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(12) **United States Patent**
Lawrence et al.(10) **Patent No.:** **US 12,390,427 B2**(45) **Date of Patent:** **Aug. 19, 2025**(54) **PLASMINOGEN ACTIVATOR INHIBITOR-1 (PAI-1) INHIBITOR AND METHOD OF USE**2007/0155747 A1 7/2007 Dasse et al.
2010/0137194 A1 6/2010 Lawrence et al.
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See application file for complete search history.(56) **References Cited**

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(57) **ABSTRACT**

Provided herein are plasminogen activator-1 (PAI-1) inhibitor compounds and uses thereof in the treatment of any disease or disorder associated with elevated PAI-1. The disclosure includes, but is not limited to, the use of such compounds to prevent or reduce thrombosis and fibrosis, to promote thrombolysis, and to modulate lipid metabolism and treat diseases or disorders associated with elevated PAI-1, cholesterol, or lipid levels.

4 Claims, 9 Drawing Sheets

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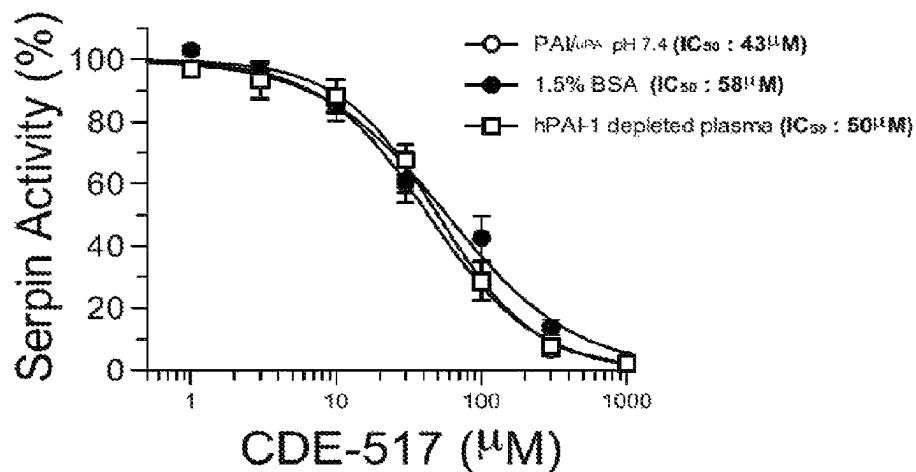


FIGURE 1

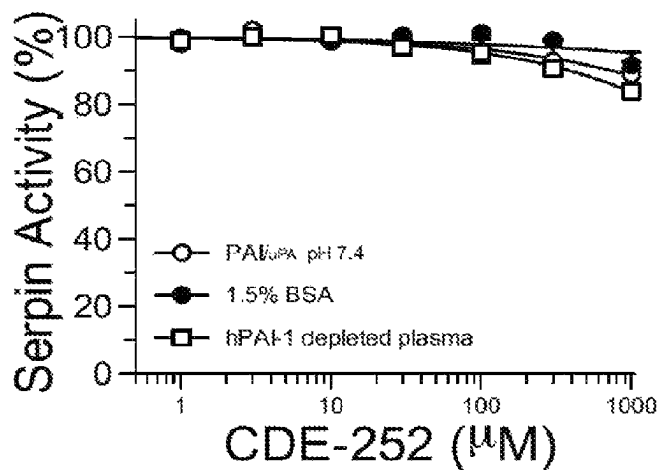


FIGURE 2

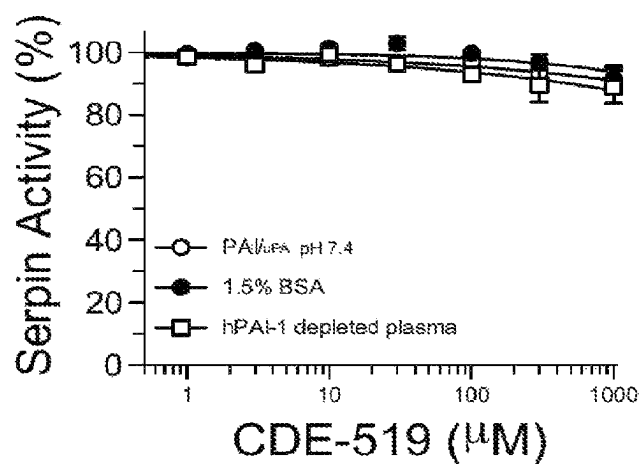


FIGURE 3

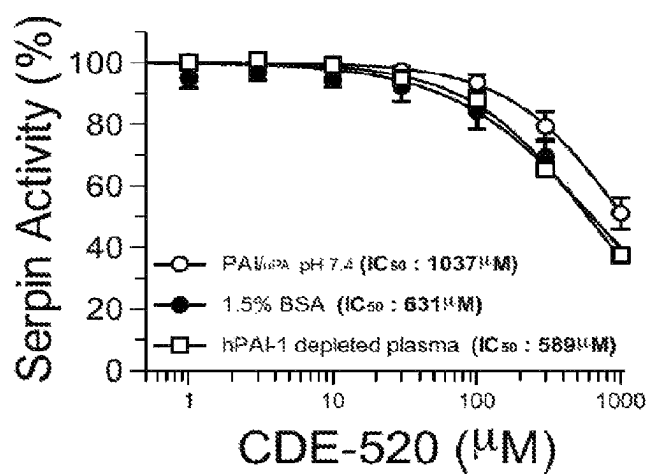


FIGURE 4

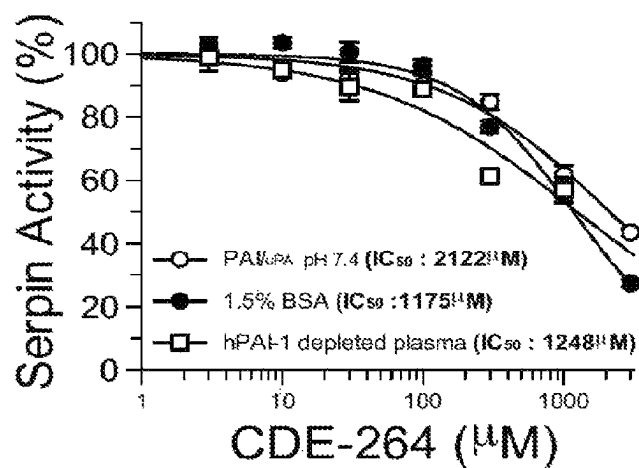


FIGURE 5

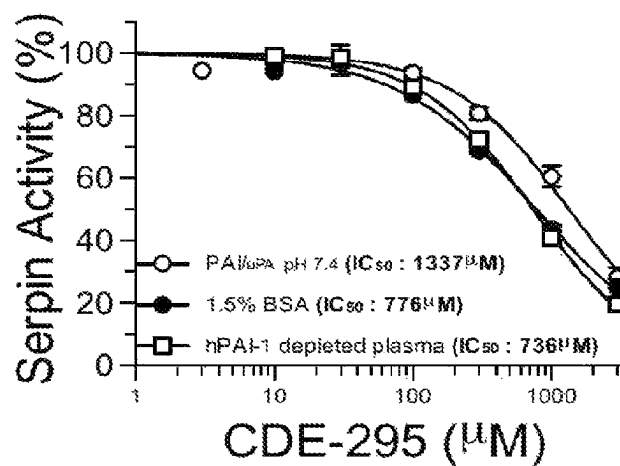


FIGURE 6

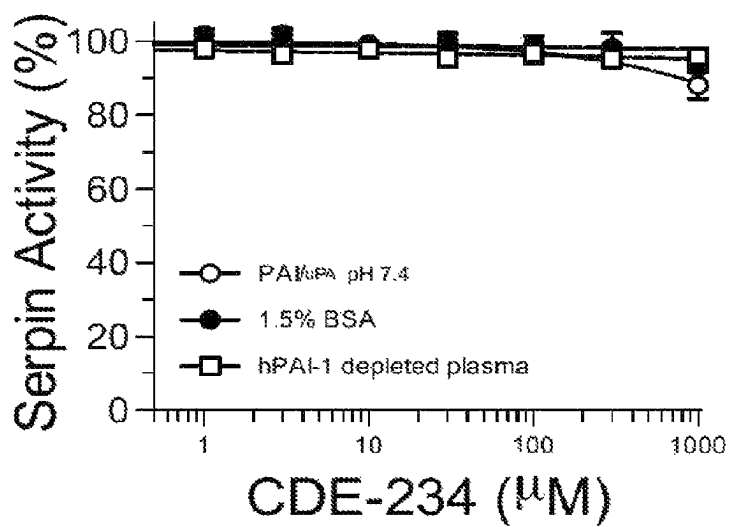


FIGURE 7

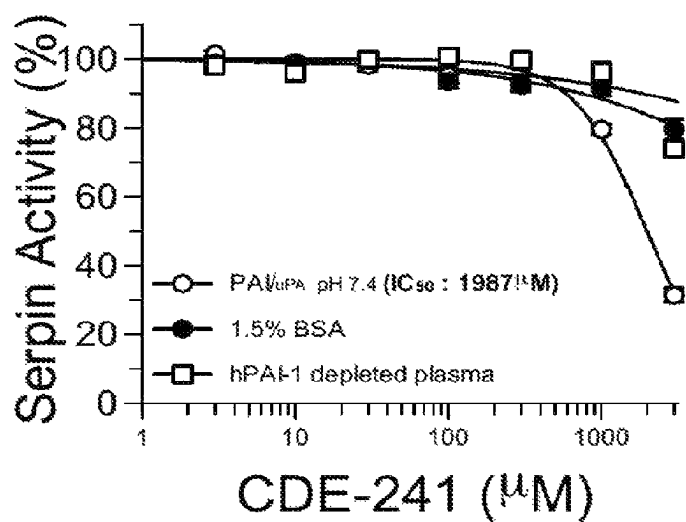


FIGURE 8

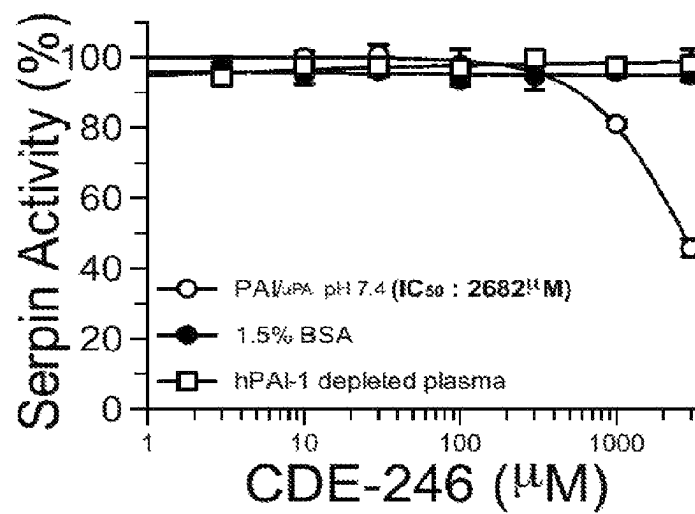


FIGURE 9

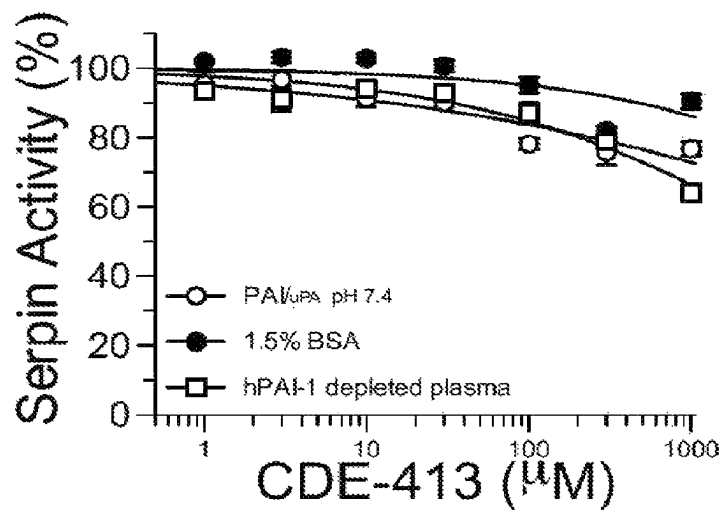


FIGURE 10

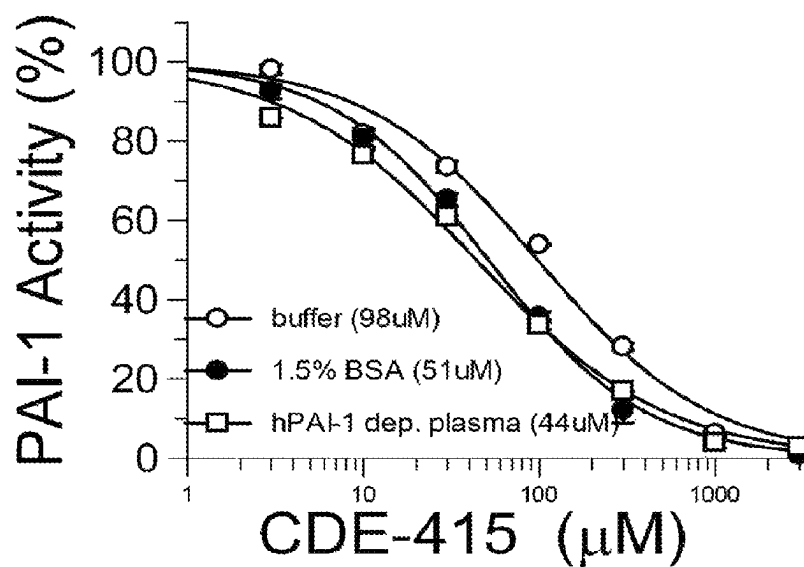


FIGURE 11

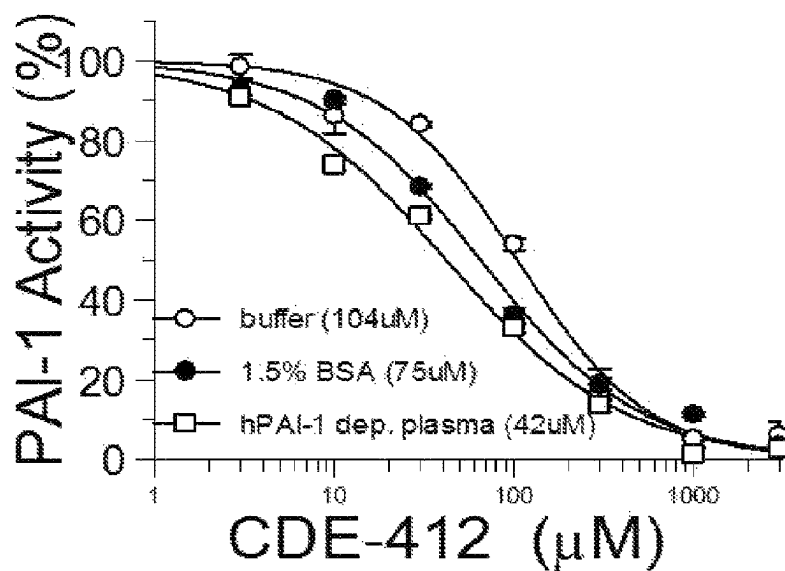


FIGURE 12

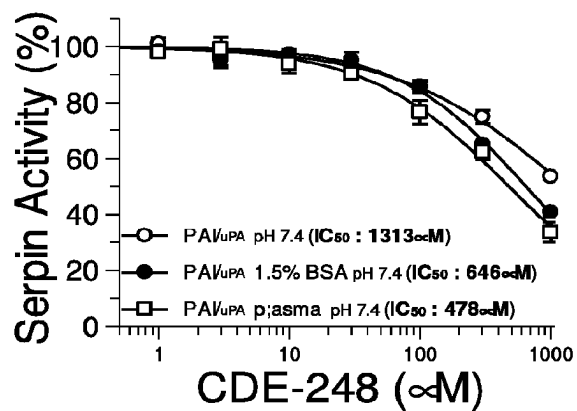


FIGURE 13

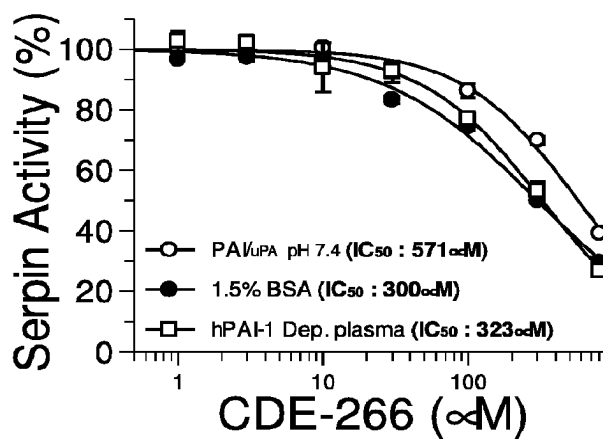
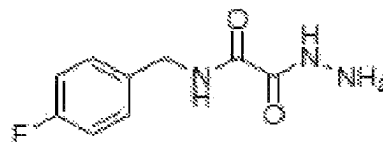


FIGURE 14

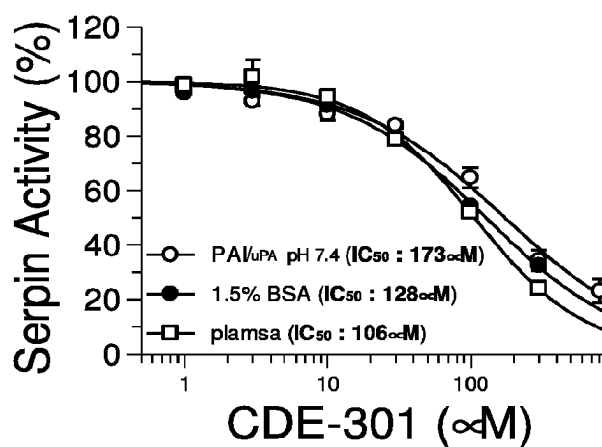
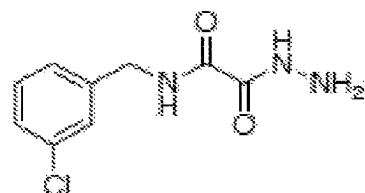
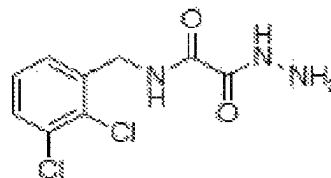


FIGURE 15



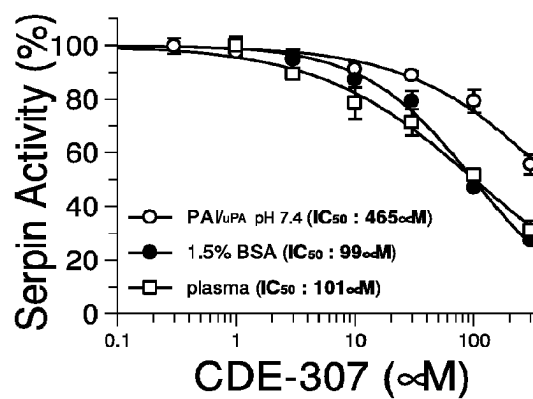


FIGURE 16

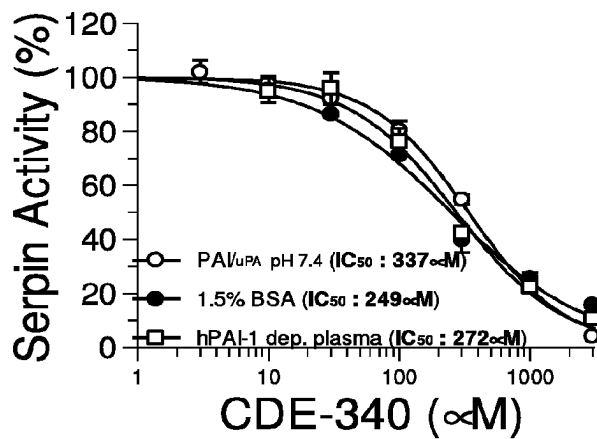
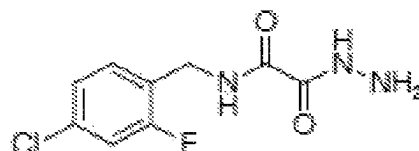


FIGURE 17

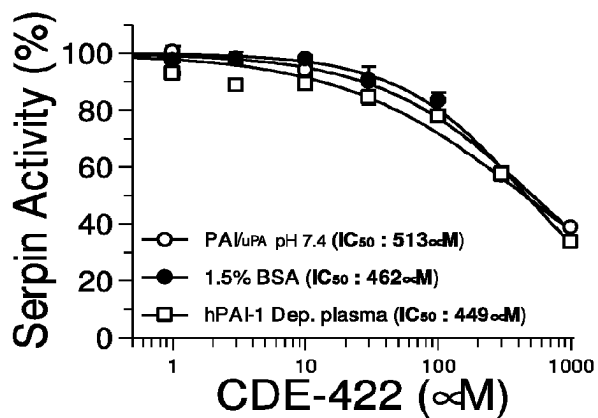
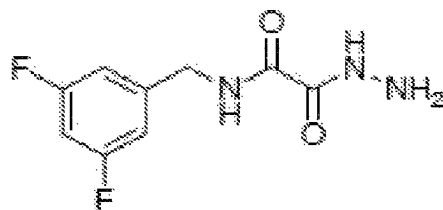
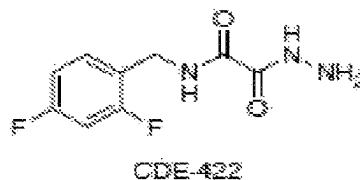


FIGURE 18



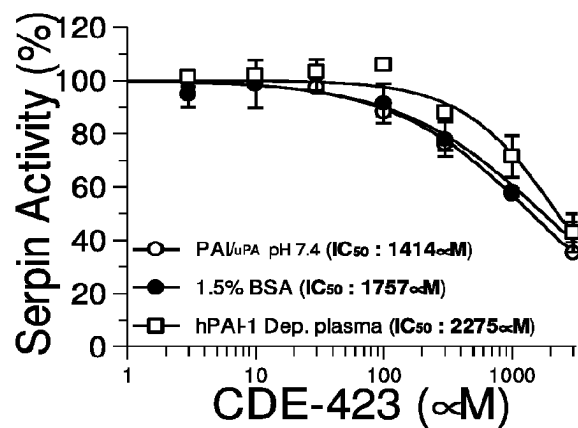


FIGURE 19

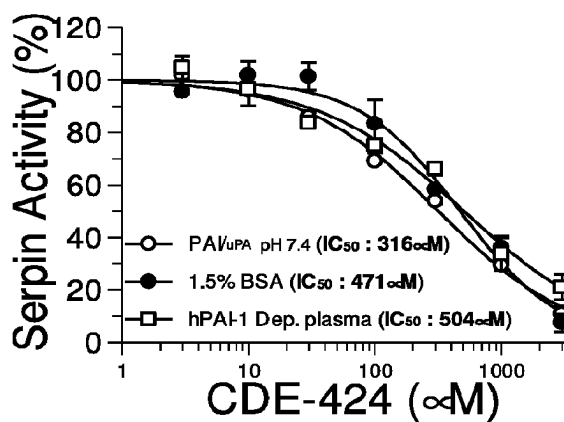
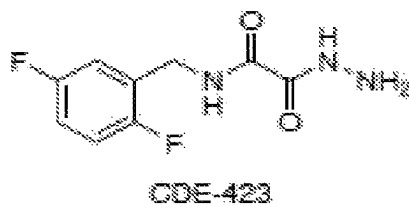


FIGURE 20

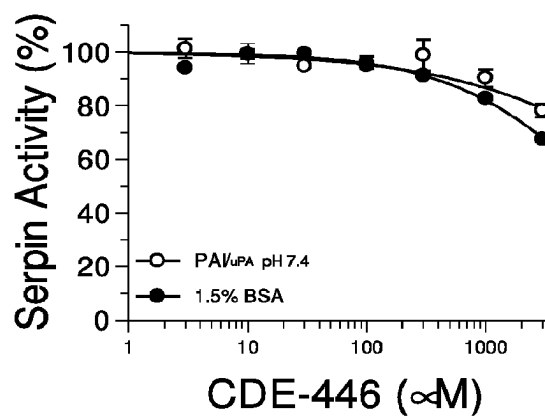
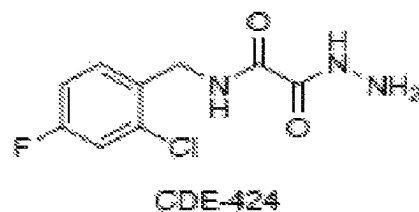
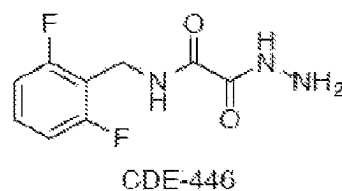


FIGURE 21



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**PLASMINOGEN ACTIVATOR INHIBITOR-1
(PAI-1) INHIBITOR AND METHOD OF USE****STATEMENT OF GOVERNMENT SUPPORT**

This invention was made with government support under HL089407 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD

Provided herein are compounds and methods for modulating plasminogen activator inhibitor-1 (PAI-1) activity. More particularly, the disclosure is directed to inhibitors of PAI-1 and the uses of such inhibitors in regulating PAI-1 activity. Also provided are uses of these inhibitors for the treatment of many diseases or disorders associated with PAI-1 activity. Such diseases or disorders include, but are not limited to, dysregulation of lipid metabolism, obesity, diabetes, polycystic ovary syndrome, bone loss induced by estrogen deficiency, fibrosis and fibrotic disease, inflammation, cell migration and migration-driven proliferation of cells, angiogenesis, and thrombosis. Such inhibitors are also contemplated to be useful for modulation of endogenous fibrinolysis, and in conjunction with pharmacologic thrombolysis.

BACKGROUND

Plasminogen activator inhibitor-1 (PAI-1) is a 50 kDa single-chain glycoprotein that is the principal inhibitor of both urokinase type plasminogen activator (uPA) and tissue type PA (tPA). PAI-1 inhibits tPA and uPA with second-order rate constants $\sim 10^7 \text{ M}^{-1} \text{ s}^{-1}$, a value that is 10-1000 times faster than the rates of PA inhibition by other PAIs. Moreover, approximately 70% of the total tPA in carefully collected normal human plasma is detected in complex with PAI-1, suggesting that inhibition of tPA by PAI-1 is a normal, ongoing process. PAI-1 can also directly inhibit plasmin. Thus, PAI-1 is the chief regulator of plasmin generation in vivo, and as such it appears to play an important role in both fibrotic and thrombotic disease. PAI-1 has three potential N-linked glycosylation sites and contains between 15 and 20% carbohydrate.

PAI-1 belongs to the Serine Protease Inhibitor super family (SERPIN), which is a gene family that includes many of the protease inhibitors found in blood, as well as other proteins with unrelated or unknown functions. Serpins are consumed in the process of protease inactivation and thus act as "suicide inhibitors." The association between a serpin and its target protease occurs at an amino acid residue, referred to as the "bait" residue, located on a surface loop of the serpin called the reactive center loop (RCL). The "bait" residue is also called the P1 residue, and is thought to mimic the normal substrate of the enzyme. Upon association of the P1 residue with the S1 site of a target protease, cleavage of the RCL occurs. This is coupled to a large conformational change in the serpin which involves rapid insertion of the RCL into the major structural feature of a serpin, β -sheet A. This results in tight docking of the protease to the serpin surface and to distortion of the enzyme structure, including its active site. RCL insertion also produces a large increase in serpin structural stability making the complex rigid and thus trapping the protease in a covalent acyl-enzyme complex with the serpin.

Native PAI-1 exists in at least two distinct conformations, an active form that is produced by cells and secreted, and an

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inactive or latent form that accumulates in cell culture medium over time. In blood and tissues, most of the PAI-1 is in the active form; however, in platelets both active and latent forms of PAI-1 are found. In active PAI-1, the RCL is exposed on the surface of the molecule, but upon reaction with a protease, the cleaved RCL integrates into the center of β sheet A. In the latent form, the RCL is intact, but instead of being exposed, the entire amino terminal side of the RCL is inserted as the central strand into the 8 sheet A. This accounts for the increased stability of latent PAI-1 as well as its lack of inhibitory activity.

Active PAI-1 spontaneously converts to the latent form with a half-life of one to two hours at 37° C., and latent PAI-1 can be converted back into the active form by treatment with denaturants. Negatively charged phospholipids can also convert latent PAI-1 to the active form, suggesting that cell surfaces may modulate PAI-1 activity. The observation that latent PAI-1 infused into rabbits is apparently converted to the active form is consistent with this hypothesis. The spontaneous reversible interconversion between the active and latent structures is unique for PAI-1 and distinguishes it from other serpins; however, the biological significance of the latent conformation remains unknown.

Other non-inhibitory forms of PAI-1 have also been identified. The first form results from oxidation of one or more critical methionine residues within active PAI-1. This form differs from latent PAI-1 in that it can be partially reactivated by an enzyme that specifically reduces oxidized methionine residues. Oxidative inactivation of PAI-1 may be an additional mechanism for the regulation of PAI-1, and oxygen radicals produced locally by neutrophils or other cells may inactivate PAI-1 and thus facilitate the generation of plasmin at sites of infection or in areas of tissue remodeling. PAI-1 also exists in two different cleaved forms. As noted above, PAI-1 in complex with a protease is cleaved in its RCL. Uncomplexed PAI-1 can also be found with its RCL cleaved, which can arise from dissociation of PAI-1-PA complexes or from cleavage of the RCL by a non-target protease at a site other than the P1. None of these forms of PAI-1 are able to inhibit protease activity; however, they may interact with other ligands.

The interaction of PAI-1 with non-protease ligands plays an essential role in PAI-1 function. PAI-1 binds with high affinity to heparin, the cell adhesion protein vitronectin, and members the endocytic low-density lipoprotein receptor (LDL-R) family, such as the lipoprotein receptor-related protein (LRP), and the very low density lipoprotein receptor (VLDL-R). These non-protease interactions are important for both PAI-1 localization and function, and they are largely conformationally controlled through structural changes associated with RCL insertion. In blood, most of the active PAI-1 circulates in complex with the glycoprotein vitronectin. The PAI-1 binding site for vitronectin has been localized to a region on the edge of β -sheet A in the PAI-1 structure. The binding site for LDL-R family members is less well characterized, but has been identified, in a region of PAI-1 associated with alpha helix D that is adjacent to the vitronectin binding domain. The heparin binding domain on PAI-1 has also been mapped. This site also localizes to alpha helix D in a region homologous to the heparin binding domain of antithrombin III, and may overlap with the binding site for LDL-R family members.

Vitronectin circulates in plasma and is present in the extracellular matrix primarily at sites of injury or remodeling. PAI-1 and vitronectin appear to have a significant

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functional interdependence. Vitronectin stabilizes PAI-1 in its active conformation, thereby increasing its biological half-life.

Vitronectin also enhances PAI-1 inhibitory efficiency for thrombin approximately 300-fold. In turn, PAI-1 binding to vitronectin alters its conformation from the native plasma form, which does not support cell adhesion, to an "activated" form that is competent to bind integrins. However, integrin binding is blocked by the presence of PAI-1. As noted above, the association of PAI-1 with vitronectin is conformationally controlled and upon inhibition of a protease, the conformational change in PAI-1 associated with RCL insertion results in a loss of high affinity for vitronectin and a gain in affinity for LDL-R family members. This is due to RCL insertion in PAI-1, disrupting the vitronectin binding site, while simultaneously exposing a cryptic receptor binding site that is revealed only when PAI-1 is in a complex with a protease, which results in an approximately 100,000-fold shift in the relative affinity of PAI-1 from vitronectin to LDL-R family members and a subsequent shift in PAI-1 localization from vitronectin to the cellular receptor. Thus, PAI-1 association with vitronectin and LDL-R is conformationally controlled.

High PAI-1 levels are associated with various diseases and disorders. For example, high PAI-1 levels are associated with acute diseases, such as sepsis and myocardial infarction, and chronic disorders, such as cancer, atherosclerosis, and type 2 diabetes. In addition, high PAI-1 levels are associated with cardiovascular disease, wherein PAI-1 expression is significantly increased in severely atherosclerotic vessels, and PAI-1 protein levels rise consistently during disease progression from normal vessels to fatty streaks to atherosclerotic plaques. Increased PAI-1 levels are also linked to obesity, and insulin resistance.

In addition, elevated plasma levels of PAI-1 have been associated with thrombotic events, and antibody neutralization of PAI-1 activity resulted in promotion of endogenous thrombolysis and reperfusion. Elevated levels of PAI-1 have also been implicated in polycystic ovary syndrome and bone loss induced by estrogen deficiency.

PAI-1 is synthesized in both murine and human adipocytes. There is also a strong correlation between the amount of visceral fat and plasma levels of PAI-1 in humans and mice. This dramatic up-regulation of PAI-1 in obesity has led to the suggestion that adipose tissue itself may directly contribute to elevated systemic PAI-1, which in turn increases the probability of vascular disease through increased thrombosis, and accelerated atherosclerosis. Notably, very recent data suggests that PAI-1 may also play a direct role in obesity.

In one study, genetically obese and diabetic ob/ob mice crossed into a PAI-1 deficient background had significantly reduced body weight and improved metabolic profiles compared to ob/ob mice with PAI-1. Likewise, nutritionally-induced obesity and insulin resistance were dramatically attenuated in mice genetically deficient in PAI-1 and in mice treated with an orally active PAI-1 inhibitor. The improved adiposity and insulin resistance in PAI-1-deficient mice may be related to the observation that PAI-1 deficient mice on a high fat diet had increased metabolic rates and total energy expenditure compared to wild-type mice, and peroxysome proliferator-activated receptor (PPAR γ) and adiponectin were maintained. However, the precise mechanism involved was not shown and may be complex, since the overexpression of PAI-1 in mice also impaired adipose tissue formation. Taken together, these observations suggest that PAI-1 plays a previously unrecognized direct role in obesity

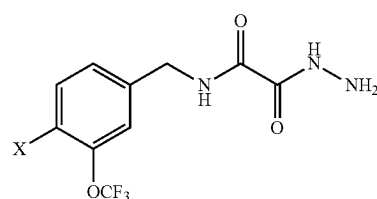
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and insulin resistance that involves interactions beyond its identified activities of modulating fibrinolysis and tissue remodeling.

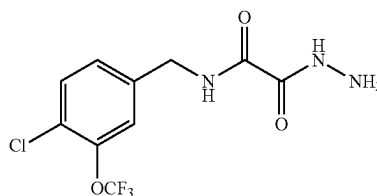
Indeed, if PAI-1 positively regulates adipose tissue development, then the association of increased PAI-1 expression with developing obesity may constitute a positive feedback loop promoting adipose tissue expansion and dysregulation of normal cholesterol homeostasis. Thus, there exists a need in the art for a greater understanding of how PAI-1 is involved in metabolism, obesity and insulin resistance.

SUMMARY

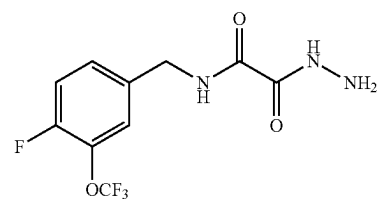
Provided herein is a compound having a structure of



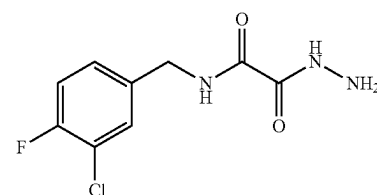
wherein X is Cl or F, or a pharmaceutically acceptable salt thereof. In some cases, the compound is



or a pharmaceutically acceptable salt thereof. In some cases, the compound is

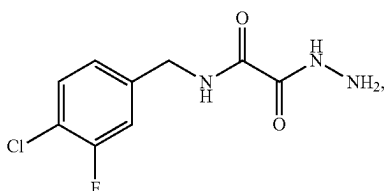


or a pharmaceutically acceptable salt thereof. Also provided is a PAI-1 inhibitor having a structure

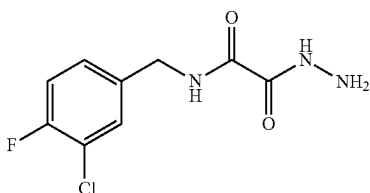


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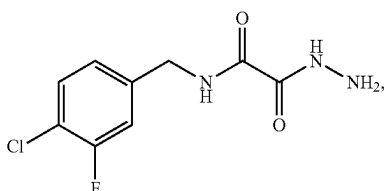
or a pharmaceutically acceptable salt thereof. Further provided is a PAI-1 inhibitor having a structure



or a pharmaceutically acceptable salt thereof. In some cases, the compound is in the form of a pharmaceutically acceptable salt. Further provided are pharmaceutical compositions of one or more of the compounds or salts disclosed herein and a pharmaceutically acceptable excipient. In some cases, the composition comprises a compound having a structure of



or pharmaceutically acceptable salt thereof. In some cases, the composition comprises a compound having a structure



or a pharmaceutically acceptable salt thereof.

Further provided are methods of inhibiting PAI-1 by contacting PAI-1 with a compound as disclosed herein. Also provided are methods of treating a disorder associated with aberrant PAI-1 activity comprising administering to a subject in need thereof a compound as disclosed herein in an amount effective to treat the disorder. In some cases, the disorder is cancer, septicemia, obesity, insulin resistance, a disease or disorder associated with dysregulation of lipid metabolism, a disease or disorder associated with an elevated level of VLDL or LDL, high cholesterol, a proliferative disease or disorder, fibrosis and fibrotic disease, inflammatory bowel disease, coagulation homeostasis, cerebrovascular disease, microvascular disease, hypertension, dementia, atherosclerosis, osteoporosis, osteopenia, arthritis, asthma, heart failure, arrhythmia, angina, hormone insufficiency, Alzheimer's disease, hypertension, inflammation, sepsis, fibrinolytic disorder, stroke, dementia, coronary heart disease, myocardial infarction, stable and unstable angina, vascular disease, peripheral arterial disease, acute vascular syndrome, thrombosis, prothrombosis, deep vein thrombosis, pulmonary embolism, cerebrovascular disease, microvascular disease, hypertension, diabetes, hyperglycemia, hyperinsulinemia, malignant lesions, premalignant lesions, gastrointestinal malignancies, liposarcoma, epithelial tumor,

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and psoriasis, an extracellular matrix accumulation disorder, neoangiogenesis, myelofibrosis, fibrinolytic impairment, polycystic ovary syndrome, bone loss induced by estrogen deficiency, angiogenesis, neoangiogenesis, myelofibrosis, or fibrinolytic impairment. In various cases, the disease or disorder involving thrombosis or prothrombosis is formation of atherosclerotic plaques, venous thrombosis, arterial thrombosis, myocardial ischemia, atrial fibrillation, deep vein thrombosis, a coagulation syndrome, pulmonary thrombosis, cerebral thrombosis, a thromboembolic complication of surgery, and peripheral arterial occlusion. In some cases, the disorder is fibrosis, and more particularly, can be pulmonary fibrosis, renal fibrosis, cardiac fibrosis, hepatic fibrosis, or scleroderma. In some cases, the disorder is inflammatory bowel disease, and more particularly, can be Crohn's disease or ulcerative colitis. In some cases, the extracellular matrix accumulation disorder is renal fibrosis, chronic obstructive pulmonary disease, polycystic ovary syndrome, restenosis, renovascular disease, diabetic nephropathy, or organ transplant rejection.

Further provided are methods of modulating cholesterol, lipid clearance, and/or lipid uptake in a subject with an elevated level of PAI-1 comprising administering to the subject an effective amount of a compound disclosed herein in an amount effective to decrease the elevated level of PAI and modulate cholesterol, lipid clearance, and/or lipid uptake in the subject. In some cases, the compound increases circulating high density lipoprotein (HDL) and/or decreases circulating very low density lipoprotein (VLDL) in the subject. In various cases, the compound inhibits apolipoprotein E (ApoE) or apolipoprotein A (ApoA) binding to VLDL-R. In various cases, the compound decreases HDL or apolipoprotein E (ApoE) or apolipoprotein A (ApoA) binding to an ApoA receptor. In various cases, the compound decreases PAI-1 binding to apolipoprotein E (ApoE). In various cases, the compound decreases PAI-1 binding to apolipoprotein A (ApoA). In various cases, the compound decreases PAI-1 binding to VLDL. In various cases, the compound binds to PAI-1 in the presence of vitronectin. In various cases, the compound binds to PAI-1 in the presence of urokinase type plasminogen activator (uPA).

In any of the methods disclosed herein, the subject can be human.

The foregoing summary is not intended to define every aspect of the invention, and additional aspects are described in other sections, such as the Detailed Description. The entire document is intended to be related as a unified disclosure, and it should be understood that all combinations of features described herein are contemplated, even if the combination of features are not found together in the same sentence, or paragraph, or section of this document.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 shows the Serpin activity in the presence of various concentrations of CDE-517.

FIG. 2 shows the Serpin activity in the presence of various concentrations of CDE-252.

FIG. 3 shows the Serpin activity in the presence of various concentrations of CDE-519.

FIG. 4 shows the Serpin activity in the presence of various concentrations of CDE-520.

FIG. 5 shows the Serpin activity in the presence of various concentrations of CDE-264.

FIG. 6 shows the Serpin activity in the presence of various concentrations of CDE-295.

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FIG. 7 shows the Serpin activity in the presence of various concentrations of CDE-234.

FIG. 8 shows the Serpin activity in the presence of various concentrations of CDE-241.

FIG. 9 shows the Serpin activity in the presence of various concentrations of CDE-246.

FIG. 10 shows the Serpin activity in the presence of various concentrations of CDE-413.

FIG. 11 shows the Serpin activity in the presence of various concentrations of CDE-415.

FIG. 12 shows the Serpin activity in the presence of various concentrations of CDE-412.

FIG. 13 shows the Serpin activity in the presence of various concentrations of CDE-248.

FIG. 14 shows the Serpin activity in the presence of various concentrations of CDE-266.

FIG. 15 shows the Serpin activity in the presence of various concentrations of CDE-301.

FIG. 16 shows the Serpin activity in the presence of various concentrations of CDE-307.

FIG. 17 shows the Serpin activity in the presence of various concentrations of CDE-340.

FIG. 18 shows the Serpin activity in the presence of various concentrations of CDE-422.

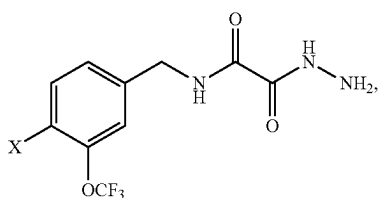
FIG. 19 shows the Serpin activity in the presence of various concentrations of CDE-423.

FIG. 20 shows the Serpin activity in the presence of various concentrations of CDE-424.

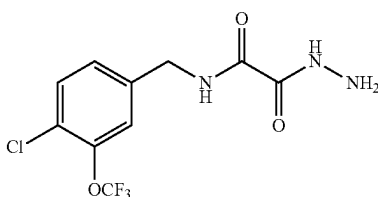
FIG. 21 shows the Serpin activity in the presence of various concentrations of CDE-446.

DETAILED DESCRIPTION

Provided herein is a PAI-1 inhibitor having a structure:

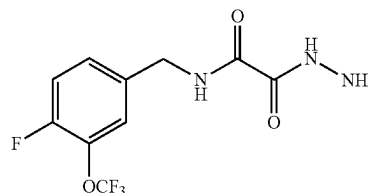


wherein X is Cl or F, or a pharmaceutically acceptable salt thereof. In some cases, the compound has a structure

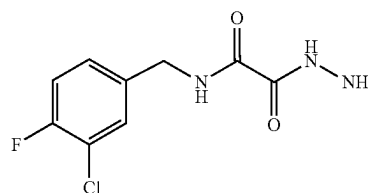


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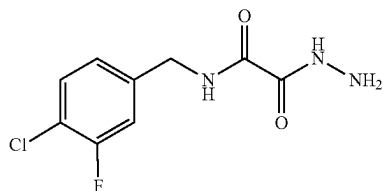
or a pharmaceutically acceptable salt thereof. In some cases, the compound has a structure



or a pharmaceutically acceptable salt thereof. Further provided is a PAI-1 inhibitor having a structure



or a pharmaceutically acceptable salt thereof. Further provided is a PAI-1 inhibitor having a structure



or a pharmaceutically acceptable salt thereof. Also provided are pharmaceutical compositions comprising one or more of these compounds or a salt thereof.

As used herein, the term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge et al. describe pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences*, 1977, 66, 1-19, which is incorporated herein by reference. Pharmaceutically acceptable salts of the compounds of this disclosure include those derived from suitable inorganic and organic acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, trifluoroacetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzene-sulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, glutamate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hy-

droxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Salts of compounds containing a carboxylic acid or other acidic functional group can be prepared by reacting with a suitable base. Such salts include, but are not limited to, alkali metal, alkaline earth metal, aluminum salts, ammonium, $N^+(C_{1-4}alkyl)_4$ salts, and salts of organic bases such as trimethylamine, triethylamine, morpholine, pyridine, piperidine, picoline, dicyclohexylamine, N,N' -dibenzylethylenediamine, 2-hydroxyethylamine, bis-(2-hydroxyethyl)amine, tri-(2-hydroxyethyl)amine, procaine, dibenzylpiperidine, dehydroabietylamine, N,N' -bis-dehydroabietylamine, glucamine, N -methylglucamine, cololidine, quinine, quinoline, and basic amino acids such as lysine and arginine. This disclosure also envisions the quaternization of any basic nitrogen-containing groups of the compounds disclosed herein. Water or oil-soluble or dispersible products may be obtained by such quaternization. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, lower alkyl sulfonate and aryl sulfonate.

Methods of Using PAI-1 Inhibitors

As mentioned herein above, it is contemplated that methods disclosed herein include treating a disease or disorder associated with elevated levels of PAI-1 comprising administering a PAI-1 inhibitor. In one aspect, the subject is a mammal. In some cases, the mammalian subject is human.

In some embodiments, provided herein are PAI-1 inhibitor compounds and methods of using the compounds in the treatment of many diseases or disorders associated with PAI-1 activity. Such conditions, e.g., diseases or disorders, include, but are not limited to, dysregulation of lipid metabolism, obesity, diabetes, polycystic ovary syndrome, bone loss induced by estrogen deficiency, fibrosis and fibrotic disease, inflammation, cell migration and migration-driven proliferation of cells, and angiogenesis or thrombosis. In some aspects, such inhibitors are also contemplated to be useful for modulation of endogenous fibrinolysis, and in conjunction with pharmacologic thrombolysis. In various aspects, provided herein are PAI-1 inhibitor compounds and methods of using the compounds in the treatment of acute diseases associated with high PAI-1 levels, such as, but not limited to, sepsis, myocardial infarction, and thrombosis, compared to PAI-1 levels in normal subjects known not to suffer from sepsis, myocardial infarction, or thrombosis. In some aspects, the PAI-1 inhibitor compounds disclosed herein are used in methods for treating diseases and disorders associated with high PAI-1 levels, such as, but not limited to, cancer, atherosclerosis, insulin resistance, type 2 diabetes, and fibrotic diseases compared to PAI-1 levels in normal subjects known not to suffer from these diseases or disorders. In various aspects, provided herein are PAI-1 inhibitor compounds for regulating lipid metabolism, including increasing circulating HDL and/or decreasing circulating VLDL in a subject.

In various aspects, a PAI-1 inhibitor is useful in the treatment of any condition, including a disease or disorder, wherein the lowering of PAI-1 levels will provide benefits.

The PAI-1 inhibitor is useful alone, or in combination with other compounds, which may act as to promote the reduction of PAI-1 levels.

The PAI-1 inhibitor can be formulated into an appropriate preparation and administered to one or more sites within the subject in a therapeutically effective amount. In some embodiments, the PAI-1 inhibitor-based therapy is effected via continuous or intermittent intravenous administration. In various aspects, the PAI-1 inhibitor-based therapy is effected via continuous or intermittent intramuscular or subcutaneous administration. In other aspects, the PAI inhibitor-based therapy is effected via oral or buccal administration. By "effective amount" what is meant is an amount of PAI-1 inhibitor compound that is sufficient to support an observable change in the level of one or more biological activities of PAI-1, plasminogen activator, HDL, LDL, or VLDL and/or an observable change in an indication for which the method of treatment is intended. The change may be reduced level of PAI-1 activity. In some aspects, the change is an increase in plasminogen activator, and/or HDL and/or a reduction in LDL and VLDL.

In various aspects, administration of the compositions is systemic or local, and in still other aspects comprises a single site injection of a therapeutically-effective amount of the PAI-1 inhibitor composition. Any route known to those of skill in the art for the administration of a therapeutic composition disclosed herein is contemplated including, for example, intravenous, intramuscular, subcutaneous, oral, or a catheter for long-term administration.

In some cases, it is contemplated that the therapeutic composition is delivered to the patient at multiple sites. The multiple administrations are rendered simultaneously or are administered over a period of several hours. It is likewise contemplated that the therapeutic composition is taken on a regular basis via oral administration. In certain cases, it is beneficial to provide a continuous flow of the therapeutic composition. Additional therapy is administered on a period basis, for example, daily, weekly, or monthly.

In addition to therapies based solely on the delivery of the PAI-1 inhibitor composition, combination therapy is specifically contemplated. It is contemplated that the PAI-1 inhibitor composition therapy is used similarly in conjunction with other agents commonly used for the treatment of elevated levels of PAI-1, LDL and VLDL.

To achieve the appropriate therapeutic outcome, using the methods and compositions disclosed herein, it is further contemplated that a composition comprising a PAI-1 inhibitor and at least one other therapeutic agent (second therapeutic agent) is administered to a subject in need thereof. Such therapeutic agents include drugs used to manage cardiovascular disease including, but not limited to, cholesterol lowering drugs, such as statins, anti-inflammatories, and ACE inhibitors. Such drugs also include drugs targeting neurological disorders including, but not limited to drugs for targeting stroke, seizures, and Alzheimer's Disease. In another aspect, the additional agents include, but are not limited to, drugs targeting diabetes. These are all disorders associated with elevated levels of PAI-1 and, therefore, it is contemplated that combination therapy may be used with PAI-1 inhibitors and other known therapies.

The combination therapy compositions are provided in a combined amount effective to produce the desired therapeutic outcome in the treatment of increased levels of PAI-1, VLDL, or LDL and/or make a detectable change in an indication as described herein. This process involves administering the PAI-1 inhibitor and the second agent(s) or factor(s) at the same time. Methods thus include adminis-

tering a single composition or pharmacological formulation that includes both agents, or administering two distinct compositions or formulations, at the same time, wherein one composition includes the PAI-1 inhibitor therapeutic composition and the other includes the second therapeutic agent.

Alternatively, the PAI-1 inhibitor treatment precedes or follows the second therapeutic agent treatment by intervals ranging from minutes to weeks. In embodiments where the second therapeutic agent and the PAI-1 inhibitor are administered separately, one generally ensures that a significant period of time did not expire between the times of each delivery, such that the second therapeutic agent and the PAI-1 inhibitor are able to exert an advantageously combined effect. In such instances, it is contemplated that one administers both modalities within about 12-24 hours of each other, or alternately, within about 6-12 hours of each other, or alternately, with a delay time of only about 12 hours. In some situations, it is desirable to extend the time period for treatment significantly, however, where several days (2, 3, 4, 5, 6 or 7) to several weeks (1, 2, 3, 4, 5, 6, 7 or 8) lapse between the respective administrations.

Systemic delivery of PAI-1 inhibitors to patients is a very efficient method for delivering a therapeutically effective amount of the compound to counteract the immediate clinical manifestations of a disease or disorder. Alternatively, local delivery of the PAI-1 inhibitor and/or the second therapeutic agent is appropriate in certain circumstances. In a certain embodiment, it is contemplated that the PAI-1 inhibitor is delivered to a patient for an extended period of time. It is further contemplated that the PAI-1 inhibitor is taken throughout a patient's lifetime to lower PAI-1, VLDL and/or LDL levels.

Pharmaceutical Compositions

As mentioned herein above, provided herein are methods using pharmaceutical compositions comprising effective amounts of PAI-1 inhibitor together with pharmaceutically acceptable excipient, such as diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers useful in PAI-1 inhibitor therapy. Such compositions include diluents of various buffer content (e.g., Tris-HCl, acetate, phosphate), pH and ionic strength; additives such as detergents and solubilizing agents (e.g., Tween 80, Polysorbate 80), antioxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., thimersol, benzyl alcohol), and bulking substances (e.g., lactose, mannitol); incorporation of the material into particulate preparations of polymeric compounds, such as polylactic acid, polyglycolic acid, etc., or in association with liposomes or micelles. Such compositions will influence the physical state, stability, rate of in vivo release, and rate of in vivo clearance of the PAI-1 inhibitor. See, e.g., Remington's Pharmaceutical Sciences, 18th Ed. (1990) Mack Publishing Co., Easton, PA, pages 1435-1712, which are herein incorporated by reference.

Sterile liquid compositions include solutions, suspensions, emulsions, syrups and elixirs. The compounds disclosed herein may be dissolved or suspended in the pharmaceutically acceptable carrier, such as sterile water, sterile organic solvent or a mixture of both. In one aspect, the liquid carrier is one suitable for parental injection. Where the compounds are sufficiently soluble they can be dissolved directly in normal saline with or without the use of suitable organic solvents, such as propylene glycol or polyethylene glycol. If desired, dispersions of the finely divided compounds can be made-up in aqueous starch or sodium carboxymethyl cellulose solution, or in a suitable oil, such as *arachis* oil. Liquid pharmaceutical compositions, which are sterile solutions or suspensions, can be utilized by intramus-

cular, intraperitoneal or subcutaneous injection. In many instances a liquid composition form may be used instead of the preferred solid oral method of administration.

It is preferred to prepare unit dosage forms of the compounds for standard administration regimens. In this way, the composition can be subdivided readily into smaller doses at the physician's direction. For example, unit dosages may be made up in packeted powders, vials or ampoules and, in one aspect, in capsule or tablet form. The active compound present in these unit dosage forms of the composition may be present in an amount of from about one gram to about fifteen grams or more, for single or multiple daily administration, according to the particular need of the patient. The daily dose of active compound will vary depending upon the route of administration, the size, age and sex of the patient, the severity of the disease state, and the response to the therapy as traced by blood analysis and the patient's recovery rate.

The precise dosage to be employed depends upon several factors including the host, whether in veterinary medicine or human medicine, the nature and severity of the condition, e.g., disease or disorder, being treated, the mode of administration and the particular active substance employed. The compounds may be administered by any conventional route, in particular enterally, and, in one aspect, orally in the form of tablets or capsules. Administered compounds can be in the free form or pharmaceutically acceptable salt form as appropriate, for use as a pharmaceutical, particularly for use in the prophylactic or curative treatment of atherosclerosis and sequelae (angina pectoris, myocardial infarction, arrhythmias, heart failure, kidney failure, stroke, peripheral arterial occlusion, and related disease states). These measures will slow the rate of progress of the disease state and assist the body in reversing the process direction in a natural manner.

PAI-1 inhibitors or derivatives thereof may be formulated for injection, or oral, nasal, pulmonary, topical, or other types of administration as one skilled in the art will recognize. The formulation may be liquid or may be solid, such as lyophilized, for reconstitution.

PAI-1 inhibitor or derivatives thereof are useful in the treatment of any of the acute or chronic diseases or disorders associated with increased levels of PAI-1, LDL, or VLDL. Conditions (e.g., diseases or disorders) alleviated or modulated by the administration of PAI-1 inhibitor, in some aspects, are those characterized by increased levels of VLDL and LDL. Such conditions may be induced as a course of therapy for other purposes, such as chemotherapy or radiation therapy. It is contemplated that such conditions may result from genetic inheritance or be the side effect of another condition or medication.

The phrase "pharmaceutically or pharmacologically acceptable" refers to molecular entities and compositions that do not produce adverse, allergic, or other untoward reactions when administered to an animal or a human. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well-known in the art. Except insofar as any conventional media or agent is incompatible with the vectors or cells, its use in therapeutic compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions.

The active compositions used in the methods disclosed herein include classic pharmaceutical preparations. Administration of these compositions will be via any common

route so long as the target tissue is available via that route. The pharmaceutical compositions may be introduced into the subject by any conventional method, e.g., by intravenous, intradermal, intramuscular, intramammary, intraperitoneal, intrathecal, retrobulbar, intrapulmonary (e.g., term release); by oral, sublingual, nasal, anal, vaginal, or transdermal delivery, or by surgical implantation at a particular site. The treatment may consist of a single dose or a plurality of doses over a period of time.

The active compounds may be prepared for administration as solutions of free base or pharmacologically acceptable salts in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions also can be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents (for example, sugars or sodium chloride). Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption (for example, aluminum monostearate and gelatin).

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle that contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well-known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions.

For oral administration of the compositions, a PAI-1 inhibitor may be incorporated with excipients and used in the form of non-ingestible mouthwashes and dentifrices. A

mouthwash may be prepared incorporating the active ingredient in the required amount in an appropriate solvent, such as a sodium borate solution (Dobell's Solution). Alternatively, the active ingredient may be incorporated into an antiseptic wash containing sodium borate, glycerin and potassium bicarbonate. The active ingredient may also be dispersed in dentifrices, including: gels, pastes, powders and slurries. The active ingredient may be added in a therapeutically effective amount to a paste dentifrice that may include water, binders, abrasives, flavoring agents, foaming agents, and humectants.

The compositions used in the methods may be formulated in a neutral or salt form. Pharmaceutically-acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups also can be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

The compositions used in the methods may be formulated in micelles or liposomes. Such formulations include sterically stabilized micelles or liposomes and sterically stabilized mixed micelles or liposomes. Such formulations can facilitate intracellular delivery, since lipid bilayers of liposomes and micelles are known to fuse with the plasma membrane of cells and deliver entrapped contents into the intracellular compartment.

Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as injectable solutions, drug release capsules and the like. For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration.

Generally, an effective amount of a PAI-1 inhibitor, or derivatives thereof, will be determined by the age, weight, and condition or severity of disease or disorder of the recipient. See, Remington's Pharmaceutical Sciences, supra, pages 697-773, herein incorporated by reference. Typically, a dosage of between about 0.001 $\mu\text{g/kg}$ body weight/day to about 1000 $\mu\text{g/kg}$ body weight/day, may be used, but more or less, as a skilled practitioner will recognize, may be used. Dosing may be one or more times daily, or less frequently, and may be in conjunction with other compositions as described herein. It should be noted that the disclosure is not limited to the dosages recited herein.

By initiating the treatment regimen with a minimal daily dose of about one gram, the blood levels of PAI-1 and the patient's symptomatic relief analysis may be used to determine whether a larger dose is indicated. One skilled in the art will appreciate that the appropriate dosage levels for treatment will thus vary depending, in part, upon the molecule delivered, the indication for which the PAI-1 inhibitor compound is being used, the route of administration, and the size (body weight, body surface or organ size) and condition (the age and general health) of the patient. Accordingly, the clinician may titrate the dosage and may modify the route of administration to obtain the optimal therapeutic effect. A typical dosage may range from about 0.1 $\mu\text{g/kg}$ to up to about 100 mg/kg or more, depending on the factors men-

tioned above. In other embodiments, the dosage may range from 0.1 µg/kg up to about 100 mg/kg; or 1 µg/kg up to about 100 mg/kg; or 5 µg/kg up to about 100 mg/kg.

"Unit dose" is defined as a discrete amount of a therapeutic composition dispersed in a suitable carrier. Parenteral administration may be carried out with an initial bolus followed by continuous infusion to maintain therapeutic circulating levels of drug product. Those of ordinary skill in the art will readily optimize effective dosages and administration regimens as determined by good medical practice and the clinical condition of the individual patient.

The frequency of dosing will depend on the pharmacokinetic parameters of the agents and the routes of administration. The optimal pharmaceutical formulation will be determined by one of skill in the art depending on the route of administration and the desired dosage. See, for example, Remington's Pharmaceutical Sciences, supra, pages 1435-1712, incorporated herein by reference. Such formulations may influence the physical state, stability, rate of in vivo release and rate of in vivo clearance of the administered agents. Depending on the route of administration, a suitable dose may be calculated according to body weight, body surface areas or organ size. Further refinement of the calculations necessary to determine the appropriate treatment dose is routinely made by those of ordinary skill in the art without undue experimentation, especially in light of the dosage information and assays disclosed herein, as well as the pharmacokinetic data observed in animals or human clinical trials.

Appropriate dosages may be ascertained through the use of established assays for determining level of myocardial infarct in conjunction with relevant dose-response data. The final dosage regimen will be determined by the attending physician, considering factors that modify the action of drugs, e.g., the drug's specific activity, severity of the damage and the responsiveness of the patient, the age, condition, body weight, sex and diet of the patient, the severity of any infection, time of administration and other clinical factors. As studies are conducted, further information will emerge regarding appropriate dosage levels and duration of treatment.

It will be appreciated that the pharmaceutical compositions and treatment methods disclosed herein are useful in fields of human medicine and veterinary medicine. Thus the subject to be treated is in one aspect a mammal. In another aspect, the mammal is a human.

In addition, further contemplated is a kit containing components comprising a composition comprising a PAI-1 inhibitor; and optionally, at least one additional factor useful in the treatment of the acute and chronic diseases and disorders discussed herein.

Uses of Compounds in the Treatment of Diseases or Disorders

Provided herein is the use of compounds as disclosed for the production of a medicament for the treatment or prevention of any disease or disorder discussed herein.

The compounds are inhibitors of the serine protease inhibitor PAI-1, and are therefore useful in the treatment or prophylaxis of those processes which involve the production and/or action of PAI-1. Thus, the compounds, in various aspects, are useful in preventing or reducing thrombosis, promoting thrombolysis, reducing fibrosis regulating lipid metabolism as described herein. In some aspects, the compounds are useful in treating high cholesterol and diseases or disorders associated with elevated levels of PAI-1. In vari-

ous aspects, the compounds are useful in treating elevated levels of VLDL or LDL. In some aspects, the compounds are useful in elevating HDL.

In some aspects, provided are the uses of these inhibitors for the treatment of a disease or disorder associated with PAI-1 activity. Such diseases or disorders include, but are not limited to, inflammation, cell migration and migration-driven proliferation of cells, and angiogenesis or thrombosis. Such inhibitors are also contemplated to be useful for modulation of endogenous fibrinolysis, and in conjunction with pharmacologic thrombolysis.

The compounds are useful in the treatment or prevention of insulin resistance, obesity, non-insulin dependent diabetes mellitus, cardiovascular disease, thrombotic events associated with coronary artery and cerebrovascular disease. The compounds are also useful for inhibiting the disease process involving the thrombotic and prothrombotic states which include, but are not limited to, formation of atherosclerotic plaques, venous and arterial thrombosis, myocardial ischemia, atrial fibrillation, deep vein thrombosis, coagulation syndromes, pulmonary thrombosis, cerebral thrombosis, thromboembolic complications of surgery (such as joint replacement), and peripheral arterial occlusion. These compounds are also useful in treating stroke associated with or resulting from atrial fibrillation.

The compounds are also used in the treatment or prophylaxis of high cholesterol and diseases or disorders associated with such a condition.

The compounds may also be used in the treatment of diseases or disorders associated with extracellular matrix accumulation, including, but not limited to, renal fibrosis, chronic obstructive pulmonary disease, polycystic ovary syndrome, restenosis, renovascular disease and organ transplant rejection.

The compounds may also be used in the treatment of fibrosis, including, but not limited to, pulmonary fibrosis, renal fibrosis, cardiac fibrosis, hepatic fibrosis, and scleroderma.

The compounds may also be used in the treatment of inflammatory bowel disease, including, but not limited to, Crohn's disease and ulcerative colitis.

The compounds may also be used in the treatment of malignancies, and diseases or disorders associated with neoangiogenesis (such as diabetic retinopathy).

The compounds may also be used in conjunction with and following processes or procedures involving maintaining blood vessel patency, including vascular surgery, vascular graft and stent patency, organ, tissue and cell implantation and transplantation.

The compounds may also be used in the treatment of Alzheimer's disease. This method may also be characterized as the inhibition of plasminogen activator by PAI-1 in a mammal, particularly a human, experiencing or subject to Alzheimer's disease. This method may also be characterized as a method of increasing or normalizing levels of plasmin concentration in a mammal, particularly those experiencing or subject to Alzheimer's disease.

The compounds may be used for the treatment of myelofibrosis with myeloid metaplasia by regulating stromal cell hyperplasia and increases in extracellular matrix proteins.

The compounds may also be used in conjunction with protease inhibitor-containing highly active antiretroviral therapy (HAART) for the treatment of diseases or disorders which originate from fibrinolytic impairment and hypercoagulability of HIV-1 infected patients receiving such therapy.

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The compounds may be used for the treatment of diabetic nephropathy and renal dialysis associated with nephropathy.

The compounds may be used to treat cancer, septicemia, proliferative diseases, such as psoriasis, improving coagulation homeostasis, cerebrovascular diseases, microvascular disease, hypertension, dementia, atherosclerosis, osteoporosis, arthritis, asthma, heart failure, arrhythmia, angina, and as a hormone replacement agent, treating, preventing or reversing progression of atherosclerosis, Alzheimer's disease, osteoporosis, osteopenia; reducing inflammatory markers, fibrinolytic disorder, reducing C-reactive protein, or preventing or treating low grade vascular inflammation, stroke, dementia, coronary heart disease, primary and secondary prevention of myocardial infarction, stable and unstable angina, primary prevention of coronary events, secondary prevention of cardiovascular events, peripheral vascular disease, peripheral arterial disease, acute vascular syndromes, deep vein thrombosis, pulmonary embolism, reducing the risk of undergoing a myocardial revascularization procedure, microvascular diseases such as nephropathy, neuropathy, retinopathy and nephrotic syndrome, hypertension, Type 1 and 2 diabetes and related diseases, obesity, insulin resistance, hyperglycemia, hyperinsulinemia, malignant lesions, premalignant lesions, gastrointestinal malignancies, liposarcomas and epithelial tumors, proliferative diseases such as psoriasis, improving coagulation homeostasis, and/or improving endothelial function, and all forms of cerebrovascular diseases.

The compounds disclosed herein can be used for the topical applications in wound healing for prevention of scarring.

The compounds disclosed herein can be used in the treatment of inflammatory diseases, septic shock and the vascular damage associated with infections and for the treatment of blood and blood products used in dialysis, blood storage in the fluid phase, especially ex vivo platelet aggregation. The compounds may also be used in combination with prothrombolytic, fibrinolytic and anticoagulant agents. The present compounds may also be added to human plasma during the analysis of blood chemistry in hospital settings to determine the fibrinolytic capacity thereof.

Further provided herein are methods for treating, preventing, ameliorating or inhibiting each of the maladies mentioned herein in a mammal, in one aspect, in a human, the method(s) each comprising administering to a mammal in need of such treatment, prevention, amelioration or inhibition a pharmaceutically or therapeutically effective amount of a compound disclosed herein, or a pharmaceutically acceptable salt thereof.

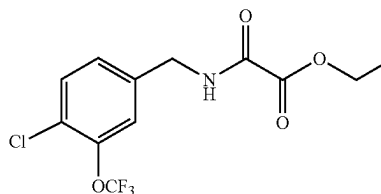
The compounds disclosed herein can also be used to treat cancer including, but not limited to, breast and ovarian cancer, and as imaging agents for the identification of metastatic cancers.

It will be understood that a pharmaceutically or therapeutically effective amount of a compound herein refers to an amount of the compound in question which will sufficiently inhibit the serine protease inhibitor PAI-1 in the mammal in need thereof to a sufficient extent to provide a desirable improvement in the condition in question or provide sufficient inhibition of the serine protease inhibitor PAI-1 to prevent, inhibit or limit the onset of the physiological basis for the malady or condition in question.

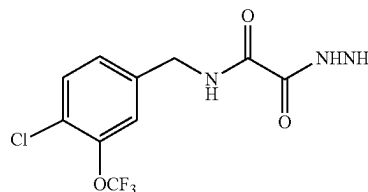
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EXAMPLES

Synthesis of Compound CDE 517:



Ethyl 2-((4-chloro-3-(trifluoromethoxy)benzyl)amino)-2-oxoacetate: A stirring solution of 4-chloro-3-(trifluoromethoxy)benzylamine (771.9 mg, 3.42 mmol) and pyridine (830 μ L, 10.26 mmol) in dichloromethane (10 mL) was cooled in an ice bath. Ethyl oxalyl chloride (385 μ L, 3.42 mmol) was added dropwise and the mixture was stirred for 24 hours at room temperature. The reaction mixture was diluted with ethyl acetate and washed with 0.2N HCl (2 \times), saturated aqueous NaHCO_3 (2 \times), and brine (1 \times). The organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo to afford 1.0726 g (96% yield) of product as a clear oil. $^1\text{H-NMR}$ (DMSO-d_6 , 400 MHz) δ 9.5 (t, $J=6$ Hz, 1H), 7.62 (d, $J=8.2$ Hz, 1H), 7.44 (bs, 1H), 7.3 (dd, $J=8.7$, 1.8 Hz, 1H), 4.33 (d, $J=6.4$ Hz, 2H), 4.21 (q, $J=6.9$ Hz, 2H), 1.23 (t, $J=6.9$ Hz, 3H).



N-(4-chloro-3-(trifluoromethoxy)benzyl)-2-hydrazinyl-2-oxoacetamide (CDE-517): To a solution of ethyl 2-((4-chloro-3-(trifluoromethoxy)benzyl)amino)-2-oxoacetate (1.0726 g, 3.29 mmol) in absolute ethanol (30 mL) was added 50% hydrazine hydrate (425 μ L, 6.59 mmol) dropwise and stirred for 2 hours. The solid was filtered, dried in vacuo, and then triturated with boiling deionized water to afford 0.7191 g (70.2% yield) of product as a white solid. $^1\text{H-NMR}$ (DMSO-d_6 , 400 MHz) δ 10.1 (bs, 1H), 9.37 (t, $J=6.4$ Hz, 1H), 7.61 (d, $J=8.2$ Hz, 1H), 7.42 (bs, 1H), 7.28 (dd, $J=8.3$, 1.8 Hz, 1H), 4.5 (d, $J=3.6$ Hz, 2H), 4.31 (d, $J=6.4$ Hz, 2H); $^{13}\text{C-NMR}$ (DMSO-d_6 , 100 MHz) δ 160.6, 158.3, 144.3, 141.1, 131.4, 128.5, 124.8, 122.4, 120.6 (q, $J=256.5$ Hz), 41.8.

Synthesis of Compound CDE-415:

N-(3-chloro-4-fluorobenzyl)-2-hydrazinyl-2-oxoacetamide (CDE-415): To a solution of 3-chloro-4-fluorobenzylamine (230 μ L, 1.83 mmol) and pyridine (296 μ L, 3.66 mmol) in dichloromethane (5 mL), ethyl 2-chloro-2-oxoacetate (215 μ L, 1.92 mmol) was added dropwise over an ice bath. The solution was removed from the ice bath after 5 minutes and left to warm to room temperature. The reaction mixture was diluted with ethyl acetate, washed with 0.2 N HCl (2 \times) and saturated NaHCO_3 (1 \times), dried with MgSO_4 , filtered and concentrated in vacuo to give 0.336 g of ethyl 2-(3-chloro-4-fluorobenzylamino)-2-oxoacetate as a white solid (71% yield). $^1\text{H NMR}$ (CDCl_3 , 400 MHz) δ 7.42 (s,

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1H), 7.34 (dd, J=2.3, 6.9 Hz, 1H), 7.17 (M, 1H), 7.10 (t, J=8.7 Hz, 1H), 4.46 (d, J=6.0 Hz, 2H), 4.35 (q, J=7.3 Hz, 2H), 1.39 (t, J=6.9 Hz, 3H). To a solution of ethyl 2-(3-chloro-4-fluorobenzylamino)-2-oxoacetate (211.9 mg, 0.816 mmol) in ethanol (6 ml), 50% hydrazine hydrate (102 μ L) was added dropwise. The reaction was stirred overnight at room temperature. The product was filtered from the mixture and dried in vacuo, providing 0.188 g of N-(3-chloro-4-fluorobenzyl)-2-hydrazinyl-2-oxoacetamide as a white solid (94% yield). ¹H NMR (DMSO-d₆, 400 MHz) δ 10.01 (s, 1H), 9.28 (t, J=6.4 Hz, 1H), 7.42 (dd, J=1.8, 5.5 Hz, 1H), 7.32 (t, J=8.7 Hz, 1H), 7.23 (m, 1H), 4.48 (s, 2H), 4.25 (d, J=6.4 Hz, 2H); ¹³C NMR (DMSO-d₆, 100 MHz) δ 160.48, 158.43, 156.75 (d, J=244 Hz), 137.32, 129.97, 128.63, 119.62 (J=18 Hz), 117.25 (J=20 Hz), 41.66.

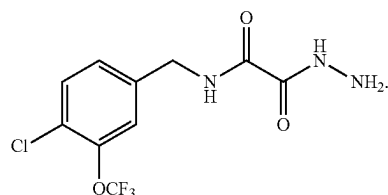
Synthesis of Compound CDE-412:

N-(4-chloro-3-fluorobenzyl)-2-hydrazinyl-2-oxoacetamide (CDE-412): To a solution of 4-chloro-3-fluorobenzylamine (225 μ L, 1.83 mmol) and pyridine (296 μ L, 3.66 mmol) in dichloromethane (5 ml), ethyl 2-chloro-2-oxoacetate (215 μ L, 1.92 mmol) was added dropwise over an ice bath. The solution was removed from the ice bath after 10 minutes and left to warm to room temperature. The reaction mixture was diluted with ethyl acetate, washed with 0.2 N HCl (2 \times) and saturated NaHCO₃ (1 \times), dried with MgSO₄, filtered and concentrated in vacuo to give 0.3878 g (82% yield) of ethyl 2-(4-chloro-3-fluorobenzylamino)-2-oxoacetate as a white solid. ¹H NMR (CDCl₃, 400 MHz) δ 7.45 (s, 1H), 7.36 (t, J=7.96 Hz, 1H), 7.09 (d, J=9.6 Hz, 1H), 7.02 (d, J=8.3 Hz, 1H), 4.48 (d, J=6.4 Hz, 2H), 4.35 (q, J=6.9 Hz, 2H), 1.38 (t, J=6.9 Hz, 3H). To a solution of ethyl 2-(4-chloro-3-fluorobenzylamino)-2-oxoacetate (119.7 mg, 0.461 mmol) in ethanol (6 ml), 50% hydrazine hydrate (57 μ L) was added dropwise. The reaction was stirred overnight at room temperature. The product was filtered from the mixture and dried in vacuo, providing 59.0 mg of N-(4-chloro-3-fluorobenzyl)-2-hydrazinyl-2-oxoacetamide as a white solid (52% yield). ¹H NMR (DMSO-d₆, 400 MHz) δ 10.02 (s, 1H), 9.30 (t, J=6.4 Hz, 1H), 7.49 (t, J=8.24 Hz, 1H), 7.24 (dd, J=1.8, 10.5 Hz, 1H), 7.08 (dd, J=1.36, 8.24 Hz, 1H), 4.51 (s, 2H), 4.27 (d, J=6.4 Hz, 2H); ¹³C NMR (DMSO-d₆, 100 MHz) δ 160.54, 158.74, 158.40, 156.29, 141.29, 141.23, 130.99, 125.08, 125.05, 118.36, 118.19, 116.31, 116.10, 41.86.

Fluorometric IC₅₀ plate assay for PAI-1 inhibitors: For assaying PAI-1 inhibitor activity in plasma, recombinant active human PAI-1 (Molecular Innovations) was added to PAI-1 depleted human plasma (Molecular Innovations) containing 10 μ g/mL aprotinin (Roche) to a concentration 20 nM. Then 10 μ L of this human plasma (with or without PAI-1) was added to wells containing 80 μ L of buffer with increasing concentrations of the PAI-1 inhibitor (Buffer: 40 mM HEPES, 100 mM NaCl, 0.005% Tween-20, pH 7.4, and 10% DMSO) and incubated for 15 min at 23° C. Next, 10 μ L of 25 nM UPA (rheotromb) (final 2.5 nM) was added to each reaction well and incubated for an additional 30 min at 24° C., the final PAI-1 concentration was 2 nM and the final uPA concentration was 2.5 nM. Following this incubation 100 μ L of buffer containing 100 mM of the uPA fluorogenic substrate Z-Gly-Gly-Arg-AMC (Calbiochem) is added for a final concentration of 50 μ M, and the residual uPA activity in each reaction mixture was determined from the rate of AMC release by uPA measured with an excitation wavelength of 370 nm and an emission wavelength of 440 nm for 10 minutes at 23° C. Data are expressed as the residual PAI-1 activity as a percent of the control PAI-1 activity.

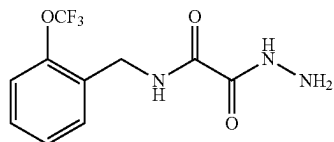
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For assays in buffer or buffer containing 1.5% bovine serum albumin (BSA) the assay was as above except that no plasma or aprotinin was added and activity was determined in either 100 mM NaCl, 40 mM HEPES, 0.005% Tween-20, 10% DMSO, pH 7.4, or in the same buffer containing 1.5% BSA. Results are shown in the figures. FIG. 1 is for a compound of structure



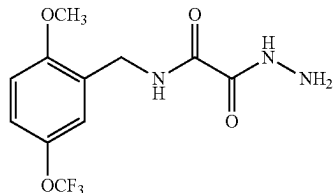
(CDE-517)

FIG. 2 is for a compound of structure



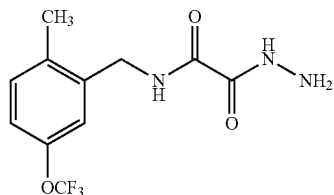
(CDE-252)

FIG. 3 is for a compound of structure



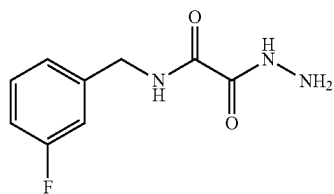
(CDE-519)

FIG. 4 is for a compound of structure



(CDE-520)

FIG. 5 is for a compound of structure



(CDE-264)

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FIG. 6 is for a compound of structure

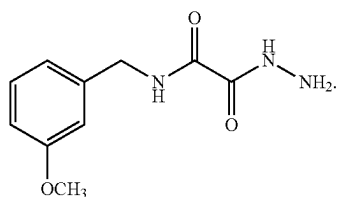


FIG. 7 is for a compound of structure

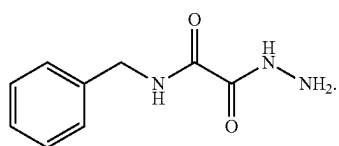


FIG. 8 is for a compound of structure

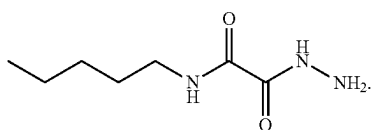


FIG. 9 is for a compound of structure

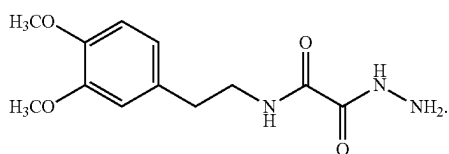


FIG. 10 is for a compound of structure

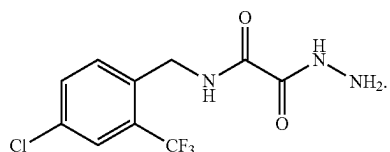
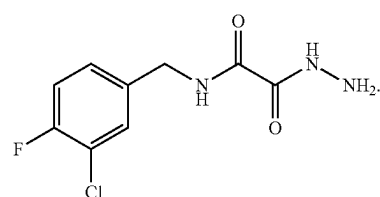
**22**

FIG. 11 is for a compound of structure

(CDE-295)

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(CDE-415)

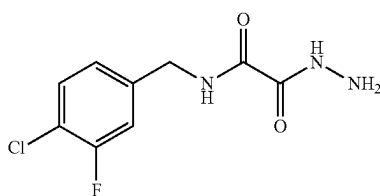
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FIG. 12 is for a compound of structure

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(CDE-234)

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(CDE-412)

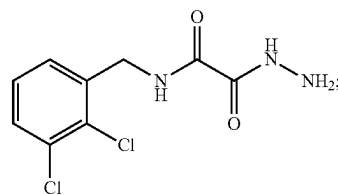
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These data compare to those of monohalophenyl compounds CDE-248 and CDE-266 (FIGS. 13 and 14, respectively), as well as to various other dihalophenyl compounds:

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(CDE-241)

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(CDE-301, FIG. 15)

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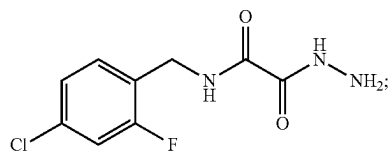
FIG. 15);

(CDE-246)

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(CDE-307, FIG. 16)

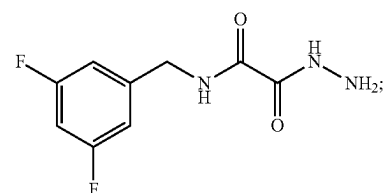
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55 FIG. 16);

(CDE-413)

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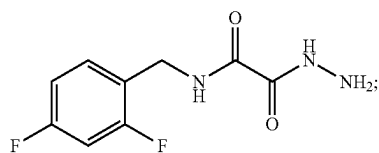


(CDE-340, FIG. 17)

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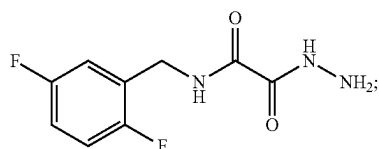
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FIG. 17);



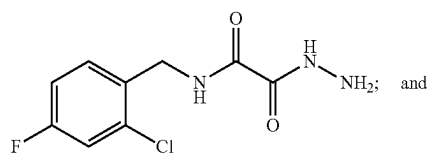
(CDE-422, FIG. 18)

FIG. 18);



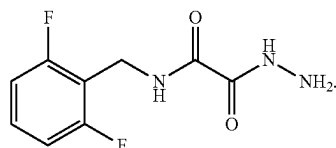
(CDE-423, FIG. 19)

FIG. 19);



(CDE-424, FIG. 20)

FIG. 20); and

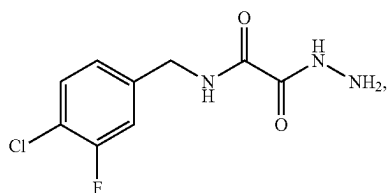


(CDE-446, FIG. 21)

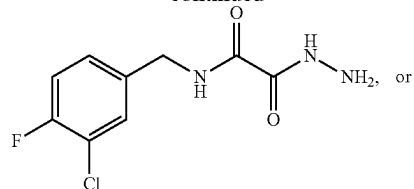
FIG. 21).

What is claimed:

1. A method of inhibiting plasminogen activator inhibitor-1 (PAI-1) comprising contacting PAI-1 with a compound which is

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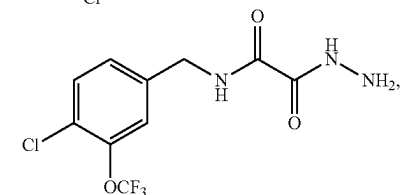
-continued



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in an amount effective to inhibit PAI-1.

2. A method of treating fibrosis, comprising administering to a subject in need thereof a compound which is

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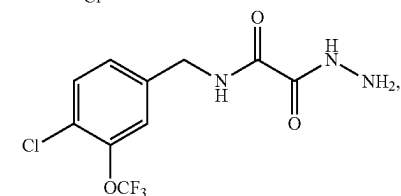
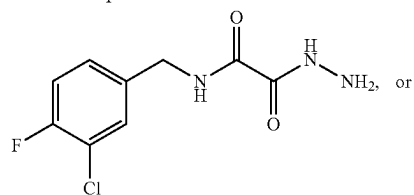
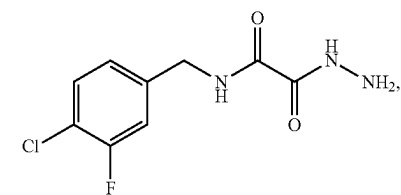
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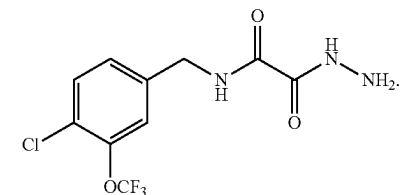
or a pharmaceutically acceptable salt thereof, in an amount effective to treat fibrosis.

3. The method of claim 2, wherein the fibrosis is pulmonary fibrosis, renal fibrosis, cardiac fibrosis, hepatic fibrosis, or scleroderma.

4. The method of claim 1, wherein the compound is

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* * * * *