

(51) International Patent Classification:
C07F 9/38 (2006.01)(21) International Application Number:
PCT/US2015/015570(22) International Filing Date:
12 February 2015 (12.02.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/938,739 12 February 2014 (12.02.2014) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

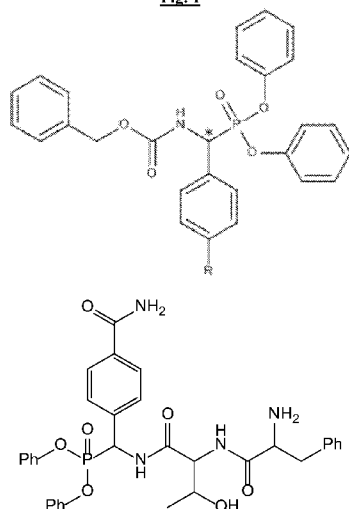
(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(54) Title: NOVEL AMINOALKYLPHOSPHONATE COMPOUNDS AND METHODS USING SAME

(57) Abstract: The present invention includes aminoalkylphosphonate compounds that are useful in preventing and/or treating prostate cancer in a male subject. The present invention further includes methods of preventing and/or treating prostate cancer in a male subject using the compounds of the invention.

Fig. 1

TITLE OF THE INVENTION

Novel Aminoalkylphosphonate Compounds and Methods Using Same

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Patent Application No. 61/938,739, filed February 12, 2014, which application is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

Prostate cancer develops in the prostate, a gland in the male reproductive system, and often affects men over the age of fifty. Prostate cancer is most common in the developed world, with increasing rates in the developing world. The American Cancer Society estimated that in 2014, over 233,000 new cases of prostate cancer would be diagnosed, and 29,480 deaths would occur due to this disease. Globally, it is the sixth leading cause of cancer-related death in men, but it is the first in the United Kingdom and the second in the U.S. However, many men with prostate cancer never develop symptoms, do not undergo therapy, and eventually die of other unrelated causes. Many factors, including genetics and diet, have been implicated in the development of prostate cancer.

Most prostate cancers are slow growing; but there are cases of aggressive prostate cancers. The cancer cells may metastasize from the prostate to other parts of the body, particularly the bones and lymph nodes. Prostate cancer may cause pain, difficulty in urinating, problems during sexual intercourse, or erectile dysfunction.

Chemotherapy has been widely used for prostate cancer treatments. Recent studies have focused on chemotherapy-induced apoptosis of tumor cells to inhibit tumor cell growth and promote cell death. Two important molecular targets for prostate cancer treatment include prostate tissue concentration of secreted growth factors, such as transforming growth factor type-P (TGF-P) and prostate-specific antigen (PSA). TGF-P regulates cell growth, differentiation, and development of a variety of functions. Another target is PSA, a serine protease that is known as gamma-seminoprotein or human glandular kallikrein-related peptidase-3 (KLK3). It is a chymotrypsin-like serine protease secreted from epithelial prostate tissue, where it is highly localized.

PSA is present in small quantities in the serum of men with healthy prostates, and is often elevated in individuals afflicted with prostate cancer or other prostate disorders.

PSA controls the growth of cancer metastasis and proliferation. Regulated by an androgen receptor-mediated transcription pathway, the primary role of PSA biologically is the liquefaction of semen via proteolysis of coagulating proteins fibronectin and semenogelin within the matrix. However, with elevated PSA levels, the protease cleaves insulin-like growth factor binding protein-3 (IGFBP-3), a modulator of mitogenic proteins insulin-like growth factors (IGF) I and II. The involvement of PSA with the IGF molecular system leads to the progression of prostate cancer, which can in turn metastasize into the patient's lymph nodes and bone, causing osteoblastic lesions via PSA-activation of latent transforming growth factor type-beta (TGF- β), and proteolysis of IGFBP-5.

PSA screening has been used to identify individuals with prostate cancer. However, PSA is an indicator of not only prostate cancer, but also prostatitis or benign prostatic hyperplasia. In fact, only 30% with high PSA have prostate cancer diagnosed after biopsy.

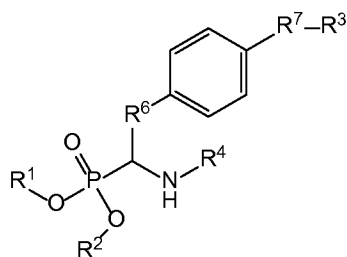
Although therapies targeting the androgen receptor, an upstream regulator of PSA, have been effective in treating prostate cancer, the disease state sometimes progresses and results in castrate-resistant prostate cancer. Alternative therapies should be pursued for prostate cancer treatment, especially in the scenario where androgen deprivation fails.

There is a need in the art for novel compounds useful for treating or preventing prostate cancer in a male subject. In one aspect, such compounds should inhibit the growth of prostate cancer cells and inhibit their dissemination to other organs and tissues in the body of the subject. In another aspect, such compounds should inhibit the release of growth factors that allow for the growth and dissemination of prostate cancer cells in the subject. The present invention addresses and meets these needs.

BRIEF SUMMARY OF THE INVENTION

The invention provides a compound of formula (I), or a salt or solvate thereof. The invention further provides a method of treating or preventing prostate cancer in a male subject in need thereof.

In certain embodiments, the compound of formula (I) is



(I), wherein in (I) R^1 and R^2 are independently selected from the

group consisting of H, C_1 - C_{10} alkyl, substituted C_1 - C_{10} alkyl, phenyl, and substituted phenyl;

R^3 is selected from the group consisting of H, F, Cl, Br, I, C_1 - C_{10} alkyl, substituted C_1 - C_{10}

5 alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, amino, substituted amino, -CN, -

OH, alkoxy, carbamoyl, -COOH, -CHO, alkoxy carbonyl, aminosulfonyl, alkylsulfonyl, and

phosphoryl; R^4 is selected from the group consisting of R^5 , a sugar, an oligosaccharide, an

amino acid and a peptide, wherein the sugar, oligosaccharide, amino acid or peptide is

optionally substituted; R^5 is selected from the group consisting of H, C_1 - C_{10} alkyl,

10 substituted C_1 - C_{10} alkyl, phenyl, substituted phenyl, acyl, alkoxy carbonyl, aminocarbonyl,

and Fmoc; R^6 and R^7 are independently selected from the group consisting of bond, $-(CH_2)_n$ -,

-O-, -O(C=O)-, -C(=O)-, -C(=O)O-, -OC(=O)O-, -NHC(=O)-, -C(=O)NH-, -C(=NH)NH-, -

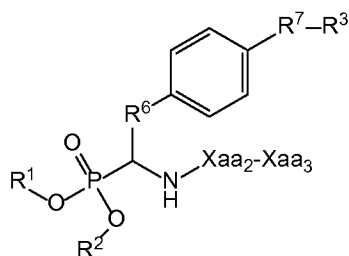
C(=NH₂)NH- and -NHC(=O)NH-; and, each occurrence of n is independently an integer

ranging from 1 to 10.

15 In certain embodiments, R^6 and R^7 are each a bond. In other embodiments, R^4

is an amino acid or a peptide. In yet other embodiments, the peptide comprises the dipeptide

Xaa₂-Xaa₃, wherein the C-terminus of Xaa₂ is covalently bound through an amide bond to the



NH group in (I) to provide (II):

(II).

In certain embodiments, Xaa₂ comprises an amino acid selected from the

20 group consisting of Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, His, Ser, Thr, Gln, Glu, Asn,

Asp, Lys, Arg, Met, Cys and cystine. In other embodiments, Xaa₃ comprises an amino acid

selected from the group consisting of Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, His, Ser,

Thr, Gln, Glu, Asn, Asp, Lys, Arg, Met, Cys and cysteine.

In certain embodiments, the N-terminus of Xaa₃ is derivatized with a group

25 selected from the group consisting of C_1 - C_6 alkyl (such as, but not limited to, methyl, ethyl,

n-propyl or isopropyl), acetyl, benzyloxycarbonyl, *tert*-butyloxycarbonyl, benzylsulfonyl, *para*-methylphenylsulfonyl, fluorenylmethyloxycarbonyl, pivaloyl, alloxycarbonyl, pentafluorophenoxycarbonyl, methoxycarbonyl, *para*-methoxybenzyloxycarbonyl, methoxyethoxymethoxycarbonyl, and trichloroethoxycarbonyl. In other embodiments, the

5 N-terminus of Xaa₃ is derivatized with two independently selected C₁-C₆ alkyl groups.

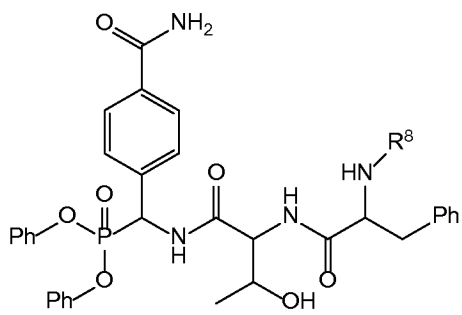
In certain embodiments, at least one of the N-H group of the peptidic groups (*i.e.*, the amide groups covalently linking Xaa₂ to Xaa₃, and Xaa₂ to the amino group alpha to the phosphonate group in (II)) is independently derivatized with a C₁-C₆ alkyl alkyl (such as, but not limited to, methyl, ethyl, *n*-propyl or isopropyl). In yet other embodiments, each one

10 of the N-H group of the peptidic groups is independently derivatized with a C₁-C₆ alkyl alkyl (such as, but not limited to, methyl, ethyl, *n*-propyl or isopropyl).

In certain embodiments, Xaa₂-Xaa₃ comprises at least one selected from the group consisting of Leu-Val, Ile-His, Tyr-Pro, Val-Tyr, Ala-Trp, Gly-Lys, Trp-Gly, Phe-Pro, Pro-Trp, Met-Pro, and Trp-Met. In other embodiments, Xaa₂-Xaa₃ comprises at least one

15 selected from the group consisting of Gly-Tyr, His-Pro, Gln-Met, Asn-Tyr, Ala-Tyr, Lys-Gly, Met-His, Gln-Val, Leu-Leu, Phe-Gly, Ser-Trp, Ser-Phe, Trp-Ala, Gly-Trp, Val-Phe, Phe-Asn, Phe-Tyr, Met-Asn, Ile-Val, Ile-Tyr, and Tyr-Tyr.

In certain embodiments, the compound is at least one selected from the group consisting of diphenyl-((2-(2-(N-R⁸)-amino-3-phenylpropanamido)-3-hydroxybutanamido)



20 (4-carbamoyl phenyl) methyl)phosphonate:

wherein R⁸ is H or a protecting selected from the group consisting of acetyl, benzyloxycarbonyl, *tert*-butyloxycarbonyl, benzylsulfonyl, *para*-methylphenylsulfonyl, fluorenylmethyloxycarbonyl, pivaloyl, alloxycarbonyl, pentafluorophenoxycarbonyl, methoxycarbonyl, *para*-methoxybenzyloxycarbonyl, methoxyethoxymethoxycarbonyl, and

25 trichloroethoxycarbonyl.

In certain embodiments, the method comprises administering to the subject a therapeutically effective amount of a compound of formula (I) or a salt or solvate thereof. In other embodiments, PSA activity in the subject is inhibited. In yet other embodiments, the growth of at least one prostate cancer cell in the subject is inhibited. In yet other

embodiments, the release of at least one growth factor selected from the group consisting of IGF-I, IGFBP-2 and IGFBP-3 is inhibited in the subject. In yet other embodiments, the subject is a mammal. In yet other embodiments, the mammal is human.

BRIEF DESCRIPTION OF THE DRAWINGS

For the purpose of illustrating the invention, there are depicted in the drawings certain embodiments of the invention. However, the invention is not limited to the precise arrangements and instrumentalities of the embodiments depicted in the drawings.

Fig. 1 illustrates (top) a general structure of diphenyl [N-(benzyloxycarbonylamino)phenylmethyl]phosphonate analogs synthesized, with varying R-groups, wherein the asterisk indicates the stereocenter, and (bottom) a non-limiting schematic illustration of a compound of the invention.

Fig. 2 illustrates a proposed mechanistic schematic of an α -aminoalkylphosphonate ester inhibitor interaction with serine proteases.

Fig. 3 is a visual representation of obtaining noncovalent binding structures of inhibitors from the Michaelis complex modeling results.

Figs. 4A-4B illustrate a covalent binding model of diphenyl [N-benzyloxycarbonylamino(4-carbamoylphenyl)methyl] phosphonate in PSA using the flexible side chain method in AutoDock4. Fig. 4A: S-Enantiomer of the inhibitor with the N-blocking group in the P2 pocket of the protein, in proximity to TYR₉₄. Fig. 4B: R-Enantiomer model showing nearly the same orientation except with the N-blocking group in the upper groove region near TRP₂₁₅.

Figs. 5A-5B illustrate noncovalent docking of diphenyl [N-benzyloxycarbonylamino(4-carbamoylphenyl)methyl] phosphonate and polar hydrogen contacts (yellow or light gray) of selected residues (red or dark gray) within P1 pocket of PSA. Amide proton of THR₁₉₀ and hydroxyl proton of SER₂₂₇ display hydrogen-bonding contacts with the C=O of the amide functional group of the inhibitor, while the carbonyl of SER₂₁₇ displays a hydrogen-bonding contact with the amide protons of the inhibitor for both the (Fig. 5A) R- and (Fig. 5B) S-enantiomer.

Fig. 6A is a graph illustrating RMSD plots of PSA-S11 complex and PSA alone. Fig. 6B illustrates a trajectory overlay of S11 over the 5 ns simulation.

Fig. 7A is a graph illustrating root-mean squared fluctuations (RMSF) of the 237 residues of PSA with the CKL residues shown in the inset. Figs. 7B-7C: RMSF representations of the protein (Fig. 7B) with and (Fig. 7C) without S11 bound. Color scale:

>0.30 nm red; >0.25 nm yellow; >0.20 nm green; >0.15 nm cyan; <0.15 nm blue. CKL side chains are displayed only. The ligand is represented as blue spheres.

Figs. 8A-8B are a set of graphs illustrating kinetic assay results of PSA amidolytic activity of chromogenic substrate S-2586 in the presence of varying

5 concentrations of inhibitor **11** from 75 nM to 1 μ M.

Figs. 9A-9B are a set of graphs illustrating a plot of log IC₅₀ vs docking score obtained from AutoDock molecular docking simulations using (Fig. 9A) a single atom constraint and conventional search protocols and (Fig. 9B) the covalent tether constraint described elsewhere herein.

10 Figs. 10A-10B are a series of images illustrating structure alignment between human PSA and trypsin, files obtained from the protein databank. Fig. 10A: Protein alignments of both proteins with RMSD < 1 Å for backbone atoms, with the ligands shown as spheres. Fig. 10B: Ligand binding structure from the 1MAX trypsin file and from docking studies. Trypsin and its associated ligand are shown in green, PSA and its corresponding
15 ligand is shown in cyan.

DETAILED DESCRIPTION OF THE INVENTION

The invention relates in part to the unexpected discovery that the aminoalkylphosphonate derivatives of the invention are useful in treating or preventing
20 prostate cancer in a male subject. In certain embodiments, the subject is a mammal. In other embodiments, the mammal is a human.

In one aspect, the compounds of the invention bind to and inhibit PSA in the subject. In another aspect, the compounds of the invention inhibit the growth of at least one prostate cancer cell and/or inhibit its dissemination to other organs and tissues in the body of
25 the subject. In yet another aspect, the compounds of the invention inhibit the release of a growth factor, such as but not limited to IGF-I, IGFBP-2 and IGFBP-3, in the subject.

Definitions

As used herein, each of the following terms has the meaning associated with it
30 in this section.

Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Generally, the nomenclature used herein and the laboratory

procedures in animal pharmacology, biochemistry, pharmaceutical science, and organic chemistry are those well-known and commonly employed in the art.

As used herein, the term “4PL” refers to 4-point log.

As used herein, the articles “a” and “an” refer to one or to more than one (*i.e.* to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

As used herein, the term “about” is understood by persons of ordinary skill in the art and varies to some extent on the context in which it is used. As used herein when referring to a measurable value such as an amount, a temporal duration, and the like, the term “about” is meant to encompass variations of $\pm 20\%$ or $\pm 10\%$, more preferably $\pm 5\%$, even more preferably $\pm 1\%$, and still more preferably $\pm 0.1\%$ from the specified value, as such variations are appropriate to perform the disclosed methods.

As used herein, the term “CI” refers to chemical ionization.

As used herein, the term “CKL” refers to classic kallikrein loop.

As used herein, a “disease” is a state of health of a subject wherein the subject cannot maintain homeostasis, and wherein if the disease is not ameliorated then the subject's health continues to deteriorate.

As used herein, a “disorder” in a subject is a state of health in which the subject is able to maintain homeostasis, but in which the subject's state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the subject's state of health.

As used herein, the term “DMSO” refers to dimethyl sulfoxide.

As used herein, the term “HRMS” refers to high-resolution mass spectrometry.

As used herein, the term “IC₅₀” refers to half-maximal inhibitory concentration.

As used herein, the term “IGF-I/II” refers to insulin-like growth factor type I/II.

As used herein, the term “IGFBP-3” refers to insulin-like growth factor binding protein type 3.

As used herein, the term “KLK3” refers to kallikrein-related peptidase-3.

As used herein, the term “LGA” refers to Lamarckian genetic algorithm.

As used herein, the term “MD” refers to molecular dynamics.

As used herein, the term “NMR” refers to nuclear magnetic resonance.

As used herein, the term “PCa” refers to prostate cancer.

As used herein, the term “pharmaceutical composition” or “composition” refers to a mixture of at least one compound useful within the invention with a pharmaceutically acceptable carrier. The pharmaceutical composition facilitates administration of the compound to a subject.

As used herein, the term “pharmaceutically acceptable” refers to a material, such as a carrier or diluent, which does not abrogate the biological activity or properties of the compound useful within the invention, and is relatively non-toxic, *i.e.*, the material may be administered to a subject without causing undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained.

As used herein, the term “pharmaceutically acceptable carrier” means a pharmaceutically acceptable material, composition or carrier, such as a liquid or solid filler, stabilizer, dispersing agent, suspending agent, diluent, excipient, thickening agent, solvent or encapsulating material, involved in carrying or transporting a compound useful within the invention within or to the subject such that it may perform its intended function. Typically, such constructs are carried or transported from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be “acceptable” in the sense of being compatible with the other ingredients of the formulation, including the compound useful within the invention, and not injurious to the subject. Some examples of materials that may serve as pharmaceutically acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; surface active agents; alginic acid; pyrogen-free water; isotonic saline; Ringer’s solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations. As used herein, “pharmaceutically acceptable carrier” also includes any and all coatings, antibacterial and antifungal agents, and absorption delaying agents, and the like that are compatible with the activity of the compound useful within the invention, and are physiologically acceptable to the subject. Supplementary active compounds may also be incorporated into the compositions. The “pharmaceutically acceptable carrier” may further include a pharmaceutically acceptable salt of the compound useful within the invention.

Other additional ingredients that may be included in the pharmaceutical compositions used in the practice of the invention are known in the art and described, for example in Remington's Pharmaceutical Sciences (Genaro, Ed., Mack Publishing Co., 1985, Easton, PA), which is incorporated herein by reference.

5 As used herein, the language “pharmaceutically acceptable salt” refers to a salt of the administered compound prepared from pharmaceutically acceptable non-toxic acids and bases, including inorganic acids, inorganic bases, organic acids, inorganic bases, solvates, hydrates, and clathrates thereof.

 As used herein, a “pharmaceutically effective amount,” “therapeutically effective amount” or “effective amount” of a compound is that amount of compound that is sufficient to provide a beneficial effect to the subject to which the compound is administered.

 As used herein, the term “PME” refers to Particle Mesh Ewald.

 The term “prevent,” “preventing” or “prevention,” as used herein, means avoiding or delaying the onset of symptoms associated with a disease or condition in a subject that has not developed such symptoms at the time the administering of an agent or compound commences. Disease, condition and disorder are used interchangeably herein.

 As used herein, the term “PSA” refers to prostate-specific antigen.

 As used herein, the term “RMSD” refers to root-mean squared deviation.

 As used herein, the term “RMSF” refers to root-mean squared fluctuation.

20 As used herein, a “subject” may be a human or non-human mammal. Non-human mammals include, for example, livestock and pets, such as ovine, bovine, porcine, canine, feline and murine mammals. In certain embodiments, the subject is human.

 As used herein, the term “TGF-P/β” refers to transforming growth factor type P/beta.

25 The term “treat,” “treating” or “treatment,” as used herein, means reducing the frequency or severity with which symptoms of a disease or condition are experienced by a subject by virtue of administering an agent or compound to the subject.

 As used herein, the term “VMD” refers to visual molecular dynamics.

 As used herein, the term “VS” refers to virtual screen.

30 As used herein, the term “alkyl,” by itself or as part of another substituent means, unless otherwise stated, a straight or branched chain hydrocarbon having the number of carbon atoms designated (*i.e.*, C₁-C₁₀ means one to ten carbon atoms) and includes straight, branched chain, or cyclic substituent groups. Examples include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, neopentyl, hexyl, and cyclopropylmethyl.

Most preferred is (C₁-C₆)alkyl, such as, but not limited to, ethyl, methyl, isopropyl, isobutyl, n-pentyl, n-hexyl and cyclopropylmethyl.

As used herein, the term “cycloalkyl,” by itself or as part of another substituent means, unless otherwise stated, a cyclic chain hydrocarbon having the number of carbon atoms designated (*i.e.*, C₃-C₆ means a cyclic group comprising a ring group consisting of three to six carbon atoms) and includes straight, branched chain or cyclic substituent groups. Examples include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl. Most preferred is (C₃-C₆)cycloalkyl, such as, but not limited to, cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

As used herein, the term “substituted alkyl,” “substituted cycloalkyl,” “substituted alkenyl” or “substituted alkynyl” means alkyl, cycloalkyl, alkenyl or alkynyl, as defined above, substituted by one, two or three substituents selected from the group consisting of halogen, -OH, alkoxy, tetrahydro-2-H-pyran-2-yl, -NH₂, -N(CH₃)₂, (1-methylimidazol-2-yl), pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, -C(=O)OH, trifluoromethyl, -C≡N, -C(=O)O(C₁-C₄)alkyl, -C(=O)NH₂, -C(=O)NH(C₁-C₄)alkyl, -C(=O)N((C₁-C₄)alkyl)₂, -SO₂NH₂, -C(=NH)NH₂, and -NO₂, preferably containing one or two substituents selected from halogen, -OH, alkoxy, -NH₂, trifluoromethyl, -N(CH₃)₂, and -C(=O)OH, more preferably selected from halogen, alkoxy and -OH. Examples of substituted alkyls include, but are not limited to, 2,2-difluoropropyl, 2-carboxycyclopentyl and 3-chloropropyl.

As used herein, the term “alkoxy” employed alone or in combination with other terms means, unless otherwise stated, an alkyl group having the designated number of carbon atoms, as defined above, connected to the rest of the molecule via an oxygen atom, such as, for example, methoxy, ethoxy, 1-propoxy, 2-propoxy (isopropoxy) and the higher homologs and isomers. Preferred are (C₁-C₃)alkoxy, such as, but not limited to, ethoxy and methoxy.

As used herein, the term “halo” or “halogen” alone or as part of another substituent means, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom, preferably, fluorine, chlorine, or bromine, more preferably, fluorine or chlorine.

As used herein, the term “aromatic” refers to a carbocycle or heterocycle with one or more polyunsaturated rings and having aromatic character, *i.e.* having (4n+2) delocalized π (pi) electrons, where n is an integer.

As used herein, the term “aryl,” employed alone or in combination with other terms, means, unless otherwise stated, a carbocyclic aromatic system containing one or more

rings (typically one, two or three rings) wherein such rings may be attached together in a pendent manner, such as a biphenyl, or may be fused, such as naphthalene. Examples include phenyl, anthracyl, and naphthyl. Preferred are phenyl and naphthyl, most preferred is phenyl.

As used herein, the term “aryl-(C₁-C₃)alkyl” means a functional group

5 wherein a one-to-three-carbon alkylene chain is attached to an aryl group, *e.g.*, -CH₂CH₂-phenyl or -CH₂-phenyl (benzyl). Preferred is aryl-CH₂- and aryl-CH(CH₃)-. The term “substituted aryl-(C₁-C₃)alkyl” means an aryl-(C₁-C₃)alkyl functional group in which the aryl group is substituted. Preferred is substituted aryl(CH₂)-. Similarly, the term “heteroaryl-(C₁-C₃)alkyl” means a functional group wherein a one to three carbon alkylene chain is attached
10 to a heteroaryl group, *e.g.*, -CH₂CH₂-pyridyl. Preferred is heteroaryl-(CH₂)-. The term “substituted heteroaryl-(C₁-C₃)alkyl” means a heteroaryl-(C₁-C₃)alkyl functional group in which the heteroaryl group is substituted. Preferred is substituted heteroaryl-(CH₂)-.

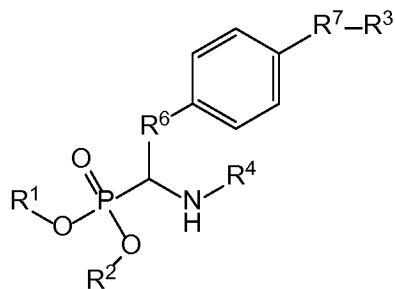
As used herein, the term “substituted” means that an atom or group of atoms has replaced hydrogen as the substituent attached to another group.

15 For a cyclic group, the term “substituted” as applied to the ring(s) of these groups refers to any level of substitution, namely mono-, di-, tri-, tetra-, or penta-substitution, where such substitution is permitted. The substituents are independently selected, and substitution may be at any chemically accessible position. In certain embodiments, the substituents vary in number between one and four. In other embodiments, the substituents
20 vary in number between one and three. In yet other embodiments, the substituents vary in number between one and two. In yet other embodiments, the substituents are independently selected from the group consisting of C₁₋₆ alkyl, -OH, C₁₋₆ alkoxy, halo, amino, acetamido and nitro. As used herein, where a substituent is an alkyl or alkoxy group, the carbon chain may be branched, straight or cyclic, with straight being preferred.

25 Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values
30 within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

Compounds

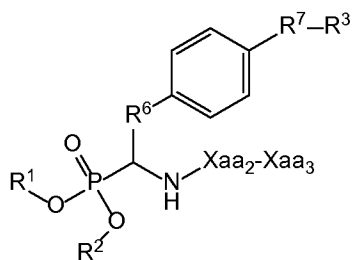
The invention includes a compound of formula (I), or a salt or solvate thereof:



- (I), wherein in (I): R^1 and R^2 are independently selected from the group consisting of H, C_1 - C_{10} alkyl, substituted C_1 - C_{10} alkyl, phenyl, and substituted phenyl; R^3 is selected from the group consisting of H, F, Cl, Br, I, C_1 - C_{10} alkyl, substituted C_1 - C_{10} alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, amino, substituted amino, -CN, -OH, alkoxy, carbamoyl, -COOH, -CHO, alkoxy carbonyl, aminosulfonyl, alkylsulfonyl, and phosphoryl; R^4 is selected from the group consisting of R^5 , a sugar, an oligosaccharide, an amino acid and a peptide, wherein the sugar, oligosaccharide, amino acid or peptide is optionally substituted; R^5 is selected from the group consisting of H, C_1 - C_{10} alkyl, substituted C_1 - C_{10} alkyl, phenyl, substituted phenyl, acyl, alkoxy carbonyl, aminocarbonyl, and Fmoc; R^6 and R^7 are independently selected from the group consisting of bond, $-(CH_2)_n$, -O-, $-O(C=O)-$, $-C(=O)-$, $-C(=O)O-$, $-OC(=O)O-$, $-NHC(=O)-$, $-C(=O)NH-$, $-C(=NH)NH-$, $-C(=NH_2)NH-$ and $-NHC(=O)NH-$; and, each occurrence of n is independently an integer ranging from 1 to 10.

In certain embodiments, the amino acid or peptide comprises a D- or L-amino acid. In other embodiments, the amino acid or peptide comprises an amino acid selected from the group consisting of Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, His, Ser, Thr, Gln, Glu, Asn, Asp, Lys, Arg, Met, Cys and cystine.

In certain embodiments, R^4 is an amino acid or a peptide. In other embodiments, the peptide comprises the dipeptide Xaa_2 - Xaa_3 , wherein the C-terminus of Xaa_2 is covalently bound through an amide bond to the NH group in (I) to provide (II):



(II).

In certain embodiments, Xaa_2 comprises a D- or L-amino acid. In other embodiments, Xaa_2 comprises an amino acid selected from the group consisting of Gly, Ala,

Val, Leu, Ile, Pro, Phe, Tyr, Trp, His, Ser, Thr, Gln, Glu, Asn, Asp, Lys, Arg, Met, Cys and cystine.

In certain embodiments, Xaa₃ comprises a D- or L-amino acid. In other embodiments, Xaa₃ comprises an amino acid selected from the group consisting of Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, His, Ser, Thr, Gln, Glu, Asn, Asp, Lys, Arg, Met, Cys and cystine.

In certain embodiments, the N-terminus of Xaa₃ is derivatized with a group selected from the group consisting of C₁-C₆ alkyl, acetyl [-C(=O)CH₃], benzyloxycarbonyl [-C(=O)OCH₂Ph], *tert*-butyloxycarbonyl [-C(=O)OC(CH₃)₃], benzylsulfonyl [-SO₂CH₂Ph], *para*-methylphenylsulfonyl [-SO₂(4-CH₃Ph)], fluorenylmethyloxycarbonyl [Fmoc], pivaloyl [-C(=O)C(CH₃)₃], alloxycarbonyl, pentafluorophenoxycarbonyl, methoxycarbonyl, *para*-methoxybenzyloxycarbonyl, methoxyethoxymethoxycarbonyl, and trichloroethoxycarbonyl. In other embodiments, the N-terminus of Xaa₃ is derivatized with two independently selected C₁-C₆ alkyl groups.

In certain embodiments, at least one of the N-H group of the peptidic groups (*i.e.*, the amide groups covalently linking Xaa₂ to Xaa₃, and Xaa₂ to the amino group alpha to the phosphonate group in (II)) is independently derivatized with a C₁-C₆ alkyl alkyl (such as, but not limited to, methyl, ethyl, *n*-propyl or isopropyl). In yet other embodiments, each one of the N-H group of the peptidic groups is independently derivatized with a C₁-C₆ alkyl alkyl (such as, but not limited to, methyl, ethyl, *n*-propyl or isopropyl).

In certain embodiments, Xaa₂-Xaa₃ comprises at least one selected from the group consisting of Leu-Val, Ile-His, Tyr-Pro, Val-Tyr, Ala-Trp, Gly-Lys, Trp-Gly, Phe-Pro, Pro-Trp, Met-Pro, and Trp-Met. In other embodiments, Xaa₂ comprises at least one selected from the group consisting of Leu, Ile, Tyr, Val, Ala, Gly, Trp, Phe, Pro, and Met. In yet other embodiments, Xaa₃ comprises at least one selected from the group consisting of Val, His, Pro, Tyr, Trp, Lys, Gly, and Trp.

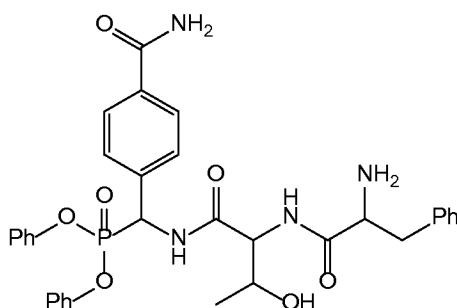
In certain embodiments, Xaa₂-Xaa₃ comprises at least one selected from the group consisting Gly-Tyr, His-Pro, Gln-Met, Asn-Tyr, Ala-Tyr, Lys-Gly, Met-His, Gln-Val, Leu-Leu, Phe-Gly, Ser-Trp, Ser-Phe, Trp-Ala, Gly-Trp, Val-Phe, Phe-Asn, Phe-Tyr, Met-Asn, Ile-Val, Ile-Tyr, and Tyr-Tyr.

In certain embodiments, Xaa₂-Xaa₃ comprises at least one selected from the group consisting of Gly-Ile, Phe-Ala, Glu-Arg, Val-Asn, Gln-Gly, Phe-Val, Thr-Pro, Phe-Leu, Tyr-Ile, Ile-Ala, Ile-Gly, Trp-Pro, Ile-Arg, Ser-Pro, Trp-His, Val-Ala, Val-Lys, Trp-Tyr, Tyr-Ala, Tyr-Asn, Trp-Phe, Lys-Ile, Pro-Pro, Asn-Pro, Asn-Thr, Phe-Trp, Val-Val, Leu-Gly,

Gln-Ile, Phe-Lys, Gln-Ala, Glu-Trp, His-His, Ile-Leu, Glu-Phe, Met-Ala, Leu-Ser, Leu-Trp, Asn-Asp, His-Gly, Phe-Ser, Gln-Trp, Lys-Tyr, Ser-Leu, Asn-Phe, His-Ala, Glu-Ile, Gly-Pro, Glu-Pro, Leu-Ala, Tyr-Val, Phe-Thr, Trp-Asp, Met-Gly, Thr-Ile, Ile-Ser, Pro-Ser, Phe-Ile, Met-Thr, Trp-Thr, Val-Thr, Ala-Ala, Lys-Pro, Pro-Ile, Gly-Phe, Tyr-Gln, Leu-Ile, Ala-Pro, Ile-Pro, Gln-Phe, Ala-His, Lys-Leu, Trp-Lys, Asn-Leu, Tyr-Trp, Phe-Asp, Gln-Gln, Trp-Trp, His-Gln, Ser-Ile, Glu-Ala, Gly-Val, Lys-Ala, Val-Gly, Asn-Ser, Ile-Met, Met-Glu, Gln-His, Phe-His, Gly-Gly, Ile-Thr, Gln-Thr, Gln-Ser, Ile-Asp, His-Trp, and Ile-Phe.

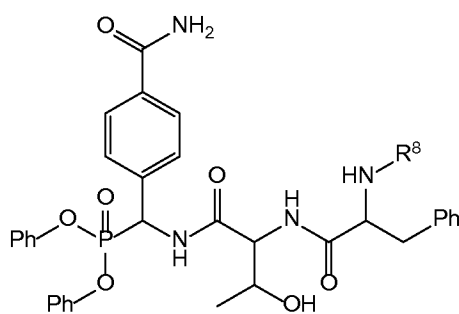
In certain embodiments, R^6 and R^7 are each a bond.

In certain embodiments, the compound is diphenyl-((2-(2-amino-3-phenylpropanamido)-3-hydroxybutanamido)(4-carbamoylphenyl) methyl)phosphonate:



In certain embodiments, the compound is selected from the group consisting of diphenyl[amino(4-carbamoylphenyl)methyl]phosphonate; diphenyl[N-(N-acetyl-D-valyl-D-leucyl)amino(4-carbamoylphenyl)methyl]phosphonate; diphenyl[N-(N-acetyl-D-alanyl-D-valyl)amino(4-carbamoylphenyl)methyl]phosphonate; and diphenyl[N-(N-acetyl)glycyl-D-phenylalanyl]amino(4-carbamoylphenyl)methyl]phosphonate.

In certain embodiments, the compound of the invention is the compound, or a



salt or solvate thereof: , wherein R^8 is H or a protecting

group. In certain amendments, R^8 is selected from the group consisting of acetyl, benzyloxycarbonyl, *tert*-butyloxycarbonyl, benzylsulfonyl, *para*-methylphenylsulfonyl, fluorenylmethyloxycarbonyl, pivaloyl, alloxycarbonyl, pentafluorophenoxycarbonyl, methoxycarbonyl, *para*-methoxybenzyloxycarbonyl, methoxyethoxymethoxycarbonyl, and trichloroethoxycarbonyl.

The compounds of the invention include prodrug, racemic, isotopically labeled, optically active, tautomeric, regioisomeric and/or stereoisomeric forms of the compounds described elsewhere herein. The compounds of the invention further include crystalline forms (also known as polymorphs), solvates, amorphous phases, and/or pharmaceutically acceptable salts of compounds described elsewhere herein.

The compounds described herein, and other related compounds having different substituents, are synthesized using techniques and materials described herein and as described, for example, in Fieser & Fieser's Reagents for Organic Synthesis, Volumes 1-17 (John Wiley and Sons, 1991); Rodd's Chemistry of Carbon Compounds, Volumes 1-5 and Supplementals (Elsevier Science Publishers, 1989); Organic Reactions, Volumes 1-40 (John Wiley and Sons, 1991), Larock's Comprehensive Organic Transformations (VCH Publishers Inc., 1989), March, Advanced Organic Chemistry 4th Ed. (Wiley 1992); Carey & Sundberg, Advanced Organic Chemistry 4th Ed., Vols. A and B (Plenum 2000, 2001), and Green & Wuts, Protective Groups in Organic Synthesis 3rd Ed. (Wiley 1999) (all of which are incorporated by reference for such disclosure). Compounds described herein are synthesized using any suitable procedures starting from compounds that are available from commercial sources, or are prepared using procedures described herein.

Salts

The compounds described herein may form salts with acids, and such salts are included in the present invention. In certain embodiments, the salts are pharmaceutically acceptable salts. The term "salts" embraces addition salts of free acids that are useful within the methods of the invention. The term "pharmaceutically acceptable salt" refers to salts that possess toxicity profiles within a range that affords utility in pharmaceutical applications. Pharmaceutically unacceptable salts may nonetheless possess properties such as high crystallinity, which have utility in the practice of the present invention, such as for example utility in process of synthesis, purification or formulation of compounds useful within the methods of the invention.

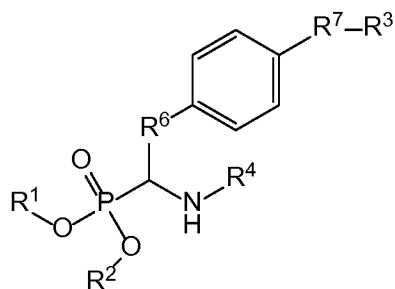
Suitable pharmaceutically acceptable acid addition salts may be prepared from an inorganic acid or from an organic acid. Examples of inorganic acids include hydrochloric, hydrobromic, hydriodic, nitric, carbonic, sulfuric (including sulfate and hydrogen sulfate), and phosphoric acids (including hydrogen phosphate and dihydrogen phosphate). Appropriate organic acids may be selected from aliphatic, cycloaliphatic, aromatic, araliphatic, heterocyclic, carboxylic and sulfonic classes of organic acids, examples of which

include formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, ascorbic, glucuronic, maleic, malonic, saccharin, fumaric, pyruvic, aspartic, glutamic, benzoic, anthranilic, 4-hydroxybenzoic, phenylacetic, mandelic, embonic (pamoic), methanesulfonic, ethanesulfonic, benzenesulfonic, pantothenic, trifluoromethanesulfonic, 2-hydroxyethanesulfonic, p-toluenesulfonic, sulfanilic, cyclohexylaminosulfonic, stearic, alginic, β -hydroxybutyric, salicylic, galactaric and galacturonic acid.

Suitable pharmaceutically acceptable base addition salts of compounds of the invention include, for example, ammonium and metallic salts including alkali metal, alkaline earth metal and transition metal salts such as, for example, calcium, magnesium, potassium, sodium and zinc salts. Pharmaceutically acceptable base addition salts also include organic salts made from basic amines such as, for example, N,N'-dibenzylethylene-diamine, chlorprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine. All of these salts may be prepared from the corresponding compound by reacting, for example, the appropriate acid or base with the compound.

Methods

The invention includes a method of treating or preventing prostate cancer in a male subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a compound of formula (I) or a salt or solvate thereof.



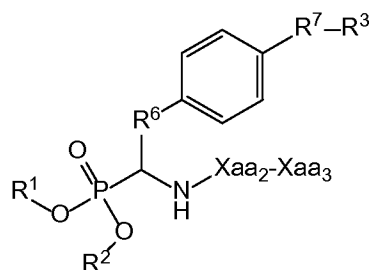
(I), wherein: R¹ and R² are independently selected from the group consisting of H, C₁-C₁₀ alkyl, substituted C₁-C₁₀ alkyl, phenyl, and substituted phenyl; R³ is selected from the group consisting of H, F, Cl, Br, I, C₁-C₁₀ alkyl, substituted C₁-C₁₀ alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, amino, substituted amino, -CN, -OH, alkoxy, carbamoyl, -COOH, -CHO, alkoxy carbonyl, aminosulfonyl, alkylsulfonyl, and phosphoryl; R⁴ is selected from the group consisting of R⁵, a sugar, an oligosaccharide, an amino acid and a peptide, wherein the sugar, oligosaccharide, amino acid or peptide is optionally substituted; R⁵ is selected from the group consisting of H, C₁-C₁₀ alkyl, substituted C₁-C₁₀ alkyl, phenyl, substituted phenyl, acyl, alkoxy carbonyl, aminocarbonyl, and Fmoc; R⁶ and R⁷ are independently selected from the group consisting of bond, -(CH₂)_n-,

-O-, -O(C=O)-, -C(=O), -C(=O)O-, -OC(=O)O-, -NHC(=O)-, -C(=O)NH-, -C(=NH)NH-, -C(=NH₂)NH- and -NHC(=O)NH-; and, each occurrence of n is independently an integer ranging from 1 to 10.

In certain embodiments, R⁶ and R⁷ are each a bond.

5 In certain embodiments, R⁴ is an amino acid or a peptide. In other embodiments, the amino acid or peptide comprises a D- or L-amino acid. In yet other embodiments, the amino acid or peptide comprises an amino acid selected from the group consisting of Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, His, Ser, Thr, Gln, Glu, Asn, Asp, Lys, Arg, Met, Cys and cystine.

10 In certain embodiments, the peptide comprises the dipeptide Xaa₂-Xaa₃, wherein the C-terminus of Xaa₂ is covalent bound through an amide bond to the NH group in



(I) to provide (II):

(II).

In certain embodiments, Xaa₂ comprises a D- or L-amino acid. In other embodiments, Xaa₂ comprises an amino acid selected from the group consisting of Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, His, Ser, Thr, Gln, Glu, Asn, Asp, Lys, Arg, Met, Cys and cystine.

In certain embodiments, Xaa₃ comprises a D- or L-amino acid. In other embodiments, Xaa₃ comprises an amino acid selected from the group consisting of Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, His, Ser, Thr, Gln, Glu, Asn, Asp, Lys, Arg, Met, Cys and cystine.

In certain embodiments, the N-terminus of Xaa₃ is derivatized with a group selected from the group consisting of acetyl, benzyloxycarbonyl, *tert*-butyloxycarbonyl, benzylsulfonyl, *para*-methylphenylsulfonyl, fluorenylmethyloxycarbonyl, pivaloyl, alloxycarbonyl, pentafluorophenoxycarbonyl, methoxycarbonyl, *para*-methoxybenzyloxycarbonyl, methoxyethoxymethoxycarbonyl, and trichloroethoxycarbonyl. In other embodiments, the N-terminus of Xaa₃ is derivatized with two independently selected C₁-C₆ alkyl groups.

In certain embodiments, at least one of the N-H group of the peptidic groups (*i.e.*, the amide groups covalently linking Xaa₂ to Xaa₃, and Xaa₂ to the amino group alpha to

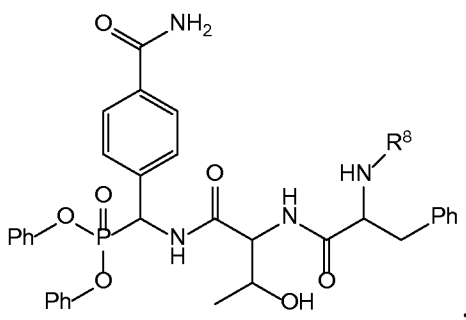
the phosphonate group in (II)) is independently derivatized with a C₁-C₆ alkyl alkyl (such as, but not limited to, methyl, ethyl, *n*-propyl or isopropyl). In other embodiments, each one of the N-H group of the peptidic groups is independently derivatized with a C₁-C₆ alkyl alkyl (such as, but not limited to, methyl, ethyl, *n*-propyl or isopropyl).

5 In certain embodiments, Xaa₂-Xaa₃ comprises at least one selected from the group consisting of Leu-Val, Ile-His, Tyr-Pro, Val-Tyr, Ala-Trp, Gly-Lys, Trp-Gly, Phe-Pro, Pro-Trp, Met-Pro, and Trp-Met. In other embodiments, Xaa₂ comprises at least one selected from the group consisting of Leu, Ile, Ty, Val, Ala, Gly, Trp, Phe, Pro, and Met. In yet other
10 embodiments, Xaa₃ comprises at least one selected from the group consisting of Val, His, Pro, Tyr, Trp, Lys, Gly, and Trp-Met.

In certain embodiments, Xaa₂-Xaa₃ comprises at least one selected from the group consisting of Gly-Tyr, His-Pro, Gln-Met, Asn-Tyr, Ala-Tyr, Lys-Gly, Met-His, Gln-Val, Leu-Leu, Phe-Gly, Ser-Trp, Ser-Phe, Trp-Ala, Gly-Trp, Val-Phe, Phe-Asn, Phe-Tyr, Met-Asn, Ile-Val, Ile-Tyr, and Tyr-Tyr.

15 In certain embodiments, Xaa₂-Xaa₃ comprises at least one selected from the group consisting of Gly-Ile, Phe-Ala, Glu-Arg, Val-Asn, Gln-Gly, Phe-Val, Thr-Pro, Phe-Leu, Tyr-Ile, Ile-Ala, Ile-Gly, Trp-Pro, Ile-Arg, Ser-Pro, Trp-His, Val-Ala, Val-Lys, Trp-Tyr, Tyr-Ala, Tyr-Asn, Trp-Phe, Lys-Ile, Pro-Pro, Asn-Pro, Asn-Thr, Phe-Trp, Val-Val, Leu-Gly, Gln-Ile, Phe-Lys, Gln-Ala, Glu-Trp, His-His, Ile-Leu, Glu-Phe, Met-Ala, Leu-Ser, Leu-Trp,
20 Asn-Asp, His-Gly, Phe-Ser, Gln-Trp, Lys-Tyr, Ser-Leu, Asn-Phe, His-Ala, Glu-Ile, Gly-Pro, Glu-Pro, Leu-Ala, Tyr-Val, Phe-Thr, Trp-Asp, Met-Gly, Thr-Ile, Ile-Ser, Pro-Ser, Phe-Ile, Met-Thr, Trp-Thr, Val-Thr, Ala-Ala, Lys-Pro, Pro-Ile, Gly-Phe, Tyr-Gln, Leu-Ile, Ala-Pro, Ile-Pro, Gln-Phe, Ala-His, Lys-Leu, Trp-Lys, Asn-Leu, Tyr-Trp, Phe-Asp, Gln-Gln, Trp-Trp, His-Gln, Ser-Ile, Glu-Ala, Gly-Val, Lys-Ala, Val-Gly, Asn-Ser, Ile-Met, Met-Glu, Gln-His,
25 Phe-His, Gly-Gly, Ile-Thr, Gln-Thr, Gln-Ser, Ile-Asp, His-Trp, and Ile-Phe.

In certain embodiments, the compound is diphenyl-((2-(2-(N-R⁸)-amino-3-phenylpropanamido)-3-hydroxybutanamido)(4-carbamoylphenyl)methyl) phosphonate:



wherein R⁸ is H or a protecting selected from the group consisting of C₁-C₆ alkyl, acetyl, benzyloxycarbonyl, *tert*-butyloxycarbonyl, benzylsulfonyl, *para*-methylphenylsulfonyl, fluorenylmethyloxycarbonyl, pivaloyl, alloxycarbonyl, pentafluorophenoxycarbonyl, methoxycarbonyl, *para*-methoxybenzyloxycarbonyl, methoxyethoxymethoxycarbonyl, and trichloroethoxycarbonyl.

In certain embodiments, the compound is selected from the group consisting of diphenyl[amino(4-carbamoylphenyl)methyl]phosphonate; diphenyl[N-(N-acetyl-D-valyl-D-leucyl)amino(4-carbamoylphenyl)methyl]phosphonate; diphenyl[N-(N-acetyl-D-alanyl-D-valyl)amino(4-carbamoylphenyl)methyl]phosphonate; and diphenyl[N-(N-acetyl)glycyl-D-phenylalanyl)amino(4-carbamoylphenyl)methyl]phosphonate.

In certain embodiments, PSA activity in the subject is inhibited. In other embodiments, the growth of at least one prostate cancer cell in the subject is inhibited. In yet other embodiments, the release of at least one growth factor selected from the group consisting of IGF-I, IGFBP-2 and IGFBP-3 is inhibited in the subject. In yet other embodiments, the subject is a human.

Combination Therapies

In certain embodiments, the compounds of the invention are useful in the methods of present invention when used concurrently with at least one additional agent useful for preventing and/or treating prostate cancer. In certain embodiments, the compound and the agent are co-administered to the subject. In other embodiments, the compound and the agent are co-formulated. In yet other embodiments, the compound and the agent have additive, complementary or synergistic effects in the prevention and/or treatment of prostate cancer.

Non-limiting examples of additional agents include docetaxel, cabazitaxel, mitoxantrone, estramustine, doxorubicin, etoposide, vinblastine, paclitaxel, carboplatin, vinorelbine, bevacizumab, thalidomide, prednisone, sipuleucel-T, abiraterone, and/or enzalutamide.

A synergistic effect may be calculated, for example, using suitable methods such as, for example, the Sigmoid-E_{max} equation (Holford & Scheiner, 19981, Clin. Pharmacokinet. 6: 429-453), the equation of Loewe additivity (Loewe & Muischnek, 1926, Arch. Exp. Pathol Pharmacol. 114: 313-326) and the median-effect equation (Chou & Talalay, 1984, Adv. Enzyme Regul. 22: 27-55). Each equation referred to above may be applied to experimental data to generate a corresponding graph to aid in assessing the effects

of the drug combination. The corresponding graphs associated with the equations referred to above are the concentration-effect curve, isobologram curve and combination index curve, respectively.

5 Administration/Dosage/Formulations

The regimen of administration may affect what constitutes an effective amount. The therapeutic formulations may be administered to the patient either prior to or after the onset of a disease or disorder. Further, several divided dosages, as well as staggered dosages may be administered daily or sequentially, or the dose may be continuously infused, or may be a bolus injection. Further, the dosages of the therapeutic formulations may be proportionally increased or decreased as indicated by the exigencies of the therapeutic or prophylactic situation.

Administration of the compositions useful within the present invention to a patient, preferably a mammal, more preferably a human, may be carried out using known procedures, at dosages and for periods of time effective to treat a disease or disorder in the patient. An effective amount of the therapeutic compound necessary to achieve a therapeutic effect may vary according to factors such as the state of the disease or disorder in the patient; the age, sex, and weight of the patient; and the ability of the therapeutic compound to treat a disease or disorder in the patient. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. A non-limiting example of an effective dose range for a therapeutic compound of the invention is from about 1 and 5,000 mg/kg of body weight/per day. One of ordinary skill in the art would be able to study the relevant factors and make the determination regarding the effective amount of the therapeutic compound without undue experimentation.

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

A medical doctor, *e.g.*, physician or veterinarian, having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than

that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

In particular embodiments, it is especially advantageous to formulate the compound in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the patients to be treated; each unit containing a predetermined quantity of therapeutic compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical vehicle. The dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the therapeutic compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding/formulating such a therapeutic compound for the treatment of a disease or disorder in a patient.

In certain embodiments, the compositions useful within the invention are formulated using one or more pharmaceutically acceptable excipients or carriers. In certain embodiments, the pharmaceutical compositions of the invention comprise a therapeutically effective amount of a compound useful within the invention and a pharmaceutically acceptable carrier.

In certain embodiments, the present invention is directed to a packaged pharmaceutical composition comprising a container holding a therapeutically effective amount of a compound of the invention, alone or in combination with a second pharmaceutical agent; and instructions for using the compound to treat, prevent, or reduce one or more symptoms of a disease or disorder in a patient.

Formulations may be employed in admixtures with conventional excipients, *i.e.*, pharmaceutically acceptable organic or inorganic carrier substances suitable for oral, parenteral, nasal, intravenous, subcutaneous, enteral, or any other suitable mode of administration, known to the art. The pharmaceutical preparations may be sterilized and if desired mixed with auxiliary agents, *e.g.*, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure buffers, coloring, flavoring and/or aromatic substances and the like. They may also be combined where desired with other active agents, *e.g.*, other analgesic agents.

Routes of administration of any of the compositions of the invention include oral, nasal, rectal, intravaginal, parenteral, buccal, sublingual or topical. The compounds for use in the invention may be formulated for administration by any suitable route, such as for oral or parenteral, for example, transdermal, transmucosal (*e.g.*, sublingual, lingual,

(trans)buccal, (trans)urethral, vaginal (*e.g.*, trans- and perivaginally), (intra)nasal and (trans)rectal), intravesical, intrapulmonary, intraduodenal, intragastrical, intrathecal, subcutaneous, intramuscular, intradermal, intra-arterial, intravenous, intrabronchial, inhalation, and topical administration.

5

Dosing

The therapeutically effective amount or dose of a compound will depend on the age, sex and weight of the patient, the current medical condition of the patient and the progression of prostate cancer in the patient being treated.

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A suitable dose of a compound of the present invention may be in the range of from about 0.01 mg to about 5,000 mg per day, such as from about 0.1 mg to about 1,000 mg, for example, from about 1 mg to about 500 mg, such as about 5 mg to about 250 mg per day. The dose may be administered in a single dosage or in multiple dosages, for example from 1 to 4 or more times per day. When multiple dosages are used, the amount of each dosage may be the same or different. For example, a dose of 1 mg per day may be administered as two 0.5 mg doses, with about a 12-hour interval between doses. It is understood that the amount of compound dosed per day may be administered, in non-limiting examples, every day, every other day, every 2 days, every 3 days, every 4 days, or every 5 days.

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In certain embodiments, the formulations of the present invention may be, but are not limited to, short-term, rapid-offset, as well as controlled, for example, sustained release, delayed release and pulsatile release formulations.

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Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures, embodiments, claims, and examples described herein. Such equivalents were considered to be within the scope of this invention and covered by the claims appended hereto. For example, it should be understood, that modifications in reaction conditions, including but not limited to reaction times, reaction size/volume, and experimental reagents, such as solvents, catalysts, pressures, atmospheric conditions, *e.g.*, nitrogen atmosphere, and reducing/oxidizing agents, with art-recognized alternatives and using no more than routine experimentation, are within the scope of the present application.

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It is to be understood that, wherever values and ranges are provided herein, the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, all values and ranges encompassed by these values and ranges are meant to be encompassed within the

scope of the present invention. Moreover, all values that fall within these ranges, as well as the upper or lower limits of a range of values, are also contemplated by the present application. The description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range and, when appropriate, partial integers of the numerical values within ranges. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

The following examples further illustrate aspects of the present invention. However, they are in no way a limitation of the teachings or disclosure of the present invention as set forth herein.

EXAMPLES

The invention is now described with reference to the following Examples. These Examples are provided for the purpose of illustration only, and the invention is not limited to these Examples, but rather encompasses all variations that are evident as a result of the teachings provided herein.

Materials:

Unless otherwise noted, all remaining starting materials were obtained from commercial suppliers and used without purification.

Synthesis and Characterization of Inhibitors:

NMR spectra for ^1H , ^{13}C , and ^{31}P nuclei were obtained on Varian INNOVA 300 MHz and 500 MHz NMR spectrometers. Broad-band proton-decoupled ^{31}P spectra were recorded using 85% phosphoric acid in a sealed capillary as an internal standard.

High-resolution mass spectrometry (HRMS) experiments were conducted using Micromass AutoSpec M magnetic sector using chemical ionization (CI) in methane.

Infrared spectra were recorded using Perkin-Elmer Spectrum One ATR FT-IR.

Inhibition Kinetics Assays:

Human PSA protein was purchased from Fitzgerald (Catalog # 30-AP14). Chromogenic substrate MeO-Suc-Arg-Pro-Tyr-pNA·HCl (S-2586) was purchased from

DiaPharma. Absorbance readings were conducted using Tecan Infinite M200 well plate reader. Sterile polystyrene-based flat bottom 96 well plates were obtained from Corning.

In typical non-limiting experiments, a 10 μ L aliquot of varying concentrations of inhibitor solutions (prepared in 99.7% dimethyl sulfoxide) was added to 75 μ L protein buffer solution (5 μ g PSA protein in 100 mM Tris·HCl, 1.5 M NaCl, pH 7.5) in a 96-well plate. 15 μ L of the substrate S-2586 in Tris buffer was added for a total concentration of 1 mM and a total volume of 100 μ L in each well. Amidolytic activity of PSA was measured by cleavage of the substrate to yield *p*-nitroanilide analyte, recording absorbance at 405 nm for 60 minutes. To determine inhibitor potency, enzymatic activities of inhibited and non-inhibited PSA kinetic readings were compared and the estimated IC₅₀ values were calculated using the four-point log (4PL) transformation analysis of the inhibitor concentration vs. enzymatic activity.

Dose-Dependent Response Assays:

To assess inhibitor potency on PSA, kinetic assays were conducted using chromogenic substrate S-2586 as a method to measure peptide cleavage via *p*-nitroaniline absorbance, the liberated chromophore. Dose-dependent responses to a range of concentrations of inhibitors were recorded and IC₅₀ values were calculated based of the average enzymatic rate of PSA as compared to the control. Final values were estimated using the 4PL method (Equation 2) to determine concentrations at which 50% PSA activity inhibition.

$$y = D + \frac{A - D}{1 + 10^{[x - \text{Log}(IC_{50})] \cdot B}} \quad (2)$$

Example 1: Synthesis of α -Aminoalkylphosphonate Ester Compounds

The synthesis of diphenyl α -aminoalkylphosphonates may be achieved using a facile Kabachnik-Fields reaction using a 3-component, 2-step, one-pot synthesis consisting of a para-substituted benzaldehyde, benzyl carbamate, and triphenylphosphite. With low solvent volume within the reaction mixture, the crude product precipitated out of solution after a given amount of time. Compounds **5**, **7**, and **12** were initially obtained as oily residues, which were dissolved in the appropriate solvent and cooled to promote crystallization or precipitation.

Compounds were used in the kinetic assays once deemed spectroscopically pure using NMR and mass spectrometry. In certain embodiments, completion of the reaction

can be monitored by ^1H NMR by observing doublet-doublet signal at 5.0-6.0 ppm range. The signal contains $^1J_{\text{P-H}}$ of ~22 Hz for heteronuclear coupling and $^1J_{\text{C-H-NH}} = 10.2$ Hz for proton-to-proton coupling. ^1H AB_{qt} splitting patterns arising from diastereotopic methylene hydrogens were also evident in the NMR spectra as indicative of reaction completion.

- 5 Heteronuclear coupling of the phosphorus to carbon atoms was also observed in the ^{13}C NMR. Long-range ^{13}C - ^{31}P couplings were observed. In certain cases, ^{13}C signal splitting by ^{31}P nuclei in the carbon NMR can be seen up through four bonds or more.

Diphenyl [N-benzyloxycarbonylamino(phenyl)methyl] phosphonate (1):

- 10 A 1 : 1 : 1.1 equivalent mixture of 110 mg benzaldehyde (1.0 mmol), 150 mg benzyl carbamate (1.0 mmol), and 0.29 mL triphenyl phosphite (1.1 mmol) was partially dissolved in 1 mL glacial acetic acid and heated to reflux for 3 hours. After completion, volatiles were removed under reduced pressure to yield a crude product. The crude was dissolved in minimal amount of hot methanol and cooled to 0°C. The voluminous solid that
15 precipitated out of solution was then collected, filtered, washed with cold methanol, and was subsequently recrystallized in methanol. The product was isolated as a colorless solid (334 mg, 71% yield).

- ^1H NMR (DMSO, 500 MHz) δ 5.10 (AB_{qt} $\Delta\nu = 42.0$ Hz, $J = 12.7$ Hz, 2H), 5.60 (dd, $^1J_{\text{P-CH}} = 22.1$ Hz & $J_{\text{CH-NH}} = 9.7$ Hz, 1H), 6.95 (d, $J = 8.3$ Hz, 2H), 7.05 (d, $J = 8.8$
20 Hz, 2H), 7.18-7.20 (m, 2H), 7.31-7.41 (m, 12H), 7.64 (d, $J = 7.3$ Hz, 2H), 8.92 (d, $J = 10.2$ Hz, 1H); ^{13}C NMR (DMSO, 300 MHz) δ 51.84, 53.91, 66.16, 120.22, 120.28, 120.33, 125.21, 125.30, 127.92, 128.24, 128.33, 128.41, 128.55, 129.77, 129.81, 134.33, 136.63, 149.71, 149.85, 150.00, 150.14, 155.93, 156.06; ^{31}P NMR (DMSO, 300 MHz) δ 15.88; HRMS (CI, methane) m/z calculated for $\text{C}_{27}\text{H}_{25}\text{NO}_5\text{P}$ (M+1) 474.147037, found 474.145015.

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Diphenyl [N-Benzyloxycarbonylamino(4-fluorophenyl)-methyl] Phosphonate (2):

- This compound was prepared in a similar manner as **1**, from 165 mg 4-fluorobenzaldehyde (1.0 mmol), 150 mg benzyl carbamate (1.0 mmol), and 0.29 mL triphenyl phosphite (1.1 mmol) in 1 mL glacial acetic acid. The product recrystallized at 0 °C
30 from methanol as colorless crystalline needles (350 mg, 66% yield).

^1H NMR (DMSO, 500 MHz) δ 5.11 (AB_{qt} $\Delta\nu = 41.8$ Hz, $J = 12.7$ Hz, 2H), 5.65 (dd, $J = 22.0$ Hz and $J = 10.2$ Hz, 1H), 6.98 (s, $J = 8.3$ Hz, 2H), 7.06 (d, $J = 8.8$ Hz, 2H), 7.18-7.20 (m, 4H), 7.30-7.38 (m, 9H), 7.70 (ddd, $J = 8.3$ Hz, 5.9 Hz, and 2.0 Hz, 2H), 8.92

(d, $J = 10.2$ Hz, 1H). ^{13}C NMR (DMSO, 300 MHz) δ 51.07, 53.18, 66.19, 115.15, 115.44, 120.19, 120.25, 120.29, 125.24, 125.31, 127.94, 128.33, 129.78, 129.83, 130.55, 130.65, 130.75, 136.59, 149.68, 149.82, 149.97, 150.09, 155.86, 155.98, 160.30. ^{31}P NMR (DMSO, 300 MHz) δ 15.56. HRMS (CI, methane) m/z calculated for $\text{C}_{27}\text{H}_{24}\text{NO}_5\text{P}$ ($M + 1$)

5 492.137615, found 492.139395.

Diphenyl [N-benzyloxycarbonylamino(4-tert-butylphenyl)methyl] phosphonate (3):

This compound was prepared in similar manner as the previous compound, from 0.13 mL 4-tert-butylbenzaldehyde (1.0 mmol), 150 mg benzyl carbamate (1.0 mmol),
10 and 0.29 mL triphenyl phosphite (1.1 mmol) in 1 mL glacial acetic acid. The product recrystallized at 0 °C from methanol as fine colorless needles (261 mg, 50% yield).

^1H NMR (DMSO, 500 MHz) δ 1.28 (s, 9 H), 5.09 (AB_{qt} $\Delta\nu = 40.5$ Hz, $J = 12.4$ Hz, 2H), 5.54 (dd, $^1J_{\text{P-CH}} = 22.0$ Hz & $J_{\text{CH-NH}} = 10.2$ Hz, 1H), 6.92 (d, $J = 8.3$ Hz, 2H), 7.04 (d, $J = 8.8$ Hz, 2H), 7.16-7.20 (m, 2H), 7.29-7.38 (m, 9H), 7.40 (d, $J = 8.3$ Hz, 2H), 7.54
15 (dd, $J = 8.3$ Hz & 2.0 Hz, 2H), 8.86 (d, $J = 10.2$ Hz, 1H). ^{13}C NMR (DMSO, 300 MHz) δ 31.03, 34.29, 51.52, 53.61, 66.12, 120.23, 120.28, 120.34, 125.24, 127.91, 128.21, 128.29, 128.33, 129.75, 131.23, 136.65, 149.89, 150.01, 150.15, 150.72, 150.76, 155.92, 156.03. ^{31}P NMR (DMSO, 300 MHz) δ 16.03; HRMS (CI, methane) m/z calculated for $\text{C}_{31}\text{H}_{33}\text{NO}_5\text{P}$ ($M+1$) 530.209637, found 530.211016.

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Diphenyl [N-Benzyloxycarbonylamino(4-hydroxyphenyl)-methyl] Phosphonate (4):

This compound was prepared in a similar manner as 1, from 610 mg 4-hydroxybenzaldehyde (5.0 mmol), 750 mg benzyl carbamate (5.0 mmol), and 1.45 mL triphenyl phosphite (5.5 mmol) in 2 mL glacial acetic acid. The product recrystallized at 0 °C
25 from ethanol/diethyl ether (3:1) as a colorless solid (1.27 g, 52% yield).

^1H NMR (DMSO, 500 MHz) δ 5.09 (AB_{qt} $\Delta\nu = 41.0$ Hz, $J = 12.6$ Hz, 2H), 5.46 (dd, $J = 21.6$ Hz and $J = 10.2$ Hz, 1H), 6.77 (d, $J = 8.3$ Hz, 2H), 6.95 (d, $J = 8.3$ Hz, 2H), 7.05 (d, $J = 8.8$ Hz, 2H), 7.19 (td, $J = 7.3$ Hz and 4.4 Hz, 2H), 7.30-7.37 (m, 9H), 7.41 (dd, $J = 8.8$ Hz and 1.9 Hz, 2H), 8.77 (d, $J = 10.2$, 1H), 9.56 (s, 1H). ^{13}C NMR (DMSO, 300 MHz)
30 δ 51.27, 53.38, 66.09, 115.18, 120.26, 120.33, 120.39, 124.32, 125.13, 125.22, 127.91, 128.33, 129.75, 129.78, 129.91, 136.70, 149.82, 149.94, 150.11, 150.23, 155.89, 156.00, 157.38, 157.41. ^{31}P NMR (DMSO, 300 MHz) δ 16.40. HRMS (CI, methane) m/z calculated for $\text{C}_{27}\text{H}_{25}\text{NO}_6\text{P}$ ($M + 1$) 490.141951, found 490.141518.

Diphenyl [N-Benzyloxycarbonylamino(4-cyanophenyl)-methyl] Phosphonate (5):

This compound was prepared in a similar manner as **1**, from 1.3 g 4-cyanobenzaldehyde (10.0 mmol), 1.5 g benzyl carbamate (10.0 mmol), and 2.9 mL triphenyl phosphite (11.0 mmol) in 2 mL glacial acetic acid. The product recrystallized at room temperature from dichloromethane/methanol as colorless crystalline needles (3.86 g, 77% yield).

^1H NMR (DMSO, 500 MHz) δ 5.12 (ABqt $\Delta\nu = 40.4$ Hz, $J = 12.5$ Hz, 2H), 5.81 (dd, $J = 23.3$ Hz and $J = 10.2$ Hz, 1H), 7.02 (d, $J = 8.3$ Hz, 2H), 7.05 (d, $J = 8.3$ Hz, 2H), 7.21 (td, $J = 7.3$ Hz and 3.4 Hz, 2H), 7.32–7.39 (m, 9H), 7.86–7.91 (m, 4H), 9.05 (d, $J = 10.2$ Hz, 1H). ^{13}C NMR (DMSO, 300 MHz) δ 51.68, 53.76, 66.35, 111.09, 118.57, 120.17, 120.23, 120.29, 125.36, 125.47, 127.96, 128.37, 129.34, 129.42, 129.85, 129.92, 132.38, 136.51, 140.05, 149.59, 149.72, 149.86, 150.00, 155.90, 156.01. ^{31}P NMR (DMSO, 300 MHz) δ 14.52. HRMS (CI, methane) m/z calculated for $\text{C}_{28}\text{H}_{24}\text{N}_2\text{O}_5\text{P}$ ($M + 1$) 499.142286, found 499.143536.

Diphenyl [N-Benzyloxycarbonylamino(4-methoxyphenyl)-methyl] Phosphonate (6):

This compound was prepared in a similar manner as **1**, from 340 mg 4-anisaldehyde (2.5 mmol), 368 mg benzyl carbamate (2.5 mmol), and 0.74 mL triphenyl phosphite (2.8 mmol) in 2 mL glacial acetic acid. The product recrystallized at 0 °C from dichloromethane/methanol as a colorless solid (554 mg, 44%).

^1H NMR (DMSO, 500 MHz) δ 3.76 (s, 3H), 5.10 (ABqt $\Delta\nu = 40.4$ Hz, $J = 12.7$ Hz, 2H), 5.54 (dd, $J = 22.1$ Hz and $J = 10.2$ Hz, 1H), 6.95–6.98 (m, 4H), 7.06 (d, $J = 8.3$ Hz, 2H), 7.19 (td, $J = 7.3$ Hz and 2.9 Hz, 2H), 7.30–7.38 (m, 9H), 7.56 (dd, $J = 8.3$ Hz and 1.9 Hz, 2H), 8.84 (d, $J = 10.2$ Hz, 1H). ^{13}C NMR (DMSO, 300 MHz) δ 51.20, 53.29, 55.13, 66.10, 113.84, 120.23, 120.29, 120.36, 125.15, 125.24, 126.12, 127.91, 128.33, 129.75, 129.80, 129.86, 136.67, 149.77, 149.91, 150.06, 150.20, 155.89, 156.00, 159.15. ^{31}P NMR (DMSO, 300 MHz) δ 16.15. HRMS (CI, methane) m/z calculated for $\text{C}_{28}\text{H}_{26}\text{NO}_6\text{P}$ (M^+) 503.149776, found 503.149148.

Diphenyl [N-Benzyloxycarbonylamino (4-(dimethylamino)phenyl)methyl] Phosphonate (7):

This compound was prepared in a similar manner as **1**, from 750 mg 4-(dimethylamino)benzaldehyde (5.0 mmol), 750 mg benzyl carbamate (5.0 mmol), and 1.45

mL triphenyl phosphite (5.5 mmol) in 1.5 mL glacial acetic acid. The product recrystallized at 0 °C from methanol as colorless needles (812 mg, 31% yield).

¹H NMR (DMSO, 500 MHz) δ 2.89 (s, 6H), 5.09 (ABqt Δν = 38.5 Hz, J = 12.7 Hz, 2H), 5.42 (dd, J = 21.1 Hz and J = 10.2 Hz, 1H), 6.71 (d, J = 8.8 Hz, 2H), 6.96 (d, J = 7.8 Hz, 2H), 7.06 (d, J = 8.3 Hz, 2H), 7.18 (td, J = 7.3 Hz and 3.4 Hz, 2H), 7.31-7.37 (m, 9H), 7.41 (dd, J = 8.8 Hz and 1.9 Hz, 2H), 8.74 (d, J = 10.2 Hz, 1H). ¹³C NMR (DMSO, 300 MHz) δ 51.26, 53.36, 66.03, 112.03, 120.26, 120.36, 120.42, 121.04, 125.07, 125.18, 127.88, 128.32, 129.24, 129.33, 129.72, 129.77, 136.73, 149.85, 149.98, 150.14, 150.26, 155.87, 156.00. ³¹P NMR (DMSO, 300 MHz) δ 16.41. HRMS (CI, methane) m/z calculated for C₂₉H₂₉N₂O₅P (M+) 516.181411, found 516.182187.

Diphenyl [N-Benzyloxycarbonylamino(4-(methylsulfonyl)-phenyl)methyl] Phosphonate (8):

This compound was prepared in a similar manner as **1**, from 185 mg 4-(methylsulfonyl)benzaldehyde (1.0 mmol), 150 mg benzyl carbamate (1.0 mmol), and 0.29 mL triphenyl phosphite (1.1 mmol) in 2 mL glacial acetic acid. The product recrystallized at 0 °C from methanol as fine colorless needles (355 mg, 65% yield).

¹H NMR (DMSO, 500 MHz) δ 3.23 (s, 3 H), 5.11 (ABqt Δν = 38.9 Hz, J = 12.3 Hz, 2H), 5.80 (dd, J = 22.6 Hz and J = 10.2 Hz, 1H), 7.02 (d, J = 7.8 Hz, 2H), 7.07 (d, J = 8.8 Hz, 2H), 7.20 (t, J = 7.7 Hz, 2H), 7.32-7.38 (m, 9H), 7.92 (dd, J = 8.2 Hz and 1.6 Hz, 2H), 7.96 (d, J = 8.3 Hz, 2H), 9.06 (d, J = 9.7 Hz, 1H). ¹³C NMR (DMSO, 300 MHz) δ 43.37, 51.58, 53.65, 66.32, 120.17, 120.23, 120.31, 125.34, 125.45, 127.05, 127.95, 128.35, 129.30, 129.36, 129.83, 129.91, 136.51, 140.30, 140.57, 140.62, 149.59, 149.71, 149.86, 150.00, 155.90, 156.01. ³¹P NMR (DMSO, 300 MHz) δ 15.31. HRMS (CI, methane) m/z calculated for C₂₈H₂₇NO₇PS (M + 1) 552.124588, found 552.122968.

Diphenyl [N-Benzyloxycarbonylamino(4-methoxycarbonylphenyl)methyl] Phosphonate (9):

This compound was prepared in a similar manner as **1**, from 165 mg methyl-4-formylbenzoate (1.0 mmol), 150 mg benzyl carbamate (1.0 mmol), and 0.29 mL triphenyl phosphite (1.1 mmol) in 1 mL glacial acetic acid. The product recrystallized at 0 °C from dichloromethane/methanol as colorless crystalline needles (350 mg, 66% yield).

¹H NMR (DMSO, 500 MHz) δ 3.86 (s, 3H), 5.11 (ABqt Δν = 41.5 Hz, J = 12.5 Hz, 2H), 5.74 (dd, J = 23.1 Hz and J = 10.2 Hz, 1H), 7.01 (d, J = 8.3 Hz, 2H), 7.06 (d, J

= 8.8 Hz, 2H), 7.18-7.21 (m, 2H), 7.32-7.38 (m, 9H), 7.80 (dd, J = 8.8 Hz and 1.9 Hz, 2H), 7.99 (d, J = 8.3 Hz, 2H), 9.03 (d, J = 10.2 Hz, 1H). ¹³C NMR (DMSO, 300 MHz) δ 51.72, 52.19, 53.78, 66.26, 120.19, 120.23, 120.31, 125.28, 125.39, 127.92, 128.33, 128.73, 128.81, 129.19, 129.39, 129.42, 129.80, 129.88, 136.54, 139.73, 149.60, 149.74, 149.89, 150.03, 155.93, 156.04, 165.84. ³¹P NMR (DMSO, 300 MHz) δ 14.92. HRMS (CI, methane) m/z calculated for C₂₉H₂₇NO₇P (M + 1) 532.152516, found 532.151548.

Diphenyl [N-Benzyloxycarbonylamino(4-carboxyphenyl)-methyl] Phosphonate (10):

This compound was prepared in a similar manner as 1, from 750 mg 4-carboxybenzaldehyde (5.0 mmol), 750 mg benzyl carbamate (5.0 mmol), and 1.45 mL triphenyl phosphite (5.5 mmol) in 10 mL glacial acetic acid. The product recrystallized at 0 °C from methanol as a colorless solid (1.47 g, 57% yield).

¹H NMR (DMSO, 500 MHz) δ 5.11 (ABqt Δν = 40.7 Hz, J = 12.6 Hz, 2H), 5.72 (dd, J = 23.1 Hz and J = 10.2 Hz, 1H), 7.00 (d, J = 8.3 Hz, 2H), 7.06 (d, J = 8.3 Hz, 2H), 7.18 (m, 2H), 7.30-7.38 (m, 9H), 7.77 (dd, J = 8.5 Hz and 1.7 Hz, 2H), 7.96 (d, J = 8.3 Hz, 2H), 9.02 (d, J = 10.2 Hz, 1H), 13.03 (s, 1H). ¹³C NMR (DMSO, 300 MHz) δ 51.73, 53.82, 66.27, 120.20, 120.26, 120.33, 125.31, 125.41, 127.94, 128.37, 128.58, 128.66, 129.36, 129.83, 129.89, 130.65, 136.57, 139.23, 149.63, 149.77, 149.92, 150.06, 155.95, 156.06, 166.93. ³¹P NMR (DMSO, 300 MHz) δ 14.95. HRMS (CI, methane) m/z calculated for C₂₈H₂₅NO₇P (M + 1) 518.136866, found 518.137054.

Diphenyl [N-Benzyloxycarbonylamino(4-carbamoylphenyl)methyl] Phosphonate (11):

Compound 2 (360 mg, 0.70 mmol) and di-tert-butylidicarbonate (450 mg, 2.06 mmol) were partially dissolved in 12 mL THF/1 mL pyridine and stirred at 50 °C for 30 min, after which the starting material completely dissolved into the reaction mixture. Ammonium carbonate (400 mg, 4.16 mmol) was added to the reaction vessel and the solution was stirred for an additional 6 h under identical conditions. After completion, solvents were removed under rotatory evaporation and the solid residue was suspended in methanol. The solution was stirred upon gentle heating until dissolution to remove excess ammonia and the solvent volume was reduced. The product precipitated out of solution at -16 °C and was subsequently recrystallized from methanol at 0 °C as fine white crystals (270 mg, 75%).

¹H NMR (DMSO, 500 MHz) δ 5.11 (ABqt Δν = 40.1 Hz, J = 12.4 Hz, 2H), 5.68 (dd, J = 22.9 Hz and J = 10.2 Hz, 1H), 6.99 (d, J = 7.8 Hz, 2H), 7.05 (d, J = 8.8 Hz, 2H), 7.20 (t, J = 7.4 Hz, 2H), 7.31-7.38 (m, 9H), 7.40/7.99 (s, cis/trans, 2H), 7.72 (dd, J = 8.7 Hz

and 1.9 Hz, 2H), 7.88 (d, J = 8.3 Hz, 2H), 8.96 (d, J = 10.2 Hz, 1H). ^{13}C NMR (DMSO, 300 MHz) δ 51.65, 53.74, 66.24, 120.20, 120.26, 120.34, 125.28, 125.39, 127.54, 127.94, 128.26, 128.37, 129.81, 129.89, 134.12, 136.59, 137.46, 149.65, 149.79, 149.95, 150.08, 155.93, 156.04, 167.44. ^{31}P NMR (DMSO, 300 MHz) δ 14.99. HRMS (CI, methane) m/z calculated for $\text{C}_{28}\text{H}_{26}\text{N}_2\text{O}_6\text{P}$ (M + 1) 517.152850, found 517.152278.

Diphenyl [N-Benzyloxycarbonylamino(4-nitrophenyl)-methyl] Phosphonate (12):

This compound was prepared in a similar manner as **1**, from 1.5 g of 4-nitrobenzaldehyde (10.0 mmol), 1.5 g benzyl carbamate (10.0 mmol), and 2.9 mL triphenyl phosphite (11.0 mmol) in 4 mL glacial acetic acid. The product recrystallized from dichloromethane/methanol at 0 °C as a colorless solid (3.66 g, 71% yield).

^1H NMR (DMSO, 500 MHz) δ 5.11 (ABqt $\Delta\nu$ = 39.2 Hz, J = 12.7 Hz, 2H), 5.87 (dd, J = 23.3 Hz and J = 10.6 Hz, 1H), 7.03 (d, J = 8.3 Hz, 2H), 7.06 (d, J = 8.3 Hz, 2H), 7.21 (m, 2H), 7.32–7.38 (m, 9H), 7.95 (dd, J = 8.8 Hz and 1.9 Hz, 2H), 8.28 (d, J = 8.8 Hz, 2H), 9.10 (d, J = 10.2 Hz, 1H). ^{13}C NMR (DMSO, 300 MHz) δ 51.52, 53.59, 66.35, 120.17, 120.23, 120.26, 120.33, 123.50, 123.53, 125.38, 125.47, 127.95, 128.35, 129.65, 129.72, 129.85, 129.92, 136.50, 142.08, 147.30, 147.34, 149.56, 149.69, 149.83, 149.97, 155.90, 156.03. ^{31}P NMR (DMSO, 300 MHz) δ 14.40. HRMS (CI, methane) m/z calculated for $\text{C}_{27}\text{H}_{24}\text{N}_2\text{O}_7\text{P}$ (M + 1) 519.132115, found 519.131005.

Diphenyl [N-Benzyloxycarbonylamino(4-aminophenyl)-methyl] Phosphonate (13):

A 1.03 g (2 mmol) portion of **12** was added to a round-bottom flask containing 6 mL ethanol and 6 mL glacial acetic acid and was placed under gentle heating until dissolution. A 2 g portion of zinc dust was added to the solution and heated to reflux overnight. The resulting mixture was filtered and the filtrate solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate and washed with saturated sodium bicarbonate solution (x3). The organic layer was extracted, dried with magnesium sulfate, filtered, and the solvent was reduced under vacuum. The sample was triturated with diethyl ether to afford a solid, which was subsequently filtered and recrystallized with methanol/diethyl ether at -16 °C to yield the product as a yellow solid (805 mg, 83%).

^1H NMR (DMSO, 500 MHz) δ 3.60 (s, br), 5.11 (ABqt $\Delta\nu$ = 41.8 Hz, J = 11.7 Hz, 2H), 5.65 (dd, J = 22.1 Hz and J = 10.2 Hz, 1H), 6.99 (d, J = 8.3 Hz, 2H), 7.06 (d, J = 8.3 Hz, 2H), 7.20 (t, J = 7.3 Hz, 2H), 7.31–7.41 (m, 11H), 7.72 (dd, J = 8.3, 1.9 Hz, 2H), 8.95 (d, J = 10.2 Hz, 1H). ^{13}C NMR (DMSO, 300 MHz) δ 51.36, 53.45, 66.26, 120.22, 120.29,

120.36, 122.60, 125.30, 125.41, 127.95, 128.37, 129.71, 129.83, 129.91, 133.31, 136.59, 149.65, 149.79, 149.94, 150.08, 155.92, 156.04. ^{31}P NMR (DMSO, 300 MHz) δ 15.18. HRMS (CI, methane) m/z calculated for $\text{C}_{27}\text{H}_{25}\text{N}_2\text{O}_5\text{P}$ (M $^{+}$) 488.150111, found 488.151111.

5 Example 2: Ligand Docking Experiments

AutoDock 4.2 uses an empirical force field calculation using two Lennard-Jones potentials to calculate pairwise potentials for the van der Waals and hydrogen-bonding terms, as well as a Coulombic electrostatic and entropic potential parameters. The AutoDock scoring function was parametrized from 30 protein-ligand complexes and their binding constants, majority of which are in the protease class of proteins. Potential binding conformations and free energies were determined in silico for the selected α -aminoalkylphosphonates. The scoring function parameters used here omitted internal electrostatics in the calculation, an option allowable in the AutoDock suite.

Molecular docking experiments of α -aminoalkylphosphonate inhibitors with PSA were conducted using the software AutoDock 4.2 with AutoDock Tools. Ligand files were prepared using HyperChem and minimized with AMBER force field. The three-dimensional structure of human PSA was retrieved from the Protein Data Bank (PDB ID: 2ZCK). Receptor file was prepared by removing the light (L) and heavy (H) chains of the monoclonal antibody as well as water molecules in the structure file. Default Gasteiger charges were assigned to the receptor.

In order to model the Michaelis complex between the α -aminoalkylphosphonate inhibitor and PSA, a slightly modified flexible side chain method for covalent docking was utilized. The inhibitors were linked via covalent bond from the OG (oxygen of the hydroxyl group) atom of SER195 to the P atom of the inhibitor while removing a phenoxide from the structure to generate the pentavalent phosphorus. Chirality on the phosphorus stereocenter was selected to be the (R)-isomer, in order for the P=O of the phosphonate ester functional group to be in spatial proximity of the amide protons in the oxyanion hole, the likely source for the stabilization of the tetrahedral intermediate. For the docking study, water was chosen as the dummy ligand to calculate the free energy change from the flexible side chain. In order to prevent interactions from the water molecule and the rigid/flexible receptors, a Gaussian map was used to restrain the oxygen atom to the following coordinates: x -65.000, y -37.475, z -21.304. The Gaussian function is employed with zero energy at the designated site using a half-width setting of 5 and an energy barrier height of 1000 kJ. The grid parameters were as follows: box center -36.636, -37.475,

–21.304 (x, y, z), box points 126, 100, 100 (x, y, z), and 0.375 Å resolution. The Lamarckian Genetic Algorithm (LGA) search function was used with 2,500,000 energy calculations/run, 25 LGA runs, and with randomized starting position (tran0), orientation (quat0), and dihedrals (dihe0) as well as default Solis–Wets local search options. Flexible side chains with the lowest internal energy were chosen as the covalent binding pose, computationally modeled as the MD-predicted crystal structure of PSA-ligand Michaelis complex. A schematic of ligand binding serving as the basis for modeling the Michaelis complex is shown in Fig. 2.

To model the noncovalent binding of diphenyl α -aminoalkylphosphonates, two methods were employed. The first involved more conventional modeling methods described. First, the covalent map function in AutoDock Tools was utilized as a positional restraint on the phosphorus atom of the ligand in close proximity to the SER₁₉₅:OG atom (coordinates x –36.925, y –35.894, z –20.439) of the protein. The LGA search method was employed by randomizing initial position, orientation, and relative dihedrals. The grid box defining the binding search space was input as x center –36.636, y center –37.475, and z center –21.304 (x, y, z) with 100 points (20 Å) in each dimension and 0.200 Å grid resolution. Default LGA search was used with 25 independent runs with 2,500,000 calculations/run. The second method utilized the covalent ligand (flexible side chain) obtained from the Michaelis complex modeling experiments. The covalent linkage acts as a tether without the use of a Gaussian well. From those structures, the covalently bound ligand was removed from the protein and the diphenyl phenoxide moiety was reestablished, the ligand was minimized using AMBER, and docked into the protein binding site using identical grid parameters. Solis-Wets local minimization was used with 200,000 energy minimizations/run and 25 runs, with the upper and lower limit of rho set to 80.0 and 0.01, respectively. Initial position, orientation, and dihedrals were all conserved in these experiments. All other parameters were kept as default. This method is visually summarized in Fig. 2.

Example 3: Molecular Dynamics Simulation

MD simulations were performed using GROMACS version 4.6.5 with the AMBER ff99SB-ILDN force field. The S-enantiomer of **11** (referred to here as **S11**) was chosen for the simulations on the basis of its AutoDock 4.2 binding score. Ligand parameters were acquired with Antechamber and the AmberTools13 package along with the AM1-BCC method through USCF Chimera to assign partial charges. GROMACS usable topologies

were acquired through conversion via ACPYPE. Ligand topology, including atom type and partial charges, can be found in Table 3.

The PSA protein structure was obtained in the same manner as described in the molecular docking experiments, with the sugar moieties removed for the simulation. The PSA-S11 complex and PSA were centered in separate cubic boxes and each solvated using the TIP3P water model and SPC216 solvent configuration. Simulation parameters were obtained from AMBER ff99SB-ILDN parametrization procedure and accommodated to fit a standard for both systems. All Histidine residues within the protein structure were kept neutral for the simulation. Histidine residues 25, 48, 70, 75, 87, 91, 101, 161, 172, and 234 were protonated at the N_ε atom, and HIS₅₇ was protonated at the N_δ position. Charges of anionic/cationic residues assigned to the PSA structure can be found in Table 4. No additional ions were needed for the system to achieve electroneutrality. Short-range nonbonded interaction cutoffs were set to 1.0 nm, while the Particle Mesh Ewald (PME) algorithm was executed as the Coulomb-type to calculate long-range electrostatics. Dispersion correction was performed to account for energy and pressure cutoffs due to the Verlet cutoff-scheme. Periodic boundary conditions were set to allow free motion along the 3D lattice.

A steepest descent minimization removed improper atom contacts. Convergence was achieved when a maximum force of less than 1000 kJ mol⁻¹ nm⁻¹ resided on any atom. Sequentially, a two-step equilibration phase was used to independently simulate both constant volume (*NVT*) and constant pressure (*NPT*) ensembles. *NVT* ensembles of 50 ps (ps) were simulated for both systems, sustaining the temperature at 310 K, through the utilization of the velocity rescaling (v-rescale) thermostat. Protein and solvent atoms were thermally coupled separately. Subsequently, *NPT* equilibration was isotropically controlled using the Parrinello–Rahman barostat. Systems were simulated at intervals of 50 ps until pressures sustained at 1.0 bar with v-rescale thermostat for maintaining 310 K. Following *NPT* equilibration, MD simulations were conducted for 5 ns using the same conditions as described elsewhere herein.

Example 4: AutoDock Ligand Binding Structure Determination

To accumulate binding structures of diphenyl aminoalkylphosphonates derivatives, a unique method to determine docking conformations was used. Due to the rather large solvent-accessible volume surrounding the catalytic triad of PSA, unconstrained

standard docking procedures would yield a myriad of binding conformations when using LGA search, despite the number of LGA runs or energy calculations. As a consequence, major clusters of binding poses within 2.0 Å could not be obtained and resulted in at least several clusters that deviated greatly from one another. This is not only due to the
5 aforementioned binding space of PSA, but also the number of rotatable torsions allowed for the ligand, which further complicates the computationally exhaustive search.

In order to model the Michaelis complex of the bound inhibitor, the flexible side chain parameters of AutoDock was exploited as a covalent tether constraint. The flexible side chain method has several advantages; it avoids the clash penalty that arises from
10 neighboring atoms when using a single Gaussian map and keeps the scoring function intact while modeling a bound conformation of the inhibitor to the protein. Inhibitory compounds were constructed into the binding site of PSA via covalent linkage between SER₁₉₅ and the phosphonate moiety of the inhibitor (Fig. 4). Using a position-constrained water molecule as a dummy ligand, Michaelis complexes of PSA were minimized and the ligands were
15 determined using LGA search and AutoDock 4.2 scoring function. Poses generated were not only consistent with the general placement of moieties seen in homologue models, top hits from individual runs had clustered better than using a Gaussian constraint alone on the ligand to SER₁₉₅. Noncovalent ligand poses were generated by first cleaving the ligand side chain from the SER₁₉₅ residue, and then constructing the diphenylphosphonate ester moiety.

Noncovalent ligand searches were performed using a local search and the AutoDock scores are summarized in Table 1. All ligand docking experiments were performed with the major
20 ionization state of each ligand at biological pH. It must be noted that the result of the flexible side chain/dummy ligand experiment does not provide free energy scores. Covalent binding structures were sorted by intramolecular energies calculated between the covalently linked
25 ligand and the protein. Top Michaelis complex structures were selected, the ligand was cleaved from the protein, rebuilt, minimized locally, and redocked as a noncovalent molecule to obtain comparable docking scores.

Due to the chiral center involved, compounds isolated from reaction steps were racemic mixtures. As a consequence, both R- and S-enantiomers of the designed
30 compounds were scored using AutoDock. Although the enantiomers have scores that differ from one another, the S-enantiomers scored generally higher, the overall trend in ranking of molecules and their respective scores remains the same (*i.e.*, **11** scoring highest for both stereoisomers, **3** the lowest). Without wishing to be limited by any theory, the difference can

be attributed to the summation of favorable and nonfavorable interactions between non-S1 moieties, which were observed by slight variations in arrangement in space. Both enantiomer scores were within 1 kJ/mol from one another, with the exception being **3**.

The compounds selected were chosen based on their variety of functional groups in order to investigate their interactions with the P1 pocket residues. It was determined by the docking studies that **11** had scored the highest out of the compounds chosen for this study, with a free energy score of $-8.29/-9.14$ kJ·mol⁻¹ for R/S, respectively. Both stereoisomers were consistent with respect to their interactions between the P1 pocket residues of THR₁₉₀, SER₂₁₇, and SER₂₂₇ and the carbamoyl moiety of the ligand (Fig. 5). The model predicts that both hydroxyls of THR₁₉₀ and SER₂₂₇ form hydrogen bonds with the C=O of the carbamoyl, with distances of 2.2 and 2.5 Å, respectively. Concurrently, the amide proton forms a 2.2 Å hydrogen bond with the carbonyl of the SER₂₁₇ amide. Without wishing to be limited by any theory, the push-pull hydrogen bonding between the S1/P1 groups of the inhibitor/protein may stabilize the binding conformation.

As a comparison, a tyrosine-like analogue **4** was constructed to compare ligand binding poses. The compound was modeled to show similar hydrogen-bonding interactions with P1 residues, although not to the same extent as **11**. The compound still obtained a significant score of $-8.12/-8.42$ kJ·mol⁻¹ for the R/S enantiomers, respectively. Compound **10** scored third, with R/S scores of $-7.63/-7.89$ kJ·mol⁻¹. All three compounds displayed favorable polar interactions with P1 residues. Ligands with bulkier groups at the para-position of the phenyl ring scored worse, due to steric clashing within the binding space of the P1 pocket. As a consequence, ligands **3**, **7**, and **8** scored lower due to the van der Waals term penalty.

Compound **5**, which contains a cyano moiety, was predicted to have a strong affinity to the P1 site. According to the computational work, it predicts the likelihood of a polar interaction between SER₁₈₉ and THR₁₉₀ hydroxyls and the nitrile group. Without wishing to be limited by any theory, the highly polar nature of the cyano group within the environment of the polar P1 pocket resulted in significant scores of $-7.97/-8.14$ kJ·mol⁻¹ (R/S). Overall, the flexible side chain method provided results that resembled crystallographic structures in homology models previously published (Fig. 10).

Example 5: MD Trajectories

MD simulations presented here were performed using the GROMACS bundle. The starting ligand binding pose of **11** was taken from AutoDock molecular docking screens,

choosing the more optimal S-enantiomer (referred to as **S11** within this Example).

Compound **S11** was modeled dynamically to test its overall binding stability, deviations of protein structure, fluctuations of local residues, and the retention of ligand hydrogen bond contacts.

5 To validate the stability of the binding complex, MD analyses were performed using the Visual Molecular Dynamics (VMD) package and the trajectory tools provided by GROMACS. Root-mean squared deviation of the PSA-**S11** complex and PSA control are shown in Fig. 6. In both simulations, global protein trajectories were nearly identical in the retention of the folded PSA structure. The average root-mean squared deviation (RMSD)
10 trajectory values were below 0.20 nm for PSA with the inhibitor retained in the binding cavity. **S11** retained localization of its moieties within the binding cavity of PSA. The p-carbamoylphenyl group was highly retained in the P1 pocket with very little fluctuation and no significant torsion twists. The N-carboxybenzyl (Cbz) side group was conserved through the Classic Kallikrein Loop (CKL) toward the P3 site of PSA. The diphenoxy groups of **S11**
15 deviate significantly throughout the simulation due to high solvent accessibility and very little interaction with PSA to stabilize its local trajectory.

Secondary structure changes were closely observed during the simulation, but no substantial early folding events were found. However, during the simulations of PSA and PSA-**S11**, the secondary structure of the CKL region temporary shifted between 3 and 10
20 helices to alpha helices/loops.

Root-mean squared fluctuation (RMSF) plots were taken to compare side chain trajectory changes upon ligand binding. Overall, RMSF of both simulations are very similar (Fig. 7). The exception to this is ARG_{95G}, a CKL residue, and its involvement in the PSA-**S11** complex was interesting when comparing the two simulations. The RMSF of the
25 CKL was significant in both runs, but ARG_{95G} fluctuated significantly less with **S11** bound. The RMSF of the residue was 0.34 and 0.17 nm for the unbound and bound runs, respectively. ARG_{95G}, a highly polar amino acid, could fluctuate tremendously with high solvent accessibility but this movement was reduced in the presence of **S11**. Without wishing to be limited by any theory, this may be caused by a hydrogen bonding contact between the
30 proton-donating guanidine and the carbonyl of the Cbz protecting group (noted here as ^{Cbz}CONH). The prevalence of these hydrogen bonding contacts are illustrated in Table 2. During the course of the simulation, the carbonyl group hydrogen bonds with the terminal and internal amines of the residue with a prevalence of 62.8% and 12.4%, respectively. Taking these contacts into consideration, reduction of the RMSF of ARG_{95G} upon ligand

binding may play a role in binding stability. B-Factors from the crystal structure of PSA were compared to the MD studies display good qualitative agreement in RMSF of residues.

Additionally, the frequency of S1/P1 hydrogen bonding interactions were mapped during the simulations. This allowed observing the retention of these contacts that were initially observed during the docking simulations, which provide only a static map of the interactions. Specifically, hydrogen bonding between P1 residues THR₁₉₀, SER₂₁₇, and SER₂₂₇ and the carbamoyl moiety (noted here as ^{Aryl}CONH₂) of the ligand were thought to be exclusively involved. The PSA-S11 hydrogen bond map was relatively consistent with the polar contacts observed in AutoDock with all three aforementioned residues in the P1 site.

Some interactions were mostly conserved during the simulation time, such as the 61.6% prevalence between the hydroxyl group of SER₂₂₇ and the carbonyl (^{Aryl}CONH₂) of S11. Some interactions were moderately retained during the simulation time, such as residue interactions with THR₁₉₀ and SER₂₁₇, with hydrogen bonding contact incidences of 20.4% and 28.4%, respectively. Contacts with low frequency of occurrence within the P1 pocket include some contacts not mapped during the docking simulations, such as SER₁₉₂. Without not wishing to be limited by any theory, this incidental contact may not be a major contributor to the stabilization of the binding complex, due to its location on the outer P1 pocket. Additionally, hydrogen bonding between GLY₁₉₃ and the phosphonate ester were highly conserved during the simulation, contributing to the retained trajectory of moiety to its local environment despite high solvent accessibility.

Interactions with P1 residues were retained during the course of the simulation with no significant ligand conformational changes evidenced. The stability of this complex throughout the duration of the simulation is consistent with the proposed binding orientation in the pose space.

Example 6: Dose-Dependent Response Assays

To assess inhibitor potency on PSA, kinetic assays were conducted using chromogenic substrate S-2586 as a method to measure peptide cleavage via p-nitroaniline as a product formation, the liberated chromophore. Dose-dependent response curves were obtained by measuring enzymatic activity of PSA with a dose of substrate and inhibitor. The control in these experiments were represented by the average kinetic rate of substrate cleavage with a blank dose of DMSO. IC₅₀ values were calculated based on the average enzymatic rate of PSA as compared to the control. Final values were estimated using the 4-

point log (4PL) method to determine concentrations at which 50% PSA activity inhibition can be extrapolated from the response curves.

The kinetic response curves for PSA with varying doses of the synthesized inhibitors (R/S mixture) were measured. As one example, Fig. 8 illustrates the kinetic response curve for **11**. By varying inhibitor concentrations from 75 nM to 1 μ M, a clear dose-dependent response was obtained. Concentrations chosen to calculate IC_{50} values were determined by initially measuring PSA activities using total inhibitor concentrations ranging from 75 nM to 250 μ M. Subsequent assays were performed to optimally determine concentrations closest to the inflection point in order to properly estimate IC_{50} values using the 4PL method. Plateau values of the sigmoidal curve where dose concentrations correlated with approximately zero or 100% activity observed were avoided in order to properly use the logistic function.

From using the 4PL method to determine IC_{50} values, a broad range of potencies was observed from this selective class of compounds (Table 1). According to its concentration response curve, compound **4** demonstrated only modest inhibition of PSA with an IC_{50} of 7.0 μ M. **4** tested as a potent inhibitor of PSA. Another compound with similar activity was **10**, with an estimated IC_{50} of 7.8 μ M. Overall, **11** displayed strong dose-dependent inhibition of PSA with an IC_{50} of approximately 250 nM. From this data, the p-carbamoyl appears to be specific in binding to PSA, which could be due to being more specific to the P1 site of the enzyme than the p-hydroxy group of **4**.

IC_{50} values for compounds **2**, **3**, and **5** were not determined. These compounds lacked the desired solubility for the designed buffer system containing 10% DMSO and concentration range to properly determine dose concentration at 50% PSA activity. As a consequence, extrapolation of a $\log IC_{50}$ value from the dose response curve could not be calculated. Instead, relative enzymatic activities at 250 μ M final concentration were reported.

Example 7: Comparison of Docking Scores vs Experimental Values

In order to assess the validity of the computational model, $\log IC_{50}$ values extrapolated from dose-dependent response curves were plotted against docking scores obtained from the AutoDock scoring algorithm. Using the method described, empirical and model results correlated well within the bounds established in the protocol (Fig. 9). The comparison of ten compounds and their scores resulted in a $R^2 = 0.732$. Since the enantiomers of the compounds were not separated and tested individually in the kinetic

assays, both R- and S-enantiomer scores of a compound were plotted vs identical log IC₅₀ values. Compounds **2**, **3**, and **5** were omitted from the plot since IC₅₀ values could not be determined due to the limits of the experiment.

The results were compared to the more naive method of molecular docking, utilizing a global search with a single atom constraint on the phosphorus atom of the inhibitor to the hydroxyl functional group of SER₁₉₅. The comparison in both instances yielded no appreciable match in trend between the model and the experimental data ($R^2 = 0.042$).

Without wishing to be limited by any theory, this may be explained by the size of the search space within the binding site of the protein. With significant volume size in a site of interest, false positives become more visible due to the expanded conformational degrees of freedom of the ligand. As a consequence, improper poses not analogous to crystallographic data are incorrectly scored.

Overall, the docking energies using the single-atom constraint scored lower than the covalent tether method. The penalty that arises from steric clashes with neighboring atoms in proximity to the Gaussian well factor into the lower scores obtained. The lack of parameter settings in the AutoDock suite hinders the accuracy of this method. Traditional methods failed to provide a top pose that resembled crystallographic structures obtained from homology models without imposing some selection bias. The flexible side-chain approach was able to circumvent these issues. As a result, the expanded flexible side-chain methodology described in this paper is a suitable choice as a predictor of drug potency.

Taken together, the present results describe an effective method for the development of novel PSA inhibitors using a convenient synthetic route to yield diphenyl α -aminoalkylphosphonates. Using the molecular docking software AutoDock, binding at the S1 position of the substrate was optimized. The modeling methodology employed demonstrated a significant correlation between in silico ligand binding energies to in vitro dose-dependent inhibition values. From the modeling, the push-pull hydrogen bonding arrangement between S1/P1 functionalities plays a role in yielding favorable docking scores, specifically the interactions between the P1 residues of THR₁₉₀, SER₂₁₇, and SER₂₂₇ and the carbamoyl group of the inhibitor. Through MD simulations via GROMACS, these interactions were consistent throughout the 5 ns simulation without major fluctuations of the ligand within the binding cavity.

The IC₅₀ of **5** was lower than predicted from the molecular docking studies. Without wishing to be limited by any theory, this may be due to assignment of partial charges in the AutoDock suite, thus leading to improperly scoring a polar interaction between P1

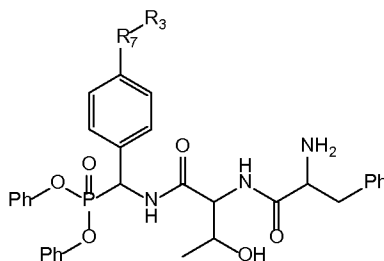
residues with the cyano moiety. Atom type assignments in AutoDock can be adjusted to better characterize the aryl-cyano group topology and resolve issues regarding false positives. As with any de novo drug design, ligand topologies may sometimes be modified to determine more probable charge distributions.

5

Example 8: Comparison of Docking Scores vs Experimental Values

Modeling studies were performed with dipeptidyl derivatives of α -aminoalkylphosphonates. Protein-ligand binding was dynamically evaluated using GROMACS MD simulation. Results are summarized in Table 5.

10 **Table 1.** AutoDock noncovalent binding scores of diphenylphosphonate ester compounds selected and their stereoisomers and IC_{50} concentrations as a comparison (IC_{50} values were obtained from the racemic mixture of each compound).



compound	R-group	binding score (kJ/mol)	IC ₅₀
1	H	R: -6.82 S: -7.41	79.9 μ M
2	F	R: -7.07 S: -7.43	43.4% @250 μ M
3	C(CH ₃) ₃	R: -4.70 S: -6.06	53.2% @250 μ M
4	OH	R: -8.12 S: -8.42	7.0 μ M
5	CN	R: -8.14 S: -7.97	68.8% @250 μ M
6	OCH ₃	R: -7.55 S: -6.61	40.8 μ M
7	N(CH ₃) ₂	R: -6.86 S: -6.67	77.4 μ M
8	SO ₂ CH ₃	R: -7.11 S: -6.45	77 μ M
9	CO ₂ CH ₃	R: -7.03 S: -7.32	91.9 μ M
10	CO ₂ H	R: -7.63 S: -7.89	7.8 μ M
11	CONH ₂	R: -8.29 S: -9.14	0.25 μ M
12	NO ₂	R: -6.92 S: -6.63	137 μ M
13	NH ₂	R: -7.51 S: -7.65	16.7 μ M

The compound diphenyl-[(2-(2-amino-3-phenylpropanamido)-3-hydroxybutanamido)(4-carbamoylphenyl)methyl]phosphonate (Fig. 1) was found to bind to PSA with an IC₅₀ of 250 nM.

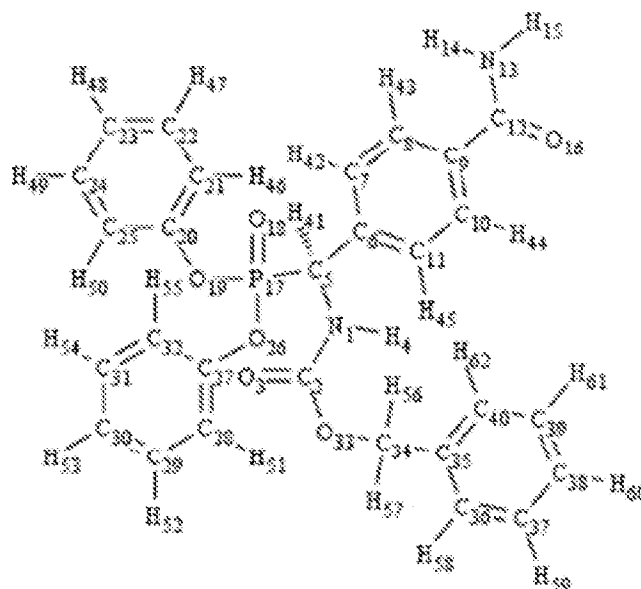
Table 2. Hydrogen-bond prevalence map between **S11** and PSA during the 5 ns MD simulation.

H-donor	donor atom	H-acceptor	acceptor atom	% existence
ligand	^{Chc} CONH	SER ₂₁₄	CONH	47.071
ligand	^{Aryl} CONH ₂	THR ₁₉₀	CONH	7.259
ligand	^{Aryl} CONH ₂	SER ₁₉₂	OH	5.679
ligand	^{Aryl} CONH ₂	SER ₂₁₇	CONH	28.454
ARG _{95G}	NHC(NH ₂) ₂ ⁺	ligand	^{Chc} CONH	12.418
ARG _{95G}	NHC(NH ₂) ₂ ⁺	ligand	^{Chc} CONH	62.827
THR ₁₉₀	OH	ligand	^{Aryl} CONH ₂	20.396
GLY ₁₉₃	CONH	ligand	PO(OPh)	59.588
GLY ₁₉₃	CONH	ligand	PO(OPh)	4.179
SER ₁₉₅	OH	ligand	PO(OPh)	2.699
SER ₂₇₇	OH	ligand	^{Aryl} CONH ₂	61.588

^aInteracting atom pairs are in bold.

5

Table 3. Atom type and partial charges for compound **S11** used in MD solution.

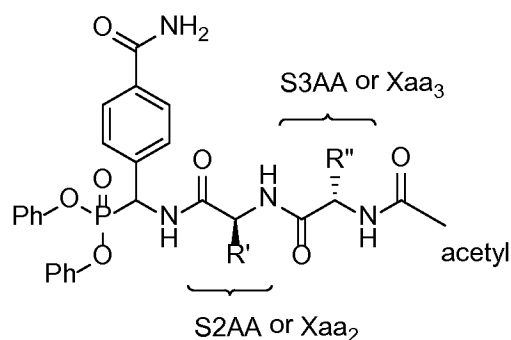


Name	Type	q	Name	Type	q
N1	n	-0.4699	C32	ca	-0.1477
C2	c	0.724101	O33	os	-0.4369
O3	o	-0.573	C34	c3	0.1917
H4	hn	0.3115	C35	ca	-0.1153
C5	c3	-0.0932	C36	ca	-0.118
C6	ca	-0.1133	C37	ca	-0.125
C7	ca	-0.11	C38	ca	-0.127
C8	ca	-0.0925	C39	ca	-0.125
C9	ca	-0.1196	C40	ca	-0.118
C10	ca	-0.0925	H41	hl	0.1267
C11	ca	-0.11	H42	ha	0.154
C12	c	0.667701	H43	ha	0.151
N13	n	-0.67	H44	ha	0.151
H14	hn	0.3155	H45	ha	0.154
H15	hn	0.3155	H46	ha	0.1517
O16	o	-0.6061	H47	ha	0.1353
P17	p5	1.495501	H48	ha	0.134
O18	o	-0.7475	H49	ha	0.1353
O19	os	-0.513	H50	ha	0.1517
C20	ca	0.1506	H51	ha	0.1517
C21	ca	-0.1477	H52	ha	0.1353
C22	ca	-0.1133	H53	ha	0.134
C23	ca	-0.139	H54	ha	0.1353
C24	ca	-0.1133	H55	ha	0.1517
C25	ca	-0.1477	H56	hl	0.0812
O26	os	-0.513	H57	hl	0.0812
C27	ca	0.1506	H58	ha	0.1355
C28	ca	-0.1477	H59	ha	0.1345
C29	ca	-0.1133	H60	ha	0.133
C30	ca	-0.139	H61	ha	0.1345
C31	ca	-0.1133	H62	ha	0.1355

Table 4. Charges of residue on PSA residues with anionic residues (formal charge -1) and cationic residues (formal charge +1). PRO₂₄₇ and ILE₁₆ were assigned formal charges of -1 and +1, respectively, due to the terminal carboxylate on PRO247 and terminal ammonium on ILE₁₆.

Anionic Residue		Cationic Residue	
GLU 21	GLU23	ILE16	LYS24
GLU77	ASP78	ARG36	ARG38
ASP95	ASP97	ARG60	LYS62
ASP98	ASP102	ARG69	LYS95E
GLU110	GLU113	ARG95G	ARG95J
ASP116	ASP122	ARG107	LYS119
GLU129	GLU145	LYS153	LYS154
GLU147	GLU148	LYS175	LYS178
ASP159	ASP166	ARG185	LYS188
ASP194	GLU218	ARG225	LYS231
GLU224	ASP241	ARG236	LYS237
PRO247		LYS240	

Table 5. Protein-ligand binding results obtained for dipeptidyl derivatives of α -aminoalkylphosphonates.



N-term (S3AA)	C-term (S2AA)	Score (SDPE1)	N-term (S3AA)	C-term (S2AA)	Score (SDPE1)
Ala	Ala	-9.32	Lys	Ala	-7.55
Ala	Arg	-7.15	Lys	Arg	-6.1
Ala	Asn	-6.91	Lys	Asn	-8.31
Ala	Asp	-8.82	Lys	Asp	-5.28
Ala	Gln	-9.53	Lys	Gln	-8.97
Ala	Glu	-9.13	Lys	Glu	-6.65
Ala	His	-9.41	Lys	His	-6.64
Ala	Ile	-9.8	Lys	Ile	-8.71
Ala	Leu	-9.39	Lys	Leu	-7.78
Ala	Lys	-9.11	Lys	Lys	-7.56
Ala	Met	-9.48	Lys	Met	-6.23
Ala	Phe	-9.96	Lys	Phe	-9.54
Ala	Pro	-8.95	Lys	Pro	-7.47
Ala	Ser	-8.12	Lys	Ser	-8.08
Ala	Thr	-7.98	Lys	Thr	-6.97
Ala	Trp	-10.2	Lys	Trp	-9.23
Ala	Tyr	-9.63	Lys	Tyr	
Ala	Val	-9.66	Lys	Val	-9.65
Ala	Gly	-8.67	Lys	Gly	-11.29
Arg	Ala	-7.45	Met	Ala	-8.62
Arg	Arg	-8.57	Met	Arg	-6.88
Arg	Asn	-8.9	Met	Asn	-8.33
Arg	Asp	-8.26	Met	Asp	-8.14
Arg	Gln	-7.94	Met	Gln	-10.5
Arg	Glu	-9.96	Met	Glu	-7.08
Arg	His	-8.28	Met	His	-8.26
Arg	Ile	-9.74	Met	Ile	-9.09
Arg	Leu	-8.34	Met	Leu	-8.81
Arg	Lys	-8.15	Met	Lys	-8.41
Arg	Met	-7.96	Met	Met	-6.92
Arg	Phe	-8.79	Met	Phe	-8.63

Arg	Pro	-8.13	Met	Pro	-7.4
Arg	Ser	-8.71	Met	Ser	-8.47
Arg	Thr	-8.47	Met	Thr	-8.59
Arg	Trp	-8.13	Met	Trp	-10.67
Arg	Tyr	-8.07	Met	Tyr	-8.17
Arg	Val	-8.46	Met	Val	-7.72
Arg	Gly		Met	Gly	-8.83
Asn	Ala	-8.8	Ser	Ala	-7.84
Asn	Arg	-7.49	Ser	Arg	-7.51
Asn	Asn	-7.64	Ser	Asn	-9.11
Asn	Asp	-7.64	Ser	Asp	-8.34
Asn	Gln	-7.56	Ser	Gln	-9.04
Asn	Glu	-6.9	Ser	Glu	-8.99
Asn	His	-8.41	Ser	His	-8.75
Asn	Ile	-8.48	Ser	Ile	-9.35
Asn	Leu	-8.3	Ser	Leu	-9.48
Asn	Lys	-8.08	Ser	Lys	-7.39
Asn	Met	-10.04	Ser	Met	-8.84
Asn	Phe	-10.12	Ser	Phe	-9.46
Asn	Pro	-8.66	Ser	Pro	-9.35
Asn	Ser	-8.08	Ser	Ser	-7.11
Asn	Thr	-7.32	Ser	Thr	-8.51
Asn	Trp	-8.48	Ser	Trp	-8.67
Asn	Tyr	-9.63	Ser	Tyr	-8.74
Asn	Val	-9.94	Ser	Val	-8.55
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Asp	Ala	-6.97	Thr	Ala	-8.17
Asp	Arg	-7.47	Thr	Arg	-8.82
Asp	Asn	-9.46	Thr	Asn	-9.57
Asp	Asp	-6.97	Thr	Asp	-6.43
Asp	Gln	-7.95	Thr	Gln	-9.06
Asp	Glu	-7.65	Thr	Glu	-8.48
Asp	His	-7.36	Thr	His	-7.41
Asp	Ile	-9.02	Thr	Ile	-9.07
Asp	Leu	-7.22	Thr	Leu	-8.85
Asp	Lys	-7.7	Thr	Lys	-6.53
Asp	Met	-8.26	Thr	Met	-9.33
Asp	Phe	-9.19	Thr	Phe	-9.36
Asp	Pro	-7.28	Thr	Pro	-8.1
Asp	Ser	-7.64	Thr	Ser	-8.53
Asp	Thr	-7.2	Thr	Thr	-8.48
Asp	Trp	-9.35	Thr	Trp	-9.33
Asp	Tyr	-8.81	Thr	Tyr	-8.93
Asp	Val	-8.47	Thr	Val	-9.33

Asp	Gly	-8.28	Thr	Gly	-7.8
Gln	Ala	-8.56	Trp	Ala	-11.3
Gln	Arg	-6.68	Trp	Arg	-7.68
Gln	Asn	-8.28	Trp	Asn	-7.97
Gln	Asp	-6.76	Trp	Asp	-6.43
Gln	Gln	-9.19	Trp	Gln	-9.46
Gln	Glu	-7.48	Trp	Glu	-9.52
Gln	His	-9.16	Trp	His	-9.01
Gln	Ile	-6.96	Trp	Ile	-8.04
Gln	Leu	-8.8	Trp	Leu	-9.48
Gln	Lys	-6.63	Trp	Lys	
Gln	Met	-8.7	Trp	Met	-8.75
Gln	Phe	-8.24	Trp	Phe	-9.57
Gln	Pro	-8.2	Trp	Pro	-10.83
Gln	Ser	-7.33	Trp	Ser	-10.28
Gln	Thr	-7.3	Trp	Thr	-8.74
Gln	Trp	-8.88	Trp	Trp	-9.18
Gln	Tyr	-9.29	Trp	Tyr	-9.2
Gln	Val	-8.02	Trp	Val	-6.03
Gln	Gly	-8.15	Trp	Gly	-10.2
Glu	Ala	-8	Tyr	Ala	-10.36
Glu	Arg	-7.63	Tyr	Arg	-6.95
Glu	Asn	-7.6	Tyr	Asn	-10.37
Glu	Asp	-7.51	Tyr	Asp	-8.36
Glu	Gln	-8.25	Tyr	Gln	-8.83
Glu	Glu	-7.21	Tyr	Glu	-8.94
Glu	His	-7.71	Tyr	His	-6.56
Glu	Ile	-7.77	Tyr	Ile	-10.01
Glu	Leu	-7.42	Tyr	Leu	-7.26
Glu	Lys	-8.18	Tyr	Lys	-9.45
Glu	Met	-9.08	Tyr	Met	-8.05
Glu	Phe	-8.44	Tyr	Phe	-10.1
Glu	Pro	-7.73	Tyr	Pro	-8.09
Glu	Ser	-7.82	Tyr	Ser	-8.83
Glu	Thr	-8.29	Tyr	Thr	-8.07
Glu	Trp	-8.24	Tyr	Trp	-9.65
Glu	Tyr	-7.54	Tyr	Tyr	-10
Glu	Val	-8.28	Tyr	Val	-11.31
Glu	Gly	-7.44	Tyr	Gly	-10.61
His	Ala	-9.24	Val	Ala	-8.71
His	Arg	-6.8	Val	Arg	-8.15
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His	Asp	-7.01	Val	Asp	-7.82
His	Gln	-9.08	Val	Gln	-10.32

His	Glu	-8.2	Val	Glu	-7.12
His	His	-9.5	Val	His	-8.06
His	Ile	-11.65	Val	Ile	-10.02
His	Leu	-7.93	Val	Leu	-12.25
His	Lys	-8.95	Val	Lys	-8.84
His	Met	-10.35	Val	Met	-8.86
His	Phe	-9.08	Val	Phe	-9.9
His	Pro	-6.83	Val	Pro	-7.64
His	Ser	-6.96	Val	Ser	-8.84
His	Thr	-7.24	Val	Thr	-8.51
His	Trp	-9.67	Val	Trp	-8.98
His	Tyr	-8.46	Val	Tyr	-9.37
His	Val	-8.19	Val	Val	-9.55
His	Gly	-8.85	Val	Gly	-9.13
Ile	Ala	-8.43	Gly	Ala	-8.43
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Ile	Asn	-8.92	Gly	Asn	-8.57
Ile	Asp	-6.99	Gly	Asp	-8.31
Ile	Gln	-9.54	Gly	Gln	-9.91
Ile	Glu	-9.41	Gly	Glu	-8.28
Ile	His	-8.32	Gly	His	-9.46
Ile	Ile	-7.92	Gly	Ile	-9.8
Ile	Leu	-9.28	Gly	Leu	-9.54
Ile	Lys	-9.61	Gly	Lys	-10.35
Ile	Met	-8.82	Gly	Met	-9.35
Ile	Phe	-9.34	Gly	Phe	-10.3
Ile	Pro	-9.31	Gly	Pro	-8.49
Ile	Ser	-9.14	Gly	Ser	-7.4
Ile	Thr	-9.35	Gly	Thr	-8.92
Ile	Trp		Gly	Trp	-10.85
Ile	Tyr	-9.82	Gly	Tyr	-8.89
Ile	Val	-8.01	Gly	Val	-9.11
Ile	Gly	-9.98	Gly	Gly	-9.07
Leu	Ala	-7.53			
Leu	Arg	-6.71			
Leu	Asn	-9.21			
Leu	Asp	-6.06			
Leu	Gln	-8.36			
Leu	Glu	-7.44			
Leu	His	-6.93			
Leu	Ile	-9.49			
Leu	Leu	-10.31			
Leu	Lys	-9.24			
Leu	Met	-7.81			

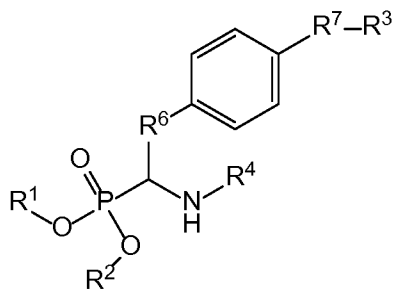
Leu	Phe	-9.84
Leu	Pro	-7.03
Leu	Ser	-9.42
Leu	Thr	-8.49
Leu	Trp	-8.14
Leu	Tyr	-8.71
Leu	Val	-8.58
Leu	Gly	-8.16

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. While this invention has been disclosed with reference to specific embodiments, it is apparent that other
5 embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

CLAIMS

What is claimed:

1. A compound of formula (I), or a salt or solvate thereof:



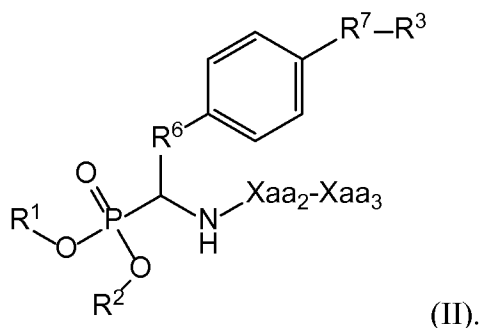
(I), wherein in (I):

- R^1 and R^2 are independently selected from the group consisting of H, C_1 - C_{10} alkyl, substituted C_1 - C_{10} alkyl, phenyl, and substituted phenyl;
- R^3 is selected from the group consisting of H, F, Cl, Br, I, C_1 - C_{10} alkyl, substituted C_1 - C_{10} alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, amino, substituted amino, -CN, -OH, alkoxy, carbamoyl, -COOH, -CHO, alkoxycarbonyl, aminosulfonyl, alkylsulfonyl, and phosphoryl;
- R^4 is selected from the group consisting of R^5 , a sugar, an oligosaccharide, an amino acid and a peptide, wherein the sugar, oligosaccharide, amino acid or peptide is optionally substituted;
- R^5 is selected from the group consisting of H, C_1 - C_{10} alkyl, substituted C_1 - C_{10} alkyl, phenyl, substituted phenyl, acyl, alkoxycarbonyl, aminocarbonyl, and Fmoc;
- R^6 and R^7 are independently selected from the group consisting of bond, $-(CH_2)_n-$, -O-, -O(C=O)-, -C(=O)-, -C(=O)O-, -OC(=O)O-, -NHC(=O)-, -C(=O)NH-, -C(=NH)NH-, -C(=NH₂)NH- and -NHC(=O)NH-; and, each occurrence of n is independently an integer ranging from 1 to 10.

2. The compound of claim 1, wherein R^6 and R^7 are each a bond.

3. The compound of claim 1, wherein R^4 is an amino acid or a peptide.

4. The compound of claim 3, wherein the peptide comprises the dipeptide Xaa₂-Xaa₃, wherein the C-terminus of Xaa₂ is covalently bound through an amide bond to the NH group in (I) to provide (II):



5. The compound of claim 4, wherein Xaa₂ comprises an amino acid selected from the group consisting of Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, His, Ser, Thr, Gln, Glu, Asn, Asp, Lys, Arg, Met, Cys and cystine.

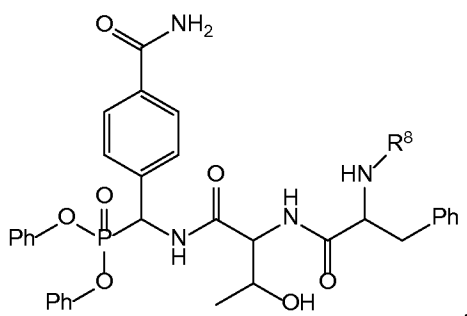
6. The compound of claim 4, wherein Xaa₃ comprises an amino acid selected from the group consisting of Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, His, Ser, Thr, Gln, Glu, Asn, Asp, Lys, Arg, Met, Cys and cysteine.

7. The compound of claim 4, wherein the N-terminus of Xaa₃ is derivatized with a group selected from the group consisting of C₁-C₆ alkyl, acetyl, benzyloxycarbonyl, *tert*-butyloxycarbonyl, benzylsulfonyl, *para*-methylphenylsulfonyl, fluorenylmethyloxycarbonyl, pivaloyl, alloxycarbonyl, pentafluorophenoxycarbonyl, methoxycarbonyl, *para*-methoxybenzyloxycarbonyl, methoxyethoxymethoxycarbonyl, and trichloroethoxycarbonyl.

8. The compound of claim 4, wherein Xaa₂-Xaa₃ comprises at least one selected from the group consisting of Leu-Val, Ile-His, Tyr-Pro, Val-Tyr, Ala-Trp, Gly-Lys, Trp-Gly, Phe-Pro, Pro-Trp, Met-Pro, and Trp-Met.

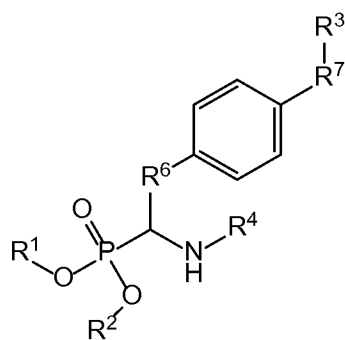
9. The compound of claim 4, wherein Xaa₂-Xaa₃ comprises at least one selected from the group consisting of Gly-Tyr, His-Pro, Gln-Met, Asn-Tyr, Ala-Tyr, Lys-Gly, Met-His, Gln-Val, Leu-Leu, Phe-Gly, Ser-Trp, Ser-Phe, Trp-Ala, Gly-Trp, Val-Phe, Phe-Asn, Phe-Tyr, Met-Asn, Ile-Val, Ile-Tyr, and Tyr-Tyr.

10. The compound of claim 1, wherein the compound is at least one selected from the group consisting of diphenyl-((2-(2-(N-R⁸)-amino-3-phenylpropanamido)-3-hydroxybutanamido)(4-carbamoyl phenyl) methyl)phosphonate:



wherein R⁸ is H or a protecting selected from the group consisting of acetyl, benzyloxycarbonyl, *tert*-butyloxycarbonyl, benzylsulfonyl, *para*-methylphenylsulfonyl, fluorenylmethyloxycarbonyl, pivaloyl, alloxycarbonyl, pentafluorophenoxycarbonyl, methoxycarbonyl, *para*-methoxybenzyloxycarbonyl, methoxyethoxymethoxycarbonyl, and trichloroethoxycarbonyl.

11. A method of treating or preventing prostate cancer in a male subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a compound of formula (I) or a salt or solvate thereof.



(I), wherein in (I):

R¹ and R² are independently selected from the group consisting of H, C₁-C₁₀ alkyl, substituted C₁-C₁₀ alkyl, phenyl, and substituted phenyl;

R³ is selected from the group consisting of H, F, Cl, Br, I, C₁-C₁₀ alkyl, substituted C₁-C₁₀ alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, amino, substituted amino, -CN, -OH, alkoxy, carbamoyl, -COOH, -CHO, alkoxycarbonyl, aminosulfonyl, alkylsulfonyl, and phosphoryl;

R⁴ is selected from the group consisting of R⁵, a sugar, an oligosaccharide, an amino acid and a peptide, wherein the sugar, oligosaccharide, amino acid or peptide is optionally substituted;

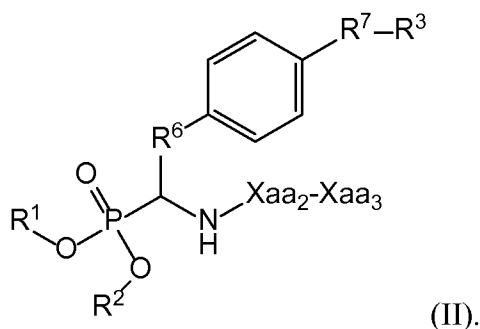
R^5 is selected from the group consisting of H, C_1 - C_{10} alkyl, substituted C_1 - C_{10} alkyl, phenyl, substituted phenyl, acyl, alkoxy carbonyl, aminocarbonyl, and Fmoc;

R^6 and R^7 are independently selected from the group consisting of bond, $-(CH_2)_n-$, $-O-$, $-O(C=O)-$, $-C(=O)-$, $-C(=O)O-$, $-OC(=O)O-$, $-NHC(=O)-$, $-C(=O)NH-$, $-C(=NH)NH-$, $-C(=NH_2)NH-$ and $-NHC(=O)NH-$; and,

each occurrence of n is independently an integer ranging from 1 to 10.

12. The method of claim 11, wherein R^4 is an amino acid or a peptide.

13. The method of claim 12, wherein the peptide comprises the dipeptide Xaa_2 - Xaa_3 , wherein the C-terminus of Xaa_2 is covalently bound through an amide bond to the NH group in (I) to provide (II):



14. The method of claim 13, wherein Xaa_2 and Xaa_3 independently comprise an amino acid selected from the group consisting of Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, His, Ser, Thr, Gln, Glu, Asn, Asp, Lys, Arg, Met, Cys and cystine.

15. The method of claim 13, wherein the N-terminus of Xaa_3 is derivatized with a group selected from the group consisting of C_1 - C_6 alkyl, acetyl, benzyloxycarbonyl, *tert*-butyloxycarbonyl, benzylsulfonyl, *para*-methylphenylsulfonyl, fluorenylmethyloxycarbonyl, pivaloyl, alloxycarbonyl, pentafluorophenoxycarbonyl, methoxycarbonyl, *para*-methoxybenzyloxycarbonyl, methoxyethoxymethoxycarbonyl, and trichloroethoxycarbonyl.

16. The method of claim 13, wherein Xaa_2 - Xaa_3 comprises at least one selected from the group consisting of Leu-Val, Ile-His, Tyr-Pro, Val-Tyr, Ala-Trp, Gly-Lys, Trp-Gly, Phe-Pro, Pro-Trp, Met-Pro, and Trp-Met.

17. The method of claim 13, wherein Xaa₂-Xaa₃ comprises at least one selected from the group consisting of Gly-Tyr, His-Pro, Gln-Met, Asn-Tyr, Ala-Tyr, Lys-Gly, Met-His, Gln-Val, Leu-Leu, Phe-Gly, Ser-Trp, Ser-Phe, Trp-Ala, Gly-Trp, Val-Phe, Phe-Asn, Phe-Tyr, Met-Asn, Ile-Val, Ile-Tyr, and Tyr-Tyr.

18. The method of claim 11, wherein PSA activity in the subject is inhibited.

19. The method of claim 11, wherein the growth of at least one prostate cancer cell in the subject is inhibited.

20. The method of claim 11, wherein the release of at least one growth factor selected from the group consisting of IGF-I, IGFBP-2 and IGFBP-3 is inhibited in the subject.

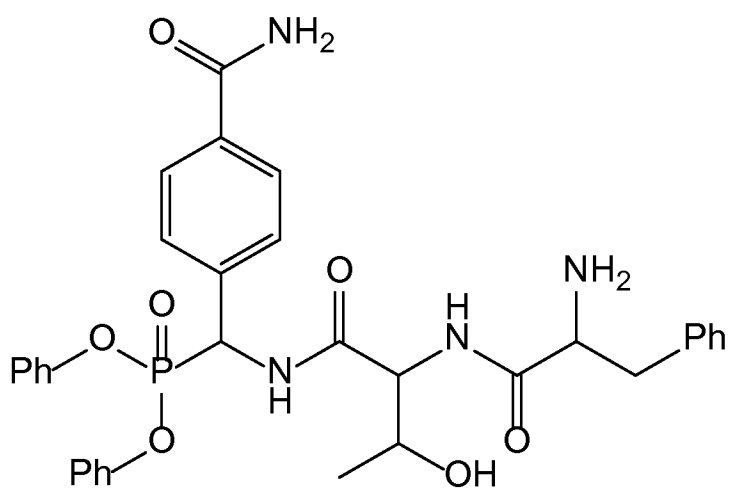
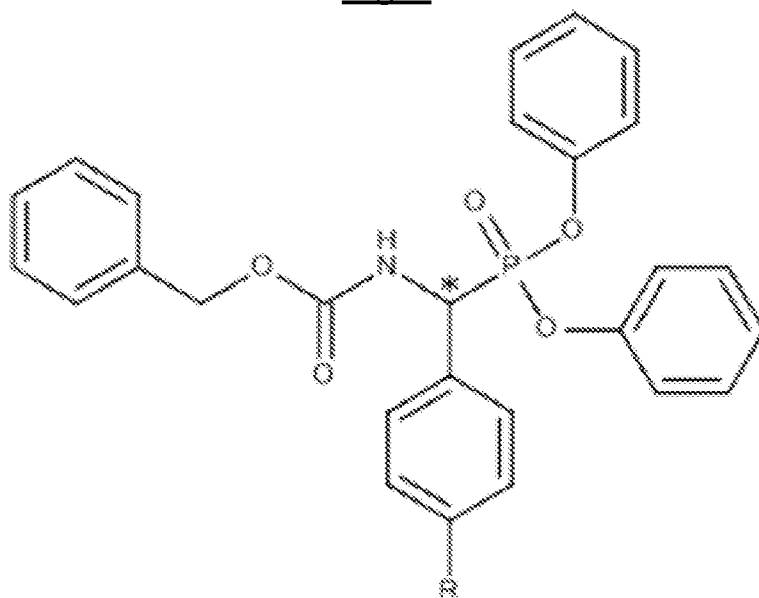
Fig. 1

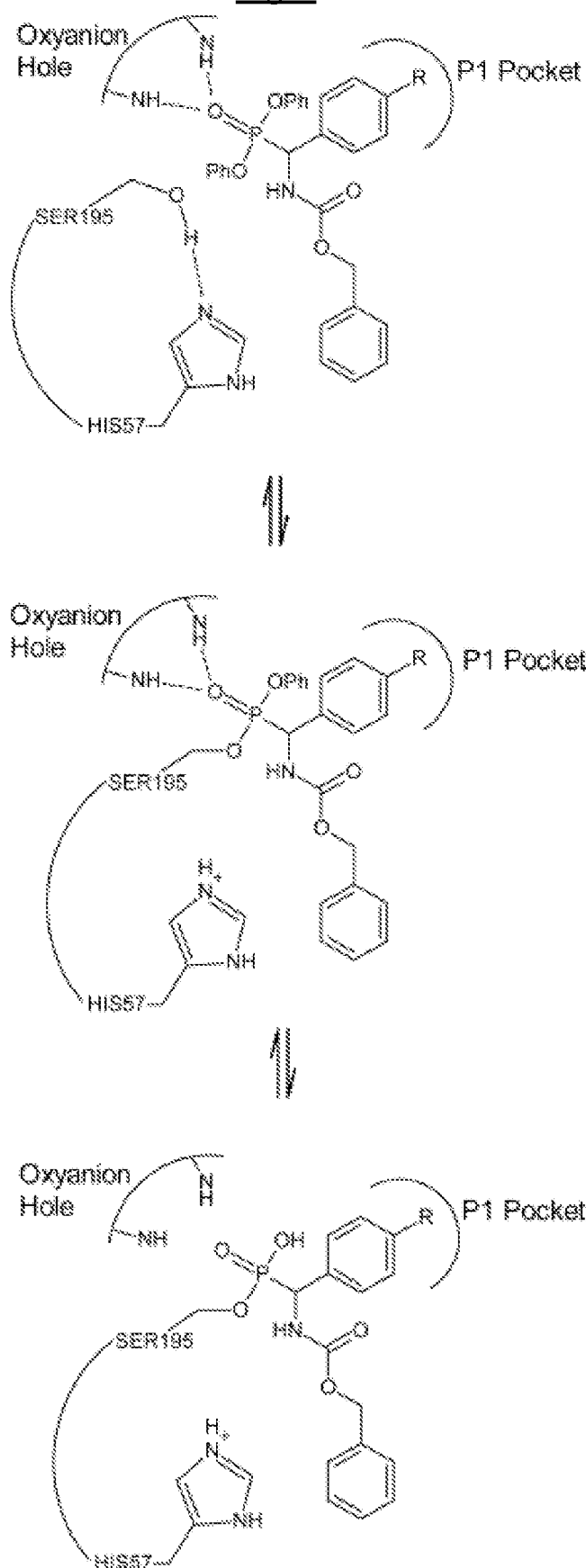
Fig. 2

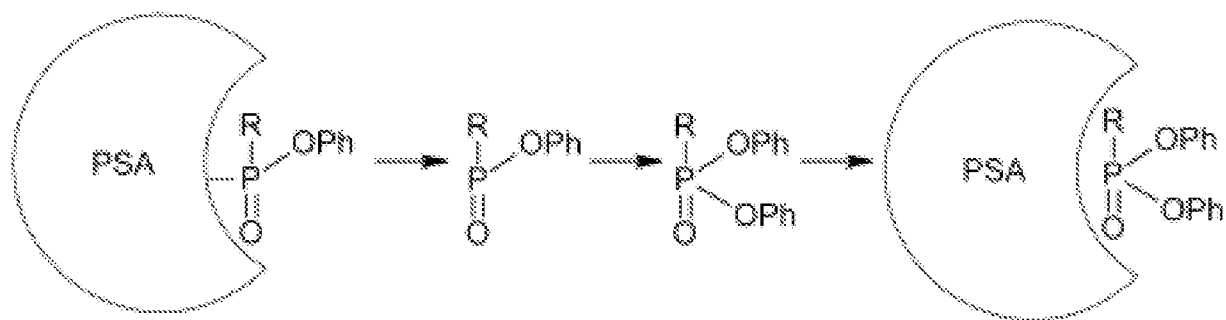
Fig. 3

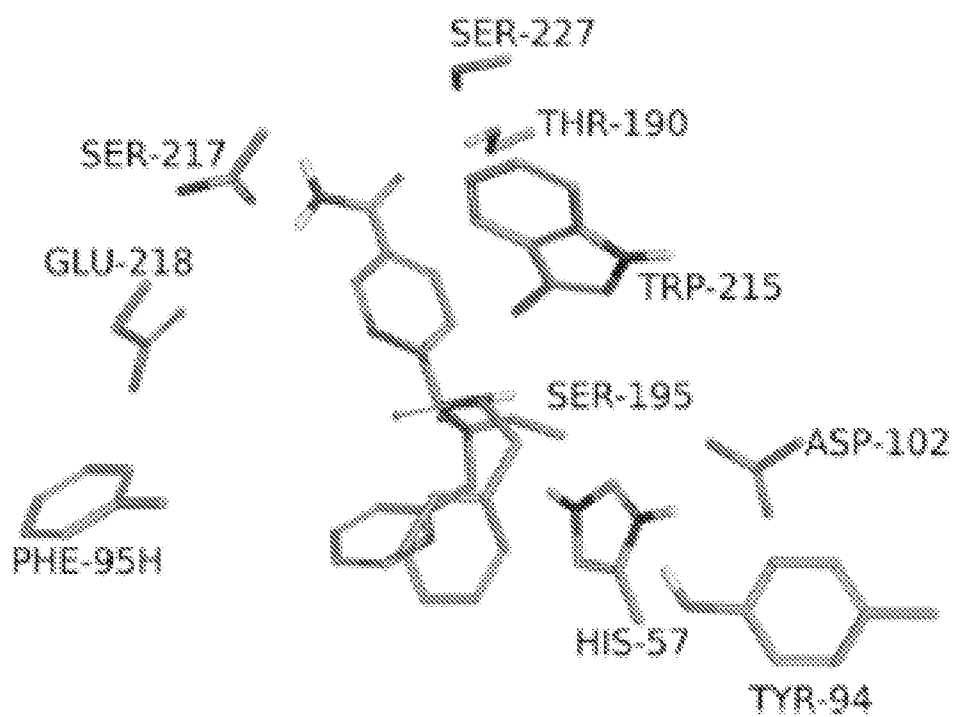
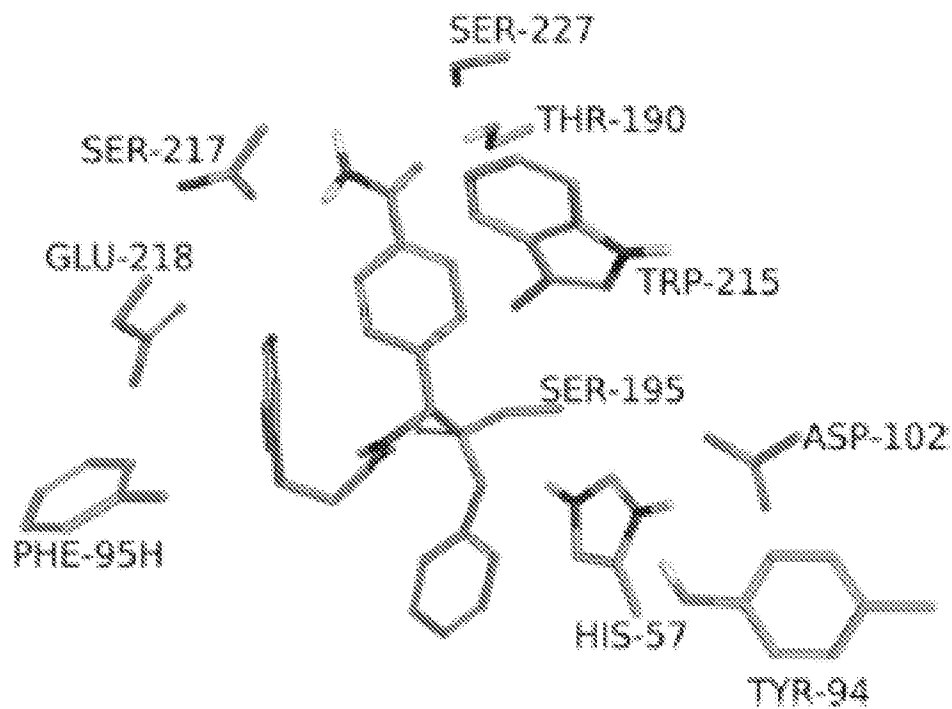
Fig. 4A**Fig. 4B**

Fig. 5A



Fig. 5B

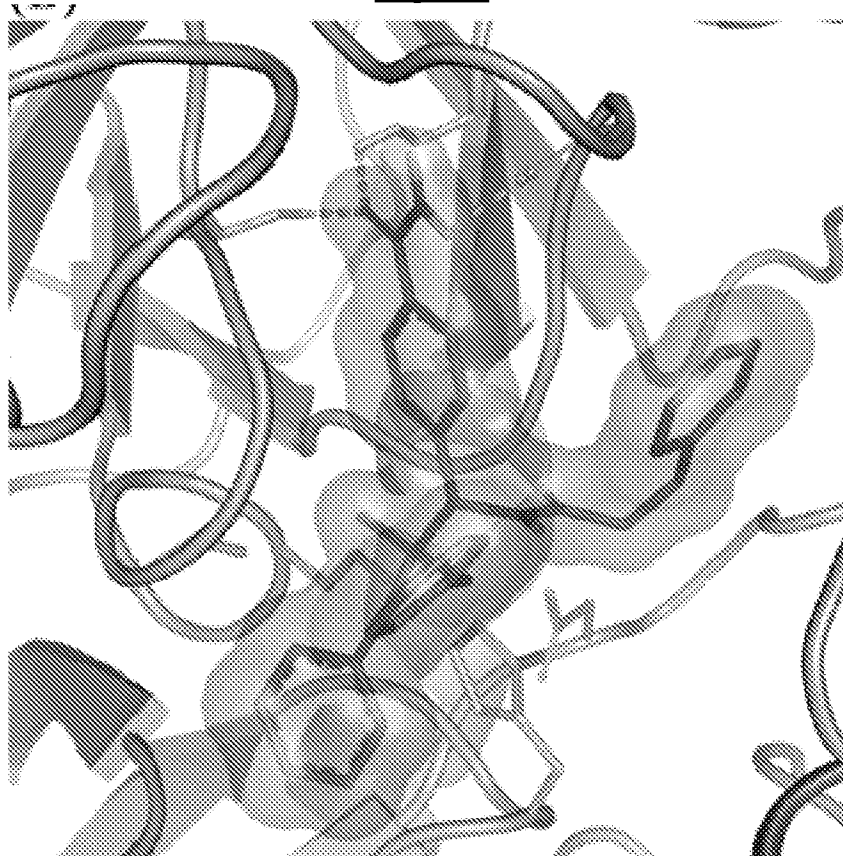


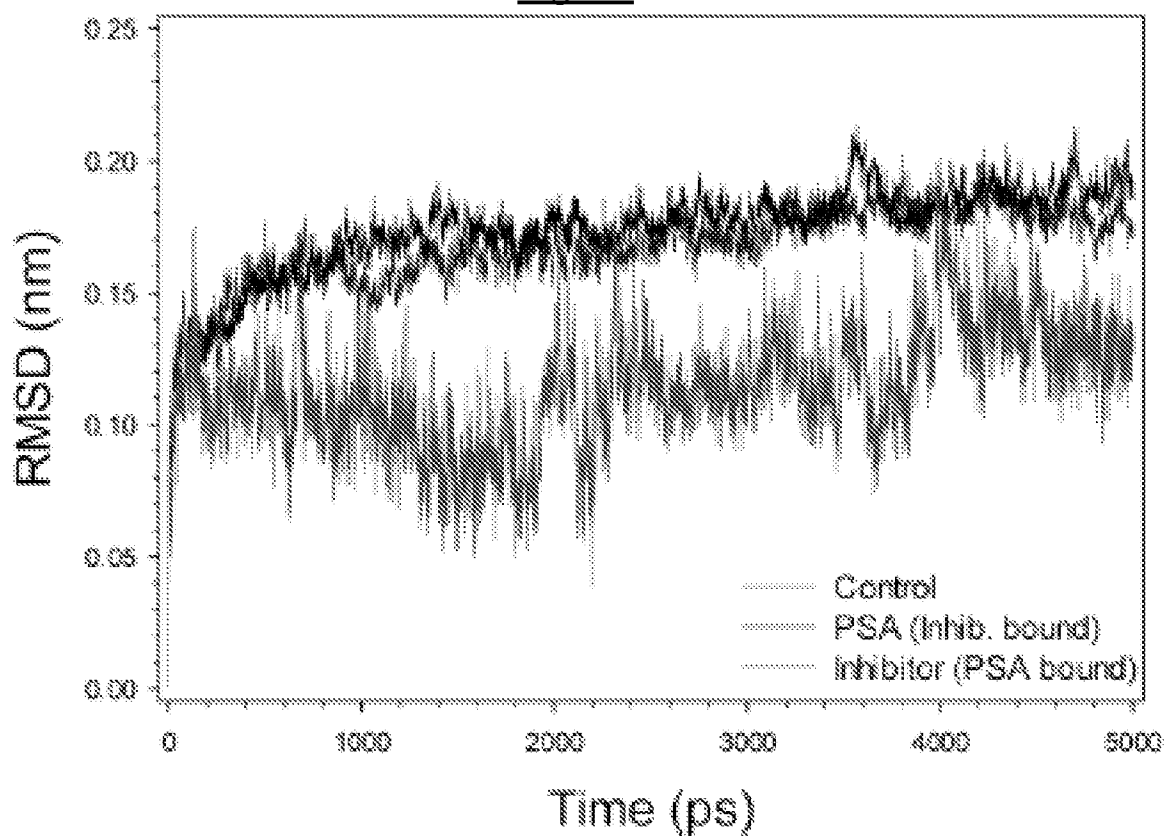
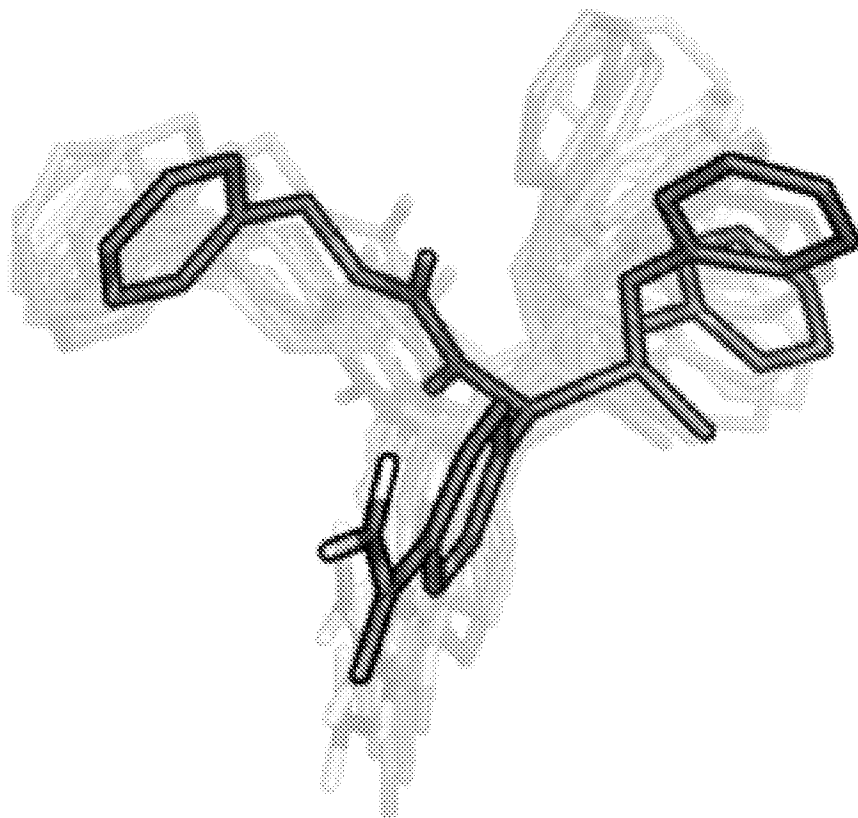
Fig. 6A**Fig. 6B**

Fig. 7A

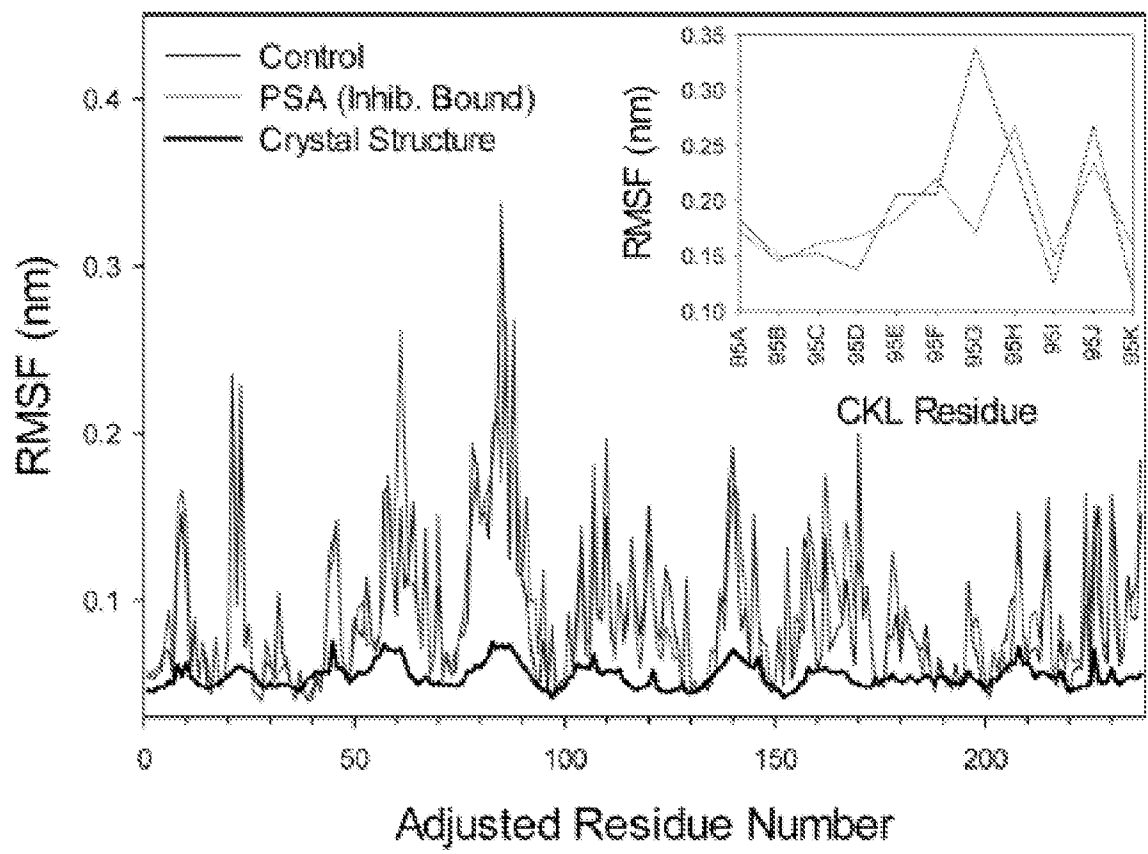


Fig. 7B



Fig. 7C



Fig. 8A

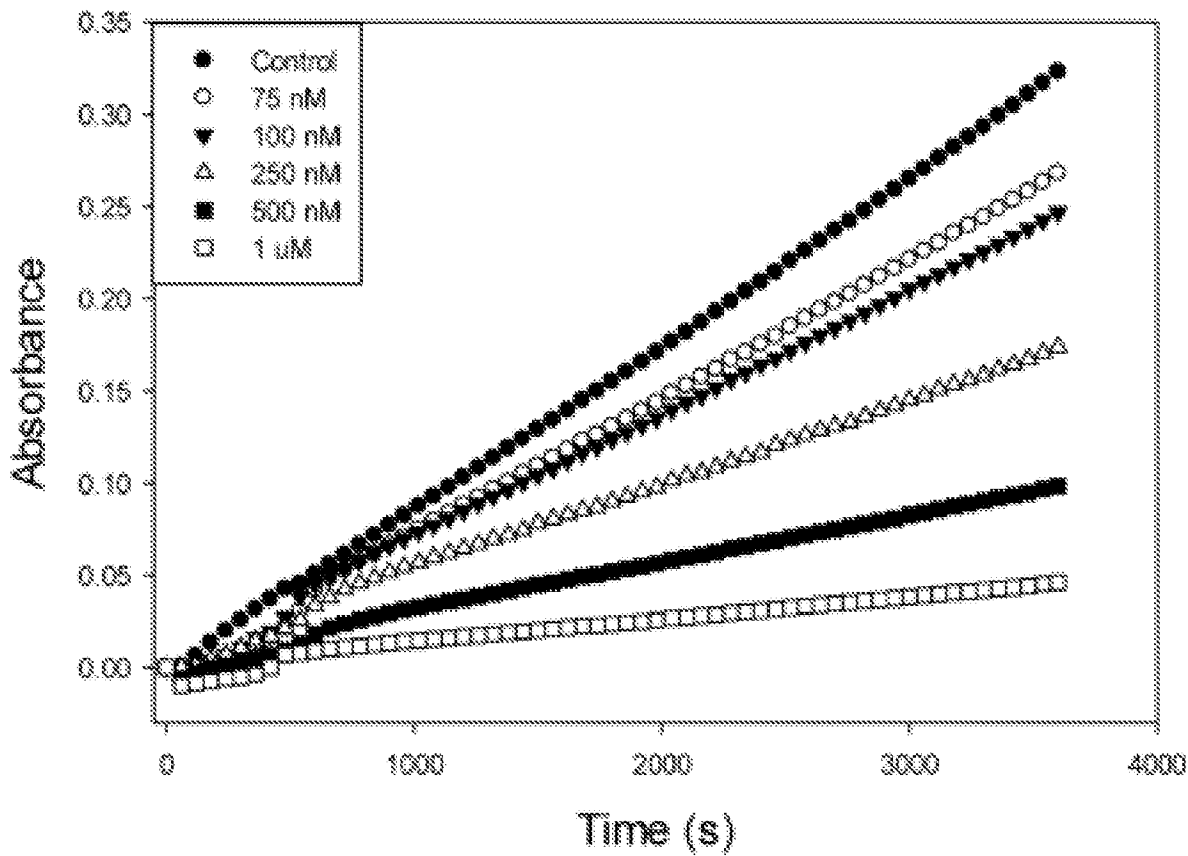


Fig. 8B

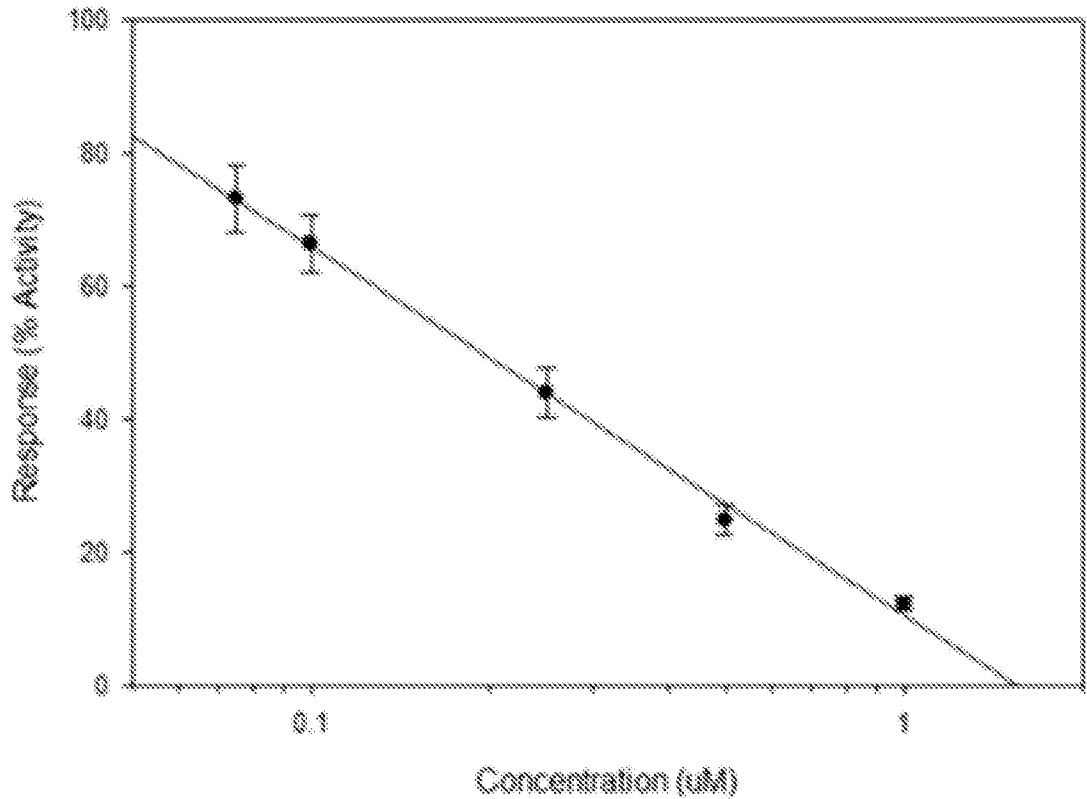


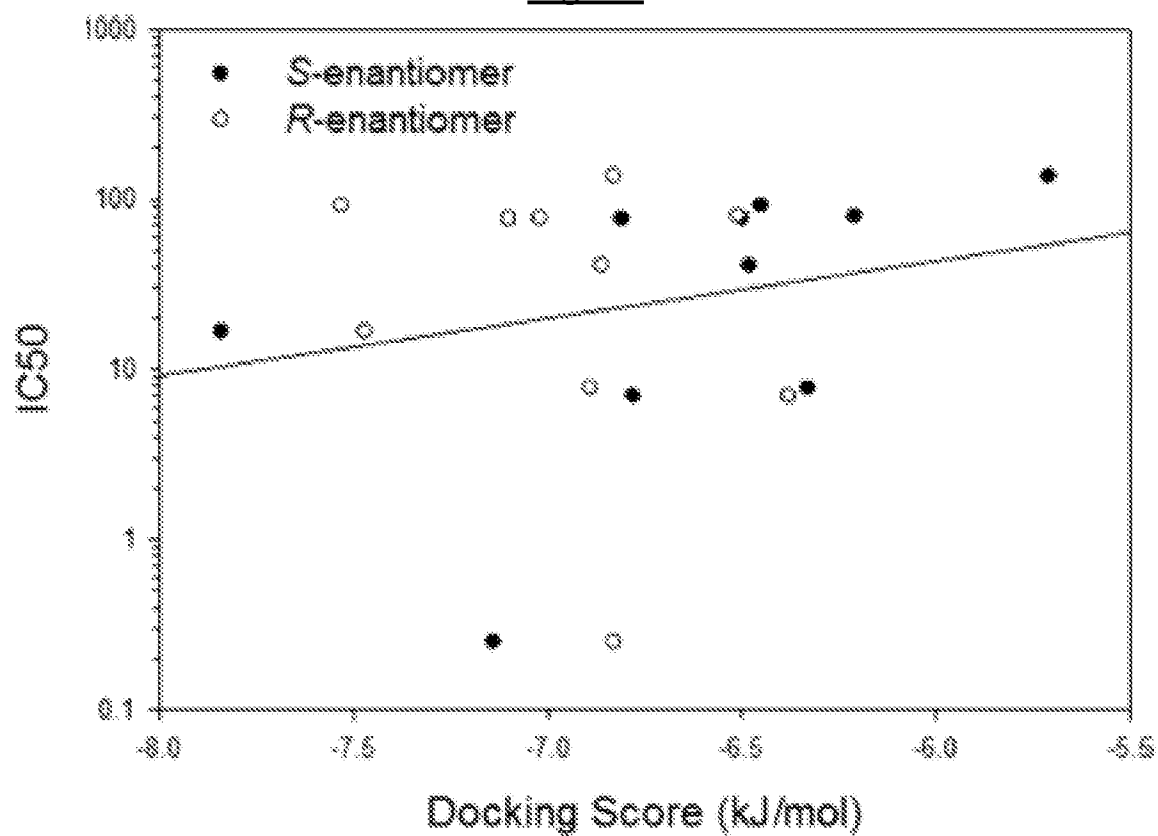
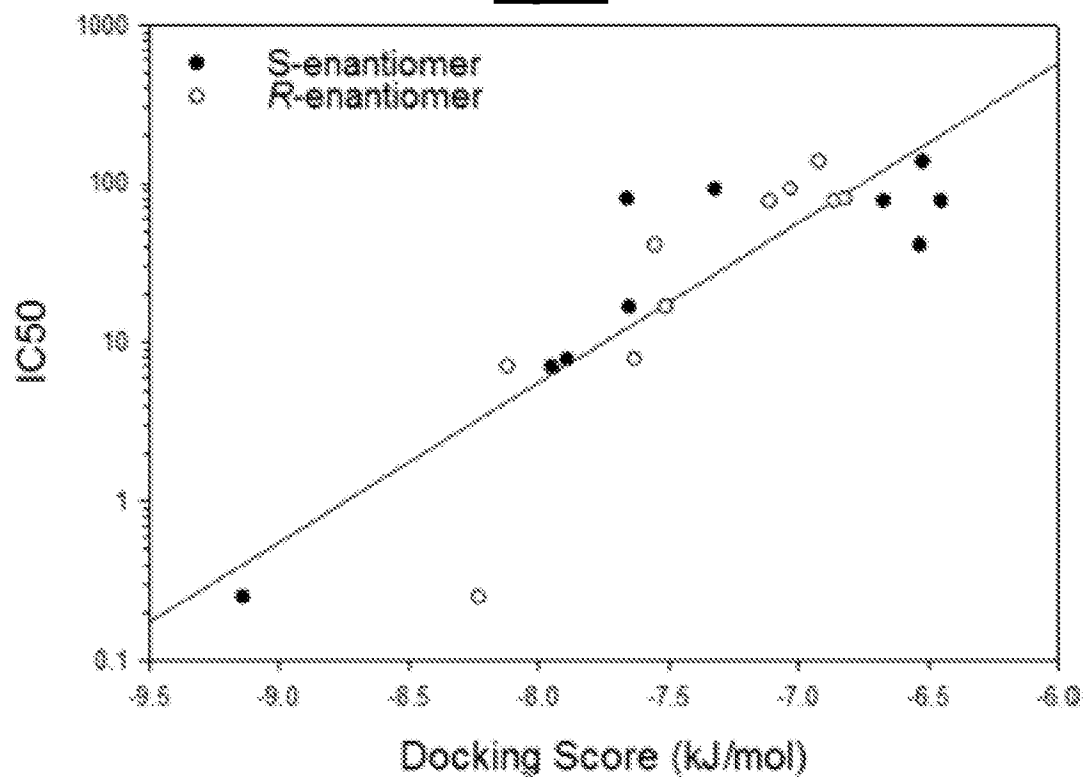
Fig. 9A**Fig. 9B**

Fig. 10A

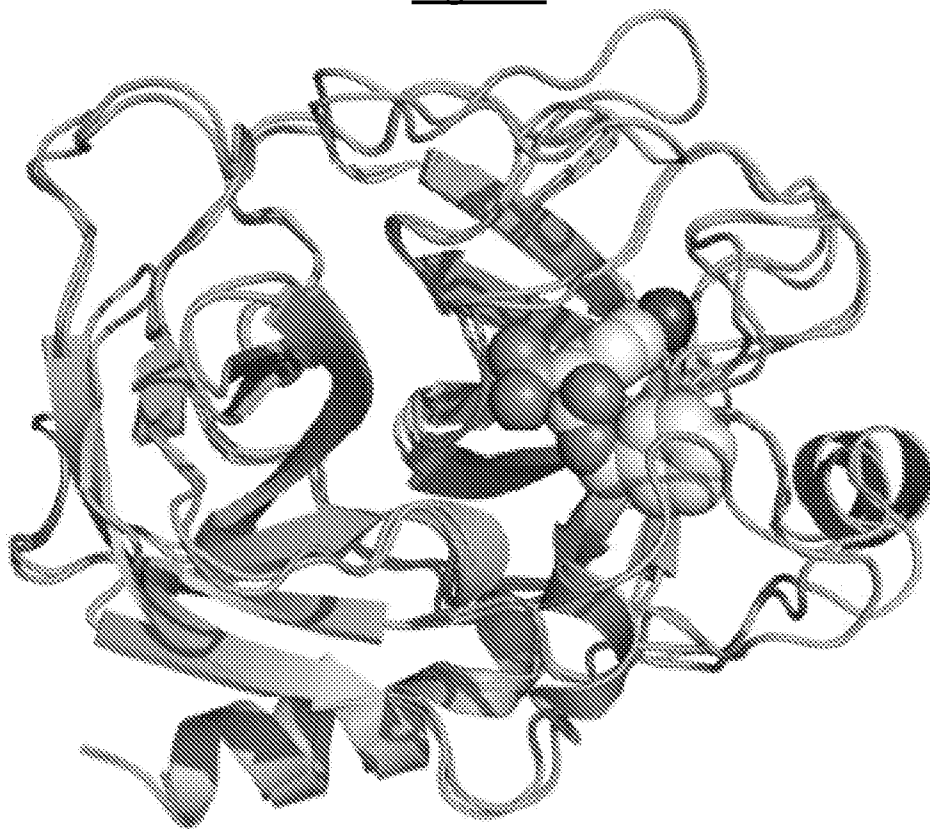
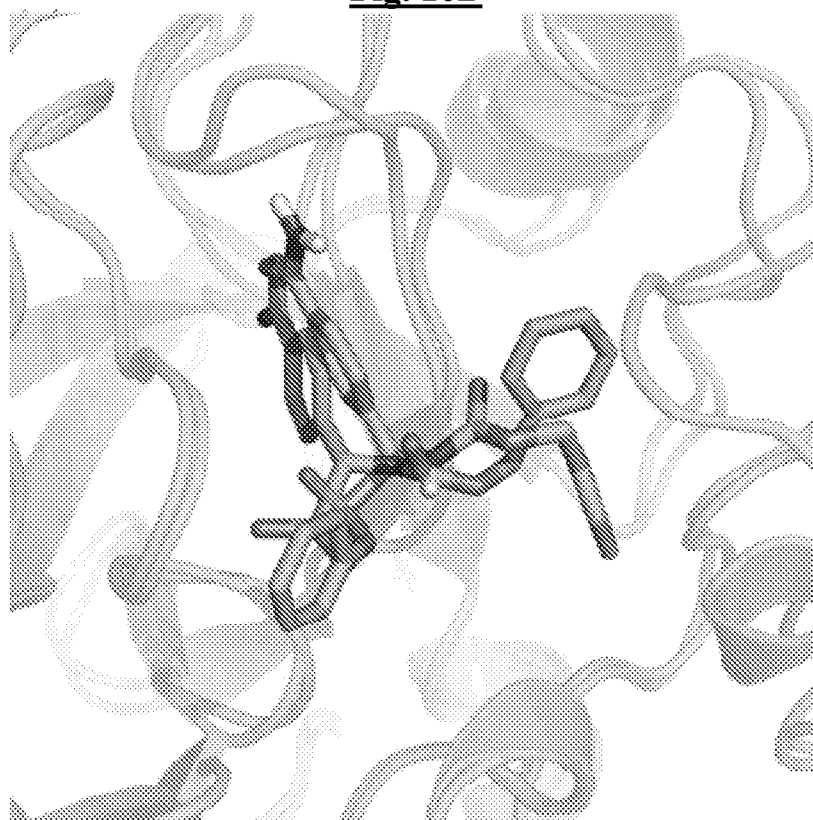


Fig. 10B



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2015/015570

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07F 9/38 (2015.01)

CPC - C07F 9/3808 (2015.05)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - A61P 35/00, 35/04; C07F 9/38 (2015.01)

CPC - A61K 31/66; C02F 5/14; C07F 9/3808, 9/3834 (2015.05)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 514/114; 562/11, 15 (keyword delimited)

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

PatBase, PubChem, STN, Google Patents, Google Scholar.

Search terms used: Aminophenyl Methyl phosphonic acid IGF IGFBP prostate cancer

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PUBCHEM, Compound Summary for CID 98449. Create Date: 2005-03-26. [retrieved on 19 June 2015]. Retrieved from the Internet. <URL: https://pubchem.ncbi.nlm.nih.gov/compound/98449?from=summary >. entire document	1, 2
Y	US 2008/0312191 A1 (AUGUSTYNS et al) 18 December 2008 (18.12.2008) entire document	11, 18, 19
Y	US 2003/0138432 A1 (GLAZIER) 24 July 2003 (24.07.2003) entire document	11, 18, 19
Y	US 2003/0138432 A1 (GLAZIER) 24 July 2003 (24.07.2003) entire document	18
A	WO 2008/018881 A1 (OSI PHARMACEUTICALS INC) 14 February 2008 (14.02.2008) entire document	20

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

23 June 2015

Date of mailing of the international search report

22 JUL 2015

Name and mailing address of the ISA/US

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Authorized officer:

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2015/015570

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Extra Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1, 2, 11, 18-20

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2015/015570

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: Claims 1-20 are drawn to a compound of formula (I) or a salt or solvate thereof, and a method thereof.

The first invention of Group I+ is restricted to a compound of formula (I): or a salt or solvate thereof, wherein R1 and R2 are independently H; R3 is H; R4 is R5; R5 is H; and R6 and R7 are independently a bond; and methods thereof. It is believed that claims 1, 2, 11, and 18-20 read on this first named invention and thus these claims will be searched without fee to the extent that they read on the above embodiment.

Applicant is invited to elect additional formula(e) for each additional compound to be searched in a specific combination by paying an additional fee for each set of election. An exemplary election would a compound of formula (I): or a salt or solvate thereof, wherein R1 is C1-C10 unsubstituted alkyl; and R2 is H; R3 is H; R4 is R5; R5 is H; and R6 and R7 are independently a bond; and methods thereof. Additional formula(e) will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+ formulae do not share a significant structural element, requiring the selection of alternatives for the compound variables R1, R2, R3, R4, R6, and R7.

The Groups I+ share the technical features of a compound having the core structure of formula (I) or a salt or solvate thereof; and a method of treating or preventing prostate cancer in a male subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a compound having the core structure of formula (I) or a salt or solvate thereof. However, these shared technical features do not represent a contribution over the prior art.

Specifically, US 2008/0312191 A1 to Augustyns et al. teach a compound having the core structure of formula (I) or a salt or solvate thereof, wherein R1, and R2 are independently phenyl; R3 is amino; R4 is R5; R5 is H; R6 is C1 alkyl (See Para. [0494], Compound 34d;...see shown structure...); and a method of treating or preventing prostate cancer in a male subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a compound having the core structure of formula (I) or a salt or solvate thereof (See Para. [0338],...the invention provides use of the compounds according to the invention for preparation of medicament for treating conditions...prostate cancer...; See Para. [0494], Compound 34d;...see shown structure...).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature.