroxyl, pyridinyl, pyridazinyl, pyrimidyl, pyrazyl, triazinyl, pyrrolyl, pyrazolyl, imidazolyl, (1,2,3)- and (1,2,4)-triazolyl, pyrazinyl, pyrimidinyl, tetrazolyl, furyl, thienyl, isoxazolyl, thiazolyl, phenyl, isoxazolyl, oxazolyl and peptides.

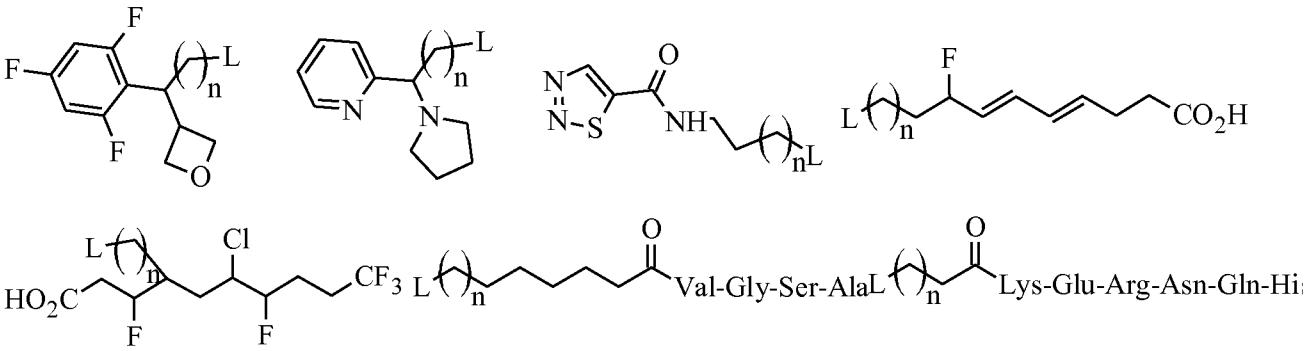
[0066] Examples of R-L (n=0-2):



[0067] In some embodiments, R can be amino acids, fatty acid, peptides, nucleotides or an aliphatic group (C_1-C_{40}), saturated or unsaturated, with linker L to have formula $R(CH_2)_nL$ (n can be 0,1, and 2), which may be substituted by any of the following groups: H, F, Cl, I, OH, SH, NH_2, COOH, SO_3H, NO_2, amino acids, fatty acids and C_1-C_{40} of alkyl, alkenyl, alkynyl, phenyl, Benzyl, haloalkyl, haloformyl, carbonyl, aldehyde, carbonate ester, carboxyl, carboxylate, carboxamide, primary amine, secondary amine, tertiary amine, primary ketimine, secondary

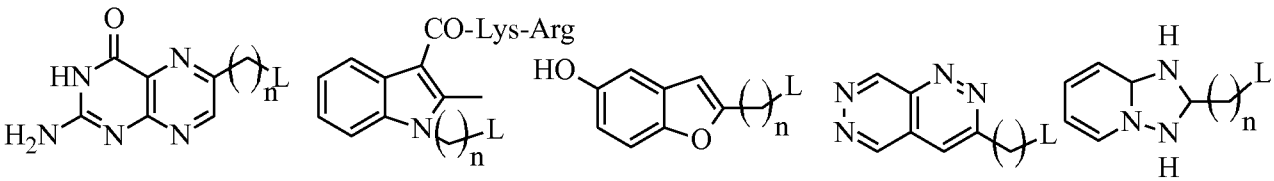
ketimine, primary aldimine, secondary aldimine, imide, azide, azo, cyanate, isocyanide, isothiocyanate, nitrate, nitrile, nitrosooxy, nitro, nitroso, pyridyl, phosphate, phosphono, sulfonyl, sulfo, sulfinyl, sulfhydryl, thiocyanate, disulfide, primary hydroxyl, secondary hydroxyl, tertiary hydroxyl, pyridinyl, pyridazinyl, pyrimidyl, pyrazyl, triazinyl, pyrrolyl, pyrazolyl, imidazolyl, (1,2,3)- and (1,2,4)-triazolyl, pyrazinyl, pyrimidinyl, tetrazolyl, furyl, thienyl, isoxazolyl, thiazolyl, phenyl, isoxazolyl, oxazolyl and peptides.

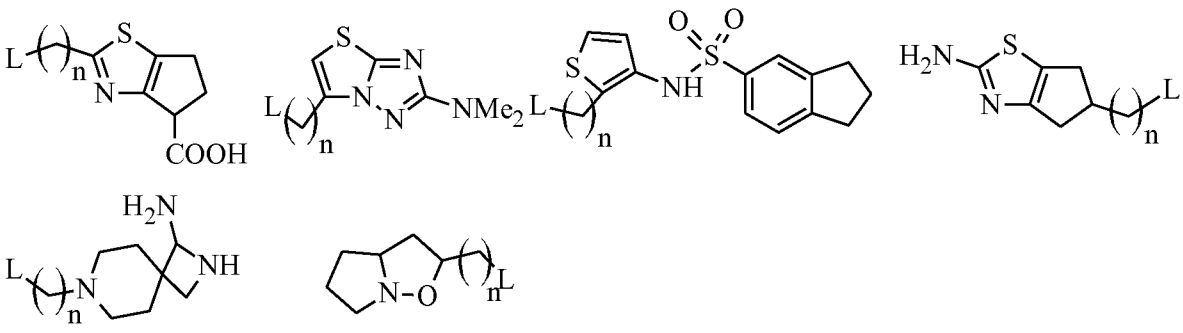
[0068] Examples of R-L (n=0-2):



[0069] In some embodiments, R comprises a fused bicyclic ring or polycyclic, saturated or unsaturated ($\leq C_{40}$), with linker L to have formula $R(CH_2)_nL$ (n can be 0,1, and 2), which may be substituted by any of the following groups: H, F, Cl, I, OH, SH, NH_2, COOH, SO_3H, NO_2, amino acids, fatty acids, nucletides and C_1-C_{40} of alkyl, alkenyl, alkynyl, phenyl, Benzyl, haloalkyl, haloformyl, carbonyl, aldehyde, carbonate ester, carboxyl, carboxylate, carboxamide, primary amine, secondary amine, tertiary amine, primary ketimine, secondary ketimine, primary aldimine, secondary aldimine, imide, azide, azo, cyanate, isocyanide, isothiocyanate, nitrate, nitrile, nitrosooxy, nitro, nitroso, pyridyl, phosphate, phosphono, sulfonyl, sulfo, sulfinyl, sulfhydryl, thiocyanate, disulfide, primary hydroxyl, secondary hydroxyl, tertiary hydroxyl, pyridinyl, pyridazinyl, pyrimidyl, pyrazyl, triazinyl, pyrrolyl, pyrazolyl, imidazolyl, (1,2,3)- and (1,2,4)-triazolyl, pyrazinyl, pyrimidinyl-, tetrazolyl, furyl, thienyl, isoxazolyl, thiazolyl, phenyl, isoxazolyl, oxazolyl and peptides.

[0070] Examples of R-L (n=0-2):





[0071] The agents can be modified to be dimer or trimer with or without spacers (A) in between to form bones of –N-N-, -O-O-, -S-S-, -B-O-B-, -B-S-B-, -B-N-B-, -N-S-N-, -C-O-C-, -C-S-C-, -C-N-C-, -N-O-N-, –N-O-, -N-S-, -C-N-, -C-O-, -B-O-, -B-S-, -B-N-, and -C-S-.

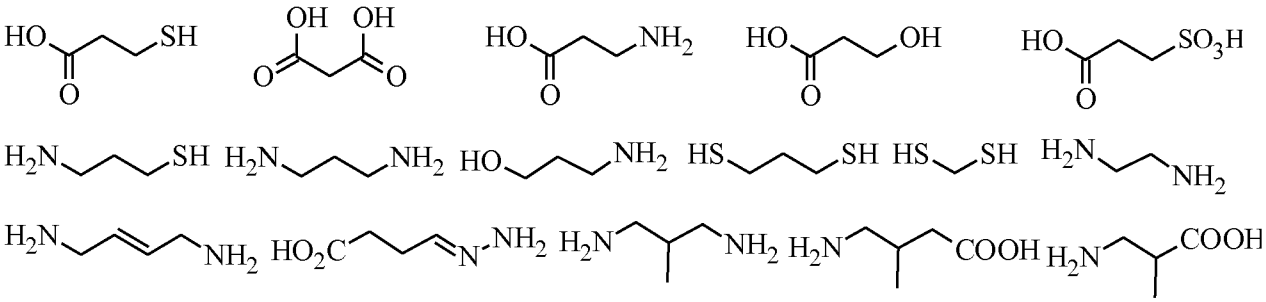
[0072] Spacer, A, is constructed as formula $X_1(CR'_m)_nY_1$, which can directly link to D and polymer/copolymer/dendrimer (P) or connect after activation of A and/or D and/or P, for example by NHS, N-hydroxy succinimide. X_1 and Y_1 can be any of the following groups or bonds: OH, SH, NH_2, N-NH2, COOH, SO_3H, CONH2, CONH-NH_2 SO2NH2, CHO, F, Cl, Br, I, -O-, -S-, CONH-, -SO_2-, SONH-, and -NH-; R' can be H, CH_3, CN; m=0 to 2; n = 0 to 30; R', m and n are individually independent, can be in any combination with each other and within; C-C bond within $(CR'_m)_n$ can be single or double or triple; Either single or double bond are between X_1 and $(CR'_m)_n$, and $(CR'_m)_n$ and Y_1.

[0073] In another construct for A, $X_2(CR'_m)_n R_s(CR'_m)_nY_2$, X_2 and Y_2 can be any moiety from –H, -CO_2H, -OH, -SO_2NH-, -SO_2NH_2, -SO_3H, -NH_2, =N-NH2, -CONH-, -$CONH_2$, -B(OH)-, -CHO, -CO-, -S-, –SH, F, Cl, Br, and I; R' can be H, CH_3, CN; m=0 to 2; n = 0 to 10; R', m and n are individually independent, can be in any combination with each other and within; C-C bond within $(CR'_m)_n$ can be single or double or triple; Either single or double bond are between X_2 and $(CR'_m)_n$, and $(CR'_m)_n$ and Y_2; R_s comprises a cyclic moiety of 4-8 membered rings or fused rings or polycyclic structures with one or more of N, O, and S, aromatic and non-aromatic, saturated and unsaturated, which may be substituted by any of the following groups: H, F, Cl, I, OH, SH, NH_2, COOH, SO_3H, NO_2,amino acids, fatty acids, nucleotides and C_1-C_{40} of alkyl, alkenyl, alkynyl, phenyl, Benzyl, haloalkyl, haloformyl, carbonyl, aldehyde, carbonate ester, carboxyl, carboxylate, carboxamide, primary amine, secondary amine, tertiary amine, primary ketimine, secondary ketimine, primary aldimine, secondary aldimine, imide, azide, azo, cyanate, isocyanide, isothiocyanate, nitrate, nitrile, nitrosooxy, nitro, nitroso, pyridyl, phosphate, phosphono, sulfonyl, sulfo, sulfinyl, sulfhydryl, thiocyanate, disulfide, primary hydroxyl, secondary hydroxyl, tertiary hydroxyl, pyridinyl, pyridazinyl, pyrimidyl, pyrazyl, triazinyl,

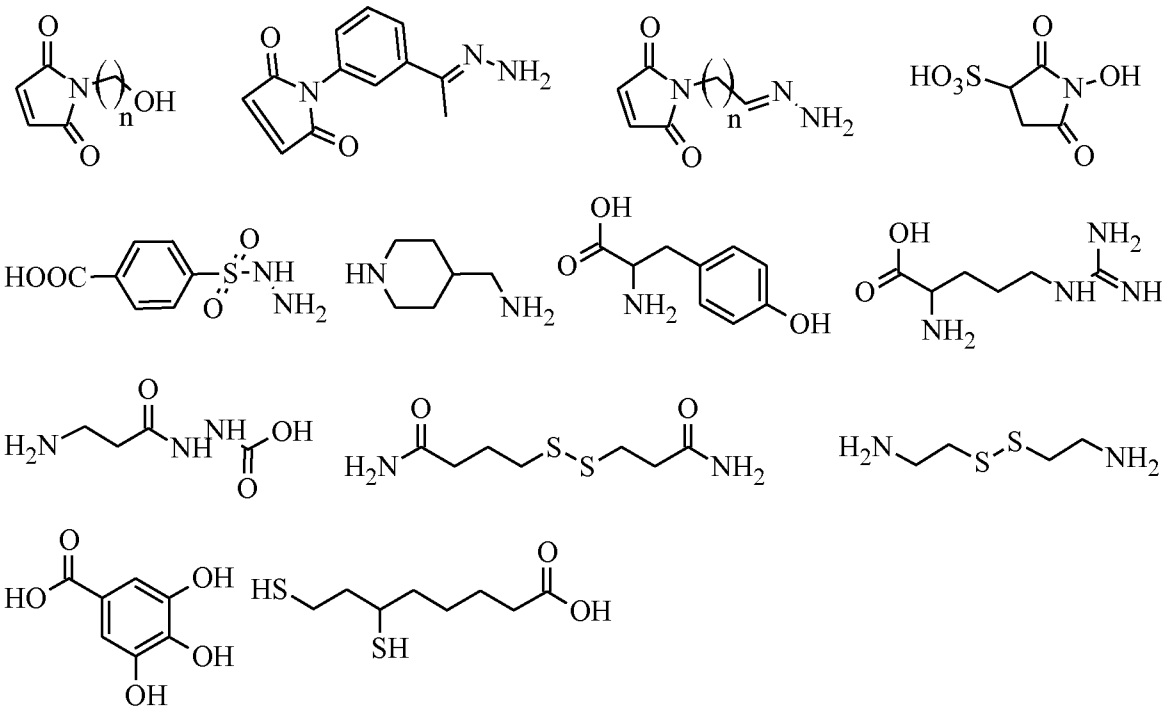
pyrrolyl, pyrazolyl, imidazolyl, (1,2,3)- and (1,2,4)-triazolyl, pyrazinyl, pyrimidinyl-, tetrazolyl, furyl, thienyl, isoxazolyl, thiazolyl, phenyl, isoxazolyl, oxazolyl and peptides.

[0074] The spacer can be any combination of A as well.

[0075] Examples of A are in the following:



[0076] Other spacers can be, but not limited to (n=0 to 3):



[0077] A can also be monodisperse PEG, as formula $X_1(CH_2CH_2O)_nCH_2CH_2Y_2$(n=0 to 5000) with any combination of end groups of X_1 and Y_1 as linkers, for example PEG2000 bis amine.

[0078] A can also be amino acids, peptides and nucleotides.

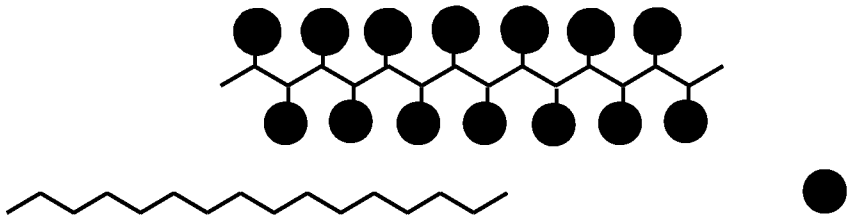
[0079] D, A, L-R and P can be activated, for example, by succinimidyl 4-formylbenzoate (SFB), succinimidyl 4-hydrazinonicotinate acetone hydrazone (SANH), and NHS.

[0080] In another aspect, polymer/copolymer (P) is used to conjugate with D with or without A, among which dendrimer and tubular shaped dendronized linear polymer/copolymer are preferred.

[0081] The selected dendrimers' scaffolds include tubular shape, star Shape, spherical and tear shape with functional groups of either –NH2 or –OH or –COOH or -SO3H or guanidine or

phosphonate or PEGylated or saccharide-terminated, and combinations of the above. The dendrimers can be cationic, anionic and neutral in physiological or tumor's acidic surrounding environment, among which neutral state is preferred. The construct should be non-toxic, non-immunogenic and biodegradable. Examples of dendrimers are triazine dendrimer-G(0-5)-NH_2, Poly(L-lysine) dendrimer-G(0-5)-NH_2, poly-ester dendrimer-G(0-5)-OH, poly-ester dendrimer-G(0-5)-COOH, PAMAM-G(0-5)-OH, PAMAM-G(0-5)-COOH, PAMAM-G(0-5)-NH_2, folate-PEG-PAMAM-G(0-5)-OH, folate-PEG-PAMAM-G(0-5)-COOH, folate-PEG-PAMAM-G(0-5)-NH_2, Polyol-G(0-5)-OH (polyol), poly propyl ether imine-G(0-5)-COOH, poly propyl ether imine-G(0-5)-OH, dendron PAMAM-G(0.5-4.5)-OH, dendron PAMAM-G(0.5-4.5)-COOH, dendron PAMAM-G(0.5-4.5)-NH_2, HDTPA-G(0-5) (poly diethylenetriamine pentaacetic acid dendrimer), and with the PEGylation of the above.

[0082] "Rigid rod and tubular" shaped dendronized linear polymer/copolymer is used to conjugate to D with or without S, as depicted in the following diagram:



Linear polymer/copolymer Dendrimer

[0083] Enlongated tubular rigid rod is better than linear, rigid globular, star-like for tissue penetration, especially for tumors. A size of bigger than 40 kDa can increase the plasma circulation time. The linear polymer can link with hundreds of dendrimers for very high payload of D.

[0084] Linear polymer/copolymer (with average n of 10 to 1000) can be poly(methacrylic acid), poly(4-vinylphenol), poly(vinyl alcohol), poly(4-styrenesulfonic acid), poly(methyl methacrylate-co-methacrylic acid), poly(N- isopropylacrylate-co-methacrylic acid), poly(2-hydroxypropyl methacrylate), poly(DL-ornithine), PLGA-PEG copolymer, Polyethylcyanoacrylate, Cellulose diacetate-g-poly(p-dioxanone) co-polymer, poly(lactic-co-glycolic acid) (PLGA), poly(sebacoyl diglyceride), poly(glycerol sebacate), polyamines, poly(L-lactic acid), poly(D-lactic acid), Poly-Glycerol-Dodecanoate, poly[(R)-3-hydroxybutyrate] (PHB), poly(epsilon-caprolactone) (PCL), azide-terminated poly(ethylene glycol), Polyhydroxyalkanoate, and poly(ethylene glycol)-peptide copolymer.

[0085] Preferably, the whole conjugate is neutral under the physiological and conditions around target tissues. Capping of dendrimers through PEGylation with different sizes, for example PEG 200 to PEG 10,000, increase the solubility, change the surface charge and reduce toxicity (when –NH_2 as the functional group on the dendrimer), change biodistribution profile,

and modulate tissue penetration and accumulation, for examples, PEGylation of poly-ester dendrimer, PEGylation of PAMAM-NH_2, and PEGylation of poly(L-lysine).

[0086] A or any combination of A can link active targeting ligand, for examples folate or peptides, to dendrimers to target delivery of conjugates.

Examples

Example 1. Improving Atorvastatin

[0087] HMG-CoA reductase inhibitors are a group of prescription drugs used to lower cholesterol. They work by slowing down the body's ability to make cholesterol in the blood. Also known as "the statins," medicines in this class include atorvastatin (brand name Lipitor with $13 billion of market share), fluvastatin (Lescol), lovastatin (Mevacor), pravastatin (Pravachol), simvastatin (Zocor), and Rosuvastatin (Crestor). Inhibition of the enzyme decreases *de novo* cholesterol synthesis, increasing expression of low-density lipoprotein receptors (LDL receptors) on hepatocytes. This increases LDL uptake by the hepatocytes, decreasing the amount of LDL-cholesterol in the blood. Statins also reduces blood levels of triglycerides and slightly increases levels of HDL-cholesterol. So far, this class of compounds is the most effective for antihyperlipidemic and may combine with other therapies such as squalene synthase inhibitor, C-reactive protein production blocker, fibrates, inhibition of apoB synthesis, thyroid agonist, angiotensin receptor blockers, PPAR-d agonist, bile acid sequestrant polymer to improve the efficacy for cardiovascular disease.

[0088] Common side effects of statins are musculoskeletal effects, such as arthralgia, disorder of muscle, musculoskeletal pain, myalgia, spasm, and severe muscle damage called rhabdomyolysis. On August 8, 2001, cerivastatin (Baycol) was withdrawn from the market because of increasing reports of rhabdomyolysis, which cause skeletal muscle cells break down and release cell content including myoglobin. This condition may lead to muscle breakdown and kidney failure since myoglobin is toxic to the kidneys. A rare, but more serious, side effect of statins is liver damage.

[0089] Atorvastatin (Lipitor) is considered as the candidate to be improved in the area of ADME through the approach claimed in the patent application. The property of ADME of atorvastatin is summarized in the followings (Database: *MICROMEDEX® 2.0;* Lennernas **2003** *Clin Pharmacokinet.* 42:1141-60; Williams **2002** *Clin Pharmacokinet.* 41: 343–70; *Lipitor: Prescribing Information.* Pfizer. June **2009**; Lins, *et al.* **2003** *Nephrol Dial Transplant.* 18:967-76; Black, *et al.* **1998** *Drug Metab Dispos.* 26:755-63; Jacobson, *et al.* **2000** *Drug Metab Dispos.* 28:1369-78; Lau, *et al.* **2006** *Drug Metab Dispos.* 34:1175-81; Black, *et al.* **1999** *Drug Metab*

Dispos. 27:916-23; Srinivas **2009** *J Clin Pharmacol.* 49:1492-3; Lau, *et al.* **2007** *Clin Pharmacol Ther.* 81:194-204).

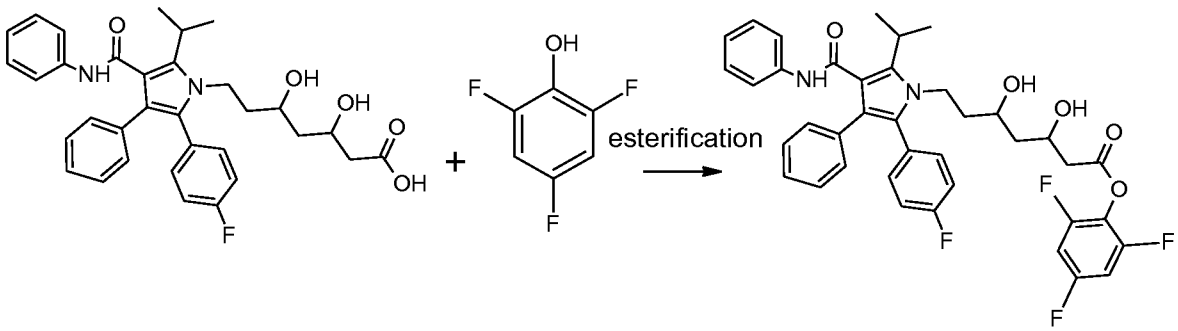
[0090] Atorvastatin undergoes rapid oral absorption, with T_{max} of 1–2 hours. The absolute bioavailability is about 14% with large volume distribution of 371L; however, the systemic availability for HMG-CoA reductase activity is about 30% including active metabolites. Atorvastatin undergoes high intestinal clearance and first-pass metabolism, which is the main cause for the low systemic availability.

[0091] The major metabolism pathways of atorvastatin is through cytochrome P450 3A4 hydroxylation to form active ortho- and parahydroxylated metabolites, as well as various beta-oxidation metabolites. The ortho- and parahydroxylated metabolites are responsible for 70% of systemic HMG-CoA reductase activity. The ortho-hydroxy metabolite undergoes further metabolism via glucuronidation.

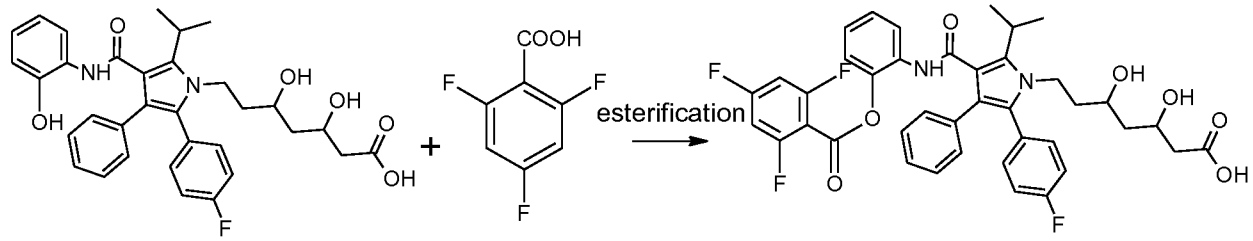
[0092] It is primarily eliminated via hepatic biliary excretion, with less than 2% of atorvastatin recovered in the urine. Atorvastatin is also a substrate of the intestinal P-glycoprotein efflux transporter, which pumps the drug back into the intestinal lumen during drug absorption.

[0093] Pharmacokinetics of atorvastatin showed age and disease state-dependency. Healthy elderly patients (> 65 years old) show a 40% and 30% greater AUC compared to young adults. Patients with A-stage liver disease show a 4-fold increase in both C_{max} and AUC. Patients with B-stage liver disease show a 16-fold increase in C_{max} and an 11-fold increase in AUC.

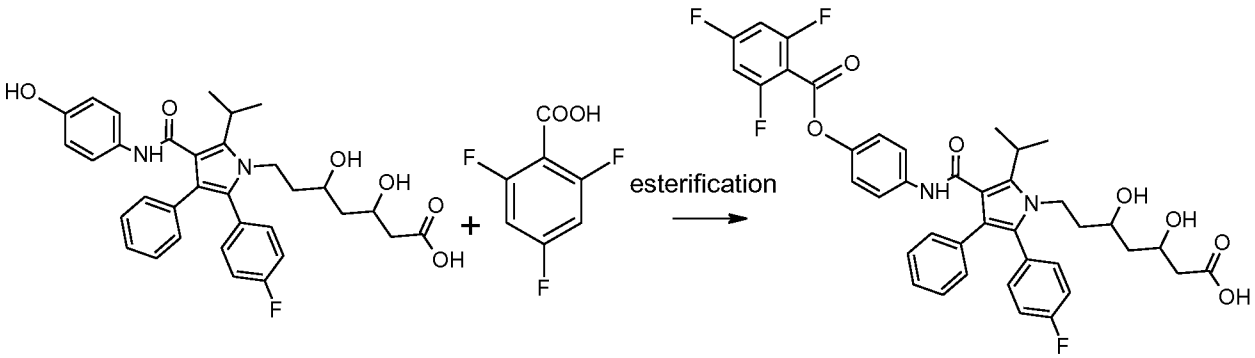
[0094] From the ADMET summary listed above, bioavailability and musculoskeletal side effects are the major concerns and/or aspects for improvements. Based on the claims in the patent, examples and synthetic schemes of prodrugs and excipients-drug conjugates of atorvastatin are listed in the following:



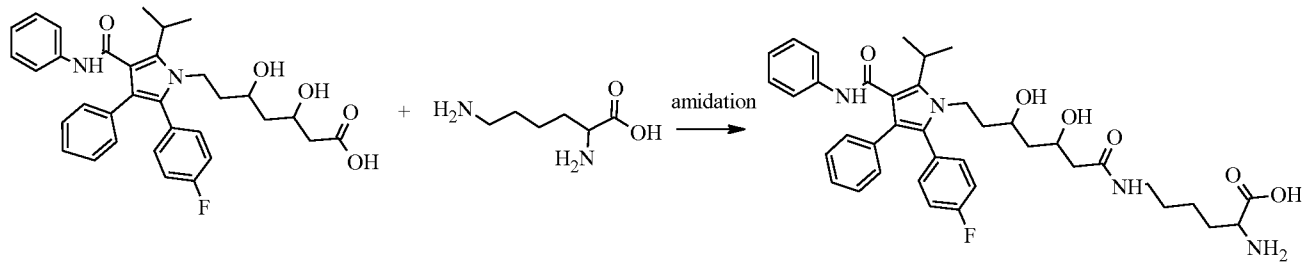
Atorvastatin 2, 4, 6-trifluorophenol (a)



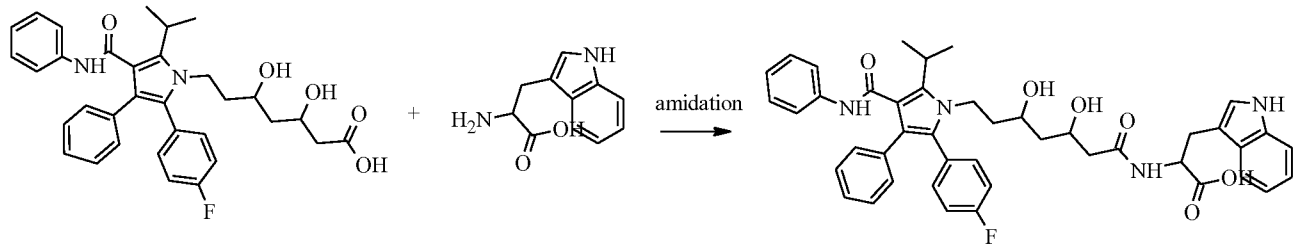
Ortho-hydroxylated atorvastatin 2,4,6-trifluorobenzoic acid (b)



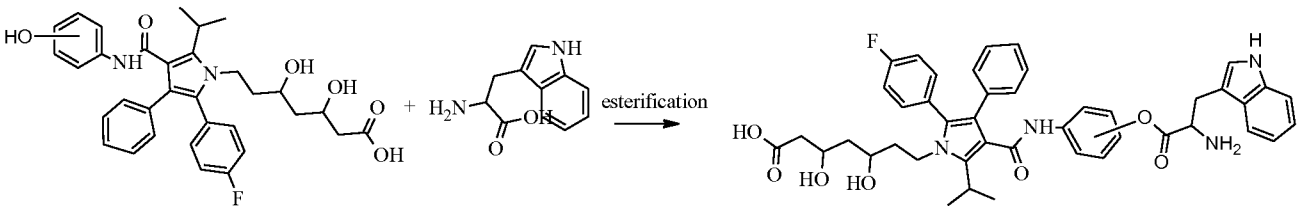
Para-hydroxylated atorvastatin 2,4,6-trifluorobenzoic acid (c)



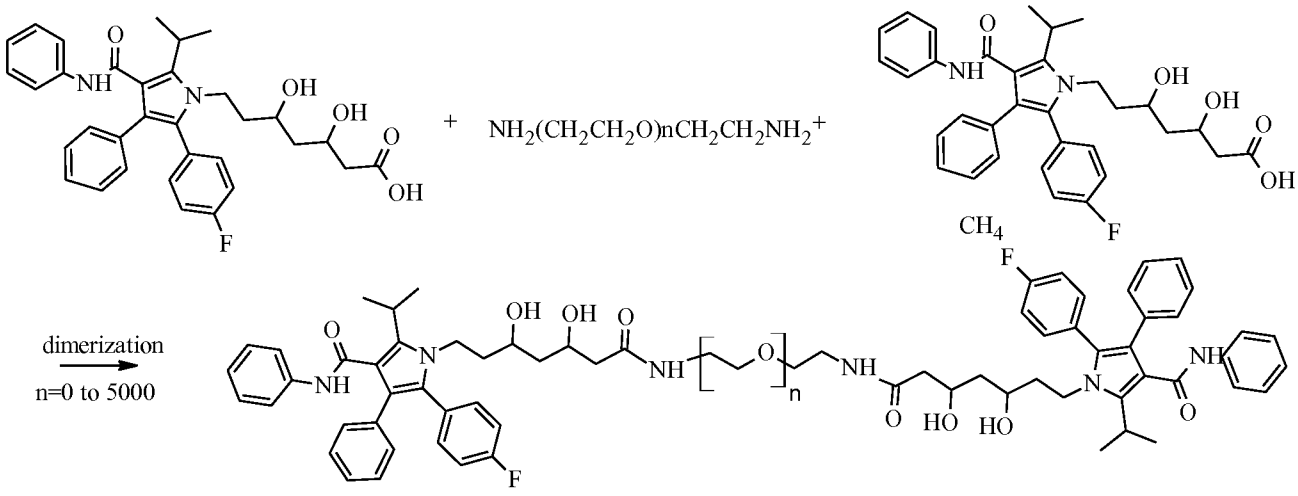
Atorvastatin Lysine (d)



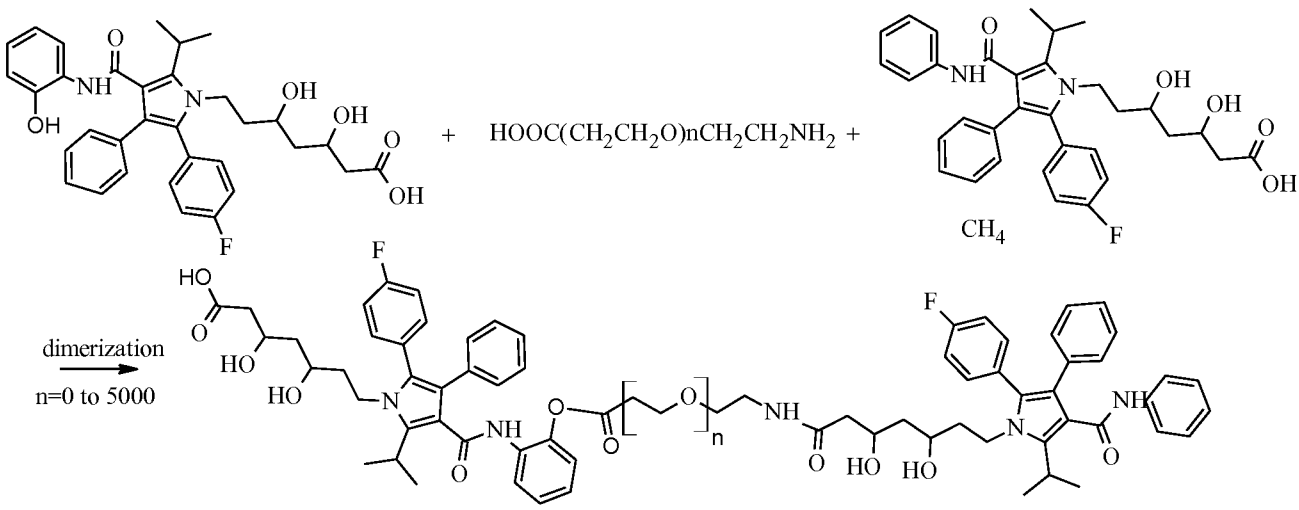
Atorvastatin Tryptophan (e)



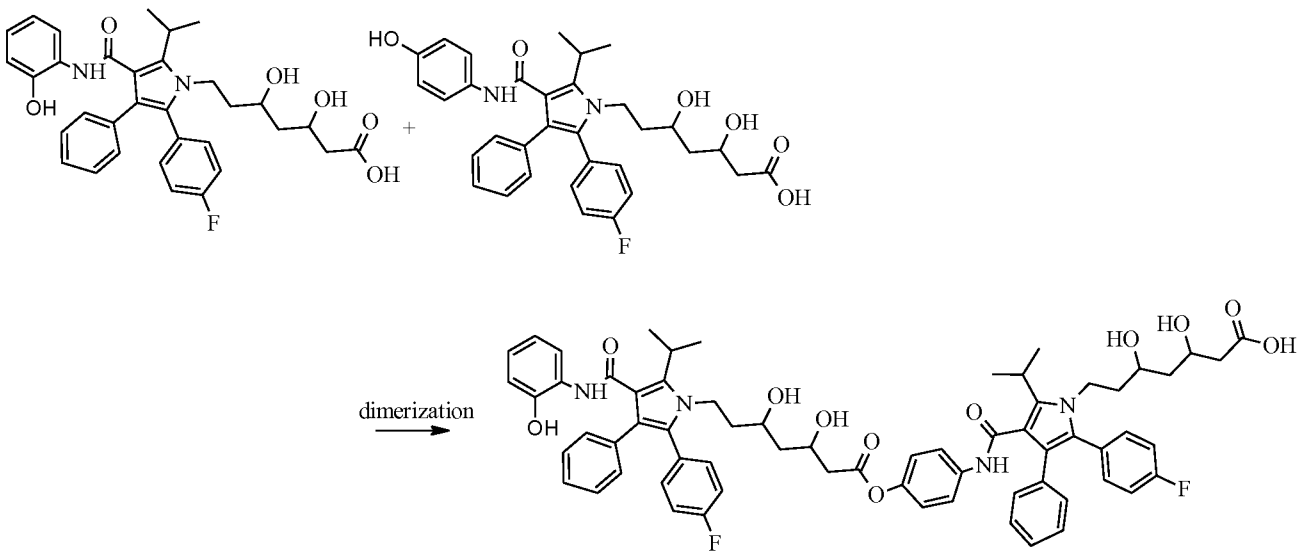
Ortho- or para- OH Atorvastatin Tryptophan (f)


dimerization
n=0 to 5000
CH4

(g)


dimerization
n=0 to 5000
CH4

(h)


dimerization

(i)

[0095] Examples of conjugated drugs (a), (b), (c), (d), (e), (f), (g), (h), and (i) show possibilities to modify atorvastatin and its ortho- and para-hydroxylated active metabolites to improve ADMET properties. The reaction schemes may not reflect the synthetic routes. Drug, modifying reagents and spacers can be activated with other functional groups, which should be not modified, protected by BOC groups first.

[0096] Prodrug (a) is to modify carboxylic acid moiety in atorvastatin through the conjugation with 2,4,6-trifluorophenol, which is metabolic stable. The passive absorption of this conjugate will be improved due to the increase in clogP (from 4.13 to 7.25). Prodrug (b) is the conjugate between the active metabolite ortho-hydroxylated atorvastatin and 2, 4, 6-trifluorobenzoic acid through the ortho-hydroxyl moiety, which is metabolic stable. The passive absorption of this conjugate will be improved due to the increase in clogP (from 4.13 to 5.08). The carboxylic acid moiety can be modified with or without the modification of ortho-hydroxyl group using the conjugation groups listed in the claims as different prodrugs. Prodrug (c) is the conjugate between the active metabolite para-hydroxylated atorvastatin and 2, 4, 6-trifluorobenzoic acid through the para-hydroxyl moiety, which is metabolic stable. The passive absorption of this conjugate will be improved due to the increase in clogP (from 3.39 to 5.13). Prodrugs (d) (e) and (f) are examples of using amino acids to change the absorption, metabolism and distribution profiles. Starting with hydroxylated metabolites, the soft spots have been blocked. Dimers of atorvastatin, ortho- and para- hydrozylated atorvastatin, including the combinations of any two forms, are exemplified in (g) and (h) with PEG as spacer, and (i) without any spacer. The conjugate (g) and (f) may become nano-particle, which greatly facilitates the absorption process, eliminates transporter efflux issue, changes metabolism, drug distribution, excretion and safety profiles. For the modified drugs listed above, the absorption is further increased if the conjugate is not the substrate of the intestinal P-glycoprotein efflux transporter. The penetration into myocytes will be decreased due to expected higher systemic exposure (AUC) and lower volume distribution, which leads to lower musculoskeletal toxicity. The expected results including proof of concept, efficacy, and toxicity can be demonstrated in the following experiments:

[0097] A: Human liver and intestinal microsome stability assay with the control of atorvastatin. Two expected outcomes. (1) 2, 4, 6-trifluorophenol or 2, 4, 6-trifluorobenzoic acid, lysine, tryptophan and PEG will be released from the conjugate, respectively, and atorvastatin including active metabolites (ortho- and para-hydroxylated atorvastatin) will be generated. LC/MS/MS methods can be developed to specifically look for atorvastatin and/or ortho- and/or para-hydroxylated atorvastatin to confirm the prodrugs can release the therapeutic agents. (2)Through the time-course experiment, the sum of AUCs of atorvastatin, ortho- and para-

hydroxylated atorvastatin from the respective conjugate should be bigger than that of the control atorvastatin.

[0098] B: In vitro cell-based selectivity experiment with control of atorvastatin in rat hepatocyte versus L6 myoblast to make sure the prodrugs/conjugates (a), (b), (c), (d), (e), (f), (g), and (i) are more "muscle sparing".

[0099] C: Rat and dog PK and tissue distribution study (monitor prodrug/conjugate, atorvastatin, ortho- and para-hydroxylated atorvastatin) to confirm the results of A and B, which combine with the metabolic profiling to make sure there is no potential reactive metabolites. If the metabolic profiling is similar to atorvastatin, the prodrug/conjugate will be tested into the animal efficacy model. Otherwise, candidate can be screened into the full toxicity panel to see if there are any off-target effects besides being tested in the efficacy model.

[00100] E: Safety, dose escalation in Guinea Pig model, exams includes hepatic biochemisty (ALT, AST), CK, renal biochemistry, hematology and urinalysis, vital signs, and electrocardiograms (ECGs).

[00101] 2, 4, 6-trifluorophenol or 2, 4, 6-trifluorobenzoic acid or lysine or tryptophan or PEG are part of the examples listed in the claims, which are metabolic stable and can be released from the ester and amide bonds in the conjugates (a) to (i). All the claimed conjugation groups with or without spacers can be adopted into atorvastatin and active metabolites (ortho-hydroxylated atorvastatin and para-hydroxylatd atorvastatin) through the moieties of three –OH and -COOH groups. Experiments A and B can be used to select the best conjugation groups, linkers and spacers. OATP1B transporter assay can be used as another HTS tier to increase the systemic exposure. (Lau, *et al.* **2007** *Clin Pharmacol Ther.* 81(2): 194-204; Istvan, *et al.* **2001** *Science* 292:1160-1164; Pfefferkorn, *et al.* **2007** *Bioorg & Med Chem Letters* 17: 4538–4544; Bratton, *et al.* **2007** *Bioorg & Med Chem* 15: 5576–5589; Park, *et al.* **2008** *Bioorg & Med Chem Letters* 18: 1151–1156; Larsen, *et al.* **2007** *Bioorg & Med Chem Letters* 17: 5567–5572; Capuzzi, *et al.* **1971** *Lipids* 6: 601-608; Capuzzi, *et al.* **1971** *Lipids* 6: 609-616; Pfefferkorn, *et al.* **2009** *Cur Opinion in Invest. Drugs* 10: 245-252; Schaefer, *et al.* **2004** *Toxicology and Applied Pharmacology* 194: 10– 23; Madsen, *et al.* **2008** *J of Pharm & Exp Therap* 324: 576-586.)

Example 2. Improving Metformin

[00102] Metformin is an oral anti-diabetic drug in the biguanide class. It is the first-line drug of choice for the treatment of type 2 diabetes, particularly in overweight and obese people and those with normal kidney function (**2009** *Diabetes Care*, 32 Suppl 1:S13–61). It helps reduce LDL cholesterol and triglyceride levels and is not associated with weight gain, and is the only anti-diabetic drug that has been conclusively shown to prevent the cardiovascular complications of diabetes and reduce mortality by about 30% when compared with insulin and sulfonylureas

(glibenclamide and chlorpropamide), and by about 40% when compared with the group only given dietary advice (**1998** *Lancet* 352:854–65; Selvin, *et al.* **2008** *Arch Intern Med.* 168:2070–80). As of 2009, metformin is one of only two essential oral anti-diabetics and the most widely prescribed (more than 40 million prescriptions) anti-diabetic drug in the world.

[00103] Metformin improves hyperglycemia primarily through its suppression of hepatic glucose production (hepatic gluconeogenesis) by over one third. Metformin activates AMP-activated protein kinase (AMPK), a liver enzyme that plays an important role in insulin signaling, whole body energy balance, and the metabolism of glucose and fats. Activation of AMPK is required for an increase in the expression of SHP, which in turn inhibits the expression of the hepatic gluconeogenic genes PEPCK and Glc-6-Pase and increases the amount of cytosolic AMP, resulting in insulin-independent glucose uptake. In addition to suppressing hepatic glucose production, metformin increases insulin sensitivity, enhances peripheral glucose uptake, increases fatty acid oxidation, and decreases absorption of glucose from the gastrointestinal tract. (Kirpichnikov, *et al.* **2002** *Ann Intern Med.* 1371:25–33; Hundal, *et al.* **2000** *Diabetes* 4912:2063–9; Towler, *et al.* **2007** *Circ Res.* 100:328–41; Zhou, *et al.* **2001** *J Clin Invest.* 108:1167–74; Kim, *et al.* **2008** *Diabetes*, 57:306–14; Zhang, *et al.* **2007** *Am J Physiol Heart Circ Physiol.* 293:H457–66; Musi, *et al.* **2002** *Diabetes* 51:2074–81; Collier, *et al.* **2006** *Am J Physiol Endocrinol Metab.* 291:E182–E189.)

[00104] Besides for type 2 diabetes, it is as effective and safe as insulin for the management of gestational diabetes, and healthier in the neonatal period for women. It also has been investigated to lower cancer risks such as pancreatic and breast. (Tertti, *et al.* **2008** *Rev Diabet Stud.* 5:95–101; Rowan, *et al.* **2008** *N Engl J Med.* 258:2003–15; Nicholson, *et al.* **2009** *Obstet Gynecol.* 113:193–205; Balani, *et al.* **2009** *Diabet Med.*, 26:798–802; Evans, *et al.* **2005** *BMJ.* 330:1304–5; Libby, *et al.* **2009** *Diabetes Care.* 32:1620–5; Berstein **2010** *Future Oncol.* 6:1313-23; Jalving, *et al.* **2010** *Eur J Cancer.* 46:2369-80; Sanli, *et al.* **2010** *Int J Radiat Oncol Biol Phys.* 78:221-9; Papanas, *et al.* **2010** *Expert Opin Investig Drugs.* 19: 913-7; Li, *et al.* **2009** *Gastroenterology* 137:482–8; Rozengurt, *et al.* **2010** *Clin Cancer Res.* 16:2505-11; Vazquez-Martin, *et al.* **2010** *Cell Cycle.* 9(18); Vazquez-Martin, *et al.* **2010** *Curr Mol Med.* 10:674-91; Koch *Nat Rev* **2010** *Endocrinol.* 6:356; Wysocki, *et al.* **2010** *Expert Rev Mol Diagn.* 10:509-19; Bodmer, *et al.* **2010** *Diabetes Care.* 33:1304-8; Gonzalez-Angulo, *et al.* **2010** *Clin Cancer Res.* 16:1695-700).

[00105] The most common adverse effect of metformin is gastrointestinal upset, including diarrhea, cramps, nausea, vomiting and increased flatulence. Metformin is more commonly associated with gastrointestinal side effects than most other anti-diabetic drugs. The most serious potential side effect of metformin is lactic acidosis that is related to comorbid conditions such as

impaired liver or kidney function. Lactate uptake by the liver is diminished with metformin administration because lactate is a substrate for hepatic gluconeogenesis, a process which metformin inhibits. In healthy individuals, metformin and lactate can be cleared by other mechanisms (including uptake by the kidneys). When there is impaired renal function, clearance of metformin (and lactate) is reduced and the drug may accumulate, leading to lactic acidosis. (Bolen, *et al.* **2007** *Ann Intern Med.* 147:386–99; Khurana, et al. **2010** *Heart.* 96:99-102; Nicholson, *et al.* **2009** *Obstet Gynecol.* 113:193-205.)

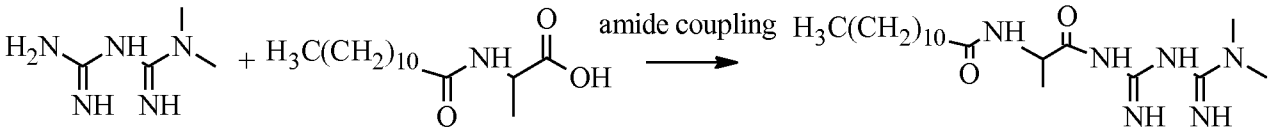
[00106] Metformin has an oral bioavailability of 33-55% under fasting conditions, and is absorbed slowly. Peak plasma concentrations (Cmax) are reached within one to three hours of taking immediate-release metformin and four to eight hours with extended-release formulations. Metformin is not metabolized by liver and cleared unchanged into urine. It is distributed and accumulated into red blood cells with an elimination of half-life of 17.6 hours. The concentration in blood plasma is too low to detect within 24 hours of a single oral dose. The average elimination half-life in plasma is 6.2 hours. The plasma protein binding of metformin is negligible with very high apparent volume of distribution (300–1000 L after a single dose). Steady state is usually reached in one or two days. Chemically, it is a hydrophilic base which exists at physiological pH as the cationic species (>99.9%). Consequently, its passive diffusion through cell membranes should be very limited. The oral absorption, hepatic uptake and renal excretion of metformin are mediated very largely by organic cation transporters (OCTs). The intersubject differences in the levels of expression of OCT1 and OCT3 in the liver are very large and may contribute more to the variations in the hepatic uptake and clinical effect of metformin, while OCT2 genotype was a significant predictor of renal clearance and the net secretion. The clearance of metformin can be affected by the co-dose of drugs which are cleared by tubular secretion, such as cimetidine and cephalexin. (Scheen, *et al.* **2011** *Clin Pharmacokinet.* 50:81-98; Robert, *et al.* **2003** *Diabetes Metab.* 29:279–83; Chen, *et al.* **2009** *Pharmacogenet Genomics.* 19:497-504; Song, *et al.* **2008** *Clin Pharmacol Ther.* 84:559-62; Somogyi, *et al.* **1987** *Br J Clin Pharmacol.*, 23:545–51; Jayasagar, *et al.* **2002** *Drug Metabol Drug Interact.* 19:41–8).

[00107] In summary, problems associated with metformin are: Moderate bioavailability, about 55±16%, due to limited absorption; Highly basic agent, protonated under physiological conditions; Slowly and incompletely adsorbed from the upper intestine after PO; Variable bioavailability; Absorption through para-cellular route, saturate, dose dependent absorption; It is projected that metformin is trapped inside the red blood cells, thus limited amount can reach liver, brain, and skeletal muscle, where AMPK is expressed. Require very high dose to see efficacy; Fast kidney excretion; Human healthy volunteers, half-life 1.52-4.5 h, 78.9-99.9% dose were excreted into urine through active renal tubular secretion, absorption was not complete

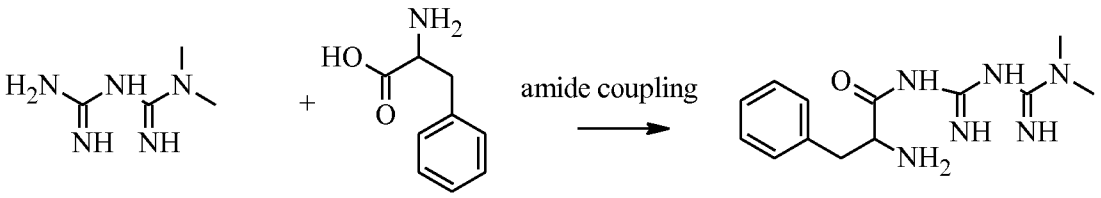
(0.5-1.5 g), about 20 to 30% go to feces due to saturable absorption process; Uncomfortable GI at effective dose of 0.5-2 g per day.

[00108] The strategies include prodrug to enhance intestinal absorption (bioavailability increased from 43% to 65%) and solve the issue of saturate and dose dependent absorption, and formulation technologies for extended release tablets to delay Tmax, improve systemic exposure and efficacy, and reduce dose frequency to once daily (Kristiina, *et al.* **2009** *J. Med. Chem.*, 52, 4142–4148; Schwartz, *et al.* **2008** *Expert Opin Drug Metab Toxicol.* 4:1235-43; Schwartz, *et al.* **2006** *Expert Opin Pharmacother.* 7:803-9).

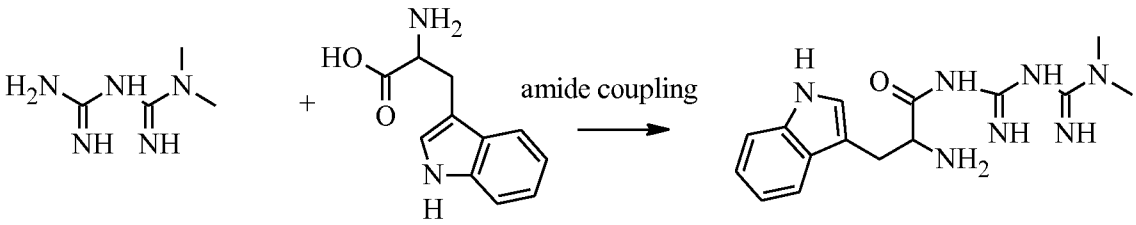
[00109] Based on the patent claims listed here, examples of modifications to metformin are documented with testing experiments and expected outcomes supported by literatures and knowledge of ADMET:



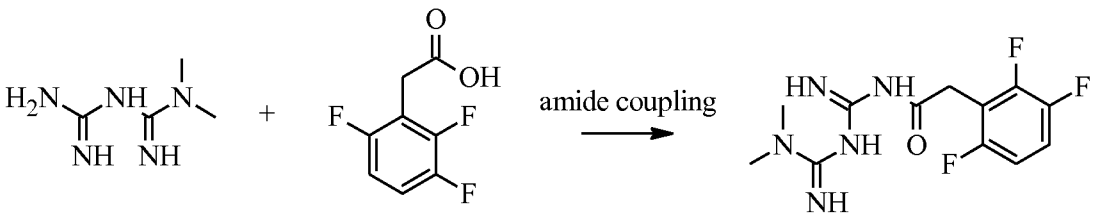
Metformin N-Lauroyl-L-alanine (a)



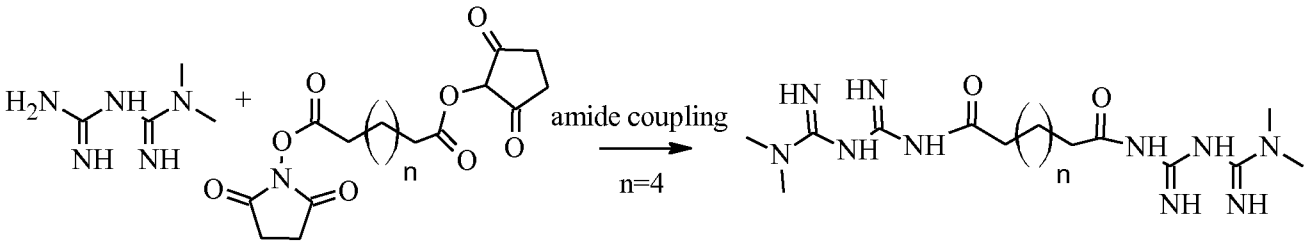
Metformin Phenylalanine (b)



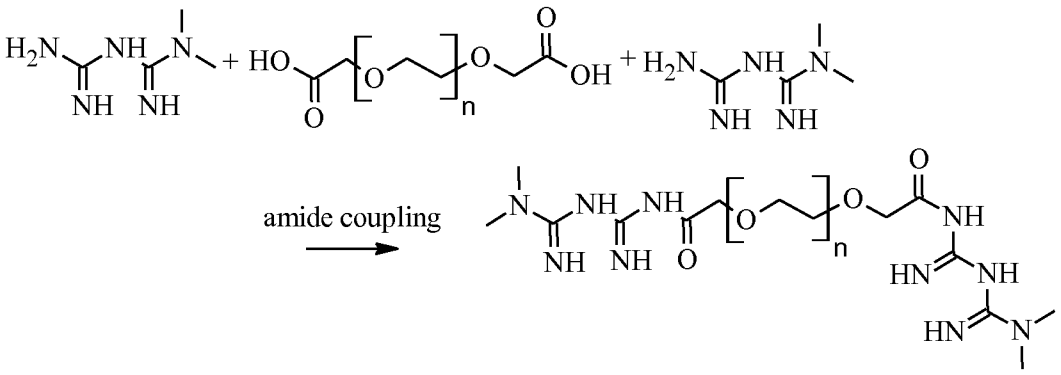
Metformin Tryptophan (c)



Metformin 2, 3, 6-Trifluorophenylacetic acid (d)



Metformin Disuccinimidyl suberate (d)



(e)

[00110] Examples of conjugated drugs (a) to (e) show possibilities to use small molecule functional groups, spacers and formulation excipients to modify metformin to solve ADMET issues. Prodrug (a) is to modify primary amine moiety in metformin through the conjugation with N-Lauroyl-L-alanine, which is a formulation excipient. The passive absorption of this conjugate will be improved due to increase in clogP (from -2.31 to 2.17). The prodrugs (b) and (c) are the conjugates of metformin and amino acids, which are pharmacologically safe. The passive absorption of these two conjugates will be improved due to the increase in clogP (from -2.31 to about -0.9). Prodrug (d) is a dimer linked through disuccinimidyl suberate. This dimer is more lipiphilic. Prodrug (e) is a dimer linked through monodisperse PEG. The size of the dimer can be screened through using different size of PEG. The screening criteria will be long systemic circulation with higher penetration in liver, brain and skeletal muscle. The PEG spacer will help the absorption process without the involvement of transporters. All the prodrugs, (a) to (e) are weak basic, and will not exist as cationic species at physiological pH values. The absorption process is expected to change due to both charge state change and MW increase to over 250. The problem of dose dependent and saturation absorption will be solved. Moreover, all the listed prodrugs (a) to (e) have larger molecular weight and are more lipophilic, which will greatly reduce the passive excretion through urine. The prodrugs will be expected to have different distribution profile without sequestration, thus, lower the efficacious dose, especially in the patients who have genetic polymorphism in the coding region of OCT1 (Wang, et al. **2002** *J. of Pharmacology and Experimental Therapeutics* 302: 510-515). Published data show that the novel prodrug strategy can improve the oral absorption and bioavailability of metformin.

(Kristiina, *et al.* **2009** *J. Med. Chem.* 52, 4142–4148). The daily doses would be reduced by approximately 30% assuming that clearances in rats will scale similarly to the human situation. Many of the unwanted gastrointestinal adverse effects could be ameliorated. The prodrug approach should be a safer option for more lipophilic analogs of metformin, since they release the active drug molecule, metformin, shortly after absorption. In conclusion, these novel prodrugs of metformin may improve the clinical usefulness of this very hydrophilic antidiabetic agent. The required daily doses could be reduced, which may also decrease the unwanted adverse effects associated with metformin therapy.

[00111] The conjugates (a) and (e) expand prodrug concept to formulation excipients, polymers, surfactants and lipids to illustrate the new concept/way to improve ADMET through both common principles and possible targeted delivery approach. Based on the size and structure of the excipient, the conjugate may become nano-particle, which greatly facilitates the absorption process, change drug distribution, excretion and safety profiles. As stated above, metformin can be used in the cancer therapy. Since the tumor physiology and structure are quite different from normal tissues, special design of the conjugate will help to target deliver the therapy to solid tumors, as illustrated in Velcade example. The conjugate (e) will stay neutral under the physiological conditions and more acidic environment surrounding tumors, which facilitates the penetration and accumulation of the conjugate. After metformin is released in the tumor, the very small size of the molecule will be beneficial to the penetration across the irregular tumor texture. Meanwhile, relatively basic environment in tumor will help the delivery of strong basic metformin within the irregular tumor structure.

[00112] All the examples of the conjugates (a) to (e) can be used for original marketed indication for type 2 diabetes. The expected results including proof of concept, absorption, distribution, bioavailability and safety can be demonstrated in the following experiments.

[00113] A: Distribution Coefficients in Octanol/Water, pKa of Metformin and Metformin Prodrugs, screen physical chemical property to help choose the right candidates.

[00114] B: In Vitro Bioconversion of Metformin Prodrugs. The bioactivation of the prodrugs can be evaluated in vitro by using human plasma, rat plasma, human liver and intestinal microsome, rat liver and intestinal microsome, and 20% rat liver homogenate at 37 °C, to make sure the release of metformin from the prodrugs.

[00115] C: In Vivo Bioconversion of Metformin Prodrugs with control of metformin. In vivo rat PK study in both intravenous and oral administration route, combine with tissue distribution study. Based on the results from this experiment (bioavailability, distribution in liver, brain and skeletal muscle), the candidates can be chosen.

[00116] D: The candidates can be tested in rat diabetes efficacy model with control of metformin. When the candidates have better efficacy compared to metformin with same and/or lower dose (best will be once daily), the candidates are forwarded into dose escalation/ safety experiment.

[00117] E: Safety, dose escalation in SD rats with metformin as control, exams includes hepatic biochemisty (ALT, AST), CK, renal biochemistry, hematology and urinalysis, vital signs, and electrocardiograms (ECGs), moribundity/mortality and clinical signs of toxicity. Other adverse findings including necrosis, inflammation of the parotid salivary gland, body weight loss need to be monitored as well. Metformin was also associated with evidence of minimal metabolic acidosis. Monitor serum lactate and beta-hydroxybutyric acid, serum bicarbonate and urine pH to see if prodrugs have lower metabolic acidosis potential. The no observable adverse effect levels (NOAEL) of the prodrugs are compared with control of metformin. The better ones (better efficacy and better safety profile) will be the clinical candidates (Sarkar, *et al.* **1996** *Pharmacol Res.* 33:1-4.)

[00118] Conjugates (a) to (e) are part of the examples listed in the patent application. All the claimed conjugation groups can be adopted into metformin through conjugation with primary amine group –NH2 to form prodrug/conjugate of therapeutic agents, and use experiments A to E to select the best conjugation groups to improve ADMET and efficacy for once daily dose. Transporter assays for OCT1, OCT2 and OCT3 can be another tier HTS for candidate selection. The prodrugs are better if they are the substrates of OCT1, OCT3, and not the substrates of OCT2.

Example 3. Improving Velcade

[00119] Nanosized materials facilitate the delivery of chemotherapy to solid tumors via enhanced permeation and retention effect with the potential of active targeting. To this point, only liposomal systems have been approved for general clinical use. Dendrimers have recently been explored as alternative systems for improved targeting compared to liposomal drug formulations (Saad M. *et al.***2008** *J. Controlled Release*130:107-114). Normally, Dendrimer is globular shape with multivalency. The monodisperse of dendrimers leads to batch-to-batch consistency, and reproducible pharmacodynamics and pharmacokinetics. Covalent attachment over noncovalent association is preferred due to higher drug payload with more controlled drug release via enzymatic or pH dependent cleavage.

[00120] Velcade (bortezomib) is a FDA approved treatment for multiple myeloma patients and mantle cell lymphoma patients who have received at least one prior therapy. It is a first-in-class proteasome inhibitor as cancer therapy, which targets 20S/26S proteasome. It can get into multiple pathways and mechanisms. There are two bottlenecks that limit the wide applications

of this novel therapy to cancer patients. One is the adverse events associated with these treatments including myelosuppression, gastrointestinal disturbance, and peripheral neuropathy. Treatment-emergent peripheral neuropathy (PN) is an important dose-limiting toxicity during treatment of multiple myeloma (MM) (Saeki, *et al.* **2010** *Nippon Rinsho* 68: 1818-22; Cavaletti, *et al.* **2010** *Leuk Lymphoma* 51:1178-87; Mateos **2010** *Cancer Treat Rev* 36 Suppl 2:S24-32). The other is the insufficient penetration and accumulation of this agent into the solid tumors, which leads no/limited efficacy for breast cancer, non-small cell lung cancer, pancreatic cancer, and etc. (Williamson, *et al.* **2009** *Mol Cancer Ther*, 8:3234-3243; Scagljotti, *et al.* **2010** *Lung Cancer* 68:420-6). However, the novel mechanism of action of Velcade makes it an attractive tool compound/therapeutic agent for cancers. It has the potential efficacies from the summation of various types of cancer targets.

[00121] The conjugation of polymer/copolymer with Velcade will benefit in two folds. One is to mask Velcade from deactivation due to cyclization at the physiological pH 7.4 (Lawrence J. Milo, et al *J. Medicinal Chemistry* 2011, 54, 4365-4377). The other is to mask the physical and chemical property of the drug. The penetration of the conjugate will not depend on the pKa and logP of the drug. Instead, through the design and selection of polymer/copolymer, the penetration and accumulation of the therapy in the solid tumor can be modulated and improved within the irregular tumor vasculature and pH gradient.

[00122] Here, suitable polymers, linkers and spacers have been designed to conjugate with Bortezomib or Velcade to target delivery the therapy to the solid tumors. The new conjugates can co-dose with complimentary therapeutic agents and/or modified agents through the current approach to further improve the efficacy and/or reduce the toxicity, for example, co-dose Velcade-dendrimer conjugate with metformin or prodrug of metformin in previous examples, but not limited to previous examples. The modified therapy is expected to increase in plasma half-life, diminish the uptake by reticuloendothelial system or macrophage or efflux transporters, increase tumor penetration and accumulation, modulate bio-distribution, reduce non-specific systemic toxicity, increase safety margin and overcome the challenges of combating cancer drug resistance.

[00123] The proof of concept (POC) of the efficacy and safety of Velcade in solid tumors through dendrimer-Velcade and dendronized linear polymer/copolymer-Velcade conjugates is summarized in the following:

[00124] The proposed dendrimers for testing include PEGylated triazine-G2 dendrimer, PEGylated Poly(L-lysine)-G5 dendrimer, poly-ester-G4 dendrimer, Poly(ester)-G4 dendronized linear poly(4-hydroxystyrene), PAMAM-G4-COOH, folate-PEG-PAMAM-G4, Polyol-G5-OH (polyol),and PAMAM-G(2.5, 3.5, 4.5). The choices of the above dendrimers is based on the

considerations of PK property for renal clearance (size cutoff), tumor physiology (pH targeting), cytotoxicity, penetration rate, shapes which fit for tumor microvasculature, nanoparticle size for long systemic circulation and freedom for tumor penetration. (Lee, *et al.* **2008** *Journal of Controlled Release* 132, 164-170; Steven, *et al.* **2009** *Nano Letters* Vol 9 No. 5, 1909-1915).

[00125] The proposed linear polymer/copolymer, which carries dendrimers or dendrons (listed above), to form tubular shape copolymers, can be poly(methacrylic acid), poly(4-vinylphenol), poly(vinyl alcohol), poly(4-styrenesulfonic acid), poly(methyl methacrylate-co-methacrylic acid), poly(N- isopropylacrylate-co-methacrylic acid), poly(2-hydroxypropyl methacrylate), poly(DL-ornithine), PLGA-PEG copolymer, Polyethylcyanoacrylate, Cellulose diacetate-g-poly(p-dioxanone) co-polymer, poly(lactic-co-glycolic acid) (PLGA), poly(sebacoyl diglyceride), poly(glycerol sebacate), polyamines, poly(L-lactic acid), poly(D-lactic acid), Poly-Glycerol-Dodecanoate, poly[(R)-3-hydroxybutyrate] (PHB), poly(epsilon-caprolactone) (PCL), azide-terminated poly(ethylene glycol), Polyhydroxyalkanoate, and poly(ethylene glycol)-peptide copolymer, with size n of 10, 30, 50, 100, 300, 500, 1000.

[00126] Preferably, the spacers for the conjugations between dendrimer and D, linear polymer/copolymer and dendrimer are amino acid, peptides, nucleotides, construct $X_1(CR'_m)_nY_1$ with n not bigger than 10, construct $X_2(CR'_m)_n R_s(CR'_m)_nY_2$ with n not bigger than 3, R_s a cyclic moiety of 5 or 6 membered ring or polycyclic structures with one or more of N, O, and S, aromatic and non-aromatic, saturated and unsaturated, with small substitute group, such as -CO_2H, -OH, -SO_2NH-, -SO_2NH_2, -SO_3H, -NH_2, =N-NH2, -CONH-, -$CONH_2$, -B(OH)-, -CHO, -CO-, -S-, -SH, -F, -Cl, -Br, and -I.

[00127] In vitro testing for dendrimer and dendronized linear polymer (P) biocompatibility and toxicity test for constructs' selection includes 72 hr MTT cytotoxicity for panel of cell lines, such as HEP G_2, 293 human embryonic kidney cells, B16F10, CCRF; red blood cell lysis and complement activation assay for haemolytic activity; cytokine release assay; CaCo-2 monolayer viability assay.

[00128] In vitro spacer selection experiments include process development for P-A; Purification using gel permeation chromatography (GPC), size exclusion (SEC) and reserve phase HPLC; The selection criteria of spacers is that no release under PBS buffer at pH 7.4, slow release in plasma and pH 6.4 of buffer environment, and fully release from linear polymer/copolymer and dendrimer in hepatocytes within 24 h. The spacers should be deuterium labeled if they can't be determined by mass spectrometry. P-A choices are narrowed down.

[00129] In vivo testing for selected P-A includes body distribution assay with time course to examine the short term fate (1 hr) and long term fate (1 month). The construct should be built using deuterated starting material. Based on the Velcade payload and MW of dendrimer, the

dose for this study is designed to be 10 times of maximum tolerated dose of Velcade at 0.8 mg/kg to see acute toxicity. This becomes metabolic fate experiment as well when collecting blood, urine, feces and major organs including heart, lung, liver, pancreas, spleen, stomach, intestine, brain, bone, and muscle at 0.5 hr, 1 hr, 4 hr, 24 hr, 72 hr, and 1 month, with three animals per time point. Basic safety observations for this acute dosing need to be recorded, such as diarrhea, blood urine, food intake, anything related to abnormal movement and/or change of reactions, etc. Fast and highly accumulated dendrimers with longer blood circulation time will be used for the conjugation experiments; immunogenicity tests for IgG, IgM induction, and cytokine induction. The dosing route can be oral or subcutaneous or IV.

[00130] Based on the constructs' bio-distribution-time course study, tumor type can be chosen for certain polymer-Velcade conjugate. If certain P-A is accumulated in specific tissues within short period of time (for example, within half an hour) with long blood circulation profile, it will have higher chances to overcome drug-efflux-pump-overexpression mediated multi-drug resistance (MDR) with excellent efficacy in vivo (Hu, *et al.* **2009** *Current Drug Metabolism* 10, 836-841).

[00131] Optimization of the Linkers between Construct and Velcade (Bio-liable versus Buffer Hydrolysis): Linkers between the construct and Velcade will affect the release rate either from pH-based hydrolysis or from enzymatic reactions. The selection criteria are that the linker with or without selected spacers will offer long systemic circulation of the conjugate with high tumor accumulation of free Velcade. Linker optimization experiments includes process development for A-Velcade; Purification using SEC and/or reserve phase HPLC; In vitro release study in PBS buffer (pH 7.4 blood and pH 6.4 cancer cell environment) and in plasma through determination of Velcade or A concentration. The selection criteria of linkers is that no release under PBS buffer at pH 7.4, slow release in plasma and pH 6.4 of buffer environment, and release fully to Velcade in hepatocytes within 24 h. When without spacers, direct conjugates of dendrimer or dendronized linear polymer with Velcade will be tested against claimed linkers. Process chemistry development with purification through GPC, SEC and reverse phased HPLC; Characterization using GPC-FTIR, LC-light scattering detector, LC-refractive index detector, LC-MALDI TOF, NMR and etc. to make sure the batch to batch reproducibility; Sterility through ultrafiltration and/or dialysis to remove potential bacterial lipopolysaccharide and cell forms of bacteria; The selection criteria of linkers is that no release under PBS buffer at pH 7.4, slow release in plasma and pH 6.4 of buffer environment, and release fully to Velcade in hepatocytes within 24 h. These selection criteria will help to make sure high accumulation of Velcade in tumor with long systemic circulation time.

[00132] Solid Tumor Efficacy Study: Based on the above two experiments, candidates of construct-Velcade conjugates will be dosed into selected solid tumor models up to six months to see efficacy, survival rate and if multi-drug resistance (MDR) has been overcome. The study includes a control group of Velcade at maximum tolerated dose of 0.8 mg/kg and current standard therapy for the corresponding cancer type. Based on the payload of Velcade on each construct, the corresponding dose of the conjugate to 0.8 mg/kg of free Velcade is given on a 2/week schedule to group of 10 animals for a period of at least 4 weeks, or until the humane end point was reached in which >50% of the mice in Velcade group have tumor over 10% of animal body weight. The conjugate will be considered as drug candidate when its efficacy is better than the current standard therapy. The tumor will be collected at the end of the study with the measurement of the concentration to rationalize the efficacy study results.

[00133] Expected outcomes from the above experiments in sequential order: Narrow down the dendrimer and dendronized linear polymers through knowing constructs' biocompatibility and toxicity; Narrow down spacer choices from the claims; Narrow down P-A construct choices; know the right tumor types for each construct, and rank the accumulation rate of the constructs with potential of combating of MDR; Narrow A-Velcade choices to generate P-A-Velcade for in vivo PK study; Narrow down linker selections for P-Velcade when without spacers, and generate P-Velcade for in vivo PK study.

[00134] In vivo rat PK study with tumor and plasma concentration measurement: Further narrow down the candidates for efficacy study.

[00135] Efficacy studies: expect to see if the candidates can sustain the therapeutic efficacy in established tumor models with the potential of evaluation of no re-occurrence within three to six months.

[00136] With POC in animal models, the efficacy is expected to translate into clinical with much greater safety margin, thus fulfill the goal of reduction of toxicity of Velcade and improve efficacy in solid tumors.

Incorporation by Reference

[00137] References and citations to other documents, such as patents, patent applications, patent publications, journals, books, papers, web contents, have been made in this disclosure. All such documents are hereby incorporated herein by reference in their entirety for all purposes.

Equivalents

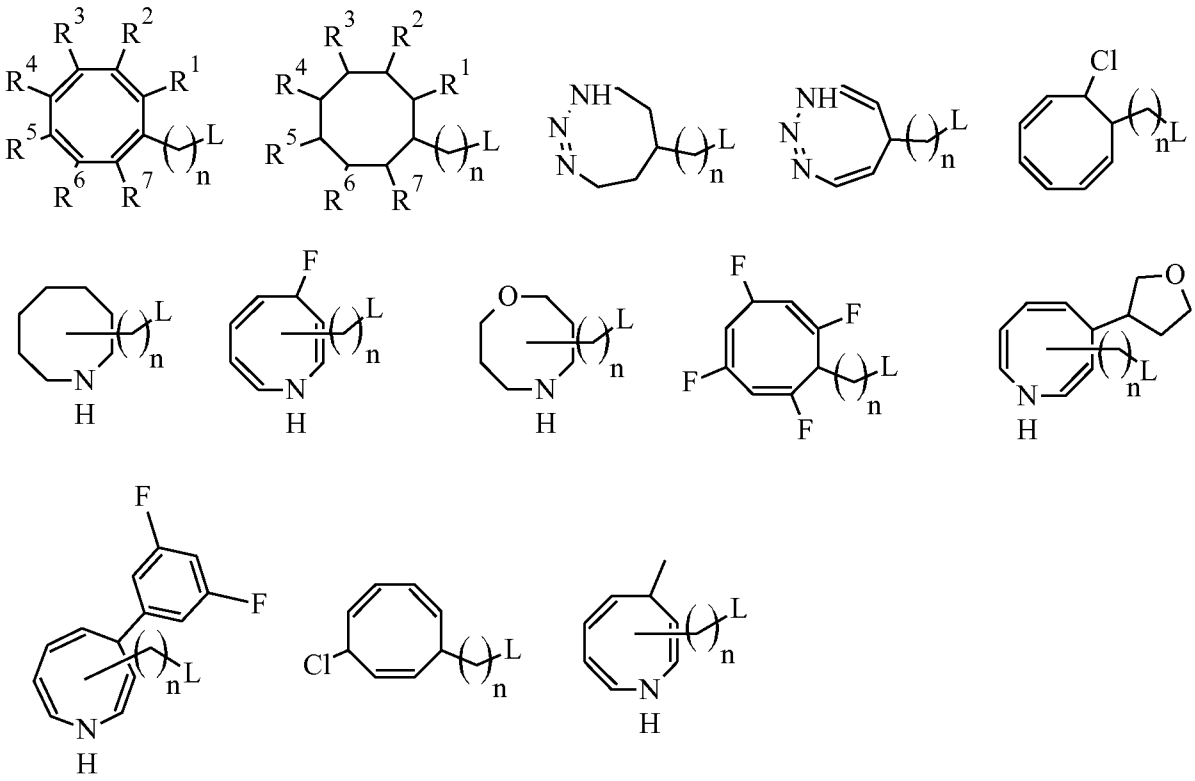
[00138] The representative examples are intended to help illustrate the invention, and are not intended to, nor should they be construed to, limit the scope of the invention. Indeed, various modifications of the invention and many further embodiments thereof, in addition to those shown and described herein, will become apparent to those skilled in the art from the full

contents of this document, including the examples and the references to the scientific and patent literature included herein. The examples contain important additional information, exemplification and guidance which can be adapted to the practice of this invention in its various embodiments and equivalents thereof.

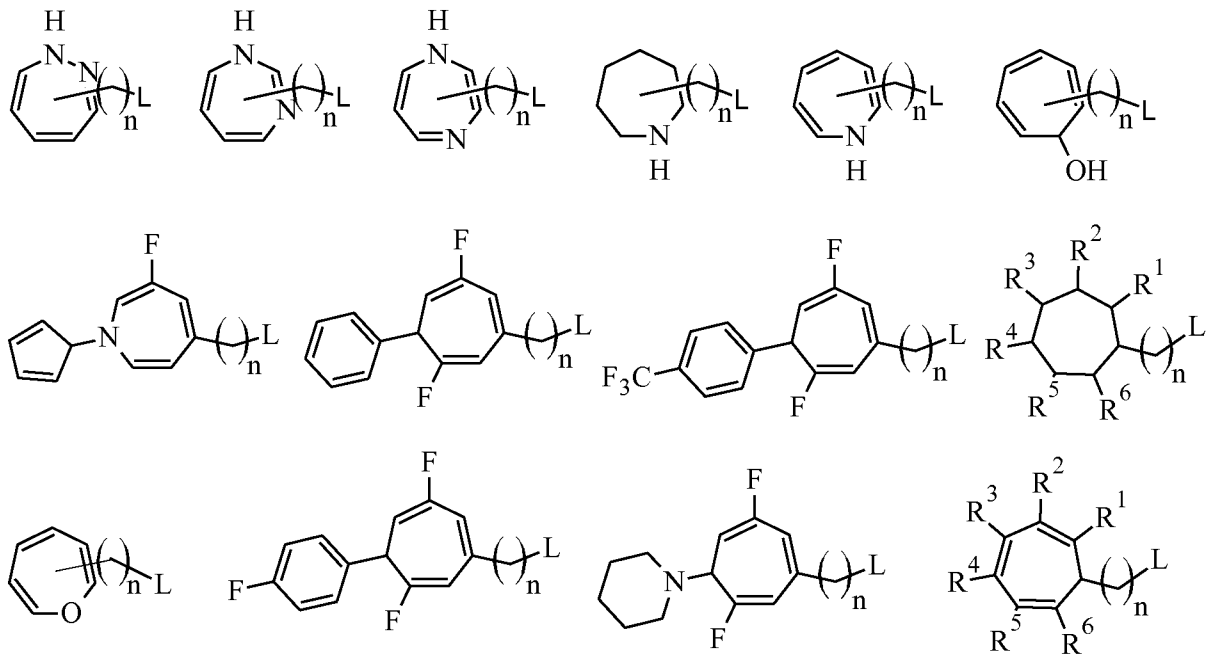
What is claimed is:

CLAIMS

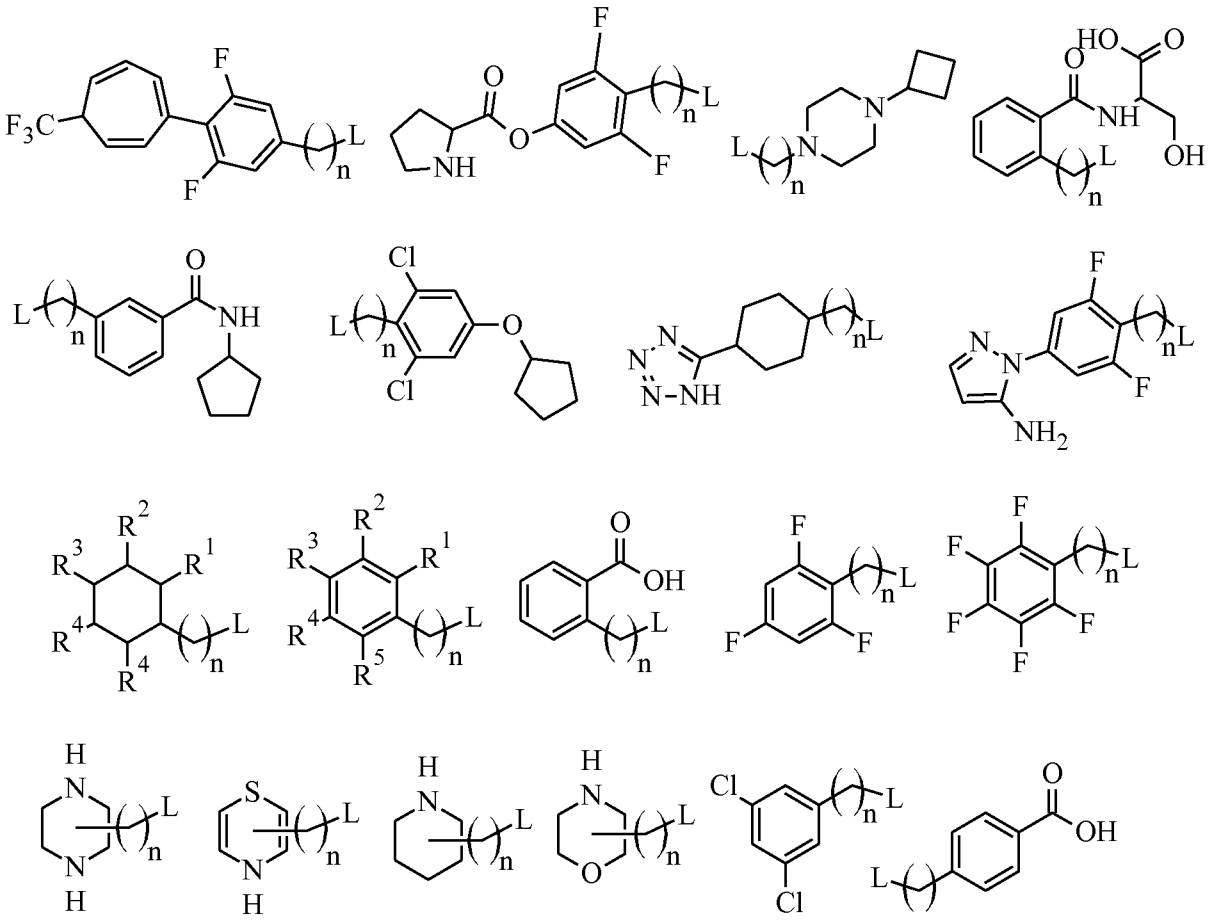
1. A method for improving one or more of a compound's ADMET properties, comprising covalently coupling the compound with one or more pendant groups, whereby the coupling resulting on one or more bones of –N-O-, -N-S-, -C-N-, -C-O-, -N-N-, -B-O-, -B-S-, -B-N- and -C-S-, wherein the one or more pendant groups comprises a cyclic moiety.
2. The method of Claim 1, wherein at least one of the one or more pendant groups is covalently coupled to the compound through one or more moieties selected from $-CO_2H$, -OH, $-SO_2NH-$, $-SO_2-$, -NH-, -CONH-, -B(OH)-, and –S-, and wherein the cyclic moiety comprises a 4-8 membered ring or fused ring structure.
3. The method of Claim 1, wherein the improved property is absorption.
4. The method of Claim 1, wherein the improved property is distribution.
5. The method of Claim 1, wherein the improved property is metabolism.
6. The method of Claim 1, wherein the improved property is excretion.
7. The method of Claim 1, wherein the improved property is toxicity.
8. The method of Claim 2, wherein one or more pendant groups comprises an 8-membered ring.
9. The method of Claim 2, wherein one or more the pendant groups comprises a 7-membered ring.
10. The method of Claim 2, wherein one or more the pendant groups comprises a 6-membered ring.
11. The method of Claim 2, wherein one or more the pendant groups comprises a 5-membered ring.
12. The method of Claim 2, wherein one or more the pendant groups comprises a 4-membered ring.
13. The method of Claim 2, wherein one or more the pendant groups comprises a fused ring.
14. The method of Claim 1, wherein the compound is a statin.
15. The method of Claim 1, wherein the statin is atorvastatin.
16. The method of Claim 1, wherein the compound is a derivative of atorvastatin.
17. The method of Claim 1, wherein the compound is metformin.
18. The method of Claim 1, wherein the compound is a derivative of metformin.
19. The method of Claim 1, wherein the compound is bortezomib.
20. The method of Claim 1, wherein at least one of the one or more pendant groups is a moiety or structural component selected from:



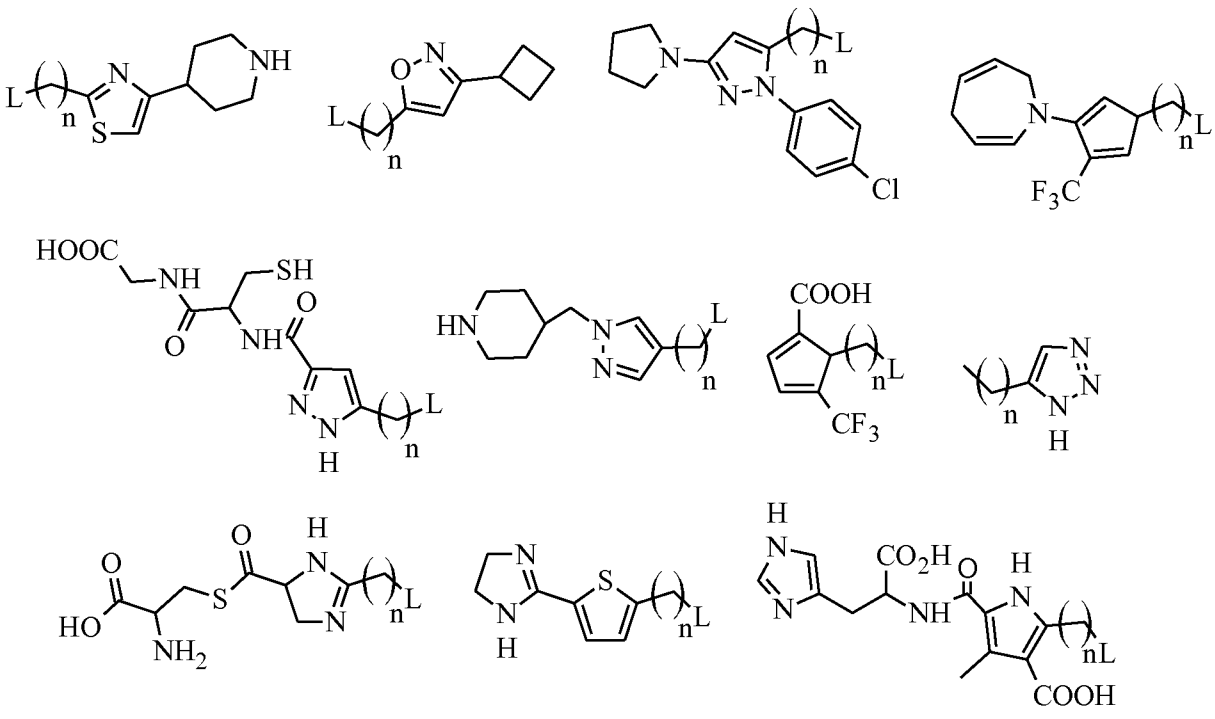
21. The method of Claim 1, wherein at least one of the one or more pendant groups is a moiety or structural component selected from:



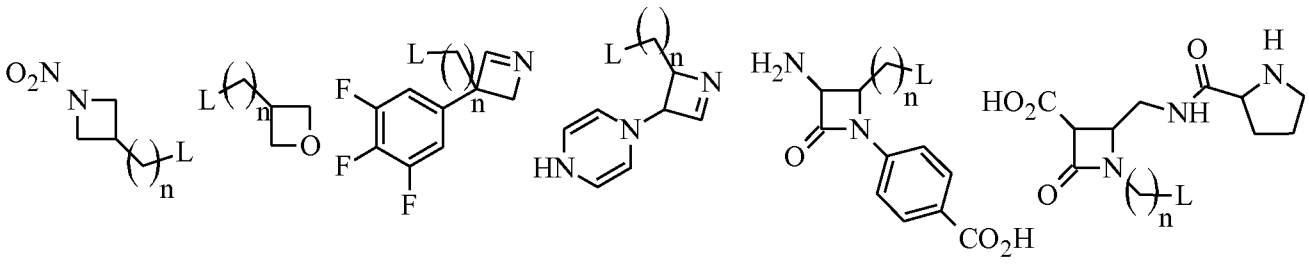
22. The method of Claim 1, wherein at least one of the one or more pendant groups is a moiety or structural component selected from:



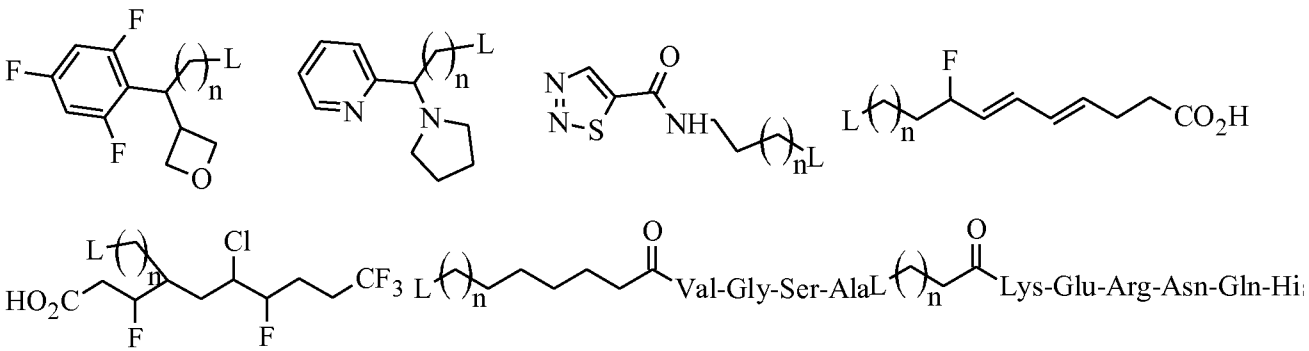
23. The method of Claim 1, wherein at least one of the one or more pendant groups is a moiety or structural component selected from:



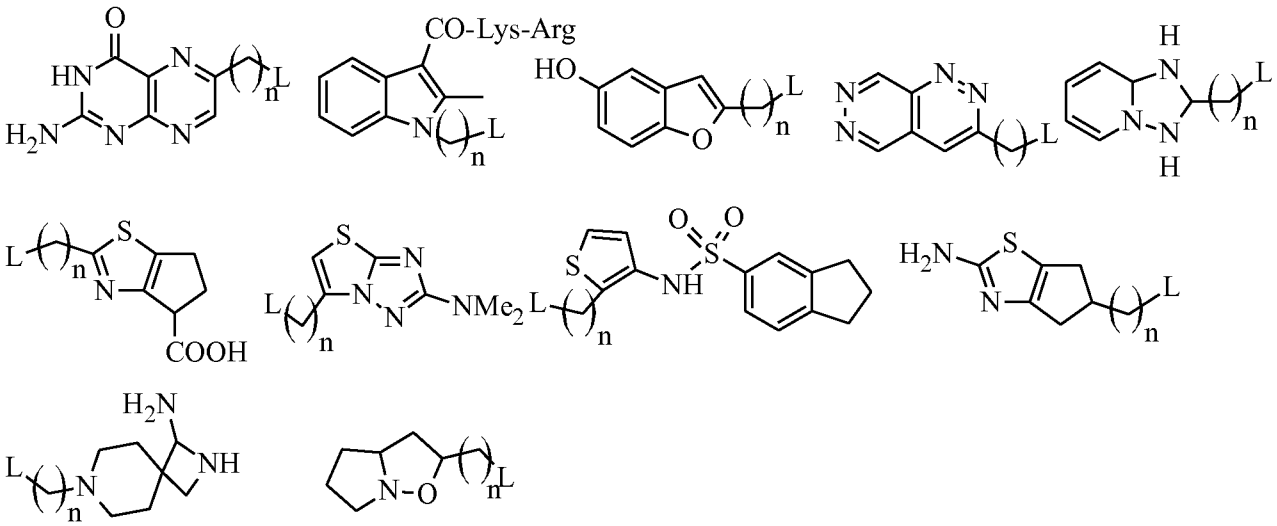
24. The method of Claim 1, wherein at least one of the one or more pendant groups is a moiety or structural component selected from:



25. The method of Claim 1, wherein at least one of the one or more pendant groups is a moiety or structural component selected from:



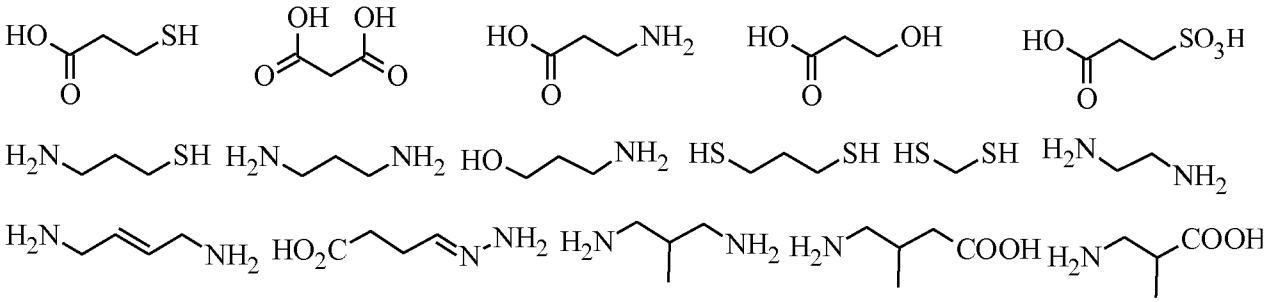
26. The method of Claim 1, wherein at least one of the one or more pendant groups is a moiety or structural component selected from:



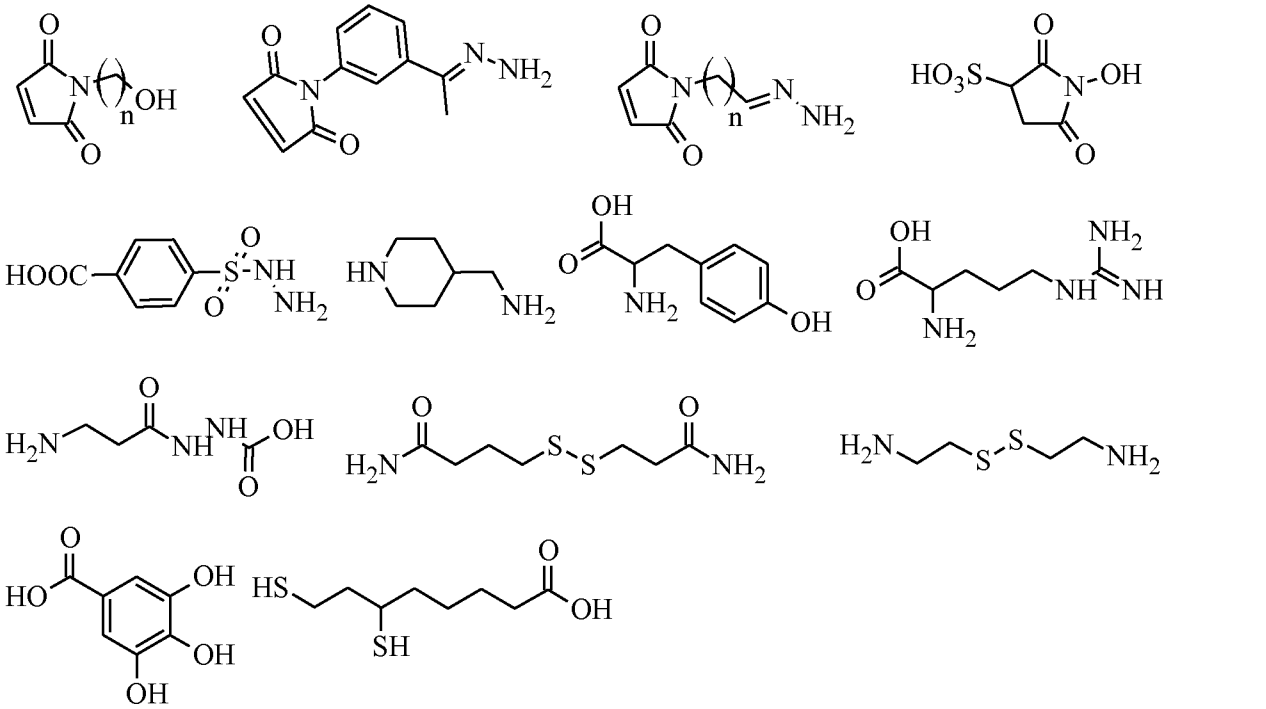
27. The method of Claim 1, wherein the agent is modified to be dimer or trimer with or without a spacer in between to form one or more bones of –N-N-, -O-O-, -S-S-, -B-O-B-, -B-S-B-, -B-N-B-, -N-S-N-, -C-O-C-, -C-S-C-, -C-N-C-, -N-O-N-, –N-O-, -N-S-, -C-N-, -C-O-, -B-O-, -B-S-, -B-N-, and -C-S-.

28. The method of Claim 1, wherein the compound is chemically coupled to the pendant group through one or more spacers.

29. The method of Claim 28, wherein the one or more spacers is selected from a moiety or structural component from:



30. The method of Claim 28, wherein the one or more spacers is selected from a moiety or structural component from:



31. The method of Claim 28, wherein the one or more spacers is selected from amino acids, peptides nucleotides and a moiety or structural component from: monodisperse PEG, as formula $X(CH_2CH_2O)_nCH_2CH_2Y$(n is an integer from 0 to 5000) with any combination of end groups of X and Y as linkers.

32. A improved chemical compound according to any method of Claims 1-31.

33. A pharmaceutical composition comprising a chemical compound obtained from any method of Claims 1-31 and a therapeutically acceptable excipient.