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## (54) Title: COMPOSITIONS AND METHODS FOR TREATING BRAIN INJURY

(57) **Abstract:** Compounds, and compositions, methods, and uses thereof, are described herein for treating brain injuries.

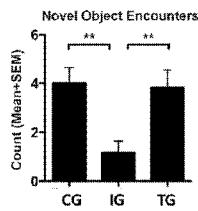
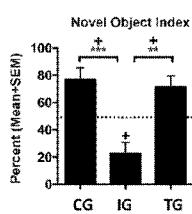
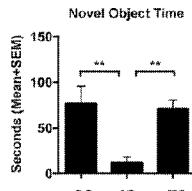


FIG. 1

**Declarations under Rule 4.17:**

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*
- *of inventorship (Rule 4.17(iv))*

**Published:**

- *with international search report (Art. 21(3))*

## TECHNICAL FIELD

5 The invention described herein pertains to compounds, and compositions, methods, and uses thereof, for treating brain injuries. For example, the invention described herein pertains to vasopressin receptor modulators, and compositions, methods, and uses thereof, for treating brain injury.

## BACKGROUND AND SUMMARY OF THE INVENTION

10 It has been reported that 2.5 million Americans per year suffer a traumatic brain injury (TBI). These injuries are reported to result in 50,000 deaths and 275,000 hospital admissions. In addition, these injuries reportedly result in an average lifetime expense of \$550,000/patient, and an annual economic burden estimated at \$86 billion. TBI contributes to a third of injury-related deaths in the US and is among the leading causes of death and disability in people under 35 and over 65.

15 Brain injuries often have a common feature in cerebral edema and swelling. Cerebral edema, often termed the secondary injury in TBI, follows the primary insult and is a major contributor to morbidity and mortality. Left untreated, brain injury can also lead to severe cognitive, learning, and memory impairment. In addition, brain injuries can lead to loss of motor function. Cerebral edema can result from brain trauma as well as other nontraumatic 20 causes such as ischemic stroke, cancer, and brain inflammation that may arise from other diseases or conditions, such as meningitis or encephalitis. A treatment that mitigates or prevents the effects of moderate to severe TBI, including closed head, non-penetrating, moderate TBIs that result from single incidents, such as car accidents, falls, and the like is needed to fill this critical gap in care. Accordingly, there is a current need for compounds, 25 compositions, and methods for treating brain injury.

It has been discovered that the selective arginine vasopressin V1a receptor (V1aR) antagonists described herein are useful in treating brain injuries. It has also been unexpectedly discovered that the compounds are efficacious in treating edema, as well as cognitive, memory, and learning deficits that arise from brain injury. It is to be understood that 30 the compositions, methods, and uses described herein are not limited by the source of the brain injury, which may instead stem from a wide variety of causes, including but not limited to, impacts, rapid acceleration or deceleration of the head, shock waves, chemotherapy, such as CAR-T therapy, or as a comorbidity with another disease.

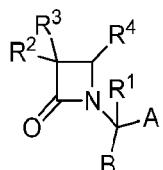
Arginine vasopressin (AVP) is a chemical signal in the brain influencing

cerebral vascular resistance and brain water permeability. Without being bound by theory, it is suggested herein that AVP is a chemical signal in the brain influencing cerebral vascular resistance and brain water permeability and may contribute to the pathophysiology following head trauma or injury. These cerebrovascular effects are mediated through the V1aR receptor, 5 which is highly expressed in cortical and subcortical brain areas across all mammals. However, it has been reported that the systemic treatment of brain injury is complicated by unwanted side effects arising from competing antagonism of peripheral V1aR receptors, such as decreases in cerebral blood flow, blood pressure, or other loss of cardiovascular function. Krieg et al., *J. Neurotrauma* 32:221-27 (2015).

10 It has been unexpectedly discovered that the compounds described herein can be administered systemically, including oral administration at doses that are therapeutically effective in the central nervous system (CNS), without clinically significant adverse affects on blood pressure or cardiovascular function.

15 In one illustrative embodiment of the invention, selective V1aR antagonists, and compositions and methods for using such vasopressin antagonists, are described herein. In another illustrative embodiment, selective V1aR antagonists, and compositions and methods for using such V1aR antagonists, that are configured to achieve or capable of generating CNS concentrations in the range from 1 nM to at least about 100 nM upon administration to a host animal are described herein. In another illustrative embodiment, selective V1aR antagonists, 20 and compositions and methods for using such V1aR antagonists, that are configured to achieve or capable of generating CNS concentrations in the range from 1 nM to at least about 10 nM, or at least about 1 nM upon administration to a host animal are described herein. In another illustrative embodiment, highly potent and selective V1aR antagonists, and compositions and methods for using such V1aR antagonists, that are configured to achieve or capable of 25 generating CNS concentrations of at least about 100 pM, at least about 10 pM, or at least about 1 pM, upon administration to a host animal are described herein.

It has been discovered herein that brain injuries are treatable with the selective V1aR antagonists described herein. In one embodiment, the vasopressin receptor antagonists are of the formula



30

and pharmaceutically acceptable salts thereof; wherein

A is a carboxylic acid, an ester, or an amide;

B is a carboxylic acid, an ester, or an amide; or B is an alcohol or thiol, or a

derivative thereof, including alkyl, aryl, or acyl derivatives thereof;

R<sup>1</sup> is hydrogen or C<sub>1</sub>-C<sub>6</sub> alkyl;

R<sup>2</sup> is hydrogen, alkyl, alkenyl, alkynyl, alkoxy, alkylthio, halo, haloalkyl, cyano, 5 formyl, alkylcarbonyl, or a substituent selected from the group consisting of -CO<sub>2</sub>R<sup>8</sup>, -CONR<sup>8</sup>R<sup>8'</sup>, and -NR<sup>8</sup>(COR<sup>9</sup>); where R<sup>8</sup> and R<sup>8'</sup> are each independently selected from hydrogen, alkyl, cycloalkyl, optionally substituted aryl, or optionally substituted arylalkyl; or R<sup>8</sup> and R<sup>8'</sup> are taken together with the attached nitrogen atom to form a heterocyclyl group; and where R<sup>9</sup> is selected from hydrogen, alkyl, cycloalkyl, alkoxyalkyl, optionally substituted 10 aryl, optionally substituted arylalkyl, optionally substituted heteroaryl, optionally substituted heteroarylalkyl, and R<sup>8</sup>R<sup>8'</sup>N-(C<sub>1</sub>-C<sub>4</sub> alkyl);

R<sup>3</sup> is an amino, amido, acylamido, or ureido group, which is optionally substituted; or R<sup>3</sup> is a nitrogen-containing heterocyclyl group attached at a nitrogen atom; and

R<sup>4</sup> is alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, alkylcarbonyl, optionally 15 substituted aryl, optionally substituted arylalkyl, optionally substituted arylhaloalkyl, optionally substituted arylalkoxyalkyl, optionally substituted arylalkenyl, optionally substituted arylhaloalkenyl, or optionally substituted arylalkynyl.

In another embodiment, pharmaceutical compositions containing one or more of the compounds are also described herein. In one aspect, the compositions include a

20 therapeutically effective amount of the one or more compounds for treating a host animal with a brain injury. It is to be understood that the compositions may include other components and/or ingredients, including, but not limited to, other therapeutically active compounds, and/or one or more carriers, diluents, excipients, and the like, and combinations thereof. In another embodiment, methods for using the compounds and pharmaceutical compositions for treating 25 host animals with a brain injury are also described herein. In one aspect, the methods include the step of administering one or more of the compounds and/or compositions described herein to the host animal. In another aspect, the methods include administering a therapeutically effective amount of the one or more compounds and/or compositions described herein for treating host animals a brain injury. In another embodiment, uses of the compounds and 30 compositions in the manufacture of a medicament for treating host animals with a brain injury are also described herein. In one aspect, the medicaments include a therapeutically effective amount of the one or more compounds and/or compositions described herein.

It is to be understood herein that the compounds described herein may be used alone or in combination with other compounds useful for treating brain injuries, including those 35 compounds that may be therapeutically effective by the same or different modes of action. In

addition, it is to be understood herein that the compounds described herein may be used in combination with other compounds that are administered to treat other symptoms of a brain injury, such as pain, inflammation, nausea, vomiting, blurred vision, faintness, and the like.

## BRIEF DESCRIPTION OF THE DRAWINGS

5 FIG. 1 shows results for the Novel Object Recognition Test; Control Group (CG), Injury Group (IG), Treatment Group (TG); \* (p<0.05), \*\* (p<0.01), \*\*\* (p<0.001).

FIG. 2A shows results for the Barnes Maze Test; Control Group (CG), Injury Group (IG), Treatment Group (TG); \* (p<0.05), \*\* (p<0.01).

10 FIG. 2B shows results for the Barnes Maze Test broken down by days; Control Group (●), Injury Group (○), Treatment Group (□); \* (p<0.05), \*\* (p<0.01).

FIG. 3 shows Resting State Functional Connectivity comparing the Injury Group to the Treatment Group.

FIG. 4A shows glass brains showing the 3D organizations of the hypoconnectivity observed in the Injury Group for Area G.

15 FIG. 4B shows glass brains showing the 3D organizations of the hyperconnectivity observed in the Treatment Group for Area G.

FIG. 5 shows scans and volumes of lateral ventricles; Control Group (CG), Injury Group (IG), Treatment Group (TG).

20 FIG. 6 shows glass brains showing the 3D organizations of the hypoconnectivity observed in the Injury Group and Treatment Group for Area M.

## DETAILED DESCRIPTION

Described herein is the use of one or more V1aR antagonists for treating brain injuries. The compounds described herein may have the potential to greatly improve the lives of those suffering from brain injuries, including, but not limited to traumatic brain injury (TBI), 25 including blast TBI, cerebral edema, chronic traumatic encephalopathy (CTE), subarachnoid hemorrhage, stroke, concussion, and falls.

Several illustrative embodiments of the invention are described by the following illustrative clauses:

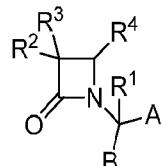
30 A method for treating a brain injury in a host animal is described, where the method comprises administering a composition comprising one or more selective V1aR antagonists described herein to the host animal.

A method for treating a brain injury in a host animal is described, where the brain injury is caused at least in part by impact, traumatic brain injury (TBI), mild TBI, blast TBI, cerebral edema, chronic traumatic encephalopathy (CTE), subarachnoid hemorrhage,

stroke, ischemic stroke, concussion, falls, or a combination thereof, and where the method comprises administering a composition comprising one or more selective V1aR antagonists described herein to the host animal.

5 The method of any one of the preceding clauses wherein the brain injury is characterized by edema, such as lateral ventricle edema, cognitive deficits, learning deficits, memory deficits, or motor deficits, or a combination of any of the foregoing.

The method of any one of the preceding clauses wherein one or more of the antagonists are selected from compounds of the formula:



10 and pharmaceutically acceptable salts thereof, wherein

A is a carboxylic acid, an ester, or an amide;

B is a carboxylic acid, an ester, or an amide; or B is an alcohol or thiol, or a derivative thereof;

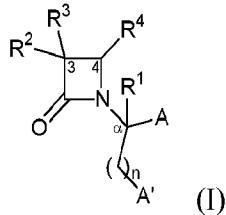
R<sup>1</sup> is hydrogen or C<sub>1</sub>-C<sub>6</sub> alkyl;

15 R<sup>2</sup> is hydrogen, alkyl, alkenyl, alkynyl, alkoxy, alkylthio, halo, haloalkyl, cyano, formyl, alkylcarbonyl, or a substituent selected from the group consisting of -CO<sub>2</sub>R<sup>8</sup>, -CONR<sup>8</sup>R<sup>8'</sup>, and -NR<sup>8</sup>(COR<sup>9</sup>); where R<sup>8</sup> and R<sup>8'</sup> are each independently selected from hydrogen, alkyl, cycloalkyl, optionally substituted aryl, or optionally substituted arylalkyl; or R<sup>8</sup> and R<sup>8'</sup> are taken together with the attached nitrogen atom to form a heterocyclyl group; and where R<sup>9</sup> is selected from hydrogen, alkyl, cycloalkyl, alkoxyalkyl, optionally substituted aryl, optionally substituted arylalkyl, optionally substituted heteroaryl, optionally substituted heteroarylalkyl, and R<sup>8</sup>R<sup>8'</sup>N-(C<sub>1</sub>-C<sub>4</sub> alkyl);

R<sup>3</sup> is an amino, amido, acylamido, or ureido group, which is optionally substituted; or R<sup>3</sup> is a nitrogen-containing heterocyclyl group attached at a nitrogen atom; and

25 R<sup>4</sup> is alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, alkylcarbonyl, optionally substituted aryl, optionally substituted arylalkyl, optionally substituted arylhaloalkyl, optionally substituted arylalkoxyalkyl, optionally substituted arylalkenyl, optionally substituted arylhaloalkenyl, or optionally substituted arylalkynyl.

The method of any one of the preceding clauses wherein one or more of the antagonists are selected from compounds of the formula:



and pharmaceutically acceptable salts thereof, wherein

A and A' are each independently selected from  $-\text{CO}_2\text{H}$ , or an ester or amide derivative thereof;

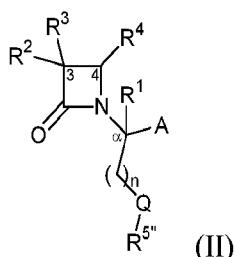
5 n is an integer selected from 0 to about 3;

$\text{R}^1$  is hydrogen or  $\text{C}_1\text{-C}_6$  alkyl;

$\text{R}^2$  is hydrogen, alkyl, alkenyl, alkynyl, alkoxy, alkylthio, halo, haloalkyl, cyano, formyl, alkylcarbonyl, or a substituent selected from the group consisting of  $-\text{CO}_2\text{R}^8$ ,  $-\text{CONR}^8\text{R}^{8'}$ , and  $-\text{NR}^8(\text{COR}^9)$ ; where  $\text{R}^8$  and  $\text{R}^{8'}$  are each independently selected from hydrogen, alkyl, cycloalkyl, optionally substituted aryl, or optionally substituted arylalkyl; or  $\text{R}^8$  and  $\text{R}^{8'}$  are taken together with the attached nitrogen atom to form an heterocycle; and where  $\text{R}^9$  is selected from hydrogen, alkyl, cycloalkyl, alkoxyalkyl, optionally substituted aryl, optionally substituted arylalkyl, optionally substituted heteroaryl, optionally substituted heteroarylalkyl, and  $\text{R}^8\text{R}^{8'}\text{N}-(\text{C}_1\text{-C}_4$  alkyl);

15  $\text{R}^3$  is an amino, amido, acylamido, or ureido group, which is optionally substituted; or  $\text{R}^3$  is a nitrogen-containing heterocyclyl group attached at a nitrogen atom; and  
 $\text{R}^4$  is alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, alkylcarbonyl, optionally substituted aryl, optionally substituted arylalkyl, optionally substituted arylhaloalkyl, optionally substituted arylalkoxyalkyl, optionally substituted arylalkenyl, optionally substituted arylhaloalkenyl, or optionally substituted arylalkynyl.

The method of any one of the preceding clauses wherein one or more of the antagonists are selected from compounds of the formula:



and pharmaceutically acceptable salts thereof, wherein

25 A is  $-\text{CO}_2\text{H}$ , or an ester or amide derivative thereof;

Q is oxygen; or Q is sulfur or disulfide, or an oxidized derivative thereof;

n is an integer from 1 to 3;

$R^1$ ,  $R^2$ ,  $R^3$ , and  $R^4$  are as defined in formula I; and

$R^5$  is selected from hydrogen, alkyl, cycloalkyl, alkoxyalkyl, optionally substituted arylalkyl, optionally substituted heterocyclyl or optionally substituted heterocyclalkyl, and optionally substituted aminoalkyl.

5 The method of any one of the preceding clauses wherein A is  $-CO_2R^5$ ; where  $R^5$  is selected from hydrogen, alkyl, cycloalkyl, alkoxyalkyl, optionally substituted arylalkyl, heterocyclyl, heterocyclyl(C<sub>1</sub>-C<sub>4</sub> alkyl), and  $R^6R^7N-(C_2-C_4\text{ alkyl})$ .

The method of any one of the preceding clauses wherein A is monosubstituted amido, disubstituted amido, or an optionally substituted nitrogen-containing heterocycllamido.

10 The method of any one of the preceding clauses wherein heterocyclyl is independently selected from tetrahydrafuryl, morpholinyl, pyrrolidinyl, piperidinyl, piperazinyl, homopiperazinyl, or quinuclidinyl; where said morpholinyl, pyrrolidinyl, piperidinyl, piperazinyl, homopiperazinyl, or quinuclidinyl is optionally N-substituted with C<sub>1</sub>-C<sub>4</sub> alkyl or optionally substituted aryl(C<sub>1</sub>-C<sub>4</sub> alkyl). It is to be understood that in each occurrence of the 15 various embodiments described herein, heterocyclyl is independently selected in each instance.

The method of any one of the preceding clauses wherein  $R^6$  is independently selected from hydrogen or alkyl; and  $R^7$  is independently selected in each instance from alkyl, cycloalkyl, optionally substituted aryl, or optionally substituted arylalkyl. The method of any one of the preceding clauses wherein  $R^6$  and  $R^7$  are taken together with the attached nitrogen 20 atom to form an optionally substituted heterocycle, such as pyrrolidinyl, piperidinyl, morpholinyl, piperazinyl, and homopiperazinyl; where said piperazinyl or homopiperazinyl is also optionally N-substituted with  $R^{13}$ ; where  $R^{13}$  is independently selected in each instance from hydrogen, alkyl, cycloalkyl, alkoxy carbonyl, optionally substituted aryloxy carbonyl, optionally substituted arylalkyl, and optionally substituted aryloyl. It is also to be understood 25 that in each occurrence of the various embodiments described herein,  $R^6$  and  $R^7$  are each independently selected in each instance.

The method of any one of the preceding clauses wherein A and/or A' is an amide. The method of any one of the preceding clauses wherein both A and A' are amides. The method of any one of the preceding clauses wherein A and/or A' is an amide of a secondary 30 amine, also referred to herein as a secondary amide. The method of any one of the preceding clauses wherein both A and A' are secondary amides. It is to be understood that secondary amides include amides of cyclic amines attached at nitrogen.

35 The method of any one of the preceding clauses wherein A is an amide. The method of any one of the preceding clauses wherein A is an amide of a secondary amine, also referred to herein as a secondary amide.

The method of any one of the preceding clauses wherein the antagonists are diesters, acid-esters, or diacids, including pharmaceutically acceptable salts thereof, where each of A and A' is independently selected. The method of any one of the preceding clauses wherein the antagonists are ester-amides, where one of A and A' is an ester, and the other is an amide.

5 The method of any one of the preceding clauses wherein the antagonists are diamides, where each of A and A' are independently selected from monosubstituted amido, disubstituted amido, and optionally substituted nitrogen-containing heterocycllamido.

The method of any one of the preceding clauses wherein A and/or A' is an independently selected monosubstituted amido of the formula C(O)NHX-, where X is selected 10 from alkyl, cycloalkyl, alkoxyalkyl, optionally substituted aryl, optionally substituted arylalkyl, heterocycl, heterocycl-(C<sub>1</sub>-C<sub>4</sub> alkyl), R<sup>6</sup>R<sup>7</sup>N-, and R<sup>6</sup>R<sup>7</sup>N-(C<sub>2</sub>-C<sub>4</sub> alkyl), where each heterocycl is independently selected.

The method of any one of the preceding clauses wherein A and/or A' is an independently selected disubstituted amido of the formula C(O)NR<sup>14</sup>X-, where R<sup>14</sup> is selected 15 from hydroxy, alkyl, alkoxy carbonyl, and benzyl; and X is selected from alkyl, cycloalkyl, alkoxyalkyl, optionally substituted aryl, optionally substituted arylalkyl, heterocycl, heterocycl-(C<sub>1</sub>-C<sub>4</sub> alkyl), R<sup>6</sup>R<sup>7</sup>N-, and R<sup>6</sup>R<sup>7</sup>N-(C<sub>2</sub>-C<sub>4</sub> alkyl), where each heterocycl is independently selected.

The method of any one of the preceding clauses wherein A and/or A' is an amide 20 of an independently selected optionally substituted nitrogen-containing heterocycle attached at a nitrogen. Illustrative nitrogen-containing heterocycles include but are not limited to pyrrolidinyl, pyrrolidinonyl, piperidinyl, piperidinonyl, piperazinyl, homopiperazinyl, triazolidinyl, triazinyl, oxazolidinyl, isoxazolidinyl, thiazolidinyl, isothiazolidinyl, 1,2-oxazinyl, 1,3-oxazinyl, morpholinyl, oxadiazolidinyl, thiadiazolidinyl, and 1,2,3,4-tetrahydroisoquinolin-2-yl; each of which is optionally substituted. Such optional substitutions include the groups R<sup>10</sup>, R<sup>12</sup>, R<sup>6</sup>R<sup>7</sup>N-, and R<sup>6</sup>R<sup>7</sup>N-(C<sub>1</sub>-C<sub>4</sub> alkyl), as defined herein.

The method of any one of the preceding clauses wherein A and/or A' is independently optionally substituted 2-(pyrrolidin-1-ylmethyl)pyrrolidin-1-yl attached at a nitrogen.

30 The method of any one of the preceding clauses wherein A and/or A' is independently optionally substituted 1,2,3,4-tetrahydroisoquinolin-2-yl attached at a nitrogen.

The method of any one of the preceding clauses wherein A and/or A' is an independently selected amide of an optionally substituted piperidinyl attached at the nitrogen. Illustrative optional substitutions include hydroxy, alkyl, cycloalkyl, alkoxy, alkoxy carbonyl, 35 hydroxy alkyloxyalkyl, including (hydroxy(C<sub>2</sub>-C<sub>4</sub> alkyloxy))-(C<sub>2</sub>-C<sub>4</sub> alkyl), R<sup>6</sup>R<sup>7</sup>N-, R<sup>6</sup>R<sup>7</sup>N-

The method of any one of the preceding clauses wherein A and/or A' is an independently selected piperidinyl substituted at the 4-position and attached at the nitrogen.

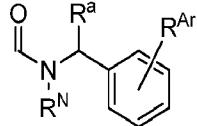
5 The method of any one of the preceding clauses wherein A and/or A' is an independently selected amide of an optionally substituted piperazinyl attached at a nitrogen. Illustrative optional substitutions include hydroxy, alkyl, cycloalkyl, alkoxy, alkoxy carbonyl, hydroxyalkyloxyalkyl, including (hydroxy( $C_2-C_4\text{ alkyl}$ ))- $(C_2-C_4\text{ alkyl})$ ,  $R^6R^7N-$ ,  $R^6R^7N-$ alkyl, including  $R^6R^7N-(C_1-C_4\text{ alkyl})$ , diphenylmethyl, optionally substituted aryl, optionally substituted aryl( $C_1-C_4\text{ alkyl}$ ), and piperidin-1-yl( $C_1-C_4\text{ alkyl}$ ). The method of any one of the preceding clauses wherein A and/or A' is an independently selected piperazinyl substituted at the 4-position and attached at a nitrogen.

10 The method of any one of the preceding clauses wherein A and/or A' is an independently selected amide of an optionally substituted homopiperazinyl attached at a nitrogen. Illustrative optional substitutions include hydroxy, alkyl, cycloalkyl, alkoxy, alkoxy carbonyl, hydroxyalkyloxyalkyl, including (hydroxy( $C_2-C_4\text{ alkyl}$ ))- $(C_2-C_4\text{ alkyl})$ ,  $R^6R^7N-$ ,  $R^6R^7N-$ alkyl, including  $R^6R^7N-(C_1-C_4\text{ alkyl})$ , diphenylmethyl, optionally substituted aryl, optionally substituted aryl( $C_1-C_4\text{ alkyl}$ ), and piperidin-1-yl( $C_1-C_4\text{ alkyl}$ ). The method of any one of the preceding clauses wherein A and/or A' is an independently selected homopiperazinyl substituted at the 4-position and attached at a nitrogen. The method of any one of the preceding clauses wherein A and/or A' is an independently selected homopiperazinyl substituted at the 4-position with alkyl, aryl, aryl( $C_1-C_4\text{ alkyl}$ ), and attached at a nitrogen.

15 The method of any one of the preceding clauses wherein A' is monosubstituted amido, disubstituted amido, or an optionally substituted nitrogen-containing heterocyclylamido.

20 The method of any one of the preceding clauses wherein A' is  $-CO_2R^5'$ ; where  $R^5'$  is selected from hydrogen, alkyl, cycloalkyl, alkoxyalkyl, optionally substituted arylalkyl, heterocycl, heterocycl( $C_1-C_4\text{ alkyl}$ ), and  $R^6R^7N-(C_2-C_4\text{ alkyl})$ ; where heterocycl is in each occurrence independently selected from tetrahydropyran, morpholinyl, pyrrolidinyl, piperidinyl, piperazinyl, homopiperazinyl, or quinuclidinyl; where said morpholinyl, pyrrolidinyl, piperidinyl, piperazinyl, homopiperazinyl, or quinuclidinyl is optionally N-substituted with  $C_1-C_4\text{ alkyl}$  or optionally substituted aryl( $C_1-C_4\text{ alkyl}$ ). The method of any one of the preceding clauses wherein  $R^5'$  is optionally substituted heterocyclalkyl or optionally substituted aminoalkyl, including  $R^6R^7N-(C_2-C_4\text{ alkyl})$ .

25 The method of any one of the preceding clauses wherein A is of the formula



where R<sup>N</sup> is hydrogen or optionally substituted alkyl, or an amide prodrug forming group; R<sup>a</sup> is hydrogen or optionally substituted alkyl; and R<sup>Ar</sup> is hydrogen or one or more aryl substituents, such as but not limited to halo, hydroxy, optionally substituted alkyl, optionally substituted

5 alkoxy, nitro, and the like. The method of any one of the preceding clauses wherein at least one of R<sup>N</sup>, R<sup>a</sup>, and R<sup>Ar</sup> is not hydrogen. The method of any one of the preceding clauses wherein at least one of R<sup>N</sup> and R<sup>a</sup> is not hydrogen. In another embodiment, A is of the formula



where R<sup>N</sup>, R<sup>a</sup>, and R<sup>Ar</sup> are as defined herein.

10 The method of any one of the preceding clauses wherein A is selected from monosubstituted amido, disubstituted amido, and optionally substituted nitrogen-containing heterocyclamido. The method of any one of the preceding clauses wherein A is an amide of optionally substituted 1-tetrahydronaphthylamine.

15 The method of any one of the preceding clauses wherein A and/or A' is a monosubstituted amido of the formula C(O)NHX, where X is selected from alkyl, cycloalkyl, alkoxyalkyl, optionally substituted aryl, optionally substituted arylalkyl, heterocyclyl, heterocyclyl-(C<sub>1</sub>-C<sub>4</sub> alkyl), R<sup>6</sup>R<sup>7</sup>N-, and R<sup>6</sup>R<sup>7</sup>N-(C<sub>2</sub>-C<sub>4</sub> alkyl), where each heterocyclyl is independently selected.

20 The method of any one of the preceding clauses wherein A and/or A' is a disubstituted amido of the formula C(O)NR<sup>14</sup>X, where R<sup>14</sup> is selected from hydroxy, alkyl, alkoxy carbonyl, and benzyl; and X is selected from alkyl, cycloalkyl, alkoxyalkyl, optionally substituted aryl, optionally substituted arylalkyl, heterocyclyl, heterocyclyl-(C<sub>1</sub>-C<sub>4</sub> alkyl), R<sup>6</sup>R<sup>7</sup>N-, and R<sup>6</sup>R<sup>7</sup>N-(C<sub>2</sub>-C<sub>4</sub> alkyl), where each heterocyclyl is independently selected.

25 The method of any one of the preceding clauses wherein A and/or A' is an amide of an optionally substituted nitrogen-containing heterocycle attached at a nitrogen. Illustrative nitrogen-containing heterocycles include but are not limited to pyrrolidinyl, pyrrolidinonyl, piperidinyl, piperidinonyl, piperazinyl, homopiperazinyl, triazolidinyl, triazinyl, oxazolidinyl, isoxazolidinyl, thiazolidinyl, isothiazolidinyl, 1,2-oxazinyl, 1,3-oxazinyl, morpholinyl, oxadiazolidinyl, thiadiazolidinyl, and 1,2,3,4-tetrahydroisoquinolin-2-yl; each of which is 30 optionally substituted. Such optional substitutions include the groups R<sup>10</sup>, R<sup>12</sup>, R<sup>6</sup>R<sup>7</sup>N-, and

The method of any one of the preceding clauses wherein A is optionally substituted 2-(pyrrolidin-1-ylmethyl)pyrrolidin-1-yl attached at a nitrogen.

5 The method of any one of the preceding clauses wherein A is optionally substituted 1,2,3,4-tetrahydroisoquinolin-2-yl attached at a nitrogen.

The method of any one of the preceding clauses wherein A and/or A' is an amide of an optionally substituted piperidinyl attached at the nitrogen. Illustrative optional substitutions include hydroxy, alkyl, cycloalkyl, alkoxy, alkoxy carbonyl, hydroxyalkyloxyalkyl, including (hydroxy(C<sub>2</sub>-C<sub>4</sub> alkyloxy))-(C<sub>2</sub>-C<sub>4</sub> alkyl), R<sup>6</sup>R<sup>7</sup>N-, R<sup>6</sup>R<sup>7</sup>N-alkyl, including R<sup>6</sup>R<sup>7</sup>N-(C<sub>1</sub>-C<sub>4</sub> alkyl), diphenylmethyl, optionally substituted aryl, optionally substituted aryl(C<sub>1</sub>-C<sub>4</sub> alkyl), and piperidin-1-yl(C<sub>1</sub>-C<sub>4</sub> alkyl). The method of any one of the preceding clauses wherein A and/or A' is piperidinyl substituted at the 4-position and attached at the nitrogen.

15 The method of any one of the preceding clauses wherein A and/or A' is an amide of an optionally substituted piperazinyl attached at a nitrogen. Illustrative optional substitutions include hydroxy, alkyl, cycloalkyl, alkoxy, alkoxy carbonyl, hydroxyalkyloxyalkyl, including (hydroxy(C<sub>2</sub>-C<sub>4</sub> alkyloxy))-(C<sub>2</sub>-C<sub>4</sub> alkyl), R<sup>6</sup>R<sup>7</sup>N-, R<sup>6</sup>R<sup>7</sup>N-alkyl, including R<sup>6</sup>R<sup>7</sup>N-(C<sub>1</sub>-C<sub>4</sub> alkyl), diphenylmethyl, optionally substituted aryl, optionally substituted aryl(C<sub>1</sub>-C<sub>4</sub> alkyl), and piperidin-1-yl(C<sub>1</sub>-C<sub>4</sub> alkyl). The method of any one of the preceding clauses wherein A and/or A' is piperazinyl substituted at the 4-position and attached at a nitrogen.

20 The method of any one of the preceding clauses wherein A and/or A' is an amide of an optionally substituted homopiperazinyl attached at a nitrogen. Illustrative optional substitutions include hydroxy, alkyl, cycloalkyl, alkoxy, alkoxy carbonyl, hydroxyalkyloxyalkyl, including (hydroxy(C<sub>2</sub>-C<sub>4</sub> alkyloxy))-(C<sub>2</sub>-C<sub>4</sub> alkyl), R<sup>6</sup>R<sup>7</sup>N-, R<sup>6</sup>R<sup>7</sup>N-alkyl, including R<sup>6</sup>R<sup>7</sup>N-(C<sub>1</sub>-C<sub>4</sub> alkyl), diphenylmethyl, optionally substituted aryl, optionally substituted aryl(C<sub>1</sub>-C<sub>4</sub> alkyl), and piperidin-1-yl(C<sub>1</sub>-C<sub>4</sub> alkyl). The method of any one of the preceding clauses wherein A and/or A' is homopiperazinyl substituted at the 4-position and attached at a nitrogen. The method of any one of the preceding clauses wherein A and/or A' is homopiperazinyl substituted at the 4-position with alkyl, aryl, aryl(C<sub>1</sub>-C<sub>4</sub> alkyl), and attached at a nitrogen.

30 The method of any one of the preceding clauses wherein A and/or A' is an amide of a heterocycle attached at a nitrogen, where the heterocycle is substituted with heterocyclyl, heterocyclalkyl, cycloalkyl, cycloalkylalkyl, aryl, arylalkyl.

35 The method of any one of the preceding clauses wherein A and/or A' is an amide of an optionally substituted benzyl, optionally substituted 1-naphthylmethyl, or optionally

substituted 2-naphthylmethyl amine. Optional substitutions include, but are not limited to, 2,3-dichloro, 2,5-dichloro, 2,5-dimethoxy, 2-trifluoromethyl, 2-fluoro-3-trifluoromethyl, 2-fluoro-5-trifluoromethyl, 2-methyl, 2-methoxy, 3,4-dichloro, 3,5-ditrifluoromethyl, 3,5-dichloro, 3,5-dimethyl, 3,5-difluoro, 3,5-dimethoxy, 3-bromo, 3-trifluoromethyl, 3-chloro-4-fluoro, 3-chloro, 3-fluoro-5-trifluoromethyl, 3-fluoro, 3-methyl, 3-nitro, 3-trifluoromethoxy, 3-methoxy, 3-phenyl, 4-trifluoromethyl, 4-chloro-3-trifluoromethyl, 4-fluoro-3-trifluoromethyl, 4-methyl, and the like.

5 The method of any one of the preceding clauses wherein A and/or A' is an amide of an optionally substituted benzyl-N-methylamine. In another embodiment, A in formula (I) or (II) is an amide of an optionally substituted benzyl-N-butylamine, including n-butyl, and t-butyl. The method of any one of the preceding clauses wherein A is an amide of an optionally substituted benzyl-N-benzylamine. Optional substitutions include, but are not limited to, 2,3-dichloro, 3,5-dichloro, 3-bromo, 3-trifluoromethyl, 3-chloro, 3-methyl, and the like.

10 The method of any one of the preceding clauses wherein A and/or A' is an amide of an optionally substituted 1-phenylethyl, 2-phenylethyl, 2-phenylpropyl, or 1-phenylbenzylamine. The method of any one of the preceding clauses wherein A and/or A' is an amide of an optionally substituted 1-phenylethyl, 2-phenylethyl, 2-phenylpropyl, 1-phenylbenzylamine-N-methylamine. The method of any one of the preceding clauses wherein A and/or A' is an amide of an optionally substituted 2-phenyl-β-alanine, or derivative thereof, 1-phenylpropanolamine, and the like. Optional substitutions include, but are not limited to, 3-trifluoromethoxy, 3-methoxy, 3,5-dimethoxy, 2-methyl, and the like.

15 The method of any one of the preceding clauses wherein A and/or A' is an amide of an optionally substituted 1-phenylcyclopropyl, 1-phenylcyclopentyl, or 1-phenylcyclohexylamine. Optional substitutions include, but are not limited to, 3-fluoro, 4-methoxy, 4-methyl, 4-chloro, 2-fluoro, and the like.

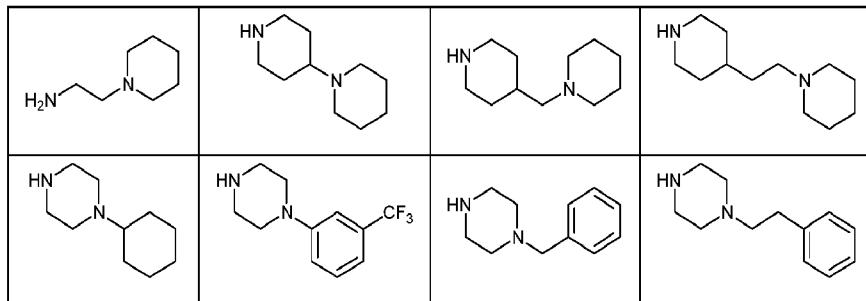
20 The method of any one of the preceding clauses wherein A and/or A' is an amide of an optionally substituted heteroarylmethylamine, including but not limited to 2-furyl, 2-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, and the like. Optional substitutions include, but are not limited to, 5-methyl, 3-chloro, 2-methyl, and the like.

25 The method of any one of the preceding clauses wherein A and/or A' is an amide of a partially saturated bicyclic aryl, including but not limited to 1-, 2-, 4-, and 5-indanylamine, 1- and 2-tetrahydronaphthylamine, indolinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, and the like, each of which is optionally substituted.

30 The method of any one of the preceding clauses wherein A and/or A' is an amide of a substituted piperidine or piperazine. Substituents on the piperidine or piperazine include

heterocyclyl, heterocyclylalkyl, optionally substituted aryl, and optionally substituted arylalkyl.

Illustrative piperidines and piperazines include the formulae:



The method of any one of the preceding clauses wherein A' is an amide of a  
 5 substituted heterocycle attached at nitrogen. Substituents include alkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, aryl, and arylalkyl. The method of any one of the preceding clauses wherein A' is an amide of a heterocycle attached at nitrogen substituted with alkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, or heterocyclylalkyl.

The method of any one of the preceding clauses wherein A' is an amide of an  
 10 optionally substituted arylheterocyclylamine, arylalkylheterocyclylamine, heterocyclylalkylamine, or heteroarylalkylamine. The method of any one of the preceding clauses wherein A' is an amide of piperidin-1-ylpiperidine or piperidin-1-ylalkylpiperidine. In another embodiment, alkyl is C<sub>1</sub>-C<sub>2</sub>-alkyl.

The method of any one of the preceding clauses wherein A is an amide of a  
 15 substituted piperidine or piperazine.

The method of any one of the preceding clauses wherein B is an alcohol or thiol, or a derivative thereof, including alkyl, aryl, or acyl derivatives thereof.

The method of any one of the preceding clauses wherein Q is oxygen or sulfur.

The method of any one of the preceding clauses wherein R<sup>5"</sup> is optionally  
 20 substituted arylalkyl.

The method of any one of the preceding clauses wherein R<sup>5"</sup> is optionally substituted aryl(C<sub>2</sub>-C<sub>4</sub> alkyl). The method of any one of the preceding clauses wherein R<sup>5"</sup> is optionally substituted aryl(C<sub>1</sub>-C<sub>2</sub> alkyl). The method of any one of the preceding clauses wherein R<sup>5"</sup> is optionally substituted benzyl. The method of any one of the preceding clauses wherein R<sup>5"</sup> is optionally substituted alkyl.

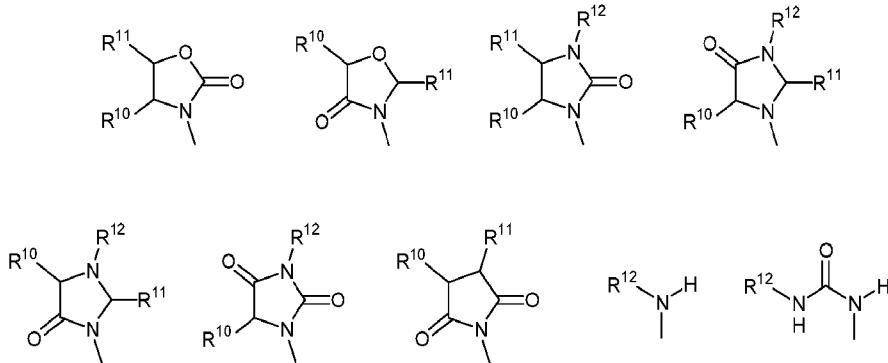
The method of any one of the preceding clauses wherein n is 1 or 2. The method of any one of the preceding clauses wherein n is 1.

The method of any one of the preceding clauses wherein R<sup>2</sup> is hydrogen, alkyl, alkoxy, alkylthio, cyano, formyl, alkylcarbonyl, or a substituent selected from the group

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consisting of  $-\text{CO}_2\text{R}^8$  and  $-\text{CONR}^8\text{R}^{8'}$ , where  $\text{R}^8$  and  $\text{R}^{8'}$  are each independently selected from hydrogen and alkyl. The method of any one of the preceding clauses wherein  $\text{R}^2$  is hydrogen or alkyl. The method of any one of the preceding clauses wherein  $\text{R}^2$  is hydrogen.

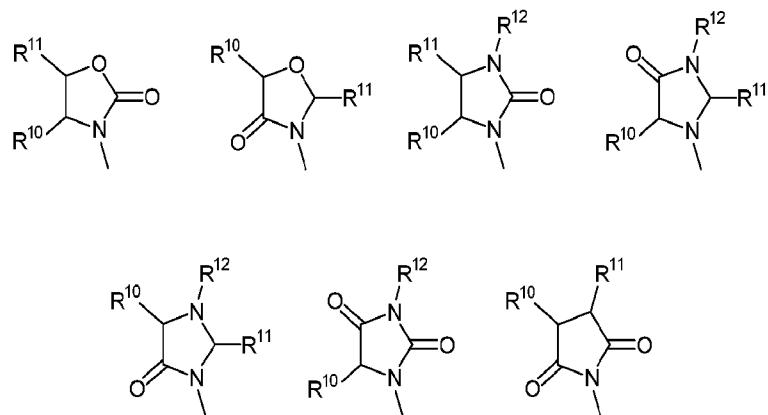
5 The method of any one of the preceding clauses wherein  $\text{R}^1$  is hydrogen. The method of any one of the preceding clauses wherein  $\text{R}^1$  is methyl. The method of any one of the preceding clauses wherein both  $\text{R}^1$  and  $\text{R}^2$  are hydrogen.

The method of any one of the preceding clauses wherein  $\text{R}^3$  is of the formulae:



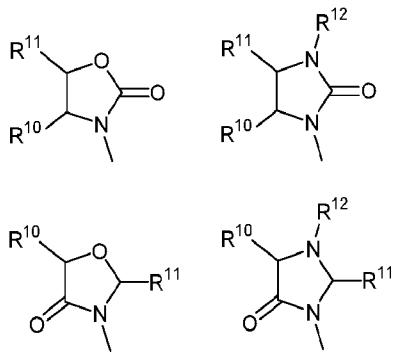
10 wherein  $\text{R}^{10}$  and  $\text{R}^{11}$  are each independently selected from hydrogen, optionally substituted alkyl, optionally substituted cycloalkyl, alkoxy carbonyl, alkyl carbonyloxy, optionally substituted aryl, optionally substituted arylalkyl, optionally substituted arylalkyloxy, optionally substituted arylalkylcarbonyloxy, diphenylmethoxy, triphenylmethoxy, and the like; and  $\text{R}^{12}$  is selected from hydrogen, alkyl, cycloalkyl, alkoxy carbonyl, optionally substituted aryloxy carbonyl, optionally substituted arylalkyl, optionally substituted aryloyl, and the like.

15 The method of any one of the preceding clauses wherein  $\text{R}^3$  is of the formulae:



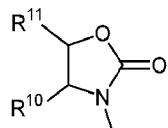
wherein  $\text{R}^{10}$ ,  $\text{R}^{11}$ , and  $\text{R}^{12}$  are as defined herein.

The method of any one of the preceding clauses wherein  $\text{R}^3$  is of the formulae:



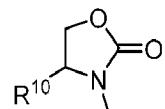
wherein R<sup>10</sup>, R<sup>11</sup>, and R<sup>12</sup> are as defined herein.

The method of any one of the preceding clauses wherein R<sup>3</sup> is of the formula:



5 wherein R<sup>10</sup> and R<sup>11</sup> are as defined herein.

The method of any one of the preceding clauses wherein R<sup>3</sup> is of the formula:



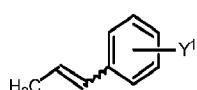
wherein R<sup>10</sup> is defined herein.

The method of any one of the preceding clauses wherein R<sup>10</sup> is alkyl, aryl, or 10 arylalkyl, each of which is optionally substituted, or wherein R<sup>10</sup> is optionally substituted aryl.

The method of any one of the preceding clauses wherein R<sup>4</sup> is of the formulae:



wherein Y is an electron withdrawing group, such as halo, and Y<sup>1</sup> is hydrogen or one or more aryl substituents, such as but not limited to halo, hydroxy, amino, nitro, optionally substituted alkyl, 15 optionally substituted alkoxy, and the like. It is to be understood that the double bond in the formulae may be all or substantially all (E), all or substantially all (Z), or a mixture thereof. The method of any one of the preceding clauses wherein the double bond in the formulae is all or substantially all (E). The method of any one of the preceding clauses wherein R<sup>4</sup> is of the formulae:



20

wherein Y<sup>1</sup> is as defined herein. In another embodiment, Y<sup>1</sup> is not hydrogen.

The method of any one of the preceding clauses wherein n is 1, the stereochemistry of the  $\alpha$ -carbon is (S) or (R), or is an epimeric mixture. The method of any one

of the preceding clauses wherein n is 1, the stereochemistry of the  $\alpha$ -carbon is (R). The method of any one of the preceding clauses wherein n is 2, the stereochemistry of the  $\alpha$ -carbon is (S).

The method of any one of the preceding clauses wherein n is 1 and Q is oxygen, the stereochemistry of the  $\alpha$ -carbon is (R). The method of any one of the preceding clauses

5 wherein n is 1 and Q is sulfur, the stereochemistry of the  $\alpha$ -carbon is (S). It is appreciated that the compounds of formulae (I) and (II) are chiral at the  $\alpha$ -carbon, except when A = A', and n = 0.

The method of any one of the preceding clauses wherein at least one compound has a vasopressin V1a receptor binding affinity (IC<sub>50</sub>) less than about 100 nM, less than about 10 50 nM, less than about 25 nM, or less than about 10 nM.

The method of any one of the preceding clauses wherein at least one compound is AVN228 (Example 233).

The method of any one of the preceding clauses wherein at least one compound is AVN246 (Example 224).

15 The method of any one of the preceding clauses wherein at least one compound is AVN251 (Example 225).

The method of any one of the preceding clauses wherein at least one compound is AVN296 (Example 232E).

20 The method of any one of the preceding clauses wherein at least one compound is AVN576 (Example 266).

The method of any one of the preceding clauses wherein the administration step includes a total daily dose of about 160 to about 700 mg total of one or more compounds of any one of the foregoing clauses, in single or divided form.

25 The method of any one of the preceding clauses wherein the administration step includes a total daily dose of about 160 to about 500 mg total of one or more compounds of any one of the foregoing clauses, in single or divided form.

The method of any one of the preceding clauses wherein the administration step includes a total daily dose of about 160 to about 400 mg total of one or more compounds of any one of the foregoing clauses, in single or divided form.

30 The method of any one of the preceding clauses wherein the administration step includes a total daily dose of about 160 to about 320 mg total of one or more compounds of any one of the foregoing clauses, in single or divided form.

The method of any one of the preceding clauses wherein the administration step includes a total daily dose of about 160 to about 240 mg total of one or more compounds of any 35 one of the foregoing clauses, in single or divided form.

The method of any one of the preceding clauses wherein the form is for oral administration.

The method of any one of the preceding clauses wherein the form is for rapid or immediate release oral administration.

5 The method of any one of the preceding clauses wherein the form is for parenteral administration.

It is understood that in emergency situations, rapid or immediate release oral administration and/or parenteral administration will provide a high Cmax with a very short Tmax. Without being bound by theory, it is believed herein that rapid or immediate release oral 10 administration and/or parenteral administration will avert downstream damage caused by injury to the brain that is left untreated, such as secondary injury events arising from cellular processes and biochemical cascades that occur in the minutes to days following the trauma. Secondary injury events include damage to the blood-brain barrier, release of factors that cause inflammation, free radical overload, excitotoxicity (excessive release of the neurotransmitter 15 glutamate), influx of calcium and sodium ions into neurons, dysfunction of mitochondria, injured axons in the brain's white matter separating from their cell bodies, changes in the blood flow to the brain, including ischemia, cerebral hypoxia, cerebral edema, and raised intracranial pressure.

The method of any one of the preceding clauses wherein the administration step 20 includes a q.d. dosing protocol.

The method of any one of the preceding clauses wherein the administration step includes a b.i.d. dosing protocol.

The method of any one of the preceding clauses wherein the administration step includes an extended release dosing protocol.

25 A pharmaceutical composition adapted for or capable of treating a brain injury or disorder in a host animal, the composition comprising one or more compounds of any one of the foregoing clauses, and optionally, one or more carriers, diluents, or adjuvants, or a combination thereof.

30 A unit dose or unit dosage form adapted for or capable of treating a brain injury or disorder in a host animal, the composition comprising one or more compounds of any one of the foregoing clauses, and optionally, one or more carriers, diluents, or adjuvants, or a combination thereof.

35 The unit dose or unit dosage form of any one of the preceding clauses comprising about 80 to about 350 mg total of one or more compounds of any one of the foregoing clauses, in single or divided form.

The unit dose or unit dosage form of any one of the preceding clauses comprising about 80 to about 250 mg total of one or more compounds of any one of the foregoing clauses, in single or divided form.

5 The unit dose or unit dosage form of any one of the preceding clauses comprising about 80 to about 200 mg total of one or more compounds of any one of the foregoing clauses, in single or divided form.

The unit dose or unit dosage form of any one of the preceding clauses comprising about 80 to about 160 mg total of one or more compounds of any one of the foregoing clauses, in single or divided form.

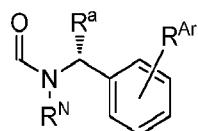
10 The unit dose or unit dosage form of any one of the preceding clauses comprising about 80 to about 120 mg total of one or more compounds of any one of the foregoing clauses, in single or divided form.

The unit dose or unit dosage form of any one of the preceding clauses adapted for oral delivery.

15 The unit dose or unit dosage form of any one of the preceding clauses adapted for extended release.

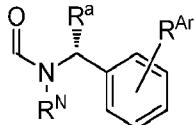
It is to be understood that each of the foregoing clauses and in each of the embodiments described herein of formula (I), the various genera, subgenera, and species of each of A, A', Y, Y<sup>1</sup>, n, R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, and the like, may be combined without limitation, 20 and therefore each such additional embodiment of the invention is thereby described by the combination. It is also to be understood that each of the foregoing clauses and in each of the embodiments described herein of formula (II), the various genera, subgenera, and species of each of A, Q, Y, Y<sup>1</sup>, n, R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>5"</sup>, and the like may be combined without limitation, and therefore each such additional embodiment of the invention is thereby described 25 by the combination. For example, the method of any one of the preceding clauses wherein compounds of formula (I) are described where

(a) A is of the formula



where R<sup>N</sup>, R<sup>a</sup>, and R<sup>Ar</sup> are as defined herein; and n is 1;

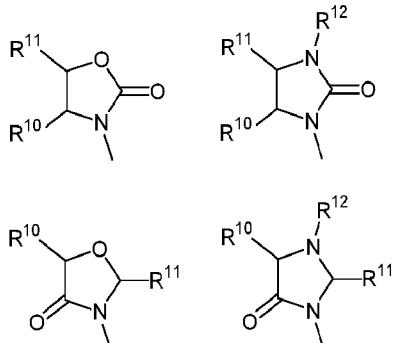
30 (b) n is 1, and R<sup>1</sup> is hydrogen;  
(c) A is of the formula



wherein  $R^N$ ,  $R^a$ , and  $R^{Ar}$  are as defined herein;  $n$  is 1; and  $R^1$  is hydrogen;

(d)  $R^1$  and  $R^3$  are both hydrogen;

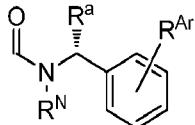
(e)  $R^1$  and  $R^2$  are both hydrogen; and  $R^3$  is of the formula



5

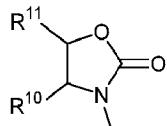
wherein  $R^{10}$ ,  $R^{11}$ , and  $R^{12}$  are as defined herein;

(f)  $A$  is of the formula



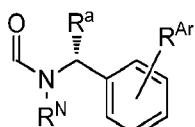
wherein  $R^N$ ,  $R^a$ , and  $R^{Ar}$  are as defined herein;  $n$  is 1;  $R^1$  and  $R^2$  are both hydrogen; and  $R^3$  is

10 of the formula

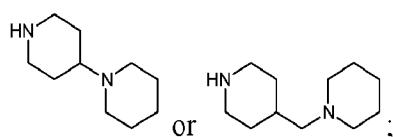


wherein  $R^{10}$  and  $R^{11}$  are as defined herein;

(g)  $A$  is of the formula



15 where  $R^N$ ,  $R^a$ , and  $R^{Ar}$  are as defined herein;  $n$  is 1;  $R^1$  and  $R^2$  are both hydrogen; and  $A'$  is of the formula



and the like.

It is appreciated that in the illustrative clauses and embodiments described

herein, A and/or A' may include a chiral center, either of the optically pure enantiomers may be included in the compounds described herein; alternatively, the racemic form may be used. For example, either or both of the following enantiomers may be included in the compounds described herein (R)-1-(3-methoxyphenyl)ethylamine, (R)-1-(3-

5 trifluoromethylphenyl)ethylamine, (R)-1,2,3,4-tetrahydro-1-naphthylamine, (R)-1-indanylamine, (R)- $\alpha$ ,N-dimethylbenzylamine, (R)- $\alpha$ -methylbenzylamine, (S)-1-(3-methoxyphenyl)ethylamine, (S)-1-(3-trifluoromethylphenyl)ethylamine, (S)-1,2,3,4-tetrahydro-1-naphthylamine, (S)-1-indanylamine, and (S)- $\alpha$ -methylbenzylamine, and the like.

In another embodiment, compounds described herein cross the blood-brain-barrier (BBB) and show high CNS permeability. In another embodiment, compounds described herein show efficacious dose levels in the brain for treating brain injuries. In another embodiment, compounds described herein exhibit plasma levels at or in excess of those necessary for clinical efficacy in treating brain injuries. In another embodiment, compounds described herein exhibit pharmacokinetics consistent with twice per day (b.i.d.) dosing. In another embodiment, compounds described herein exhibit pharmacokinetics consistent with once per day (q.d.) dosing. It is appreciated herein that both b.i.d. and q.d. dosing may be an important feature in improving patient compliance, leading to overall enhanced clinical effectiveness. In another embodiment, compounds described herein are metabolically stable in stomach and blood. In another embodiment, compounds described herein exhibit cardiovascular safety profiles both in vivo and in vitro consistent with the treatment of brain injuries. In another embodiment, compounds described herein exhibit respiratory safety in vivo.

In another embodiment, compounds described herein, and pharmaceutical compositions and medicaments containing them, exhibit high plasma levels and high brain levels, including with oral administration. In another embodiment, compounds described herein, and pharmaceutical compositions and medicaments containing them, capable of crossing the blood brain barrier (BBB), including with oral administration. In another embodiment, compounds described herein, and pharmaceutical compositions and medicaments containing them, exhibit high CNS bioavailability and high affinity without significant or competitive binding to other predetermined GPCRs, or other predetermined receptors, including but not limited to neurotransmitter related receptors, steroid receptors, ion channels, second messenger receptors, prostaglandin receptors, growth factor and hormone receptors, other brain and gastrointestinal tract peptide receptors, other enzymes, and the like. In one aspect, compounds described herein, and pharmaceutical compositions and medicaments containing them, are inactive or substantially inactive at 100 nM against a standard panel of 64 receptors including 35 GPCRs (Novascreen panel), including neurotransmitter related receptors, steroidal

receptors, ion channels, second messenger receptors, prostaglandin receptors, growth factor receptors, hormonal receptors, brain/gut peptides (not including vasopressin 1), and enzymes.

Without being bound by theory, it is believed herein that AVP and related peptides represent a family of chemical signals in vertebrates and serve an important function in the control of social behaviors and emotions. AVP is synthesized in neurons in the hypothalamus of all mammals. It is released from nerve endings in the median eminence and transported to the pituitary gland, where it enhances the release of adrenocorticotropic hormone (ACTH) and ultimately the level of stress hormones in the circulation through its actions at pituitary AVP receptors. From nerve endings in the pituitary, AVP also enters the general blood stream where it acts on the heart and blood vessels to affect cardiac performance and on the kidneys to decrease urine volume. AVP neurons and nerve fibers also are found throughout the limbic system of the brain. AVP exerts its physiological and behavioral effects by binding to specific G-Protein Coupled Receptors (GPCRs) in the central nervous system and certain peripheral tissues/sites. Three distinct AVP receptor subtypes have been identified -- V1a, V1b, and V2. V1a is the predominant AVP receptor found in the limbic system and cortex, V1b receptor is located in limbic system and pituitary gland, although it is less widespread than V1a. The V2 receptor is localized in kidney, where it mediates the antidiuretic effects of vasopressin. It is generally believed herein that V2 is not expressed in the nervous systems of adult animals or humans.

In another embodiment, compounds described herein are selectively active at the V1a AVP receptor. In another embodiment, compounds described herein are selectively active at the V1a AVP receptor, and are less active, substantially less active, and/or inactive at other AVP receptors, such as the V1b and/or V2 subtypes of AVP receptors. In another embodiment, compounds described herein are 10-fold selective for the V1a receptor compared to the V1b and/or V2 receptor. In another embodiment, compounds described herein are 100-fold selective for the V1a receptor compared to the V1b and/or V2 receptor. In another embodiment, compounds described herein are 1000-fold selective for the V1a receptor compared to the V1b and/or V2 receptor. In another embodiment, compounds described herein are 10,000-fold selective for the V1a receptor compared to the V1b and/or V2 receptor.

In another embodiment, compounds described herein are selectively active at the V1a AVP receptor compared to other G-protein coupled receptors (GPCRs). In another embodiment, compounds described herein are selectively active at the V1a AVP receptor, and are less active, substantially less active, and/or inactive at other GPCRs. In another embodiment, compounds described herein are 10-fold selective for the V1a receptor compared to other GPCRs. In another embodiment, compounds described herein are 100-fold selective

for the V1a receptor compared to other GPCRs. In another embodiment, compounds described herein are 1000-fold selective for the V1a receptor compared to other GPCRs. In another embodiment, compounds described herein are 10,000-fold selective for the V1a receptor compared to other GPCRs.

5           In another embodiment, compounds described herein, and pharmaceutical compositions and medicaments containing them, have specific behavioral effects that are context dependent (*see, for example*, Ferris & Pogal *Physiology and Behavior*, 44:235-239 (1988)). For example, in another embodiment, compounds described herein, and pharmaceutical compositions and medicaments containing them are effective in modulating 10 neuropsychiatric disorders, but have little or no effect on sexual behavior.

In each of the foregoing clauses and each of the embodiments described herein, it is to be understood that the formulae include and represent not only all pharmaceutically acceptable salts of the compounds, but also include any and all hydrates and/or solvates of the compound formulae. It is appreciated that certain functional groups, such as the hydroxy, 15 amino, and like groups form complexes and/or coordination compounds with water and/or various solvents, in the various physical forms of the compounds. Accordingly, the above formulae are to be understood to be a description of such hydrates and/or solvates, including pharmaceutically acceptable solvates.

20           In each of the clauses and embodiments described herein, it is also to be understood that the formulae include and represent each possible isomer, such as stereoisomers and geometric isomers, both individually and in any and all possible mixtures. In each of the foregoing and each of the following embodiments, it is also to be understood that the formulae include and represent any and all crystalline forms, partially crystalline forms, and non-crystalline and/or amorphous forms of the compounds.

25           As used herein, the term “solvates” refers to compounds described herein complexed with a solvent molecule. It is appreciated that compounds described herein may form such complexes with solvents by simply mixing the compounds with a solvent, or dissolving the compounds in a solvent. It is appreciated that where the compounds are to be used as pharmaceuticals, such solvents are pharmaceutically acceptable solvents. It is further 30 appreciated that where the compounds are to be used as pharmaceuticals, the relative amount of solvent that forms the solvate should be less than established guidelines for such pharmaceutical uses, such as less than International Conference on Harmonization (ICH) Guidelines. It is to be understood that the solvates may be isolated from excess solvent by evaporation, precipitation, and/or crystallization. In some embodiments, the solvates are amorphous, and in other 35 embodiments, the solvates are crystalline.

The compounds described herein may contain one or more chiral centers, or may otherwise be capable of existing as multiple stereoisomers. It is to be understood that in one embodiment, the invention described herein is not limited to any particular stereochemical requirement, and that the compounds, and compositions, methods, uses, and medicaments that include them may be optically pure, or may be any of a variety of stereoisomeric mixtures, including racemic and other mixtures of enantiomers, other mixtures of diastereomers, and the like. It is also to be understood that such mixtures of stereoisomers may include a single stereochemical configuration at one or more chiral centers, while including mixtures of stereochemical configuration at one or more other chiral centers.

Similarly, the compounds described herein may include geometric centers, such as cis, trans, E, and Z double bonds. It is to be understood that in another embodiment, the invention described herein is not limited to any particular geometric isomer requirement, and that the compounds, and compositions, methods, uses, and medicaments that include them may be pure, or may be any of a variety of geometric isomer mixtures. It is also to be understood that such mixtures of geometric isomers may include a single configuration at one or more double bonds, while including mixtures of geometry at one or more other double bonds.

As used herein, the term “alkyl” includes a chain of carbon atoms, which is optionally branched. As used herein, the terms “alkenyl” and “alkynyl” each include a chain of carbon atoms, which is optionally branched, and include at least one double bond or triple bond, respectively. It is to be understood that alkynyl may also include one or more double bonds. It is to be further understood that in certain embodiments, alkyl is advantageously of limited length, including C<sub>1</sub>-C<sub>24</sub>, C<sub>1</sub>-C<sub>12</sub>, C<sub>1</sub>-C<sub>8</sub>, C<sub>1</sub>-C<sub>6</sub>, and C<sub>1</sub>-C<sub>4</sub>, and C<sub>2</sub>-C<sub>24</sub>, C<sub>2</sub>-C<sub>12</sub>, C<sub>2</sub>-C<sub>8</sub>, C<sub>2</sub>-C<sub>6</sub>, and C<sub>2</sub>-C<sub>4</sub>, and the like. Illustratively, such particularly limited length alkyl groups, including C<sub>1</sub>-C<sub>8</sub>, C<sub>1</sub>-C<sub>6</sub>, and C<sub>1</sub>-C<sub>4</sub>, and C<sub>2</sub>-C<sub>8</sub>, C<sub>2</sub>-C<sub>6</sub>, and C<sub>2</sub>-C<sub>4</sub>, and the like may be referred to as lower alkyl. It is to be further understood that in certain embodiments alkenyl and/or alkynyl may each be advantageously of limited length, including C<sub>2</sub>-C<sub>24</sub>, C<sub>2</sub>-C<sub>12</sub>, C<sub>2</sub>-C<sub>8</sub>, C<sub>2</sub>-C<sub>6</sub>, and C<sub>2</sub>-C<sub>4</sub>, and C<sub>3</sub>-C<sub>24</sub>, C<sub>3</sub>-C<sub>12</sub>, C<sub>3</sub>-C<sub>8</sub>, C<sub>3</sub>-C<sub>6</sub>, and C<sub>3</sub>-C<sub>4</sub>, and the like. Illustratively, such particularly limited length alkenyl and/or alkynyl groups, including C<sub>2</sub>-C<sub>8</sub>, C<sub>2</sub>-C<sub>6</sub>, and C<sub>2</sub>-C<sub>4</sub>, and C<sub>3</sub>-C<sub>8</sub>, C<sub>3</sub>-C<sub>6</sub>, and C<sub>3</sub>-C<sub>4</sub>, and the like may be referred to as lower alkenyl and/or alkynyl. It is appreciated herein that shorter alkyl, alkenyl, and/or alkynyl groups may add less lipophilicity to the compound and accordingly will have different pharmacokinetic behavior. In embodiments of the invention described herein, it is to be understood, in each case, that the recitation of alkyl refers to alkyl as defined herein, and optionally lower alkyl. In embodiments of the invention described herein, it is to be understood, in each case, that the recitation of alkenyl refers to alkenyl as defined herein, and optionally lower alkenyl. In embodiments of the invention

described herein, it is to be understood, in each case, that the recitation of alkynyl refers to alkynyl as defined herein, and optionally lower alkynyl. Illustrative alkyl, alkenyl, and alkynyl groups are, but not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, pentyl, 2-pentyl, 3-pentyl, neopentyl, hexyl, heptyl, octyl, and the like, and the 5 corresponding groups containing one or more double and/or triple bonds, or a combination thereof.

As used herein, the term “alkylene” includes a divalent chain of carbon atoms, which is optionally branched. As used herein, the term “alkenylene” and “alkynylene” includes a divalent chain of carbon atoms, which is optionally branched, and includes at least one double 10 bond or triple bond, respectively. It is to be understood that alkynylene may also include one or more double bonds. It is to be further understood that in certain embodiments, alkylene is advantageously of limited length, including C<sub>1</sub>-C<sub>24</sub>, C<sub>1</sub>-C<sub>12</sub>, C<sub>1</sub>-C<sub>8</sub>, C<sub>1</sub>-C<sub>6</sub>, and C<sub>1</sub>-C<sub>4</sub>, and C<sub>2</sub>-C<sub>24</sub>, C<sub>2</sub>-C<sub>12</sub>, C<sub>2</sub>-C<sub>8</sub>, C<sub>2</sub>-C<sub>6</sub>, and C<sub>2</sub>-C<sub>4</sub>, and the like. Illustratively, such particularly limited 15 length alkylene groups, including C<sub>1</sub>-C<sub>8</sub>, C<sub>1</sub>-C<sub>6</sub>, and C<sub>1</sub>-C<sub>4</sub>, and C<sub>2</sub>-C<sub>8</sub>, C<sub>2</sub>-C<sub>6</sub>, and C<sub>2</sub>-C<sub>4</sub>, and the like may be referred to as lower alkylene. It is to be further understood that in certain 20 embodiments, alkenylene and/or alkynylene may each be advantageously of limited length, including C<sub>2</sub>-C<sub>24</sub>, C<sub>2</sub>-C<sub>12</sub>, C<sub>2</sub>-C<sub>8</sub>, C<sub>2</sub>-C<sub>6</sub>, and C<sub>2</sub>-C<sub>4</sub>, and C<sub>3</sub>-C<sub>24</sub>, C<sub>3</sub>-C<sub>12</sub>, C<sub>3</sub>-C<sub>8</sub>, C<sub>3</sub>-C<sub>6</sub>, and C<sub>3</sub>-C<sub>4</sub>, and the like. Illustratively, such particularly limited length alkenylene and/or alkynylene 25 groups, including C<sub>2</sub>-C<sub>8</sub>, C<sub>2</sub>-C<sub>6</sub>, and C<sub>2</sub>-C<sub>4</sub>, and C<sub>3</sub>-C<sub>8</sub>, C<sub>3</sub>-C<sub>6</sub>, and C<sub>3</sub>-C<sub>4</sub>, and the like may be referred to as lower alkenylene and/or alkynylene. It is appreciated herein that shorter alkylene, alkenylene, and/or alkynylene groups may add less lipophilicity to the compound and accordingly will have different pharmacokinetic behavior. In embodiments of the invention 30 described herein, it is to be understood, in each case, that the recitation of alkylene, alkenylene, and alkynylene refers to alkylene, alkenylene, and alkynylene as defined herein, and optionally lower alkylene, alkenylene, and alkynylene. Illustrative alkyl groups are, but not limited to, methylene, ethylene, n-propylene, isopropylene, n-butylene, isobutylene, sec-butylene, pentylene, 1,2-pentylene, 1,3-pentylene, hexylene, heptylene, octylene, and the like.

As used herein, the term “cycloalkyl” includes a chain of carbon atoms, which is optionally branched, where at least a portion of the chain is cyclic. It is to be understood that 35 cycloalkylalkyl is a subset of cycloalkyl. It is to be understood that cycloalkyl may be polycyclic. Illustrative cycloalkyl include, but are not limited to, cyclopropyl, cyclopentyl, cyclohexyl, 2-methylcyclopropyl, cyclopentyleth-2-yl, adamantyl, and the like. As used herein, the term “cycloalkenyl” includes a chain of carbon atoms, which is optionally branched, and includes at least one double bond, where at least a portion of the chain is cyclic. It is to be understood that the one or more double bonds may be in the cyclic portion of cycloalkenyl

and/or the non-cyclic portion of cycloalkenyl. It is to be understood that cycloalkenylalkyl and cycloalkylalkenyl are each subsets of cycloalkenyl. It is to be understood that cycloalkyl may be polycyclic. Illustrative cycloalkenyl include, but are not limited to, cyclopentenyl, cyclohexylethen-2-yl, cycloheptenylpropenyl, and the like. It is to be further understood that 5 chain forming cycloalkyl and/or cycloalkenyl is advantageously of limited length, including C<sub>3</sub>-C<sub>24</sub>, C<sub>3</sub>-C<sub>12</sub>, C<sub>3</sub>-C<sub>8</sub>, C<sub>3</sub>-C<sub>6</sub>, and C<sub>5</sub>-C<sub>6</sub>. It is appreciated herein that shorter alkyl and/or alkenyl chains forming cycloalkyl and/or cycloalkenyl, respectively, may add less lipophilicity to the compound and accordingly will have different pharmacokinetic behavior.

As used herein, the term “heteroalkyl” includes a chain of atoms that includes 10 both carbon and at least one heteroatom, and is optionally branched. Illustrative heteroatoms include nitrogen, oxygen, and sulfur. In certain variations, illustrative heteroatoms also include phosphorus, and selenium. As used herein, the term “cycloheteroalkyl” including heterocycl and heterocycle, includes a chain of atoms that includes both carbon and at least one heteroatom, such as heteroalkyl, and is optionally branched, where at least a portion of the 15 chain is cyclic. Illustrative heteroatoms include nitrogen, oxygen, and sulfur. In certain variations, illustrative heteroatoms also include phosphorus, and selenium. Illustrative cycloheteroalkyl include, but are not limited to, tetrahydrafuryl, pyrrolidinyl, tetrahydropyranyl, piperidinyl, morpholinyl, piperazinyl, homopiperazinyl, quinuclidinyl, and the like.

As used herein, the term “aryl” includes monocyclic and polycyclic aromatic 20 carbocyclic groups, each of which may be optionally substituted. Illustrative aromatic carbocyclic groups described herein include, but are not limited to, phenyl, naphthyl, and the like. As used herein, the term “heteraryl” includes aromatic heterocyclic groups, each of which may be optionally substituted. Illustrative aromatic heterocyclic groups include, but are not limited to, pyridinyl, pyrimidinyl, pyrazinyl, triazinyl, tetrazinyl, quinolinyl, quinazolinyl, 25 quinoxalinyl, thienyl, pyrazolyl, imidazolyl, oxazolyl, thiazolyl, isoxazolyl, isothiazolyl, oxadiazolyl, thiadiazolyl, triazolyl, benzimidazolyl, benzoxazolyl, benzthiazolyl, benzisoxazolyl, benzisothiazolyl, and the like.

As used herein, the term “amino” includes the group NH<sub>2</sub>, alkylamino, and 30 dialkylamino, where the two alkyl groups in dialkylamino may be the same or different, i.e. alkylalkylamino. Illustratively, amino includes methylamino, ethylamino, dimethylamino, methylethylamino, and the like. In addition, it is to be understood that when amino modifies or is modified by another term, such as aminoalkyl, or acylamino, the above variations of the term 35 amino are included therein. Illustratively, aminoalkyl includes H<sub>2</sub>N-alkyl, methylaminoalkyl, ethylaminoalkyl, dimethylaminoalkyl, methylethylaminoalkyl, and the like. Illustratively, acylamino includes acylmethylamino, acylethylamino, and the like.

As used herein, the term “amino and derivatives thereof” includes amino as described herein, and alkylamino, alkenylamino, alkynylamino, heteroalkylamino, heteroalkenylamino, heteroalkynylamino, cycloalkylamino, cycloalkenylamino, cycloheteroalkylamino, cycloheteroalkenylamino, arylamino, arylalkylamino, 5 arylalkenylamino, arylalkynylamino, heteroarylarnino, heteroarylkylamino, heteroarylklenylamino, heteroarylalkynylamino, acylamino, and the like, each of which is optionally substituted. The term “amino derivative” also includes urea, carbamate, and the like.

As used herein, the term “hydroxy and derivatives thereof” includes OH, and alkyloxy, alkenyloxy, alkynyoxy, heteroalkyloxy, heteroalkenyloxy, heteroalkynyoxy, 10 cycloalkyloxy, cycloalkenyloxy, cycloheteroalkyloxy, cycloheteroalkenyloxy, aryloxy, arylalkyloxy, arylalkenyloxy, arylalkynyoxy, heteroaryloxy, heteroarylalkyloxy, heteroarylklenyloxy, heteroarylalkynyoxy, acyloxy, and the like, each of which is optionally substituted. The term “hydroxy derivative” also includes carbamate, and the like.

As used herein, the term “thio and derivatives thereof” includes SH, and 15 alkylthio, alkenylthio, alkynylthio, heteroalkylthio, heteroalkenylthio, heteroalkynylthio, cycloalkylthio, cycloalkenylthio, cycloheteroalkylthio, cycloheteroalkenythio, arylthio, arylalkylthio, arylalkenylthio, arylalkynylthio, heteroarylthio, heteroarylalkylthio, heteroarylklenythio, heteroarylalkynylthio, acylthio, and the like, each of which is optionally substituted. The term “thio derivative” also includes thiocarbamate, and the like.

20 As used herein, the term “acyl” includes formyl, and alkylcarbonyl, alkenylcarbonyl, alkynylcarbonyl, heteroalkylcarbonyl, heteroalkenylcarbonyl, heteroalkynylcarbonyl, cycloalkylcarbonyl, cycloalkenylcarbonyl, cycloheteroalkylcarbonyl, cycloheteroalkenylcarbonyl, arylcarbonyl, arylalkylcarbonyl, arylalkenylcarbonyl, arylalkynylcarbonyl, heteroarylcarbonyl, heteroarylalkylcarbonyl, heteroarylklenykarbonyl, heteroarylalkynylcarbonyl, acylcarbonyl, and the like, each of which is optionally substituted.

25 As used herein, the term “carbonyl and derivatives thereof” includes the group C(O), C(S), C(NH) and substituted amino derivatives thereof.

As used herein, the term “carboxylic acid and derivatives thereof” includes the group CO<sub>2</sub>H and salts thereof, and esters and amides thereof, and CN.

30 As used herein, the term “sulfinic acid or a derivative thereof” includes SO<sub>2</sub>H and salts thereof, and esters and amides thereof.

As used herein, the term “sulfonic acid or a derivative thereof” includes SO<sub>3</sub>H and salts thereof, and esters and amides thereof.

35 As used herein, the term “sulfonyl” includes alkylsulfonyl, alkenylsulfonyl, alkynylsulfonyl, heteroalkylsulfonyl, heteroalkenylsulfonyl, heteroalkynylsulfonyl,

cycloalkylsulfonyl, cycloalkenylsulfonyl, cycloheteroalkylsulfonyl, cycloheteroalkenylsulfonyl, arylsulfonyl, arylalkylsulfonyl, arylalkenylsulfonyl, arylalkynylsulfonyl, heteroarylsulfonyl, heteroarylalkylsulfonyl, heteroarylalkenylsulfonyl, heteroarylalkynylsulfonyl, acylsulfonyl, and the like, each of which is optionally substituted.

5 As used herein, the term "hydroxylamino and derivatives thereof" includes NHOH, and alkyloxyNH alkenyloxyNH alkynylloxyNH heteroalkyloxyNH heteroalkenylloxyNH heteroalkynylloxyNH cycloalkyloxyNH cycloalkenylloxyNH cycloheteroalkyloxyNH cycloheteroalkenylloxyNH aryloxyNH arylalkyloxyNH arylalkenylloxyNH arylalkynylloxyNH heteroaryloxyNH heteroarylalkyloxyNH heteroarylalkenylloxyNH acyloxy, and the like, each of which is 10 optionally substituted.

As used herein, the term "hydrazino and derivatives thereof" includes alkylNHNH, alkenylNHNH, alkynylNHNH, heteroalkylNHNH, heteroalkenylNHNH, heteroalkynylNHNH, cycloalkylNHNH, cycloalkenylNHNH, cycloheteroalkylNHNH, 15 cycloheteroalkenylNHNH, arylNHNH, arylalkylNHNH, arylalkenylNHNH, arylalkynylNHNH, heteroarylNHNH, heteroarylalkylNHNH, heteroarylalkenylNHNH, heteroarylalkynylNHNH, acylNHNH, and the like, each of which is optionally substituted.

The term "optionally substituted" as used herein includes the replacement of 20 hydrogen atoms with other functional groups on the radical that is optionally substituted. Such other functional groups illustratively include, but are not limited to, amino, hydroxyl, halo, thiol, alkyl, haloalkyl, heteroalkyl, aryl, arylalkyl, arylheteroalkyl, heteroaryl, heteroarylalkyl, heteroarylheteroalkyl, nitro, sulfonic acids and derivatives thereof, carboxylic acids and 25 derivatives thereof, and the like. Illustratively, any of amino, hydroxyl, thiol, alkyl, haloalkyl, heteroalkyl, aryl, arylalkyl, arylheteroalkyl, heteroaryl, heteroarylalkyl, heteroarylheteroalkyl, and/or sulfonic acid is optionally substituted.

As used herein, the terms "optionally substituted aryl" and "optionally substituted heteroaryl" include the replacement of hydrogen atoms with other functional groups 30 on the aryl or heteroaryl that is optionally substituted. Such other functional groups illustratively include, but are not limited to, amino, hydroxy, halo, thio, alkyl, haloalkyl, heteroalkyl, aryl, arylalkyl, arylheteroalkyl, heteroaryl, heteroarylalkyl, heteroarylheteroalkyl, nitro, sulfonic acids and derivatives thereof, carboxylic acids and derivatives thereof, and the like. Illustratively, any of amino, hydroxy, thio, alkyl, haloalkyl, heteroalkyl, aryl, arylalkyl, arylheteroalkyl, heteroaryl, heteroarylalkyl, heteroarylheteroalkyl, and/or sulfonic acid is 35 optionally substituted.

Illustrative substituents include, but are not limited to, a radical -(CH<sub>2</sub>)<sub>x</sub>Z<sup>X</sup>,

where  $x$  is an integer from 0-6 and  $Z^X$  is selected from halogen, hydroxy, alkanoyloxy, including  $C_1$ - $C_6$  alkanoyloxy, optionally substituted aroyloxy, alkyl, including  $C_1$ - $C_6$  alkyl, alkoxy, including  $C_1$ - $C_6$  alkoxy, cycloalkyl, including  $C_3$ - $C_8$  cycloalkyl, cycloalkoxy, including  $C_3$ - $C_8$  cycloalkoxy, alkenyl, including  $C_2$ - $C_6$  alkenyl, alkynyl, including  $C_2$ - $C_6$  alkynyl, 5 haloalkyl, including  $C_1$ - $C_6$  haloalkyl, haloalkoxy, including  $C_1$ - $C_6$  haloalkoxy, halocycloalkyl, including  $C_3$ - $C_8$  halocycloalkyl, halocycloalkoxy, including  $C_3$ - $C_8$  halocycloalkoxy, amino,  $C_1$ - $C_6$  alkylamino,  $(C_1$ - $C_6$  alkyl) $(C_1$ - $C_6$  alkyl)amino, alkylcarbonylamino,  $N$ -( $C_1$ - $C_6$  alkyl)alkylcarbonylamino, aminoalkyl,  $C_1$ - $C_6$  alkylaminoalkyl,  $(C_1$ - $C_6$  alkyl) $(C_1$ - $C_6$  alkyl)aminoalkyl, alkylcarbonylaminoalkyl,  $N$ -( $C_1$ - $C_6$  alkyl)alkylcarbonylaminoalkyl, cyano, 10 and nitro; or  $Z^X$  is selected from  $-CO_2R^4$  and  $-CONR^5R^6$ , where  $R^4$ ,  $R^5$ , and  $R^6$  are each independently selected in each occurrence from hydrogen,  $C_1$ - $C_6$  alkyl, aryl- $C_1$ - $C_6$  alkyl, and heteroaryl- $C_1$ - $C_6$  alkyl.

The term “prodrug” as used herein generally refers to any compound that when administered to a biological system generates a biologically active compound as a result of one 15 or more spontaneous chemical reaction(s), enzyme-catalyzed chemical reaction(s), and/or metabolic chemical reaction(s), or a combination thereof. In vivo, the prodrug is typically acted upon by an enzyme (such as esterases, amidases, phosphatases, and the like), simple biological chemistry, or other process in vivo to liberate or regenerate the more pharmacologically active drug. This activation may occur through the action of an endogenous host enzyme or a non- 20 endogenous enzyme that is administered to the host preceding, following, or during administration of the prodrug. Additional details of prodrug use are described in U.S. Pat. No. 5,627,165. It is appreciated that the prodrug is advantageously converted to the original drug as soon as the goal, such as targeted delivery, safety, stability, and the like is achieved, followed by the subsequent rapid elimination of the released remains of the group forming the prodrug.

25 Prodrugs may be prepared from the compounds described herein by attaching groups that ultimately cleave in vivo to one or more functional groups present on the compound, such as  $-OH$ -,  $-SH$ -,  $-CO_2H$ -,  $-NR_2$ . Illustrative prodrugs include but are not limited to carboxylate esters where the group is alkyl, aryl, arylalkyl, heteroaryl, heteroarylalkyl, acyloxyalkyl, alkoxycarbonyloxyalkyl as well as esters of hydroxyl, thiol and amines where the 30 group attached is an acyl group, an alkoxy carbonyl, aminocarbonyl, phosphate or sulfate. Illustrative esters, also referred to as active esters, include but are not limited to 1-indanyl,  $N$ -oxysuccinimide; acyloxyalkyl groups such as acetoxy methyl, pivaloyloxymethyl,  $\beta$ -acetoxyethyl,  $\beta$ -pivaloyloxyethyl, 1-(cyclohexylcarbonyloxy)prop-1-yl, (1-aminoethyl)carbonyloxy methyl, and the like; alkoxy carbonyloxyalkyl groups, such as 35 ethoxycarbonyloxymethyl,  $\alpha$ -ethoxycarbonyloxyethyl,  $\beta$ -ethoxycarbonyloxyethyl, and the like;

alkylaminoalkyl groups, including di-lower alkylamino alkyl groups, such as dimethylaminomethyl, dimethylaminoethyl, diethylaminomethyl, diethylaminoethyl, and the like; 2-(alkoxycarbonyl)-2-alkenyl groups such as 2-(isobutoxycarbonyl) pent-2-enyl, 2-(ethoxycarbonyl)but-2-enyl, and the like; and lactone groups such as phthalidyl, 5 dimethoxyphthalidyl, and the like.

Further illustrative prodrugs contain a chemical moiety, such as an amide or phosphorus group functioning to increase solubility and/or stability of the compounds described herein. Further illustrative prodrugs for amino groups include, but are not limited to, (C<sub>3</sub>-C<sub>20</sub>)alkanoyl; halo-(C<sub>3</sub>-C<sub>20</sub>)alkanoyl; (C<sub>3</sub>-C<sub>20</sub>)alkenoyl; (C<sub>4</sub>-C<sub>7</sub>)cycloalkanoyl; (C<sub>3</sub>-C<sub>6</sub>)-10 cycloalkyl(C<sub>2</sub>-C<sub>16</sub>)alkanoyl; optionally substituted aroyl, such as unsubstituted aroyl or aroyl substituted by 1 to 3 substituents selected from the group consisting of halogen, cyano, trifluoromethanesulphonyloxy, (C<sub>1</sub>-C<sub>3</sub>)alkyl and (C<sub>1</sub>-C<sub>3</sub>)alkoxy, each of which is optionally further substituted with one or more of 1 to 3 halogen atoms; optionally substituted aryl(C<sub>2</sub>-C<sub>16</sub>)alkanoyl and optionally substituted heteroaryl(C<sub>2</sub>-C<sub>16</sub>)alkanoyl, such as the aryl or 15 heteroaryl radical being unsubstituted or substituted by 1 to 3 substituents selected from the group consisting of halogen, (C<sub>1</sub>-C<sub>3</sub>)alkyl and (C<sub>1</sub>-C<sub>3</sub>)alkoxy, each of which is optionally further substituted with 1 to 3 halogen atoms; and optionally substituted heteroarylalkanoyl having one to three heteroatoms selected from O, S and N in the heteroaryl moiety and 2 to 10 carbon atoms in the alkanoyl moiety, such as the heteroaryl radical being unsubstituted or 20 substituted by 1 to 3 substituents selected from the group consisting of halogen, cyano, trifluoromethanesulphonyloxy, (C<sub>1</sub>-C<sub>3</sub>)alkyl, and (C<sub>1</sub>-C<sub>3</sub>)alkoxy, each of which is optionally further substituted with 1 to 3 halogen atoms. The groups illustrated are exemplary, not exhaustive, and may be prepared by conventional processes.

It is understood that the prodrugs themselves may not possess significant 25 biological activity, but instead undergo one or more spontaneous chemical reaction(s), enzyme-catalyzed chemical reaction(s), and/or metabolic chemical reaction(s), or a combination thereof after administration *in vivo* to produce the compound described herein that is biologically active or is a precursor of the biologically active compound. However, it is appreciated that in some cases, the prodrug is biologically active. It is also appreciated that prodrugs may often serve to 30 improve drug efficacy or safety through improved oral bioavailability, pharmacodynamic half-life, and the like. Prodrugs also refer to derivatives of the compounds described herein that include groups that simply mask undesirable drug properties or improve drug delivery. For example, one or more compounds described herein may exhibit an undesirable property that is advantageously blocked or minimized may become pharmacological, pharmaceutical, or 35 pharmacokinetic barriers in clinical drug application, such as low oral drug absorption, lack of

site specificity, chemical instability, toxicity, and poor patient acceptance (bad taste, odor, pain at injection site, and the like), and others. It is appreciated herein that a prodrug, or other strategy using reversible derivatives, can be useful in the optimization of the clinical application of a drug.

5 As used herein, the term “leaving group” refers to a reactive functional group that generates an electrophilic site on the atom to which it is attached such that nucleophiles may be added to the electrophilic site on the atom. Illustrative leaving groups include, but are not limited to, halogens, optionally substituted phenols, acyloxy groups, sulfonyloxy groups, and the like. It is to be understood that such leaving groups may be on alkyl, acyl, and the like.

10 Such leaving groups may also be referred to herein as activating groups, such as when the leaving group is present on acyl. In addition, conventional peptide, amide, and ester coupling agents, such as but not limited to PyBop, BOP-Cl, BOP, pentafluorophenol, isobutylchloroformate, and the like, form various intermediates that include a leaving group, as defined herein, on a carbonyl group.

15 It is to be understood that in every instance disclosed herein, the recitation of a range of integers for any variable describes the recited range, every individual member in the range, and every possible subrange for that variable. For example, the recitation that n is an integer from 0 to 8, describes that range, the individual and selectable values of 0, 1, 2, 3, 4, 5, 6, 7, and 8, such as n is 0, or n is 1, or n is 2, etc. In addition, the recitation that n is an integer from 0 to 8 also describes each and every subrange, each of which may for the basis of a further embodiment, such as n is an integer from 1 to 8, from 1 to 7, from 1 to 6, from 2 to 8, from 2 to 7, from 1 to 3, from 2 to 4, etc.

20

25 As used herein, the terms “treating”, “contacting” or “reacting” when referring to a chemical reaction generally mean to add or mix two or more reagents under appropriate conditions that allows a chemical transformation or chemical reaction to take place, and/or to produce the indicated and/or the desired product. It is to be understood that the reaction which produces the indicated and/or the desired product may not necessarily result directly from the combination of two reagents which were initially added. In other words, there may be one or more intermediates which are produced in the mixture which ultimately leads to the formation 30 of the indicated and/or the desired product.

35 As used herein, the term “composition” generally refers to any product comprising the specified ingredients in the specified amounts, as well as any product which results, directly or indirectly, from combinations of the specified ingredients in the specified amounts. It is to be understood that the compositions described herein may be prepared from isolated compounds described herein or from salts, solutions, hydrates, solvates, and other

forms of the compounds described herein. It is also to be understood that the compositions may be prepared from various amorphous, non-amorphous, partially crystalline, crystalline, and/or other morphological forms of the compounds described herein. It is also to be understood that the compositions may be prepared from various hydrates and/or solvates of the compounds 5 described herein. Accordingly, such pharmaceutical compositions that recite compounds described herein are to be understood to include each of, or any combination of, the various morphological forms and/or solvate or hydrate forms of the compounds described herein. In addition, it is to be understood that the compositions may be prepared from various co-crystals of the compounds described herein.

10 Illustratively, compositions may include one or more carriers, diluents, and/or excipients. The compounds described herein, or compositions containing them, may be formulated in a therapeutically effective amount in any conventional dosage forms appropriate for the methods described herein. The compounds described herein, or compositions containing them, including such formulations, may be administered by a wide variety of conventional 15 routes for the methods described herein, and in a wide variety of dosage formats, utilizing known procedures (see generally, Remington: The Science and Practice of Pharmacy, (21<sup>st</sup> ed., 2005)).

20 The term "therapeutically effective amount" as used herein, refers to that amount of active compound or pharmaceutical agent that elicits the biological or medicinal response in a tissue system, animal or human that is being sought by a researcher, veterinarian, medical doctor or other clinician, which includes alleviation of the symptoms of the disease or disorder being treated. In one aspect, the therapeutically effective amount is that which may treat or 25 alleviate the disease or symptoms of the disease at a reasonable benefit/risk ratio applicable to any medical treatment. However, it is to be understood that the total daily usage of the compounds and compositions described herein may be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically-effective dose level for any particular patient will depend upon a variety of factors, including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed; the age, body weight, general health, gender and diet of the 30 patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidentally with the specific compound employed; and like factors well known to the researcher, veterinarian, medical doctor or other clinician of ordinary skill.

35 It is also appreciated that the therapeutically effective amount, whether referring to monotherapy or combination therapy, is advantageously selected with reference to any

toxicity, or other undesirable side effect, that might occur during administration of one or more of the compounds described herein. Further, it is appreciated that the co-therapies described herein may allow for the administration of lower doses of compounds that show such toxicity, or other undesirable side effect, where those lower doses are below thresholds of toxicity or 5 lower in the therapeutic window than would otherwise be administered in the absence of a cotherapy.

In addition to the illustrative dosages and dosing protocols described herein, it is to be understood that an effective amount of any one or a mixture of the compounds described herein can be readily determined by the attending diagnostician or physician by the use of 10 known techniques and/or by observing results obtained under analogous circumstances. In determining the effective amount or dose, a number of factors are considered by the attending diagnostician or physician, including, but not limited to the species of mammal, including human, its size, age, and general health, the specific disease or disorder involved, the degree of or involvement or the severity of the disease or disorder, the response of the individual patient, 15 the particular compound administered, the mode of administration, the bioavailability characteristics of the preparation administered, the dose regimen selected, the use of concomitant medication, and other relevant circumstances.

The dosage of each compound of the claimed combinations depends on several factors, including: the administration method, the condition to be treated, the severity of the 20 condition, whether the condition is to be treated or prevented, and the age, weight, and health of the person to be treated. Additionally, pharmacogenomic (the effect of genotype on the pharmacokinetic, pharmacodynamic or efficacy profile of a therapeutic) information about a particular patient may affect the dosage used.

It is to be understood that in the methods described herein, the individual 25 components of a co-administration, or combination can be administered by any suitable means, contemporaneously, simultaneously, sequentially, separately or in a single pharmaceutical formulation. Where the co-administered compounds or compositions are administered in separate dosage forms, the number of dosages administered per day for each compound may be the same or different. The compounds or compositions may be administered via the same or 30 different routes of administration. The compounds or compositions may be administered according to simultaneous or alternating regimens, at the same or different times during the course of the therapy, concurrently in divided or single forms.

The term “administering” as used herein includes all means of introducing the compounds and compositions described herein to the host animal, including, but are not limited 35 to, oral (po), intravenous (iv), intramuscular (im), subcutaneous (sc), transdermal, inhalation,

buccal, ocular, sublingual, vaginal, rectal, and the like. The compounds and compositions described herein may be administered in unit dosage forms and/or formulations containing conventional nontoxic pharmaceutically-acceptable carriers, adjuvants, and/or vehicles.

In making the pharmaceutical compositions of the compounds described herein, 5 a therapeutically effective amount of one or more compounds in any of the various forms described herein may be mixed with one or more excipients, diluted by one or more excipients, or enclosed within such a carrier which can be in the form of a capsule, sachet, paper, or other container. Excipients may serve as a diluent, and can be solid, semi-solid, or liquid materials, which act as a vehicle, carrier or medium for the active ingredient. Thus, the formulation 10 compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders. The compositions may contain anywhere from about 0.1% to about 99.9% active ingredients, depending upon the selected dose and dosage form.

15 The term "antagonist," as used herein, refers to a full or partial antagonist. While a partial antagonist of any intrinsic activity may be useful, the partial antagonists illustratively show at least about 50% antagonist effect, or at least about 80% antagonist effect. The term also includes compounds that are full antagonists of one or more vasopressin receptors. It is appreciated that illustrative methods described herein require therapeutically 20 effective amounts of vasopressin receptor antagonists; therefore, compounds exhibiting partial antagonism at one or more vasopressin receptors may be administered in higher doses to exhibit sufficient antagonist activity to inhibit the effects of vasopressin or a vasopressin agonist.

The effective use of the compounds, compositions, and methods described herein for treating or ameliorating one or more effects of a brain injury using one or more 25 compounds described herein may be based upon animal models, such as murine, canine, porcine, and non-human primate animal models of disease. For example, it is understood that brain injuries in humans may be characterized by a loss of function, and/or the development of symptoms, each of which may be elicited in animals, such as mice, and other surrogate test animals. In particular the mouse models described herein may be used to evaluate the methods 30 of treatment and the pharmaceutical compositions described herein to determine the therapeutically effective amounts described herein.

Each publication cited herein is incorporated herein by reference.

The following examples further illustrate specific embodiments of the invention; however, the following illustrative examples should not be interpreted in any way to limit the 35 invention.

## METHOD EXAMPLES

EXAMPLE. Controlled cortical impact injury model. Briefly, test animals are initially anesthetized with isoflurane (4.5%), intubated, and mechanically ventilated with a gas mixture of N<sub>2</sub>O (66%), O<sub>2</sub> (32%), and isoflurane (1.5–2.0%). Rectal temperature is maintained at 37.0 ± 0.5°C using a self-adjusting heating pad. Catheters are placed into the femoral artery and vein. Mean arterial blood pressure (mABP) is monitored continuously using a data acquisition system, and arterial blood gas parameters (pH, pO<sub>2</sub>, pCO<sub>2</sub> and plasma Na<sup>+</sup> concentration) are obtained at 15-min intervals. The femoral vein is used to administer vehicle, dimethyl sulfoxide (DMSO), or one or more compounds described herein. Animals are secured in a stereotactic frame. A mid-line scalp incision is made, and the skin and periosteum are retracted from the skull surface. A 10-mm-diameter craniotomy is made midway between Bregma and Lambda on the right side, 1-mm lateral to the midline. Injury is produced using a pneumatic impactor (e.g., 5 mm diameter) mounted at an angle (e.g. 10°) from the vertical plane. A single impact (e.g. velocity 6 m/s; deformation depth 3.0 mm; dwell time, 0.3 s) is delivered to the right parietal cortex. After injury, the excised skull section is replaced, sealed with bone wax and the skin incision is closed. Sham operated animals are exposed to identical surgical procedures without any injury from a pneumatic impactor. Additional details are described in Dixon et al., “A fluid percussion model of experimental brain injury in the rat” J Neurosurg 67:110–119 (1987) and Taya et al., “Modulation of AQP4 expression by the selective V1a receptor antagonist, SR49059, decreases trauma-induced brain edema” Acta Neurochir 102 (Suppl.): S425–S429 (2008).

EXAMPLE. Momentum exchange impact model. This model features several advantageous translational differences over some head injury models, such as the controlled cortical impact injury model. For example, the head, neck and body can move with impact, and the velocity of head movement and energy transfer can be calculated and scaled mild to provide range from moderate to severe concussions. Studies are conducted with moderate impacts with neuroradiological evidence of contusions. Male rats are concussed twice with one day. Additional details are described in Viano et al., “Evaluation of three animal models for concussion and serious brain injury” Ann Biomed Eng. 40(1):213-26 (2012). Within 24 hrs of the first concussion test animals are given vehicle or test compound, such as a highly selective V1a receptor antagonist that crosses the blood brain barrier. Sham non-concussed test animals are given vehicle or test compound. Test animals are treated twice daily for 5 consecutive days. At two weeks post concussion cognitive behaviors are assessed, using for example, the Barnes

maze and/or novel recognition models. In addition, motor behaviors are assessed, using for example, beam walk and/or rotarod tests. In each case, neuroradiological MRI is also performed.

EXAMPLE. Imaging Neuroanatomy. At the beginning of each imaging session, a high-resolution anatomical data set is collected using the RARE pulse sequence (20 slice; 1 mm; field of vision [FOV] 3.0 cm; 256 × 256; repetition time [TR] 2.5 sec; echo time [TE] 12.4 msec; NEX 6; 6.5-minute acquisition time).

EXAMPLE. T2 Relaxometry measurement of edema. MRI was conducted 24-28 days after the injury to characterize edematous volume in the lateral ventricles using T2 relaxometry. The time of imaging was based on the rat model used for TBI assessment by Charles River. Images are acquired using a multi-slice multi-echo (MSME) pulse sequence. The echo time (TE) is illustratively 11 ms, and 16 echoes are acquired during imaging with a recovery time (TR) of 2500 ms. Images are acquired with a field of view [FOV] 3 cm<sup>2</sup>, data matrix = 256×256×20 slices, thickness = 1 mm. Values for longitudinal relaxation time (T2) are obtained from all the slices using ParaVision 5.1 software. T2 is used to characterize the edematous volume in the lateral ventricle. The T2 values are used for segmentation and quantification of the ventricle volume. The T2 values are computed using the equation;  $y = A + C \exp(-t/T2)$  (S.D. weighted) obtained from the Paravision 5.1 software, where, A = absolute bias, C = signal intensity, t = echo time and T2 = spin-spin relaxation time. The ventricle is identified as a hyperintensity on the T2 map over three 1 mm sections. The volume is calculated using a snake region growth algorithm in itk-SNAP ([www.itksnap.org](http://www.itksnap.org)). The threshold is illustratively set at 6300 to 9000 as absolute pixel intensity. A point is seeded within the ventricle and the algorithm run until segmentation is complete.

EXAMPLE. Resting state functional connectivity. Resting-state fMRI scan is collected using spin-echo triple-shot EPI sequence (imaging parameters: matrix size = 96x96x20, TR/TE=3000/15 msec, voxel size=0.312x0.312x0.12 mm, slice thickness = 1mm. Preprocessing is illustratively accomplished by combining Analysis of Functional NeuroImages (AFNI\_17.1.12, <http://afni.nimh.nih.gov/afni/>), FMRIB Software library (FSL, v5.0.9, <http://fsl.fmrib.ox.ac.uk/fsl/>), Deformable Registration via Attribute Matching and Mutual-Saliency Weighting (DRAMMS 1.4.1, <https://www.cbica.upenn.edu/sbia/software/dramms/index.html>) and MATLAB (Mathworks, Natick, MA). Brain tissue masks for resting-state functional images are manually drawn using 3DSlicer (<https://www.slicer.org/>) and applied for skull-stripping. Normalization is completed by registering functional data to the MRI Rat Brain Template (Ekam Solutions LLC, Boston, MA) using affine registration through DRAMMS. The region-to-region functional connectivity

method is illustratively performed to measure the correlations in spontaneous BOLD fluctuations. A network is comprised of nodes and edges; nodes being the brain region of interest (ROI) and edges being the connections between regions. Nodes are defined using the ROIs segmented from an appropriate MRI RAT Brain Atlas. Illustratively, 171 nodes are defined. Voxel time series data are averaged in each node based on the residual images using the nuisance regression procedure. Pearson's correlation coefficients across all pairs of nodes (14535 pairs for 171 nodes) are computed for each subject among all three groups to assess the interregional temporal correlations. The r-values (ranging from -1 to 1) are z-transformed using the Fisher's Z transform to improve normality. For 171 nodes, 171 x 171 symmetric connectivity matrices are constructed with each entry representing the strength of edge. Group-level analysis is performed to look at the functional connectivity in controls, one hit and three hit groups. The resulting Z-score matrices from one-group t-tests are clustered using the K-nearest neighbors clustering method to identify how nodes cluster together and form resting state networks. A Z-score threshold of  $|Z|=2.3$  is applied to remove spurious or weak node connections for visualization purposes.

EXAMPLE. Treatment in the momentum exchange impact model using compounds described herein. Male Sprague Dawley rats (n=19; 250-270g) aged 65 days, are obtained from Charles River (Worcester, MA). Test animals are maintained on a 12:12 hour light:dark cycle with a lights on at 0700 hours and allowed access to food and water ad libitum. The protocols used in this study comply with the regulations of the Institutional Animal Care and Use Committee at the Northeastern University.

The pneumatic pressure drive is replicated, 50g compactor, as described by Viano and reproduced consistently, the 7.4, 9.3, and 11.2 m/s impact velocities described for mild, medium, and severe head injury, respectively. The data described herein are obtained from a 9.12 meters/sec impact velocity, as determined using high-speed video recordings.

Beginning on postnatal (P)70, animals are divided into 3 groups (n = 6-7/group; Sham Control Group (Control, CG), Injury Group (IG), and Treatment Group (TG) and begin their head impact, and drug regimens. All groups are anaesthetized with 4% isoflurane in oxygen, and Injury group and Treatment group receive the first of two total head impacts) using a custom setup from Animal Imaging Research (AIR; Holden, MA) on P70. The day after the first impact (P71), test compound, such as AVN576, is administered to the Treatment group and the Sham Control group. In addition, a vehicle only control group (VCG) is administered saline. All administrations are administered to the Control and Treatment groups twice per day at 0700 and 1400 (IP @10mg/kg). The drug regimen is continued over the following 4 days (5 days total). The second head impact is delivered two days following the first on P72. Two

weeks post impact, animals are imaged and tested for cognitive and motor behaviors. In all evaluations made, there was not a significant difference between the sham control group (CG) receiving test compounds and vehicle-only sham control group (VCG) receiving vehicle.

Concussed, untreated animals (Injury group) showed significant deficits in cognitive behavior, altered patterns of functional connectivity, and indices of anisotropy localized to the hindbrain and amygdala. Treatment group animals concussed and treated with Example 266 (AVN576) showed no deficits in learning and memory and there was not a significant difference compared to the sham Control group. Similarly, there was not a significant difference in overall motor behavior between Control and Treatment groups.

10 Treatment group animals also showed enhanced functional connectivity, even compared to Control group in network circuitry associated with learning, cognition, memory, and attention.

Repetitive moderate traumatic brain injury using a momentum exchange model produced clear neuroradiological evidence of contusions, signs of inflammation, and white matter damage. The injury to the brain in untreated animals caused significant deficits in cognition, learning, and memory.

Injury to the brain in untreated test animals was associated with enlarged lateral ventricular volumes indicative of enhanced inflammation and edema. Treatment with compounds described herein for 5 days reduced the edematous enlargement of the lateral ventricles. Treatment group animals also showed a significant reduction in injury-evoked edema near the site of impact. There was not a statistical difference between the Treatment group and Control groups, indicating a complete recovery or cure from the impact. The complete recovery/cure of edema was profoundly unexpected. Though total brain water content has been reportedly decreased by 1-2% in other studies, neither a decrease in a specific edema, nor a complete recovery to baseline has been reported. Kleindienst et al., *Acta Neurochir* 25 155:151-64 (2013); Marmarou et al. *Brain Research* 1581:89-102 (2014).

Injury to the brain in untreated test animals also resulted in hypoconnectivity in important brain networks, such as the hippocampal neurocircuitry. Treatment with compounds described herein promoted hyperconnectivity in hippocampal neurocircuitry. Treatment within 24 h after the first concussion with compounds described herein prevented and provided complete recovery from the cognitive, learning, and memory deficits that occur following brain injury. The Treatment group showed behavioral measures that were not significantly different from the Control groups, indicating a complete recovery or cure from the impact. The complete recovery/cure from cognitive, learning, and memory deficits was profoundly unexpected. It has not heretofore been reported that the cognitive, learning, or memory deficits arising from brain injury are improved by Va1R antagonism.

Without being bound by theory, it is suggested that the compounds described herein may treat the cognitive and neurobiological effects of moderate to severe brain injury by not only reducing the inflammation and edema caused by the head injury, but by also promoting neuroadaptive changes in functional connectivity to compensate for the trauma. For example, 5 the Treatment group animals showed a hyperconnectivity even compared to Sham Control animals that were not injured, yet received treatment. The observed hyperconnectivity is relative to the default mode network represented by the control groups, where the Injury group show severe hypoconnectivity. Without being bound by theory, it is suggested that the 10 hyperconnectivity, resulting from treatment with the compounds described herein may reflect a reorganization of integrated neural networks that preserve function in response to brain injury. Further, disrupted connectivity is an indication of diffuse axonal injury, and therefore, the 15 results obtained herein for impact models, such as rmTBI, are applicable to other brain injuries, including but not limited to traumatic brain injury, blast TBI, cerebral edema, chronic traumatic encephalopathy (CTE), subarachnoid hemorrhage, stroke, ischemic stroke, concussion, and falls.

EXAMPLE. Novel Object Recognition test results. This test assesses episodic learning and memory-related stimulus recognition. FIG. 1 shows that animals in the Injury group (IG) spent significantly less time (recognition index), compared to chance (50% of total object investigation) investigating the novel object ( $t(5) = 3.393, p < 0.05$ ). In contrast, both the 20 Control group (CG) ( $t(6) = 3.08, p < 0.05$ ) and the Treatment group (TG) ( $t(5) = 2.61, p < 0.05$ ) groups spent significantly greater than a chance amount of time with the novel object. When compared between groups, all groups spent a significantly longer percentage of time with the novel object compared to the Injury group (CG:  $P = 0.0006$ , TG:  $P = 0.003$ ; Tukey post hoc following One-way ANOVA,  $F_{(3,21)} = 9.14, P = 0.0005$ ). Similarly, both the Control group ( $P = 0.006$ ) and Treatment group ( $P = 0.02$ ) spent significantly more total time with the novel 25 object compared to the Injury group animals (One-way ANOVA,  $F_{(3,21)} = 5.78, P = 0.005$ ). The exploration index (novel exploration instances/total exploration instances of either object) showed that groups performed differently as a function of AVN576 exposure (One-way ANOVA,  $F_{(3,21)} = 4.48, P = 0.01$ ), with only Control group animals showing a significantly 30 greater number of exploration instances than the Injury group animals ( $P = 0.01$ ), and the Treatment group animals showing only a trend towards significance ( $P = 0.06$ ).

EXAMPLE. Barnes Maze test results. This test assesses spatial learning and memory. The injury group had significant deficits in behavioral measures of cognition, while the Treatment group did not show any deficits in learning and memory and did not differ from 35 either control group (CG and/or VCG), as shown in FIG. 2A and FIG. 2B.

When collapsed across acquisition days, Barnes maze testing showed that the IG animals had longer latencies to find the goal box than either control group (VCG:  $P = 0.03$ , CG:  $P = 0.01$ ) and the Treatment group ( $P = 0.04$ ; Tukey's post hoc following One-way ANOVA,  $F_{(3,21)} = 4.88$ ,  $P = 0.009$ ). Similarly, Injury group animals traveled a significantly greater distance searching the maze before finding the goal box compared to both CG ( $P = 0.009$ ) and TG animals ( $P = 0.03$ ). Injury group animals also spent significantly more time exploring the wrong holes (error duration) compared to CG animals ( $P = 0.03$ ) and TG animals ( $P = 0.03$ ; Tukey's post hoc following One-way ANOVA,  $F_{(3,21)} = 3.95$ ,  $P = 0.02$ ). Evaluation of the distance travelled gave identical results (data not shown). When error duration is broken down across days, there are additional differences between groups and across days (Mixed Design ANOVA, Main effect of Day:  $F_{(3,63)} = 9.10$ ,  $P < 0.0001$ ; and Group:  $F_{(3,21)} = 3.95$ ,  $P = 0.02$ ). Injury group animals committed significantly more errors on Day 1 compared to CG animals ( $P = 0.07$ ); and on Day 2 compared to CG animals ( $P = 0.01$ ) and TG animals ( $P = 0.02$ ). Evaluation of the distance travelled, and broken down across days, gave identical results (data not shown).

EXAMPLE. Resting State Functional Connectivity. FIG. 3 shows correlation matrices of 166 rat brain areas for resting state functional connectivity (rsFC) comparing the Injury group to the Treatment group. The brain areas with significant correlations often appear as clusters because they are contiguous in their neuroanatomy and function. The clusters include areas:

- A: Hypothalamus
- B: Dorsal Hippocampus – CA1, CA3, dentate
- C: Thalamus
- D: Sensory Motor CTX & Dorsal Striatum – (motor CTX, anterior cingulate, primary SS CTX), dorsal striatum
- E: Prefrontal CTX & Ventral Striatum – (prelimbic ctx, infralimbic ctx, ventral & lateral orbital), (ventral striatum)
- F: Amygdala & Piriform/Insular CTX
- G: Ventral Hippocampus & Temporal CTX - CA1, CA3, dentate
- H: Cerebellum – All lobes, Crus1&2, medial cerebellar n., (fastigial)
- I: Reticular Activating System Medulla & Cerebellum – (interpose n., copula, 10<sup>th</sup> lobule), (gigantocellularis, parabrachial, parvocellular reticular, solitary tract n. vestibular n., principle sensory n. trigeminal)
- J: Cerebellum & Medulla & Entorhinal CTX – (paramedian lobule, paraflocculus cerebellum, flocculus), (ventral subiculum, entorhinal ctx, ectorhinal ctx), (median

raphe, locus ceruleus, trapezoid body, dorsomedial tegmentum, facial n. sub ceruleus,  
pontine reticular n. cochlear n. dorsal paragigantocellularis)

K. Midbrain Dopamine – substantia nigra compacta, substantia nigra reticulare, ventral  
tegmentum

5 L. Hypothalamus connections, Amygdala

M. Reticular Activating System connections, Cerebellum connections, Raphe  
connections, Hippocampus, Reticular Activating System

N. Amygdala connections, Medulla

O. Midbrain Dopamine connections, Ventral Hippocampus

10 P. Midbrain Dopamine connections, Hypothalamus

The diagonal line separates the different experimental groups, and the pixels locations are mirror images across the diagonal (Areas G and M marked with arrows for illustration). Areas A-P show highlighted clusters of brain regions that have functional relationships and illustrate the default mode network in the Control group (data not shown). The brain areas with  
15 significant correlations may also appear as clusters because they are contiguous in their neuroanatomy and function. In general it is observed that the Injury group network shows hypoconnectivity, whereas the Treatment group network shows hyperconnectivity. The Injury group network also shows hypoconnectivity compared to the Control group (data not shown). The Treatment group network also shows hyperconnectivity compared to the Control group  
20 (data not shown).

Referring to Area G in FIG 3, it is observed that the ventral hippocampus shows hypoconnectivity in the Injury group compared to the Control group (data not shown), whereas the Treatment group shows hyperconnectivity compared to Injury group and Control group. The specific network connections, including the Control group are shown in the table.

Treatment Group (TG).

Brain region		CG	IG	TG
Ventral hippocampus	ventral CA1	X	X	X
	ventral CA2	X	X	X
	ventral dentate	X	X	X
Adjacent amygdala	cortical amygdala	X		X
	basal amygdala	X		X
	intercalated amygdala	X	X	X
	medial amygdala	X		X
	central amygdala	X		X
	lateral amygdala	X	X	X
Limbic cortex	caudal piriform cortex	X	X	X
	rostral piriform cortex			X
	endopiriform cortex			X
	temporal cortex	X		X
	entorhinal cortex	X	X	X
	ventral subiculum	X	X	X
Reticular nuclei of the midbrain	reticular nuclei midbrain	X		X
	pedunculopontine tegmentum			X
	pontine reticular nuclei			X
	parvicellular reticular nuclei			X
	parabrachial nuclei			X
	median raphe		X	X
	periaqueductal gray			X
Other brain regions	ventral tegmental area			X
	substria nigra compact			X
	substria nigra reticularis			X

The 3D organization of these brain areas and differences in connectivity are also illustrated by the glass brains in FIG. 4A (Injury group) and FIG. 4B (Treatment group). As shown, there is loss of connectivity in the Injury group, and hyperconnectivity in the Treatment group for key nodes of the ventral hippocampus, especially with the amygdala, limbic cortex, and reticular n. of the midbrain. Hyperconnectivity is also observed in the Treatment group for key nodes of the ventral hippocampus and the temporal cortex.

10 Resting state functional connectivity analysis is useful in identifying subtle alterations in traumatically injured brain organization linked to cognitive and emotional symptoms of brain injury. The loss of connectivity and clustering in the Injury group shortens the pathway length or aggregate neural connections, reducing the metabolic cost of signal transduction. Without treatment, the amygdala remains largely disconnected from the ventral hippocampus and adjacent areas. Hypoconnectivity is well-established to be strongly associated with cognitive impairment and neurodegeneration. Area G is associated with cognition, learning, and memory. The hyperconnectivity observed in the Treatment group

15

may reflect a reorganization of integrated neural networks to preserve function in response to brain injury.

Referring to Area M in FIG. 3, it is observed that the hippocampus shows hypoconnectivity in the Injury group compared to the Control group (data not shown), whereas 5 the Treatment group shows hyperconnectivity compared to Injury group and Control group. The specific network connections, including the Control group are shown in the table.

Area M – Hippocampus/Reticular Activating System; Control Group (CG), Injury Group (IG), Treatment Group (TG).

Brain region		CG	IG	TG
Reticular activating system	principle sensory nuclei trigeminal	X	X	X
	parvicellular reticular nuclei	X	X	X
	giganitocellular reticular nuclei	X	X	X
	parabrachial nuclei	X	X	X
Motor system	raphe magnus	X		X
	raphe obscurus	X	X	X
	olivary nuclei	X	X	X
	periolivary nuclei			X
	dentate nuclei cerebellum	X	X	X
	1 <sup>st</sup> cerebellar lobule	X	X	X
	interpeduncular nuclei			X
	medial geniculate			X
Ventral hippocampus	temporal cortex		X <sup>(*)</sup>	X
	CA1 ventral hippocampus			X
	CA3 ventral hippocampus			X
	dentate, ventral hippocampus			X

10 (\*) Low level connectivity observed.

The 3D organization of these brain areas and differences in connectivity are also illustrated by the glass brains in FIG. 6. As shown, there is loss of connectivity in the Injury group, and hyperconnectivity in the Treatment group for key nodes of the ventral hippocampus and reticular activating system.

15 Without treatment, the reticular activating system remains largely disconnected from the ventral hippocampus and adjacent areas. Hypoconnectivity is well-established to be strongly associated with cognitive impairment, memory, and attention. Area M is associated with cognition, learning, and memory. The hyperconnectivity observed in the Treatment group, including compared to the Control group, may reflect a reorganization of integrated 20 neural networks to preserve function in response to brain injury and provides a therapeutic benefit leading to recovery of function.

EXAMPLE. Edema and Ventricular Volumes. FIG. 5 shows the volume of lateral ventricles at the level of impact (hippocampal fimbria/septum) calculated and compared

using a single tailed T-test. There is a significant increase in ventricular volume in the Injury group compared to the Control group. The Treatment group showed a complete response/cure where ventricular volume returns to normal levels, where there is not any significant difference between the Control group and the Treatment group.

5 EXAMPLE. Motor behavior. Using a standard balance beam performance test, there was not any difference in total foot fault frequency (One-way ANOVA,  $F_{(3,21)} = 1.42, P = 0.26$ ) or total beam traversal time (One-way ANOVA,  $F_{(3,21)} = 2.40, P = 0.1$ ) for the Balance beam task. However, there were significant differences in the faults per wide and middle segments ( $p < 0.0001$ ). Using a standard rotarod performance test, the Injury group animals  
10 showed significantly shorter latencies to fall ( $69.8 \pm 5.5$  sec) compared to the Control group ( $105.4 \pm 7.6$  sec,  $P = 0.01$ ). The Treatment group showed an improvement in latency to fall ( $84.0 \pm 9.0$  sec), with a trend toward significance ( $P = 0.2$ ). However, the differences observed in the NOR test do not appear to be a function of altered motor behavior; a significant difference in the distances traveled was not observed in any group due to any motor  
15 impairment. (One-way ANOVA,  $F_{(3,21)} = 1.22, P = 0.32$ ).

EXAMPLE. Human vasopressin V<sub>1a</sub> receptor binding assay. A cell line expressing the human V<sub>1a</sub> receptor in CHO cells (henceforth referred to as the hV<sub>1a</sub> cell line) was obtained from Dr. Michael Brownstein, NIMH, Bethesda, MD, USA. The hV<sub>1a</sub> cDNA sequence is described by Thibonnier et al., Journal of Biological Chemistry, 269, 3304-3310  
20 (1994), and the expression method was the same as described by Morel et al. (1992). The hV<sub>1a</sub> cell line was grown in alpha-MEM with 10% fetal bovine serum and 250ug/ml G418 (Gibco, Grand Island, NY, USA). For competitive binding assay, hV<sub>1a</sub> cells were plated into 6-well culture plate at 1:10 dilution from a confluence flask, and maintained in culture for at least two days. Culture medium was then removed, cells were washed with 2ml binding buffer (25mM  
25 Hepes, 0.25% BSA, 1x DMEM, PH = 7.0). To each well, 990  $\mu$ l binding buffer containing InM 3H-AVP was added, and followed by 10  $\mu$ l series diluted Example compounds dissolved in DMSO. All incubations were in triplicate, and dose-inhibition curves consisted of total binding (DMSO) and 5 concentrations (0.1, 1.0, 10, 100, and 1000 nM) of test agents encompassing the IC<sub>50</sub>. 100 nM cold AVP (Sigma) was used to assess non-specific binding.  
30 Cells were incubated for 45 minutes at 37 °C, assay mixture was removed and each well was washed three times with PBS (pH = 7.4). 1ml 2% SDS was added per well and plates were let sit for 30 minutes. The whole content in a well was transferred to a scintillation vial. Each well was rinsed with 0.5ml PBS which was then added to the corresponding vial. Scintillation fluid (Ecoscint, National Diagnostics, Atlanta, Georgia) was then added at 3ml per vial. Samples  
35 were counted in a liquid scintillation counter (Beckman LS3801). IC<sub>50</sub> values were calculated

All of the alkanedioic esters and amides exemplified in the foregoing examples dissolved in DMSO were tested in this assay. Binding curves were generated according to methods described by Thibonnier et al. (1994). [<sup>3</sup>H]-AVP was added to the hV1a cell cultures 5 followed by 10-fold dilutions of each test compound. All active compounds showed a dose-dependent competitive binding curve, with IC<sub>50</sub> and K<sub>i</sub> values characteristic of high affinity binding to V<sub>1a</sub> receptors in CHO cells expressing the human V<sub>1a</sub> receptor (the hV1a cell line). For example, Example 225 showed a dose-dependent competitive binding curve, with IC<sub>50</sub> (1.86-2.13 nM) and K<sub>i</sub> (1.14-1.30 nM) values.

10 Binding affinities (IC<sub>50</sub>) and inhibition constants (K<sub>i</sub>) for illustrative compounds are shown in the following Table.

Example	V <sub>1a</sub> Binding Affinity IC <sub>50</sub> (nM)	V <sub>1a</sub> K <sub>i</sub> (nM)
18	35	-
19	35	-
20	35	-
35	1.9	1.17
37	5.5	3.39
38	< 25	85
39	23	13.3
40	11	6.5
41	< 20	18.2
42	< 20	26.4
42A	1.77	1.17
44	3.1	1.89
47	~ 50	-
59	< 100	-
63	1.84	1.13
66	~ 50	-
77	< 100	-
78	< 100	-
81	< 100	-
82	< 50	5.12
85	5.87	3.6
86A	9.79	6
87	15	-
88	2.4	1.45
91	3.24	1.99
95	1.76	1.08

Example	V <sub>1a</sub> Binding Affinity IC <sub>50</sub> (nM)	V <sub>1a</sub> K <sub>i</sub> (nM)
215	0.61	0.38
216	1.83	1.12
217	3.17	1.94
218	7.7	4.7
219	0.63	0.39
220	5.3	3.26
221	5.1	3.1
221A	2.71	1.66
221B	0.59	0.36
221C	3	1.84
221D	2.41	1.48
221E	20.2	12.4
221F	1.7	1.04
221G	1.5	0.93
221H	4	2.5
221I	12	7.4
221K	~ 5	-
221O	8.4	5.1
221P	1.7	1.1
221Q	18.1	11.1
221R	5.13	3.14
221S	5.03	3.08
221X	11.6	7.2
221Y	7.6	4.7
221AB	< 10	-
221AC	< 10	-

Example	V <sub>1a</sub> Binding Affinity IC <sub>50</sub> (nM)	V <sub>1a</sub> K <sub>i</sub> (nM)
96	4.35	2.66
100	< 100	-
101	~ 100	-
102	< 100	-
103	0.81	0.49
104	1.85	1.13
106	~ 100	-
107	< 50	-
108	~ 100	-
109	~ 100	-
110	0.49	0.27
111	1.31	0.82
112	1.34	0.8
120	0.75	0.46
120A	16.2	9.9
120B	2.93	1.79
120E	3.2	1.95
120H	2.75	1.68
132D	6.3	3.9
132F	4.8	3
133	2.43	1.49
134A	12.9	7.9
134B	44.8	27.5
134C	9.1	5.58
134G	6	3.7
134J	5.29	3.25
135	~ 50	-
136	11	33
137	17	10.5
138	21	13
139	9.5	5.84
172	4.5	2.78
173	< 100	-
174	1.46	0.89
175	4.56	2.79

Example	V <sub>1a</sub> Binding Affinity IC <sub>50</sub> (nM)	V <sub>1a</sub> K <sub>i</sub> (nM)
221AD	~ 50	-
221AE	~ 50	-
221AI	~ 50	-
221AL	~ 100	-
221AM	-	2.7
221AP	-	3.8
221AO	~ 100	-
221AQ	~ 50	-
221AS	~ 20	-
221AX	83	51
221AY	~ 30	-
221BD	2.7	1.66
221BI	56	35
222	1.83	1.13
224 (SRX246) (AVN246)	0.49	0.3
225 (SRX251) (AVN251)	1.08	0.66
225-HCl	-	1.36
225-MeI	4.8	3
226	0.49	0.3
227	11	6.71
228	13.6	8.35
229	1.53	0.94
230	7.07	4.33
230F	~ 100	-
230L	12.7	7.8
231	6.12	3.75
232	1.37	0.84
232D	2.04	1.25
232E (SRX296) (AVN296)	0.28	0.17
233 (SRX228) (AVN228)	0.56	0.34
233A	-	11.6
234	2.37	1.45
234A	8.6	5.25
235	37	23
236	1.68	1.03

Example	V <sub>1a</sub> Binding Affinity IC <sub>50</sub> (nM)	V <sub>1a</sub> K <sub>i</sub> (nM)
176	0.61	0.38
177	0.67	0.41
178	< 50	-
179	0.81	0.51
180	0.33	0.2
181	< 50	-
182	1.52	0.93
183	< 10	-
184	< 10	-
185	1.27	0.82
186	< 10	-
187	1	0.66
188	7.26	4.45
189	1.7	1.04
190	0.88	0.54
191	2.92	1.79
192	< 10	-
193	1.17	0.72
194	< 100	-
195	< 50	-
196	< 100	-
198	~ 100	-
199	< 10	-
200	5.08	3.11
201	10.5	6.43
203	2.46	1.5
204	6	3.7
205	0.34	0.21
206	1.58	0.97
207	4.48	2.74
208	16.3	10
209	16	9.8
210	29.5	18.1
211	5.37	3.29
212	0.95	0.58
213	0.78	0.48
214	1.86	1.14

Example	V <sub>1a</sub> Binding Affinity IC <sub>50</sub> (nM)	V <sub>1a</sub> K <sub>i</sub> (nM)
236A	9	5.5
238	0.11	0.07
239	6.6	4
240	25	15.5
241	2.0	1.24
242	2.2	1.36
243	0.5	0.3
244	3.4	2.1
245	1.1	0.68
246	2.1	1.3
247	0.6	0.39
248	5.3	3.3
249	1.7	1
250	6.5	4
251	0.5	0.3
252	1.8	1.1
253	9.5	5.8
254	10	6.2
255	1.9	1.2
256	2.8	1.7
266 (SRX576) (AVN576)	1.8	1.1
559	0.12	0.073
594	-	19
597	6.2	3.8
599	1.2	0.73
600	14.4	8.8
601	1	0.62
606	0.53	0.32
617	-	0.69
623	-	0.85
626	-	0.27
670	-	3.1
672	-	1.1
677	-	3
682	-	0.9
778	-	0.63

EXAMPLE. Human vasopressin V<sub>1b</sub> receptor-expressing cells. Human vasopressin receptor 1b (hV1b) cDNA (see, Lolait et al., "Extrapatuitary expression of the rat V1b vasopressin receptor gene" Proc. Natl. Acad. Sci. U S A. 92:6783-7 (1995); de Keyzer et al., "Cloning and characterization of the human V3(V1b) pituitary vasopressin receptor" FEBS Lett. 356:215-20 (1994); Sugimoto et al., "Molecular cloning and functional expression of a cDNA encoding the human V1b vasopressin receptor" J. Biol. Chem. 269:27088-92 (1994)) was inserted into a mammalian cell expression vector PCI-neo (Promega) at EcoR1 site. The recombinant plasmid carrying hV1b cDNA was identified from transformed *E. Coli* clones and used for the transfection of Chinese hamster ovary cell (CHO-K1, ATCC). Two micrograms of hV1b receptor DNA was introduced into 10<sup>5</sup> CHO cells cultured in 6-well plate, using Fugene-6 mediated transfection technique (Boehringer Mannheim). Twenty-four hrs post transfection, Cells were then cultured under selection of G-418 (0.25mg/ml) supplemented to the culture medium. Three days later, limited dilution was carried out to obtain single cell clones in 96-well plates. After a period of 2-weeks of growth, monoclones were expanded into two sets of 12-well plates. When confluence was reached, one set of wells were assayed for their ability to bind tritium-labeled arginine-vasopressin (NEN). Nine positive clones were initially identified out of 60 clones screened, and clones that demonstrated highest AVP binding were saved as permanent cell lines for hV1b affinity screening.

EXAMPLE. Human or rat vasopressin V<sub>1b</sub> cell-based receptor binding assay.

The V1b cell lines (cells expressing either the human or rat V<sub>1b</sub> receptor) were grown in alpha-MEM medium supplemented with 10% fetal bovine serum and 250ug/ml G418 (Gibco, Grand Island, NY) in 75 cm<sup>2</sup> flask. For competitive binding assay, hV1b cells were dissociated with enzyme-free, PBS based cell dissociation solution (Specialty Media, Phillipursburg, NJ), following the manufacturer's protocol. Cells were plated into 12-well culture plates at a rate of one flask to 18 plates (rate should be adjusted according to the extent of confluence), and maintained in culture for 2-3 days. Culture medium was then removed, cells were washed once with 2ml binding buffer (25mM Hepes, 0.25% BSA, 1x DMEM, PH=7.0) at room temperature. To each well, 990ul binding buffer containing 1nM <sup>3</sup>H-AVP was added, and followed by the addition of 10ul series diluted testing compounds or cold AVP, all dissolved in DMSO. All incubations were in triplicate, and dose-inhibition curves consisted of total binding (DMSO only) and 5 concentrations (0.1, 1.0, 10, 100, and 1000nm) of test agent, or cold AVP, encompassing the IC50. Cells were incubated for 30 min at 37°C in a moisturized incubator. Assay mixture was then removed and each well was washed three times with PBS (pH=7.4). After washing, 1ml 2% SDS was added per well and plates were let sit for 15 min at RT. Gently pat the plate to make sure that lysed cells were detached. The whole content in a well

was transferred to a scintillation vial. Each well was then rinsed with 0.5ml PBS and added to the corresponding vial. Scintillation fluid (Ecoscint, National Diagnostics, Atlanta, Georgia) was then added at 3ml per vial. Samples were counted in a liquid scintillation counter (Beckman LS3801). IC50 and Ki values were calculated using Prism Curve fitting software.

5 Illustrative compounds shown in the previous table show a binding constant greater than 100 nM, or greater than 1000 nM. Illustrative inhibition data (Ki, nM) are shown in the following table for selected Example compounds.

Receptor	Example 224 (AVN246)	Example 225 (AVN251)	Example 266 (AVN576)
V1a	0.30	0.66	1.1
V1b	>1000	>1000	>100
V2	>1000	>1000	>1000

EXAMPLE. Inhibition of phosphatidylinositol turnover (V<sub>1a</sub>). The 10 physiological effects of vasopressin are mediated through specific G-protein coupled receptors. The V<sub>1a</sub>R is coupled to the G<sub>q</sub>/G<sub>11</sub> family of G proteins and mediates phosphatidylinositol turnover. The agonist or antagonist character of the compounds of the invention may be determined by their ability to inhibit vasopressin-mediated turnover of phosphatidylinositol by the procedure described in the following paragraphs. Illustrative compounds, Examples 35, 44, 15 88, 110, and 133, were tested in this assay and found to be vasopressin V<sub>1a</sub> antagonists.

EXAMPLE. Inhibition of vasopressin V<sub>1b</sub>-mediated phosphatidylinositol turnover, a functional assay for antagonist activity. The physiological effects of vasopressin are mediated through specific G-protein coupled receptors. The vasopressin V<sub>1b</sub> receptor is coupled to a G protein, which is coupled to cAMP. The agonist or antagonist character of the 20 compounds described herein may be determined by their ability to inhibit vasopressin-mediated turnover of phosphatidylinositol by using conventional methods, including the procedure described in the following paragraphs.

Cell culture and labeling of cells. Three days prior to the assay, near-confluent cultures of hV1a or hV1b cells were dissociated and seeded in 6-well tissue culture plates, 25 about 100 wells being seeded from each 75 cm<sup>2</sup> flask (equivalent to 12:1 split ratio). Each well contained 1 mL of growth medium with 2 µCi of [<sup>3</sup>H]myo-inositol (American Radiolabeled Chemicals, St. Louis, MO, USA).

Cells expressing the human or rat V<sub>1b</sub> receptors are grown in alpha-modified minimal essential medium containing 10% fetal bovine serum and 0.25 mg/ml G418. Three 30 days prior to the assay, near-confluent cultures are dissociated and seeded in 6-well tissue culture plates, about 100 wells being seeded from each 75 cm<sup>2</sup> flask (equivalent to 12:1 split

Incubations (V<sub>1a</sub> and V<sub>1b</sub>). All assays were in triplicate except for basal and 10 nM AVP (both n = 6). AVP ((arginine vasopressin), Peninsula Labs, Belmont, CA, USA (#8103)) was dissolved in 0.1N acetic acid. Test agents were dissolved in DMSO and diluted in DMSO to 200 times the final test concentration. Test agents and AVP (or corresponding volumes of DMSO) were added separately as 5  $\mu$ L in DMSO to 12x75 mm glass tubes containing 1 mL of assay buffer (Tyrode's balanced salt solution containing 50 mM glucose, 10 mM LiCl, 15 mM HEPES pH 7.4, 10  $\mu$ M phosphoramidon, and 100  $\mu$ M bacitracin). The order 10 of incubations was randomized. Incubations were initiated by removing the prelabeling medium, washing the monolayer once with 1 mL of 0.9% NaCl, and transferring the contents of the assay tubes to corresponding wells. The plates were incubated for 1 hour at 37 °C. Incubations were terminated by removing the incubation medium and adding 500  $\mu$ L of ice cold 5% (w/v) trichloroacetic acid and allowing the wells to stand for 15 min.

15 Measurement of [<sup>3</sup>H]inositol phosphates (V<sub>1a</sub> and V<sub>1b</sub>). BioRad Poly-Prep Econo-Columns were packed with 0.3 mL of AG 1 X-8 100-200 formate form resin. Resin was mixed 1:1 with water and 0.6 mL added to each column. Columns were then washed with 10 mL water. Scintillation vials (20mL) were placed under each column. For each well, the contents were transferred to a minicolumn, after which the well was washed with 0.5 mL 20 distilled water, which was also added to the minicolumn. The columns were then washed twice with 5 mL of 5 mM myo-inositol to elute free inositol. Aliquots (1 mL) were transferred to 20 mL scintillation vials and 10 mL of Beckman Ready Protein Plus added. After the myo-inositol wash was complete, empty scintillation vials were placed under the columns, and [<sup>3</sup>H]inositol phosphates were eluted with three additions of 1 mL 0.5 M ammonium formate containing 0.1 N formic acid. Elution conditions were optimized to recover inositol mono-, bis-, and 25 trisphosphates, without eluting the more metabolically inert tetrakis-, pentakis-, and hexakis-phosphates. To each sample was added 10 mL of a high salt capacity scintillation fluid such as Tru-Count High Salt Capacity or Packard Hionic-Fluor. Inositol lipids were measured by adding 1 mL of 2% sodium dodecyl sulfate (SDS) to each well, allowing the wells to stand for 30 at least 30 min., and transferring the solution to 20 mL scintillation vials, to which 10 mL Beckman Ready Protein Plus scintillation fluid was then added. Samples were counted in a Beckman LS 3801 liquid scintillation counter for 10 min. Total inositol incorporation for each well was calculated as the sum of free inositol, inositol phosphates, and inositol lipids.

35 Data analysis (V<sub>1a</sub> and V<sub>1b</sub>): concentration-inhibition experiments.  
Concentration-response curves for AVP and concentration-inhibition curves for test agents

versus 10 nM AVP were analyzed by nonlinear least-squares curve-fitting to a 4-parameter logistic function. Parameters for basal and maximal inositol phosphates, EC<sub>50</sub> or IC<sub>50</sub>, and Hill coefficient were varied to achieve the best fit. The curve-fitting was weighted under the assumption that the standard deviation was proportional to dpm of radioactivity. Full concentration-response curves for AVP were run in each experiment. IC<sub>50</sub> values were converted to K<sub>i</sub> values, which reflect the antagonistic activities against AVP in the production of signaling molecule IP3, by application of the Cheng-Prusoff equation, based on the EC<sub>50</sub> for AVP in the same experiment. Inositol phosphates were expressed as dpm per 10<sup>6</sup> dpm of total inositol incorporation.

10 Data analysis (V<sub>1a</sub> and V<sub>1b</sub>): competitiveness experiments. Experiments to test for V<sub>1a</sub> competitiveness of test agents consisted of concentration-response curves for AVP in the absence and presence of two or more concentrations of test agent. Experiments to test for V<sub>1b</sub> competition by test agents consist of concentration-response curves for AVP in the absence and presence of at least five concentrations of test agent. Data were fit to a competitive logistic equation

$$Y = B + \frac{M \times \{A / [E + (D / K)]\}^Q}{1 + \{A / [E + (D / K)]\}^Q}$$

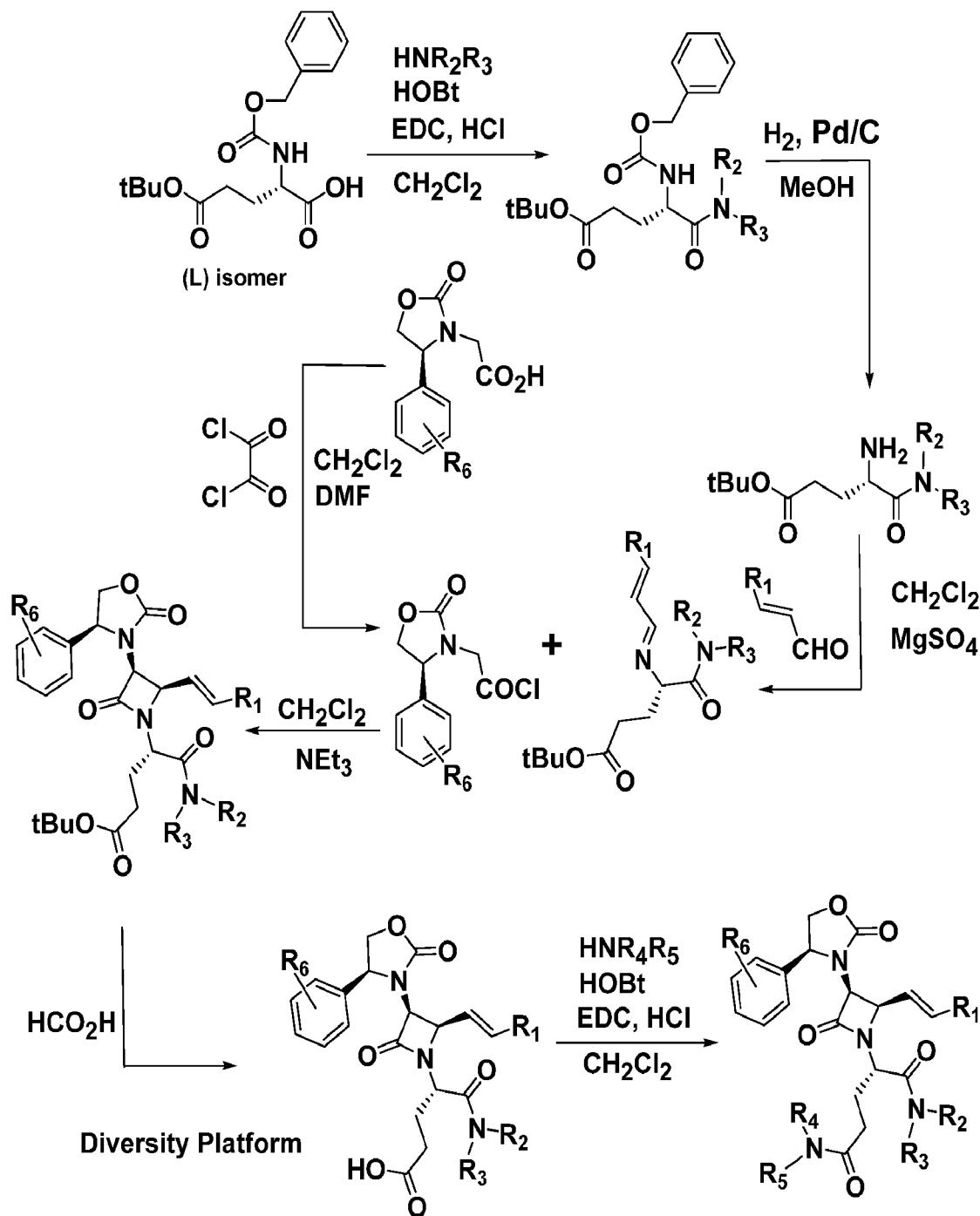
15 where Y is dpm of inositol phosphates, B is concentration of basal inositol phosphates, M is the maximal increase in concentration of inositol phosphates, A is the concentration of agonist (AVP), E is the EC<sub>50</sub> for agonist, D is the concentration of antagonist (test agent), K is the K<sub>i</sub> for antagonist, and Q is the cooperativity (Hill coefficient).

Compound Example 225 produces a dose-dependent suppression of the action of AVP with IC<sub>50</sub> (2.68 nM) and K<sub>i</sub> (0.05 nM). These values are consistent with high affinity binding of Example 225 and its inhibition of inositol lipid synthesis via the human V<sub>1a</sub> receptor.

20 EXAMPLE. Pharmacokinetics. Compounds described herein are rapidly absorbed after oral administration. Compounds described herein cross the blood-brain-barrier and achieve therapeutically effective concentrations in the CNS. Compounds described herein may be dosed according to a wide variety of protocols, including but not limited to q.d., b.i.d., and the like. Compounds described herein exhibit dose-related increases in C<sub>max</sub> and AUC when dosed according to various protocols, including but not limited to q.d., b.i.d. For example, b.i.d. dosing shows a 1.7-fold accumulation and improved T<sub>1/2</sub> for AVN246.

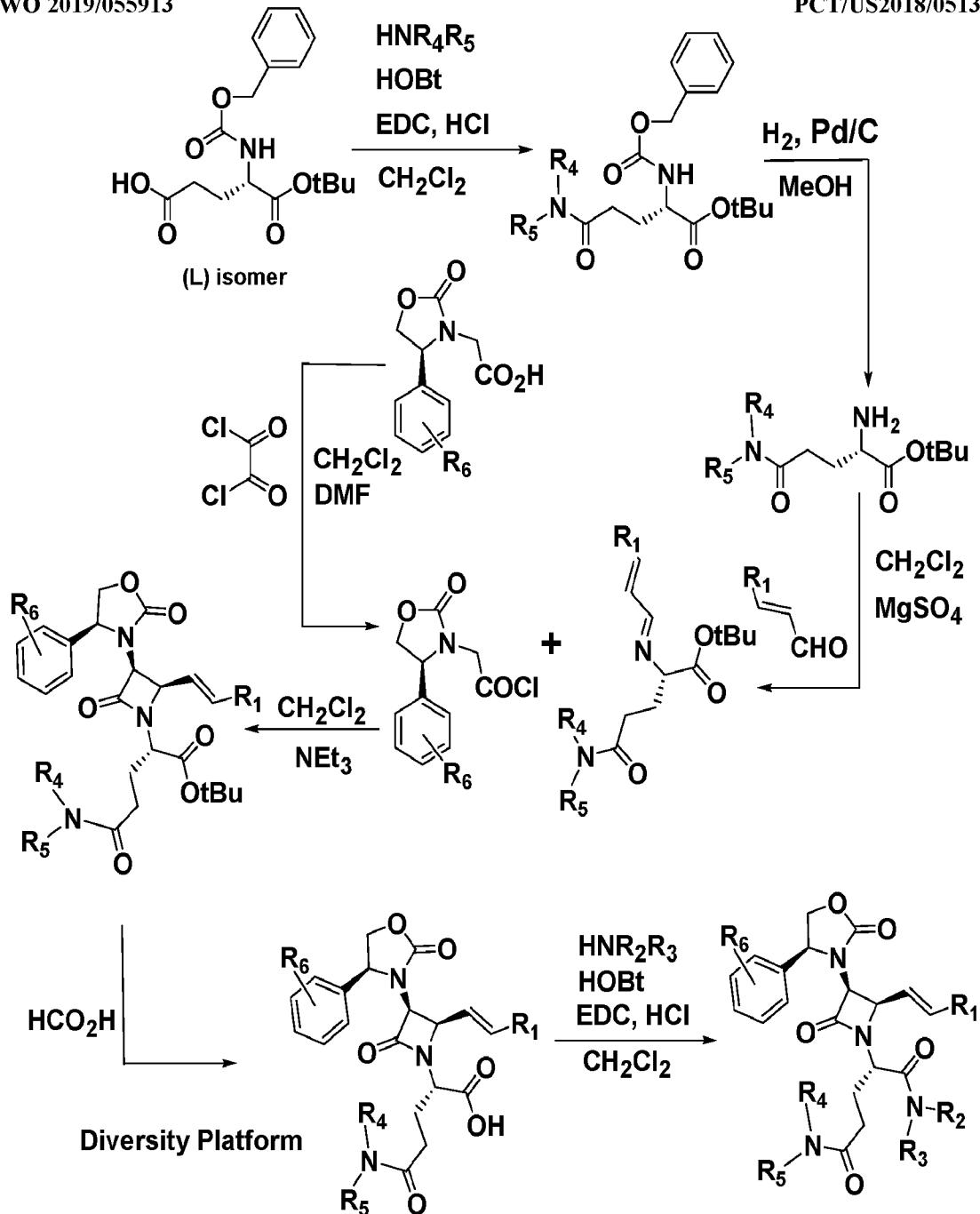
EXAMPLE. General Synthetic Routes. Proximal amide approach which permits synthetic variation at the distal amide site; proximal amide is set first, followed by distal amide diversity by parallel synthesis.

5

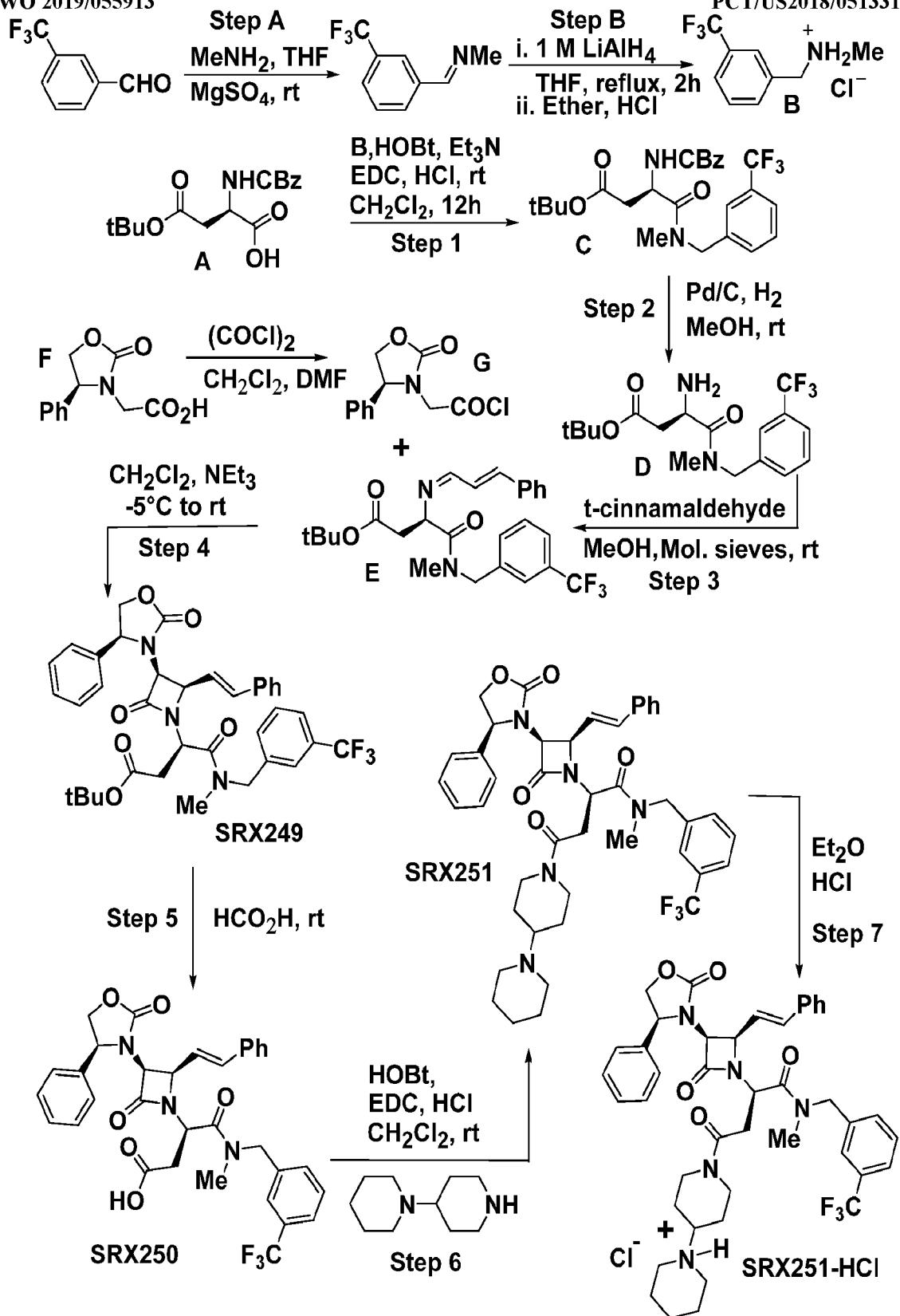


Distal amide approach which permits synthetic variations at the proximal site; distal amide is set first, followed by proximal amide diversity by parallel synthesis.

10



Synthesis of AVN251 (SRX251) and AVN251•HCl is shown below. All other compounds are prepared in an analogous manner with the appropriate selection of starting materials.



Additional details and alternative syntheses for preparing compounds described herein are described in U.S. patent No. 7,119,083, the disclosure of which are incorporated herein by reference in their entirety. The compounds described herein may be formulated and administered according to the processes described in U.S. patent No. 7,119,083. Additional 5 details are described in Guillon, C.D., et al., Azetidinones as vasopressin V1a antagonists. Bioorg Med Chem, 15(5):2054-80 (2007).

## COMPOUND EXAMPLES

Example 1. (4(S)-phenyloxazolidin-2-on-3-yl)acetyl chloride. A solution of 1.0 equivalent of (4(S)-phenyloxazolidin-2-on-3-yl)acetic acid (Evans, U.S. Patent No. 4,665,171) 10 and 1.3 equivalent of oxalyl chloride in 200 mL dichloromethane was treated with a catalytic amount of anhydrous dimethylformamide (85  $\mu$ L / milliequivalent of acetic acid derivative) resulting in vigorous gas evolution. After 45 minutes all gas evolution had ceased and the reaction mixture was concentrated under reduced pressure to provide the title compound as an off-white solid after drying for 2 h under vacuum.

Example 1A. (4(R)-phenyloxazolidin-2-on-3-yl)acetyl chloride. Example 1A was prepared following the procedure of Example 1, except that (4(R)-phenyloxazolidin-2-on-3-yl)acetic acid was used instead of (4(S)-phenyloxazolidin-2-on-3-yl)acetic acid (see, Evans & Sjogren, Tetrahedron Lett. 26:3783 (1985)).

Example 1B. Methyl (4(S)-phenyloxazolidin-2-on-3-yl)acetate. A solution of 20 (4(S)-phenyloxazolidin-2-on-3-yl)acetic acid (1 g, 4.52 mmol) (prepared according to Evans in U.S. Patent No. 4,665,171) in 20 mL of anhydrous methanol was treated hourly with 5 equivalents of acetyl chloride, for a total of 20 equivalents. The resulting solution was stirred overnight. The residue obtained after evaporation of the MeOH was redissolved in 30 mL of CH<sub>2</sub>Cl<sub>2</sub> and treated with 50 mL of saturated aqueous Na<sub>2</sub>CO<sub>3</sub>. The organic layer was 25 evaporated and dried (MgSO<sub>4</sub>) to yield the title compound as a colorless oil (1.001g, 94%); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  3.37 (d, J=18.0 Hz, 1H), 3.69 (s, 3H), 4.13 (t, J=8.3 Hz, 1H), 4.28 (d, J=18.0 Hz, 1H), 4.69 (t, J=8.8 Hz, 1H), 5.04 (t, J=8.4 Hz, 1H), 7.26-7.29 (m, 2H), 7.36-7.42 (m, 3H).

Example 1C. Methyl 2-(4(S)-phenyloxazolidin-2-on-3-yl)propanoate. A 30 solution of methyl (4(S)-phenyloxazolidin-2-on-3-yl)acetate (1 g, 4.25 mmol) in 10 mL of anhydrous THF at -78 °C was treated with 4.68 mL (4.68 mmol) of a 1 M solution of lithium bis(trimethylsilyl)amide in THF. The reaction mixture was stirred for 1 h. at about -70 °C before adding MeI (1.59 mL, 25.51 mmol). Upon complete conversion of the azetidinone, the reaction was quenched with saturated aqueous NH<sub>4</sub>Cl and partitioned between EtOAc and water. The organic layer was washed sequentially with saturated aqueous sodium bisulfite, and

saturated aqueous NaCl. The resulting organic layer was dried ( $\text{MgSO}_4$ ) and evaporated to afford the title compound (a mixture of diasteromers) as a white solid (1.06g, 93%);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.07/1.53 (d/d,  $J=7.5$  Hz, 3H), 3.59/3.74 (s/s, 3H), 3.85/4.48 (q/q,  $J=7.5$  Hz, 1H), 4.10-4.14 (m, 1H), 4.60-4.64/4.65-4.69 (m/m, 1H), 4.88-4.92/4.98-5.02 (m/m, 1H), 7.24-7.40 (m, 5H).

Example 1D. 2-(4(S)-Phenyloxazolidin-2-on-3-yl)propanoic acid. To a solution of methyl 2-(4(S)-phenyloxazolidin-2-on-3-yl)propanoate (1 g, 4.01 mmol) in 35 mL of MeOH was added, at 0°C, 14.3 mL (12.04 mmol) of a 0.84 M solution of LiOH in water. The reaction mixture was then stirred for 3 h. at ambient temperature. Upon complete hydrolysis of the azetidinone, the MeOH was removed by evaporation, the crude residue dissolved in  $\text{CH}_2\text{Cl}_2$  and treated with saturated aqueous NaCl. The resulting organic layer was dried ( $\text{MgSO}_4$ ) and evaporated to afford the title compound (racemic mixture) as a white solid (0.906g, 96%);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.13/1.57 (d/d,  $J=7.5$  Hz, 3H), 3.75/4.50 (q/q,  $J=7.5$  Hz, 1H), 4.10-4.16 (m, 1H), 4.62-4.72 (m, 1H), 4.92-5.03 (m, 1H), 7.32-7.43 (m, 5H).

Example 1E. 2-(4(S)-Phenyloxazolidin-2-on-3-yl)propanoyl chloride. A solution of 1 equivalent of Example 1D and 1.3 equivalent of oxalyl chloride in 200 mL  $\text{CH}_2\text{Cl}_2$  (150 mL / g of propanoic acid derivative) was treated with a catalytic amount of anhydrous DMF (85  $\mu\text{L}$  / mmole of propanoic acid derivative) resulting in vigorous gas evolution. After 45 min., all gas evolution had ceased and the reaction mixture was concentrated under reduced pressure to provide the title compound as an off-white solid after drying for 2 h. under vacuum.

Example 2. General procedure for amide formation from an activated ester derivative. N-Benzylloxycarbonyl-L-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide. A solution of N-benzylloxycarbonyl-L-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -N-hydroxysuccinimide ester (1.95 g, 4.64 mmol, Advanced ChemTech) in 20 mL of dry tetrahydrofuran was treated with 0.68 mL (4.74 mmol) of 3-(trifluoromethyl)benzyl amine. Upon completion (TLC, 60:40 hexanes/ethyl acetate), the mixture was evaporated, and the resulting oil was partitioned between dichloromethane and a saturated aqueous solution of sodium bicarbonate. The organic layer was evaporated to give 2.23 g (quantitative yield) of the title compound as a white solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.39 (s, 9H), 2.61 (dd,  $J=6.5$  Hz,  $J=17.2$  Hz, 1H), 2.98 (dd,  $J=3.7$  Hz,  $J=17.0$  Hz, 1H), 4.41 (dd,  $J=5.9$  Hz,  $J=15.3$  Hz, 1H), 4.50-4.57 (m, 2H), 5.15 (s, 2H), 5.96-5.99 (m, 1H), 6.95 (s, 1H), 7.29-7.34 (m, 5H), 7.39-7.43 (m, 2H), 7.48-7.52 (m, 2H).

Examples 2A-2C and 3-5 were prepared according to the procedure of Example 2, except that N-benzylloxycarbonyl-L-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -N-hydroxysuccinimide

ester was replaced by the appropriate amino acid derivative, and 3-(trifluoromethyl)benzyl amine was replaced with the appropriate amine.

Example 2A. N-Benzylloxycarbonyl-L-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -[4-(2-phenylethyl)]piperazinamide. N-benzylloxycarbonyl-L-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -N-hydroxysuccinimide ester (5.0 g, 12 mmol, Advanced ChemTech) and 5 4-(phenylethyl)piperazine 2.27 mL (11.9 mmol) gave 5.89 g (quantitative yield) of the title compound as an off-white oil;  $^1$ H NMR (CDCl<sub>3</sub>)  $\delta$  1.40 (s, 9H), 2.45-2.80 (m, 10H), 3.50-3.80 (m, 4H), 4.87-4.91 (m, 1H), 5.08 (s, 2H), 5.62-5.66 (m, 1H), 7.17-7.33 (m, 10H).

Example 2B. N-Benzylloxycarbonyl-L-glutamic acid  $\gamma$ -*t*-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide. N-benzylloxycarbonyl-L-glutamic acid  $\beta$ -*t*-butyl ester  $\alpha$ -N-hydroxysuccinimide ester (4.83 g, 11.1 mmol, Advanced ChemTech) and 3-10 (trifluoromethyl)benzylamine 1.63 mL (11.4 mmol) gave 5.41 g (98%) of the title compound as an off-white solid;  $^1$ H NMR (CDCl<sub>3</sub>)  $\delta$  1.40 (s, 9H), 1.88-1.99 (m, 1H), 2.03-2.13 (m, 1H), 2.23-2.33 (m, 1H), 2.38-2.47 (m, 1H), 4.19-4.25 (s, 1H), 4.46-4.48 (m, 2H), 5.05-5.08 (m, 2H), 15 5.67-5.72 (m, 1H), 7.27-7.34 (m, 5H), 7.39-7.43 (m, 2H), 7.48-7.52 (m, 2H).

Example 2C. N-Benzylloxycarbonyl-L-glutamic acid  $\gamma$ -*t*-butyl ester  $\alpha$ -[4-(2-phenylethyl)]piperazinamide. N-benzylloxycarbonyl-L-glutamic acid  $\gamma$ -*t*-butyl ester  $\alpha$ -N-hydroxysuccinimide ester (5.0 g, 12 mmol, Advanced ChemTech) and 4-20 (phenylethyl)piperazine 2.19 mL (11.5 mmol) gave 5.87 g (quantitative yield) of the title compound as an off-white oil;  $^1$ H NMR (CDCl<sub>3</sub>)  $\delta$  1.43 (s, 9H); 1.64-1.73 (m, 1H); 1.93-2.01 (m, 1H); 2.23-2.40 (m, 2H); 2.42-2.68 (m, 6H); 2.75-2.85 (m, 2H); 3.61-3.74 (m, 4H); 4.66-4.73 (m, 1H); 5.03-5.12 (m, 2H); 5.69-5.72 (m, 1H); 7.16-7.34 (m, 10H).

Example 3. N-Benzylloxycarbonyl-L-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -[4-(2-phenylethyl)]piperazinamide. N-benzylloxycarbonyl-L-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -N-hydroxysuccinimide ester (5.0 g, 12 mmol, Advanced ChemTech) and 25 4-(phenylethyl)piperazine 2.27 mL (11.9 mmol) gave 5.89 g (quantitative yield) of the title compound as an off-white oil;  $^1$ H NMR (CDCl<sub>3</sub>)  $\delta$  1.40 (s, 9H), 2.45-2.80 (m, 10H), 3.50-3.80 (m, 4H), 4.87-4.91 (m, 1H), 5.08 (s, 2H), 5.62-5.66 (m, 1H), 7.17-7.33 (m, 10H).

Example 4. N-Benzylloxycarbonyl-L-glutamic acid  $\gamma$ -*t*-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide. N-benzylloxycarbonyl-L-glutamic acid  $\beta$ -*t*-butyl ester  $\alpha$ -N-hydroxysuccinimide ester (4.83 g, 11.1 mmol, Advanced ChemTech) and 30 3-(trifluoromethyl)benzylamine 1.63 mL (11.4 mmol) gave 5.41 g (98%) of the title compound as an off-white solid;  $^1$ H NMR (CDCl<sub>3</sub>)  $\delta$  1.40 (s, 9H), 1.88-1.99 (m, 1H), 2.03-2.13 (m, 1H), 2.23-2.33 (m, 1H), 2.38-2.47 (m, 1H), 4.19-4.25 (s, 1H), 4.46-4.48 (m, 2H), 5.05-5.08 (m, 2H),

Example 5. N-Benzylloxycarbonyl-L-glutamic acid  $\gamma$ -t-butyl ester  $\alpha$ -[4-(2-phenylethyl)]piperazinamide. N-benzylloxycarbonyl-L-glutamic acid  $\gamma$ -t-butyl ester  $\alpha$ -N-hydroxysuccinimide ester (5.0 g, 12 mmol, Advanced ChemTech) and 4-(phenylethyl)piperazine 2.19 mL (11.5 mmol) gave 5.87 g (quantitative yield) of the title compound as an off-white oil;  $^1$ H NMR (CDCl<sub>3</sub>)  $\delta$  1.43 (s, 9H); 1.64-1.73 (m, 1H); 1.93-2.01 (m, 1H); 2.23-2.40 (m, 2H); 2.42-2.68 (m, 6H); 2.75-2.85 (m, 2H); 3.61-3.74 (m, 4H); 4.66-4.73 (m, 1H); 5.03-5.12 (m, 2H); 5.69-5.72 (m, 1H); 7.16-7.34 (m, 10H).

Example 5A. N-[(9H-Fluoren-9-yl)methoxycarbonyl]-O-(benzyl)-D-serine *t*-Butyl ester. N-[(9H-Fluoren-9-yl)methoxycarbonyl]-O-(benzyl)-D-serine (0.710 g, 1.70 mmole) in dichloromethane (8 mL) was treated with *t*-butyl acetate (3 mL) and concentrated sulfuric acid (40  $\mu$ L) in a sealed flask at 0 °C. Upon completion (TLC), the reaction was quenched with dichloromethane (10 mL) and saturated aqueous potassium bicarbonate (15 mL). The organic layer was washed with distilled water, and evaporated. The resulting residue was purified by flash column chromatography (98:2 dichloromethane/methanol) to yield the title compound as a colorless oil (0.292 g, 77%);  $^1$ H NMR (CDCl<sub>3</sub>)  $\delta$  1.44 (s, 9H); 3.68 (dd, J=2.9 Hz, J=9.3 Hz, 1H); 3.87 (dd, J=2.9 Hz, J=9.3 Hz, 1H); 4.22 (t, J=7.1 Hz, 1H); 4.30-4.60 (m, 5H); 5.64-5.67 (m, 1H); 7.25-7.39 (m, 9H); 7.58-7.61 (m, 2H); 7.73-7.76 (m, 2H).

Example 5B. O-(Benzyl)-D-serine *t*-Butyl ester. Example 5A (0.620 g, 1.31 mmol) in dichloromethane (5 mL) was treated with tris(2-aminoethyl)amine (2.75 mL) for 5 h. The resulting mixture was washed twice with a phosphate buffer (pH=5.5), once with saturated aqueous potassium bicarbonate, and evaporated to give 0.329 g (quantitative yield) of the title compound as an off-white solid;  $^1$ H NMR (CD<sub>3</sub>OD)  $\delta$  1.44 (s, 9H); 3.48 (dd, J=J'=4.2 Hz, 1H); 3.61 (dd, J=4.0 Hz, J=9.2 Hz, 1H); 3.72 (dd, J=4.6 Hz, J=9.2 Hz, 1H); 4.47 (d, J=12.0 Hz, 1H); 4.55 (d, J=12.0 Hz, 1H); 7.26-7.33 (m, 5H).

Example 6. General procedure for amide formation from a carboxylic acid. N-Benzylloxycarbonyl-D-aspartic acid  $\beta$ -t-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide. A solution of 1 g (2.93 mmol) of N-benzylloxycarbonyl-D-aspartic acid  $\beta$ -t-butyl ester monohydrate (Novabiochem) in 3-4 mL of dichloromethane was treated by sequential addition of 0.46 mL (3.21 mmol) of 3-(trifluoromethyl)benzylamine, 0.44 g (3.23 mmol) of 1-hydroxy-7-benzotriazole, and 0.62 g (3.23 mmol) of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride. After at least 12 hours at ambient temperature or until complete as determined by thin layer chromatography (95:5 dichloromethane/methanol eluent), the reaction mixture was washed sequentially with a saturated aqueous sodium bicarbonate solution and with

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distilled water. The organic layer was evaporated to give 1.41 g (quantitative yield) of the title compound as an off-white solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.39 (s, 9H); 2.61 (dd,  $J=6.5$  Hz,  $J=17.2$  Hz, 1H); 2.98 (dd,  $J=4.2$  Hz,  $J=17.2$  Hz, 1H); 4.41 (dd,  $J=5.9$  Hz,  $J=15.3$  Hz, 1H); 4.50-4.57 (m, 2H); 5.10 (s, 2H); 5.96-6.01 (m, 1H); 6.91-7.00 (m, 1H); 7.30-7.36 (m, 5H); 7.39-7.43 (m, 2H); 7.48-7.52 (m, 2H).

Examples 7-7H were prepared according to the procedure of Example 6, except that N-benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate was replaced by the appropriate amino acid derivative, and 3-(trifluoromethyl)benzyl amine was replaced with the appropriate amine.

10 Example 7. N-Benzyloxycarbonyl-D-glutamic acid  $\gamma$ -*t*-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide. N-benzyloxycarbonyl-D-glutamic acid  $\gamma$ -*t*-butyl ester (1.14 g, 3.37 mmol) and 0.53 mL (3.70 mmol, Novabiochem) of 3-(trifluoromethyl)benzylamine gave 1.67 g (quantitative yield) of Example 7 as an off-white solid. Example 7 exhibited an  $^1\text{H}$  NMR spectrum consistent with the assigned structure.

15 Example 7A. N-Benzyloxycarbonyl-L-glutamic acid  $\alpha$ -*t*-butyl ester  $\gamma$ -(4-cyclohexyl)piperazinamide. N-benzyloxycarbonyl-L-glutamic acid  $\alpha$ -*t*-butyl ester (1.36 g, 4.03 mmol) and 0.746g (4.43 mmol) of 1-cyclohexylpiperazine gave 1.93 g (98%) of Example 7A as an off-white solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.02-1.12 (m, 5H); 1.43 (s, 9H), 1.60-1.64 (m, 1H); 1.80-1.93 (m, 5H); 2.18-2.52 (m, 8H); 3.38-3.60 (m, 4H); 4.20-4.24 (m, 1H); 5.03-5.13 (m, 2H); 5.53-5.57 (m, 1H); 7.28-7.34 (m, 5H).

20 Example 7B. N-Benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -(2-fluoro-3-trifluoromethyl)benzylamide. N-benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate (Novabiochem) (0.25 g, 0.73 mmol) and 0.12 mL of (2-fluoro-3-trifluoromethyl)benzylamine gave 0.365 g (quantitative yield) of Example 7B as an off-white solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.38 (s, 9H); 2.59 (dd,  $J=6.5$  Hz,  $J=17.0$  Hz, 1H); 2.95 (dd,  $J=4.3$  Hz,  $J=17.0$  Hz, 1H); 4.46-4.56 (m, 3H); 5.11 (s, 2H); 5.94-5.96 (m, 1H); 7.15 (t,  $J=8.0$  Hz, 1H); 7.30-7.36 (m, 5H); 7.47-7.52 (m, 2H).

25 Example 7C. N-Benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -[(S)- $\alpha$ -methylbenzyl]amide. N-benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate (Novabiochem) (0.25 g, 0.73 mmol) and 0.094 mL of (S)- $\alpha$ -methylbenzylamine gave 0.281 g (90%) of Example 7C as an off-white solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.41 (s, 9H); 1.44 (d,  $J=7.0$  Hz, 3H); 2.61 (dd,  $J=7.0$  Hz,  $J=17.0$  Hz, 1H); 2.93 (dd,  $J=4.0$  Hz,  $J=17.5$  Hz, 1H); 4.50-4.54 (m, 1H); 5.04-5.14 (m, 3H); 5.94-5.96 (m, 1H); 6.76-6.80 (m, 1H); 7.21-7.37 (m, 10H).

30 Example 7D. N-Benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -[(R)- $\alpha$ -

methylbenzyl]amide. N-benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate (Novabiochem) (0.25 g, 0.73 mmol) and 0.094 mL of (R)- $\alpha$ -methylbenzylamine gave 0.281 g (90%) of Example 7D as an off-white solid;  $^1$ H NMR (CDCl<sub>3</sub>)  $\delta$  1.38 (s, 9H); 1.43 (d, J=6.9 Hz, 3H); 2.54 (dd, J=7.3 Hz, J=17.2 Hz, 1H); 2.87 (dd, J=4.1 Hz, J=17.3 Hz, 1H); 4.46-4.50 (m, 1H); 4.99-5.15 (m, 3H); 5.92-5.96 (m, 1H); 6.78-6.82 (m, 1H); 7.21-7.33 (m, 10H).

5 Example 7E. N-Benzyloxycarbonyl-D-aspartic acid  $\gamma$ -*t*-butyl ester  $\alpha$ -[N-methyl-N-(3-trifluoromethylbenzyl)]amide. N-benzyloxycarbonyl-D-aspartic acid  $\gamma$ -*t*-butyl ester (0.303 g, 0.89 mmol, Novabiochem) and 0.168 g (0.89 mmol,) of N-methyl-N-(3-trifluoromethylbenzyl)amine gave 0.287 g (65%) of Example 7E as an off-white solid;  $^1$ H NMR (CDCl<sub>3</sub>)  $\delta$  1.40 (s, 9H); 2.55 (dd, J=5.8 Hz, J=15.8 Hz, 1H); 2.81 (dd, J=7.8 Hz, J=15.8 Hz, 1H); 3.10 (s, 3H); 4.25 (d, J=15.0 Hz, 1H); 4.80 (d, J=15.5 Hz, 1H); 5.01-5.13 (m, 3H); 5.52-5.55 (m, 1H); 7.25-7.52 (m, 10H).

10 Example 7F. N-Benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -[(S)-1-(3-trifluoromethylphenyl)ethyl]amide. N-benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate (Novabiochem) (84 mg, 0.25 mmol) and 47 mg of (S)-1-(3-trifluoromethylphenyl)ethylamine gave 122 mg (quantitative yield) of Example 7F as an off-white solid. Example 7F exhibited an  $^1$ H NMR spectrum consistent with the assigned structure.

15 Example 7G. N-Benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -[(R)-1-(3-trifluoromethylphenyl)ethyl]amide. N-benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate (Novabiochem) (150 mg, 0.44 mmol) and 83 mg of (R)-1-(3-trifluoromethylphenyl)ethylamine gave 217 mg (quantitative yield) of Example 7G as an off-white solid. Example 7G exhibited an  $^1$ H NMR spectrum consistent with the assigned structure.

20 Example 7H. N-Benzyloxycarbonyl-D-glutamic acid  $\alpha$ -methyl ester  $\gamma$ -(3-trifluoromethyl)benzylamide. N-benzyloxycarbonyl-D-glutamic acid  $\alpha$ -methyl ester (508 mg, 1.72 mmol) and 317 mg (1.81 mmol) of 3-(trifluoromethyl)benzylamine gave 662 mg (85%) of Example 7H as an off-white solid. Example 7H exhibited an  $^1$ H NMR spectrum consistent with the assigned structure.

25 Example 8. General procedure for hydrogenation of a benzyloxycarbonyl amine. L-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide. A suspension of 2.23 g (4.64 mmol) of N-benzyloxycarbonyl-L-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide and palladium (5% wt. on activated carbon, 0.642 g) in 30 mL of methanol was held under an atmosphere of hydrogen until complete conversion as determined by thin layer chromatography (95:5 dichloromethane/methanol eluent). The reaction was filtered to remove the palladium over carbon and the filtrate was evaporated to give 1.52 g

(96%) of the title compound as an oil;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.42 (s, 9H); 2.26 (brs, 2H); 2.63-2.71 (m, 1H); 2.82-2.87 (m, 1H); 3.75-3.77 (m, 1H); 4.47-4.50 (m, 2H); 7.41-7.52 (m, 4H); 7.90 (brs, 1H).

Examples 9-13P were prepared according to the procedure of Example 8, except that N-benzyloxycarbonyl-L-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide was replaced by the appropriate amino acid derivative.

Example 9. L-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -[4-(2-phenylethyl)]piperazinamide. N-benzyloxycarbonyl-L-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -[4-(2-phenylethyl)]piperazinamide (5.89 g, 11.9 mmol) gave 4.24 g (98%) of Example 9 as an off-white oil;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  1.42 (s, 9H); 2.61-2.95 (m, 10H); 3.60-3.90 (m, 4H); 4.35-4.45 (m, 1H); 7.17-7.29 (m, 5H).

Example 10. D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide. N-benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide (1.41 g, 2.93 mmol) gave 0.973 g (96%) of Example 10 as an off-white oil;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  1.42 (s, 9H); 2.21 (brs, 2H); 2.67 (dd,  $J=7.1$  Hz,  $J=16.8$  Hz, 1H); 2.84 (dd,  $J=3.6$  Hz,  $J=16.7$  Hz, 1H); 3.73-3.77 (m, 1H); 4.47-4.50 (m, 2H); 7.41-7.52 (m, 4H); 7.83-7.87 (m, 1H).

Example 11. L-glutamic acid  $\gamma$ -*t*-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide. N-benzyloxycarbonyl-L-glutamic acid  $\gamma$ -*t*-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide (5.41 g, 10.9 mmol) gave 3.94 g (quantitative yield) of Example 11 as an off-white oil;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  1.41 (s, 9H); 1.73-1.89 (m, 3H); 2.05-2.16 (m, 1H); 2.32-2.38 (m, 2H); 3.47 (dd,  $J=5.0$  Hz,  $J=7.5$  Hz, 1H); 4.47-4.49 (m, 2H); 7.36-7.54 (m, 4H); 7.69-7.77 (m, 1H).

Example 12. L-glutamic acid  $\gamma$ -*t*-butyl ester  $\alpha$ -[4-(2-phenylethyl)]piperazinamide. N-benzyloxycarbonyl-L-glutamic acid  $\gamma$ -*t*-butyl ester  $\alpha$ -[4-(2-phenylethyl)]piperazinamide (5.86 g, 11.50 mmol) gave 4.28 g (99%) of Example 12 as an off-white oil;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.39 (s, 9H); 2.00-2.08 (m, 1H); 2.38-2.46 (m, 1H); 2.55-2.90 (m, 9H); 3.61-3.82 (m, 4H); 4.48-4.56 (m, 1H); 7.17-7.26 (m, 5H).

Example 13. D-glutamic acid  $\gamma$ -*t*-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide. N-benzyloxycarbonyl-D-glutamic acid  $\gamma$ -*t*-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide (1.667 g, 3.37 mmol) gave 1.15 g (94%) of Example 13 as an off-white oil;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.41 (s, 9H); 1.80-2.20 (m, 4H); 2.31-2.40 (m, 2H); 3.51-3.59 (m, 1H); 4.47-4.49 (m, 2H); 7.39-7.52 (m, 4H); 7.71-7.79 (m, 1H).

Example 13A. L-glutamic acid  $\alpha$ -*t*-butyl ester  $\gamma$ -(4-cyclohexyl)piperazinamide. N-Benzyloxycarbonyl-L-glutamic acid  $\alpha$ -*t*-butyl ester  $\gamma$ -(4-cyclohexyl)piperazinamide (1.93 g, 3.96 mmol) gave 1.30 g (93%) of Example 13A as an off-white oil;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.02-

Example 13B. D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -(2-fluoro-3-trifluoromethyl)benzylamide. N-benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -(2-fluoro-3-trifluoromethyl)benzylamide (0.36 g, 0.72 mmol) gave 0.256 g (92%) of Example 13B as an off-white oil;  $^1$ H NMR (CDCl<sub>3</sub>)  $\delta$  1.39 (s, 9H); 2.50 (brs, 2H); 2.74 (dd, J=7.0 Hz, J=16.5 Hz, 1H); 2.86 (dd, J=4.8 Hz, J=16.8 Hz, 1H); 3.89 (brs, 2H); 4.47-4.57 (m, 2H); 7.16 (t, J=7.8 Hz, 1H); 7.48 (t, J=7.3 Hz, 1H); 7.56 (t, J=7.3 Hz, 1H); 7.97-8.02 (m, 1H).

Example 13C. D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -[(S)- $\alpha$ -methyl]benzylamide. N-benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -[(S)- $\alpha$ -methylbenzyl]amide (0.275 g, 0.65 mmol) gave 0.17 g (90%) of Example 13C as an off-white oil;  $^1$ H NMR (CDCl<sub>3</sub>)  $\delta$  1.40 (s, 9H); 1.47 (d, J=6.9 Hz, 3H); 1.98 (brs, 2H); 2.49 (dd, J=7.9 Hz, J=17.7 Hz, 1H); 2.83 (dd, J=3.6 Hz, J=16.7 Hz, 1H); 3.69 (brs, 1H); 4.99-5.10 (m, 1H); 7.19-7.33 (m, 5H); 7.65-7.68 (m, 1H).

Example 13D. D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -[(R)- $\alpha$ -methylbenzyl]amide. N-benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -[(R)- $\alpha$ -methylbenzyl]amide (0.273 g, 0.64 mmol) gave 0.187 g (quantitative yield) of Example 13D as an off-white oil;  $^1$ H NMR (CDCl<sub>3</sub>)  $\delta$  1.38 (s, 9H); 1.46 (d, J=6.9 Hz, 3H); 1.79 (brs, 2H); 2.51 (dd, J=7.8 Hz, J=17.5 Hz, 1H); 2.87 (dd, J=3.6 Hz, J=16.9 Hz, 1H); 4.19 (brs, 1H); 4.99-5.11 (m, 1H); 7.18-7.34 (m, 5H); 7.86-7.90 (m, 1H).

Example 13E. D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -[N-methyl-N-(3-trifluoromethylbenzyl)]amide. N-benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -[N-methyl-N-(3-trifluoromethylbenzyl)]amide (0.282 g, 0.57 mmol) gave 0.195 g (95%) of Example 13E as an off-white oil. Example 13E exhibited an  $^1$ H NMR spectrum consistent with the assigned structure.

Example 13F. L-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -[4-(2-phenylethyl)]piperazinamide. N-benzyloxycarbonyl-L-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -[4-(2-phenylethyl)]piperazinamide (5.89 g, 11.9 mmol) gave 4.24 g (98%) of Example 13F as an off-white oil;  $^1$ H NMR (CDCl<sub>3</sub>):  $\delta$  1.42 (s, 9H); 2.61-2.95 (m, 10H); 3.60-3.90 (m, 4H); 4.35-4.45 (m, 1H); 7.17-7.29 (m, 5H).

Example 13G. D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide. N-benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide (1.41 g, 2.93 mmol) gave 0.973 g (96%) of Example 13G as an off-white oil;  $^1$ H NMR (CDCl<sub>3</sub>):  $\delta$  1.42 (s, 9H); 2.21 (brs, 2H); 2.67 (dd, J=7.1 Hz, J=16.8 Hz, 1H); 2.84 (dd, J=3.6 Hz, J=16.7 Hz, 1H); 3.73-3.77 (m, 1H); 4.47-4.50 (m, 2H); 7.41-7.52 (m, 4H); 7.83-7.87 (m, 1H).

Example 13H. L-glutamic acid  $\gamma$ -*t*-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide. N-benzyloxycarbonyl-L-glutamic acid  $\gamma$ -*t*-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide (5.41 g, 10.9 mmol) gave 3.94 g (quantitative yield) of Example 13H as an off-white oil;  $^1$ H NMR (CDCl<sub>3</sub>)  $\delta$  1.41 (s, 9H); 1.73-1.89 (m, 3H); 2.05-2.16 (m, 1H); 2.32-2.38 (m, 2H); 3.47 (dd, J=5.0 Hz, J=7.5 Hz, 1H); 4.47-4.49 (m, 2H); 7.36-7.54 (m, 4H); 7.69-7.77 (m, 1H).

Example 13I. L-glutamic acid  $\gamma$ -*t*-butyl ester  $\alpha$ -[4-(2-phenylethyl)piperazinamide. N-benzyloxycarbonyl-L-glutamic acid  $\gamma$ -*t*-butyl ester  $\alpha$ -[4-(2-phenylethyl)piperazinamide (5.86 g, 11.50 mmol) gave 4.28 g (99%) of Example 13I as an off-white oil;  $^1$ H NMR (CDCl<sub>3</sub>)  $\delta$  1.39 (s, 9H); 2.00-2.08 (m, 1H); 2.38-2.46 (m, 1H); 2.55-2.90 (m, 9H); 3.61-3.82 (m, 4H); 4.48-4.56 (m, 1H); 7.17-7.26 (m, 5H).

Example 13J. D-glutamic acid  $\gamma$ -*t*-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide. N-benzyloxycarbonyl-D-glutamic acid  $\gamma$ -*t*-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide (1.667 g, 3.37 mmol) gave 1.15 g (94%) of Example 13J as an off-white oil;  $^1$ H NMR (CDCl<sub>3</sub>)  $\delta$  1.41 (s, 9H); 1.80-2.20 (m, 4H); 2.31-2.40 (m, 2H); 3.51-3.59 (m, 1H); 4.47-4.49 (m, 2H); 7.39-7.52 (m, 4H); 7.71-7.79 (m, 1H).

Example 13K. L-glutamic acid  $\alpha$ -*t*-butyl ester  $\gamma$ -(4-cyclohexyl)piperazinamide. N-Benzyloxycarbonyl-L-glutamic acid  $\alpha$ -*t*-butyl ester  $\gamma$ -(4-cyclohexyl)piperazinamide (1.93 g, 3.96 mmol) gave 1.30 g (93%) of Example 13K as an off-white oil;  $^1$ H NMR (CDCl<sub>3</sub>)  $\delta$  1.02-1.25 (m, 5H); 1.41 (s, 9H); 1.45-1.50 (m, 1H); 1.56-1.60 (m, 1H); 1.69-1.80 (m, 6H); 3.30 (dd, J=4.8 Hz, J=8.5 Hz, 1H); 3.44 (t, J=9.9 Hz, 2H); 3.56 (t, J=9.9 Hz, 2H).

Example 13L. D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -(2-fluoro-3-trifluoromethyl)benzylamide. N-benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -(2-fluoro-3-trifluoromethyl)benzylamide (0.36 g, 0.72 mmol) gave 0.256 g (92%) of Example 13L as an off-white oil;  $^1$ H NMR (CDCl<sub>3</sub>)  $\delta$  1.39 (s, 9H); 2.50 (brs, 2H); 2.74 (dd, J=7.0 Hz, J=16.5 Hz, 1H); 2.86 (dd, J=4.8 Hz, J=16.8 Hz, 1H); 3.89 (brs, 2H); 4.47-4.57 (m, 2H); 7.16 (t, J=7.8 Hz, 1H); 7.48 (t, J=7.3 Hz, 1H); 7.56 (t, J=7.3 Hz, 1H); 7.97-8.02 (m, 1H).

Example 13M. D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -[(S)-1-(3-trifluoromethylphenyl)ethyl]amide. N-benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -[(S)-1-(3-trifluoromethylphenyl)ethyl]amide (120 mg, 0.24 mmol) gave 91 mg (91%) of Example 13M as an off-white oil, and exhibited an  $^1$ H NMR spectrum consistent with the assigned structure.

Example 13N. D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -[(R)-1-(3-trifluoromethylphenyl)ethyl]amide. N-benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -

[(R)-1-(3-trifluoromethylphenyl)ethyl]amide (217 mg, 0.44 mmol) gave 158 mg (quantitative yield) of Example 13N as an off-white oil, and exhibited an <sup>1</sup>H NMR spectrum consistent with the assigned structure.

Example 13O. D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -[N-methyl-N-(3-trifluoromethylbenzyl)]amide. N-benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -[N-methyl-N-(3-trifluoromethylbenzyl)]amide (0.282 g, 0.57 mmol) gave 0.195 g (95%) of Example 13O as an off-white oil, and exhibited an <sup>1</sup>H NMR spectrum consistent with the assigned structure.

Example 13P. D-glutamic acid  $\alpha$ -methyl ester  $\gamma$ -(3-trifluoromethyl)benzylamide. N-Benzyloxycarbonyl-D-glutamic acid  $\alpha$ -methyl ester  $\gamma$ -(3-trifluoromethyl)benzylamide (764 mg, 1.69 mmol) gave g (516mg, 96%) of Example 13P as an off-white oil, and exhibited an <sup>1</sup>H NMR spectrum consistent with the assigned structure.

Example 14. General procedure for formation of a 2-azetidinone from an imine and an acetyl chloride.

Step 1: General procedure for formation of an imine from an amino acid derivative. A solution of 1 equivalent of an  $\alpha$ -amino acid ester or amide in dichloromethane is treated sequentially with 1 equivalent of an appropriate aldehyde, and a dessicating agent, such as magnesium sulfate or silica gel, in the amount of about 2 grams of dessicating agent per gram of starting  $\alpha$ -amino acid ester or amide. The reaction is stirred at ambient temperature until all of the reactants are consumed as measured by thin layer chromatography. The reactions are typically complete within an hour. The reaction mixture is then filtered, the filter cake is washed with dichloromethane, and the filtrate concentrated under reduced pressure to provide the desired imine that is used as is in the subsequent step.

Step 2: General procedure for the 2+2 cycloaddition of an imine and an acetyl chloride. A dichloromethane solution of the imine (10 mL dichloromethane/1 gram imine) is cooled to 0 °C. To this cooled solution is added 1.5 equivalents of an appropriate amine, typically triethylamine, followed by the dropwise addition of a dichloromethane solution of 1.1 equivalents of an appropriate acetyl chloride, such as that described in Example 1 (10 mL dichloromethane/1 gm appropriate acetyl chloride). The reaction mixture is allowed to warm to ambient temperature over 1 h and is then quenched by the addition of a saturated aqueous solution of ammonium chloride. The resulting mixture is partitioned between water and dichloromethane. The layers are separated and the organic layer is washed successively with 1N hydrochloric acid, saturated aqueous sodium bicarbonate, and saturated aqueous sodium chloride. The organic layer is dried over magnesium sulfate and concentrated under reduced

pressure. The residue may be used directly for further reactions, or purified by chromatography or by crystallization from an appropriate solvent system if desired. In each case, following the 2+2 reaction, the stereochemistry of the  $\beta$ -lactam may be confirmed by circular dichroism/optical rotary dispersion (CD/ORD). Illustratively, examples of the ( $\alpha R,3S,4R$ ) and ( $\alpha S,3S,4R$ )  $\beta$ -lactam platform stereochemical configurations from prior syntheses may be used as CD/ORD standards.

5 Example 15. *tert*-Butyl [3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetate. Using the procedure of Example 14, the imine prepared from 4.53 g (34.5 mmol) glycine *tert*-butyl ester and cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (Example 1) to give 5.5 g (30%) of Example 15 as colorless crystals (recrystallized, *n*-chlorobutane); mp 194-195 °C.

10 Example 16. General procedure for acylation of an azetidin-2-on-1-ylacetate. A solution of (azetidin-2-on-1-yl)acetate in tetrahydrofuran (0.22 M in azetidinone) is cooled to -78 °C and is with lithium bis(trimethylsilyl)amide (2.2 equivalents). The resulting anion is 15 treated with an appropriate acyl halide (1.1 equivalents). Upon complete conversion of the azetidinone, the reaction is quenched with saturated aqueous ammonium chloride and partitioned between ethyl acetate and water. The organic phase is washed sequentially with 1N hydrochloric acid, saturated aqueous sodium bicarbonate, and saturated aqueous sodium chloride. The resulting organic layer is dried (magnesium sulfate) and evaporated. The residue 20 is purified by silica gel chromatography with an appropriate eluent, such as 3:2 hexane/ethyl acetate.

Example 17. 2,2,2-Trichloroethyl 2(RS)-(tert-butoxycarbonyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetate.

25 Using the procedure of Example 16, 9.0 g (20 mmol) of Example 15 was acylated with 4.2 g (20 mmol) of trichloroethylchloroformate to give 7.0 g (56%) of Example 17; mp 176-178 °C.

30 Example 18. 2(RS)-(tert-Butoxycarbonyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide. A solution of 0.20 g (0.32 mmol) of Example 17 and 52  $\mu$ L (0.36 mmol) of (3-trifluoromethylbenzyl)amine in THF was heated at reflux. Upon complete conversion (TLC), the solvent was evaporated and the residue was recrystallized (chloroform/hexane) to give 0.17 g (82%) of Example 18 as a white solid; mp 182-184 °C.

35 Example 18A. 2(RS)-(tert-Butoxycarbonyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(2-fluoro-3-trifluoromethylbenzyl)amide. Example 18A was prepared according to the procedure of

Example 18, using 2-fluoro-3-(trifluoromethyl)benzylamine instead of (3-trifluoromethylbenzyl)amine. Example 18A was obtained as a white solid (140 mg, 41%), and exhibited an <sup>1</sup>H NMR spectrum consistent with the assigned structure.

Examples 19-25AF were prepared according to the procedure of Example 14, 5 where the appropriate amino acid derivative and aldehyde were used in Step 1, and the appropriate acetyl chloride was used in Step 2.

Example 19. 2(S)-(tert-Butoxycarbonylmethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide.

The imine prepared from 1.52 g (4.39 mmol) of L-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide and cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (Example 1) to give 2.94 g of an orange-brown oil that gave, after flash column chromatography purification (70:30 hexanes/ethyl acetate), 2.06 g (70%) of Example 19 as a white solid; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.39 (s, 9H); 2.46 (dd, J=11.1 Hz, J=16.3 Hz, 1H); 3.18 (dd, J=3.8 Hz, J=16.4 Hz, 1H); 4.12-4.17 (m, 1H); 4.26 (d, J=5.0 Hz, 1H); 15 4.45 (dd, J=6.0 Hz, J=14.9 Hz, 1H); 4.54 (dd, J=5.3 Hz, J=9.8 Hz, 1H); 4.58-4.66 (m, 3H); 4.69-4.75 (m, 1H); 4.81 (dd, J=3.8 Hz, J=11.1 Hz, 1H); 6.25 (dd, J=9.6 Hz, J=15.8 Hz, 1H); 6.70 (d, J=15.8 Hz, 1H); 7.14-7.17 (m, 2H); 7.28-7.46 (m, 11H); 7.62 (s, 1H); 8.27-8.32 (m, 1H).

Example 19A. 2(S)-(tert-Butoxycarbonylmethyl)-2-[3(R)-(4(R)-phenyloxazolidin-2-on-3-yl)-4(S)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide. Example 19A was prepared according to the method of Example 19 except that 2-(4(R)-phenyloxazolidin-2-on-3-yl) acetyl chloride (Example 1A) was used instead of 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride. Example 19A was obtained as a white solid (41 mg, 13%); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.37 (s, 9H); 3.11 (dd, J=3.7 Hz, J=17.8 Hz, 1H); 3.20 (dd, J=10.6 Hz, J=17.8 Hz, 1H); 4.02 (dd, J=3.7 Hz, J=10.6 Hz, 1H); 4.10-4.17 (m, 1H); 4.24 (d, J=4.9 Hz, 1H); 4.4652-4.574 (dd, J=5.9 Hz, J=15.1 Hz, 1H); 4.58-4.76 (m, 4H); 6.27 (dd, J=9.6 Hz, J=15.8 Hz, 1H); 6.79 (d, J=15.8 Hz, 1H); 7.23-7.53 (m, 13H); 7.63 (s, 1H); 8.51-8.55 (m, 1H).

Example 20. 2(S)-(tert-Butoxycarbonylethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide. The imine prepared from 3.94 g (10.93 mmol) of L-glutamic acid  $\gamma$ -*t*-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide and cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (Example 1) to give 5.53 g (75%) of Example 20 after flash column chromatography purification (70:30 hexanes/ethyl acetate); <sup>1</sup>H NMR

(CDCl<sub>3</sub>) δ 1.36 (s, 9H); 1.85-1.96 (m, 1H); 2.18-2.49 (m, 3H); 4.14-4.19 (m, 1H); 4.30 (d, J=4.9 Hz, 2H); 4.44 (dd, J=6.1 Hz, J=14.9 Hz, 1H); 4.56-4.67 (m, 4H); 4.71-4.75 (m, 1H); 6.26 (dd, J=9.6 Hz, J=15.8 Hz, 1H); 6.71 (d, J=15.8 Hz, 1H); 7.16-7.18 (m, 2H); 7.27-7.49 (m, 11H); 7.60 (s, 1H); 8.08-8.12 (m, 1H).

5 Example 21. 2(S)-(tert-Butoxycarbonylmethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[4-(2-phenylethyl)]piperazinamide.

The imine prepared from 4.20 g (11.6 mmol) of L-aspartic acid  $\beta$ -t-butyl ester  $\alpha$ -[4-(2-phenylethyl)]piperazinamide and cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (Example 1) to give 4.37 g (55%) of Example 21 after flash column chromatography purification (50:50 hexanes/ethyl acetate); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.34 (s, 9H); 2.26-2.32 (m, 1H); 2.46-2.63 (m, 4H); 2.75-2.89 (m, 4H); 3.24-3.32 (m, 1H); 3.49-3.76 (m, 3H); 4.07-4.13 (m, 1H); 4.30 (d, J=4.6 Hz, 1H); 4.22-4.48 (m, 1H); 4.55-4.61 (m, 1H); 4.69-4.75 (m, 1H); 5.04-5.09 (m, 1H); 6.15 (dd, J=9.3 Hz, J=15.9 Hz, 1H); 6.63 (d, J=15.8 Hz, 1H); 7.18-7.42 (m, 15H).

15 Example 22. 2(S)-(tert-Butoxycarbonyl ethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[4-(2-phenylethyl)]piperazinamide.

The imine prepared from 2.54 g (6.75 mmol) of L-glutamic acid  $\gamma$ -t-butyl ester  $\alpha$ -[4-(2-phenylethyl)]piperazinamide and cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (Example 1) to give 3.55 g (76%) of Example 22 after flash column chromatography purification (50:50 hexanes/ethyl acetate); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.32 (s, 9H); 1.96-2.07 (m, 1H); 2.15-2.44 (m, 6H); 2.54-2.62 (m, 2H); 2.69-2.81 (m, 3H); 3.28-3.34 (m, 1H); 3.59-3.68 (m, 1H); 4.08-4.13 (m, 1H); 4.33-4.44 (m, 2H); 4.48-4.60 (m, 2H); 4.67-4.77 (m, 1H); 6.14 (dd, J=8.9 Hz, J=16.0 Hz, 1H); 6.62 (d, J=16.0 Hz, 1H); 7.16-7.42 (m, 15 H).

25 Example 23. 2(R)-(tert-Butoxycarbonylmethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide. The imine prepared from 0.973 g (2.81 mmol) of D-aspartic acid  $\beta$ -t-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide and cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (Example 1) to give 1.53 g (82%) of

30 Example 23 after flash column chromatography purification (70:30 hexanes/ethyl acetate); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.37 (s, 9H); 3.10 (dd, J=3.7 Hz, J=17.8 Hz, 1H); 3.20 (dd, J=10.7 Hz, J=17.8 Hz, 1H); 4.02 (dd, J=3.6 Hz, J=10.6 Hz, 1H); 4.11-4.17 (m, 1H); 4.24 (d, J=4.9 Hz, 1H); 4.46 (dd, J=5.8 Hz, J=15.1 Hz, 1H); 4.58-4.67 (m, 3H); 4.70-4.76 (m, 1H); 6.27 (dd, J=9.5 Hz, J=15.8 Hz, 1H); 6.79 (d, J=15.8 Hz, 1H); 7.25-7.50 (m, 13H); 7.63 (s, 1H); 8.50-8.54 (m, 1H).

Example 23A. 2(R)-(tert-Butoxycarbonylmethyl)-2-[3(R)-(4(R)-phenyloxazolidin-2-on-3-yl)-4(S)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide. Example 23A was prepared according to the method of Example 23 except that 2-(4(R)-phenyloxazolidin-2-on-3-yl) acetyl chloride (Example 1A) was used instead of 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride. Example 23A was obtained as a white solid (588 mg, 49%);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.39 (s, 9H); 2.47 (dd,  $J=11.2$  Hz,  $J=16.3$  Hz, 1H); 3.18 (dd,  $J=3.8$  Hz,  $J=16.3$  Hz, 1H); 4.15 (t,  $J=8.25$  Hz, 1H); 4.26 (d,  $J=5.0$  Hz, 1H); 4.45 (dd,  $J=6.0$  Hz,  $J=15.0$  Hz, 1H); 4.52-4.57 (m, 3H); 4.63 (t,  $J=9$  Hz, 1H); 4.70 (t,  $J=8$  Hz, 1H); 4.81 (dd,  $J=3.8$  Hz,  $J=10.8$  Hz, 1H); 6.25 (dd,  $J=9.8$  Hz,  $J=15.8$  Hz, 1H); 6.70 (d,  $J=15.8$  Hz, 1H); 7.15-7.17 (m, 2H); 7.27-7.51 (m, 11H); 7.62 (s, 1H); 8.27-8.32 (m, 1H).

Example 24. 2(R)-(tert-Butoxycarbonyl ethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide.

The imine prepared from 1.15 g (3.20 mmol) of D-glutamic acid  $\gamma$ -*t*-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide and cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (Example 1) to give 1.84 g (85%) of Example 24 after flash column chromatography purification (70:30 hexanes/ethyl acetate);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.37 (s, 9H); 2.23-2.39 (m, 4H); 3.71-3.75 (m, 1H); 4.13-4.18 (m, 1H); 4.31 (d,  $J=4.9$  Hz, 1H); 4.44-4.51 (m, 2H); 4.56-4.68 (m, 2H); 4.71-4.76 (m, 1H); 6.26 (dd,  $J=9.5$  Hz,  $J=15.8$  Hz, 1H); 6.71 (d,  $J=15.8$  Hz, 1H); 7.25-7.52 (m, 13H); 7.63 (s, 1H); 8.25-8.30 (m, 1H).

Example 25. 2(S)-(tert-Butoxycarbonyl ethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(4-cyclohexyl)piperazinamide. The imine prepared from 2.58 g (5.94 mmol) of L-glutamic acid  $\gamma$ -*t*-butyl ester  $\alpha$ -(4-cyclohexyl)piperazinamide and cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (Example 1) to give 3.27 g (94%) of Example 25 after flash column chromatography purification (95:5 dichloromethane/methanol);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.32 (s, 9H); 1.10-1.18 (m, 1H); 1.20-1.31 (m, 2H); 1.38-1.45 (m, 2H); 1.61-1.66 (m, 1H); 1.84-1.89 (m, 2H); 1.95-2.01 (m, 1H); 2.04-2.14 (m, 3H); 2.20-2.24 (m, 1H); 2.29-2.35 (m, 1H); 2.85-2.92 (m, 1H); 3.24-3.32 (m, 1H); 3.36-3.45 (m, 2H); 3.80-3.86 (m, 1H); 4.08 (t,  $J=8.3$  Hz, 1H); 4.27 (d,  $J=5.0$  Hz, 1H); 4.31-4.55 (m, 4H); 4.71 (t,  $J=8.3$  Hz, 1H); 4.83-4.90 (m, 1H); 6.18 (dd,  $J=9.1$  Hz,  $J=15.9$  Hz, 1H); 6.67 (d,  $J=15.9$  Hz, 1H); 7.25-7.44 (m, 10H); 8.22 (brs, 1H).

Example 25A. *tert*-Butyl 2(S)-(2-(4-cyclohexyl)piperazinylcarbonyl)ethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetate. The imine prepared from 1.282 g (3.63 mmol) of L-glutamic acid  $\alpha$ -*t*-butyl ester  $\gamma$ -(4-cyclohexyl)piperazinamide and cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-

2-on-3-yl) acetyl chloride (Example 1) to give 1.946 g (80%) of Example 25A after flash column chromatography purification (50:50 hexanes/ethyl acetate);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.15-1.26 (m, 6H); 1.39 (s, 9H); 1.55-1.64 (m, 2H); 1.77-1.83 (m, 3H); 2.22-2.35 (m, 2H); 2.40-2.50 (m, 6H); 2.75-2.79 (m, 1H); 3.43-3.48 (m, 1H); 3.56-3.60 (m, 2H); 3.75-3.79 (m, 1H); 4.10 (t,  $J=8.3$  Hz, 1H); 4.31-4.35 (m, 2H); 4.58 (t,  $J=8.8$  Hz, 1H); 4.73 (t,  $J=8.4$  Hz, 1H); 6.17 (dd,  $J=8.6$  Hz,  $J=16.0$  Hz, 1H); 6.65 (d,  $J=16.0$  Hz, 1H); 7.27-7.42 (m, 10H).

Example 25B. 2(R)-(tert-Butoxycarbonylmethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(2-fluoro-3-trifluoromethylbenzyl)amide. The imine prepared from 0.256 g (0.70 mmol) of D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -(2-fluoro-3-trifluoromethyl)benzylamide and cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (Example 1) to give 0.287 g (60%) of Example 25B after flash column chromatography purification (70:30 hexanes/ethyl acetate);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.38 (s, 9H); 3.12 (dd,  $J=4.0$  Hz,  $J=17.8$  Hz, 1H); 3.20 (dd,  $J=10.4$  Hz,  $J=17.8$  Hz, 1H); 4.05 (dd,  $J=3.9$  Hz,  $J=10.4$  Hz, 1H); 4.14 (dd,  $J=J'=8.2$  Hz, 1H); 4.25 (d,  $J=4.9$  Hz, 1H); 4.59-4.67 (m, 4H); 4.74 (t,  $J=8.3$  Hz, 1H); 6.36 (dd,  $J=9.6$  Hz,  $J=15.8$  Hz, 1H); 6.83 (d,  $J=15.8$  Hz, 1H); 7.02-7.07 (m, 1H); 7.28-7.55 (m, 12H); 8.44-8.48 (m, 1H).

Example 25C. 2(R)-(tert-Butoxycarbonylmethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[(S)- $\alpha$ -methylbenzyl]amide. The imine prepared from 0.167 g (0.57 mmol) of D-aspartic acid  $\beta$ -*t*-butyl ester [(S)- $\alpha$ -methylbenzyl]amide and cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (Example 1) to give 0.219 g (63%) of Example 25C after flash column chromatography purification (70:30 hexanes/ethyl acetate);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.35 (s, 9H); 1.56 (d,  $J=7.0$  Hz, 3H); 2.97 (dd,  $J=3.5$  Hz,  $J=18.0$  Hz, 1H); 3.15 (dd,  $J=11.0$  Hz,  $J=17.5$  Hz, 1H); 4.01 (dd,  $J=3.0$  Hz,  $J=11.0$  Hz, 1H); 4.14 (t,  $J=8.5$  Hz, 1H); 4.24 (d,  $J=5.0$  Hz, 1H); 4.57 (dd,  $J=5.0$  Hz,  $J=9.5$  Hz, 1H); 4.64 (t,  $J=8.8$  Hz, 1H); 5.07 (t,  $J=8.5$  Hz, 1H); 5.03-5.09 (m, 1H); 6.43 (dd,  $J=9.5$  Hz,  $J=16.0$  Hz, 1H); 6.83 (d,  $J=16.0$  Hz, 1H); 7.16-7.20 (m, 1H); 7.27-7.49 (m, 14H); 8.07-8.10 (m, 1H).

Example 25D. 2(R)-(tert-Butoxycarbonylmethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[(R)- $\alpha$ -methylbenzyl]amide. The imine prepared from 0.187 g (0.46 mmol) of D-aspartic acid  $\beta$ -*t*-butyl ester [(R)- $\alpha$ -methylbenzyl]amide and cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (Example 1) to give 0.25 g (64%) of Example 25D after flash column chromatography purification (70:30 hexanes/ethyl acetate);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.36 (s, 9H); 1.59 (d,  $J=7.1$  Hz, 3H); 3.10 (dd,  $J=3.5$  Hz,  $J=17.8$  Hz, 1H); 3.22 (dd,

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J=10.9 Hz, J=17.8 Hz, 1H); 3.93 (dd, J=3.5 Hz, J=10.8 Hz, 1H); 4.14 (t, J=8.1 Hz, 1H); 4.24 (d, J=5.0 Hz, 1H); 4.58 (dd, J=5.0 Hz, J=9.5 Hz, 1H); 4.65 (t, J=8.7 Hz, 1H); 4.74 (t, J=8.2 Hz, 1H); 5.06-5.14 (m, 1H); 6.32 (dd, J=9.5 Hz, J=15.8 Hz, 1H); 6.74 (d, J=15.8 Hz, 1H); 7.19-7.43 (m, 15H); 8.15-8.18 (m, 1H).

5 Example 25E. 2(R)-(tert-Butoxycarbonylmethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-methyl-N-(3-trifluoromethylbenzyl)amide. The imine prepared from 0.195 g (0.41 mmol) of D-aspartic acid  $\beta$ -t-butyl ester  $\alpha$ -[N-methyl-N-(3-trifluoromethylbenzyl)]amide and cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (Example 1) to give 0.253 g 10 (69%) of Example 25E after flash column chromatography purification (70:30 hexanes/ethyl acetate);  $^1$ H NMR (CDCl<sub>3</sub>)  $\delta$  1.36 (s, 9H); 2.53 (dd, J=4.0 Hz, J=17.0 Hz, 1H); 3.06 (dd, J=10.8 Hz, J=16.8 Hz, 1H); 3.13 (s, 3H); 4.12 (dd, J=8.0 Hz, J=9.0 Hz, 1H); 4.26 (d, J=5.0 Hz, 1H); 4.38 (d, J=15.0 Hz, 1H); 4.46 (dd, J=5.0 Hz, J=9.5 Hz, 1H); 4.56 (t, J=6.8 Hz, 1H); 4.70-4.79 (m, 2H); 5.27 (dd, J=4.0 Hz, J=11.0 Hz, 1H); 6.22 (dd, J=9.3 Hz, J=15.8 Hz, 1H); 6.73 (d, J=15.8 Hz, 1H); 7.33-7.45 (m, 14H).

15 Example 25F. 2(S)-(tert-Butoxycarbonylethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-chlorostyryl-2-yl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide. The imine prepared from 1.62 g (4.44 mmol) of L-glutamic acid  $\gamma$ -t-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide and  $\alpha$ -chlorocinnamaldehyde was combined 20 with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (Example 1) to give 0.708 g (22%) of Example 25F after flash column chromatography purification (70:30 hexanes/ethyl acetate);  $^1$ H NMR (CDCl<sub>3</sub>)  $\delta$  1.35 (s, 9H); 1.68 (brs, 1H); 2.19-2.35 (m, 2H); 2.40-2.61 (m, 2H); 4.13 (dd, J=7.5 Hz, J=9.0 Hz, 1H); 4.22 (t, J=7.0 Hz, 1H); 4.34 (d, J=4.5 Hz, 1H); 4.45 (dd, J=5.5 Hz, J=15.0 Hz, 1H); 4.51-4.60 (m, 3H); 4.89 (dd, J=7.5 Hz, J=8.5 Hz, 1H); 6.89 (s, 1H); 7.28-7.54 (m, 14H).

25 Example 25G. 2(R)-(tert-Butoxycarbonylmethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2'-methoxystyryl-2-yl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide. The imine prepared from 0.34 g (0.98 mmol) of D-aspartic acid  $\beta$ -t-butyl ester  $\alpha$ -(3-trifluoromethylbenzyl)amide and 2'-methoxy cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (Example 1) to give 0.402 g 30 (59%) of Example 25G after flash column chromatography purification (70:30 hexanes/ethyl acetate);  $^1$ H NMR (CDCl<sub>3</sub>)  $\delta$  1.35 (s, 9H); 1.68 (brs, 1H); 2.19-2.35 (m, 2H); 2.40-2.61 (m, 2H); 4.13 (dd, J=7.5 Hz, J=9.0 Hz, 1H); 4.22 (t, J=7.0 Hz, 1H); 4.34 (d, J=4.5 Hz, 1H); 4.45 (dd, J=5.5 Hz, J=15.0 Hz, 1H); 4.51-4.60 (m, 3H); 4.89 (dd, J=7.5 Hz, J=8.5 Hz, 1H); 6.89 (s,

Example 25H. *tert*-Butyl (2R)-(Benzylloxymethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetate. The imine prepared from 0.329 g (1.31 mmol) of O-(benzyl)-D-serine *t*-butyl ester (Example 5B) and cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (Example 1) to give 0.543 g (73%) of Example 25H after flash column chromatography purification (90:10 hexanes/ethyl acetate); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.39 (s, 9H); 3.56 (dd, J=2.7 Hz, J=9.5 Hz, 1H); 3.82 (dd, J=4.8 Hz, J=9.5 Hz, 1H); 4.11 (t, J=8.3 Hz, 1H); 4.21-4.29 (m, 2H); 4.50-4.58 (m, 3H); 4.71-4.78 (m, 2H); 6.19 (dd, J=9.1 Hz, J=16.0 Hz, 1H); 6.49 (d, J=16.0 Hz, 1H); 7.07-7.11 (m, 1H); 7.19-7.40 (m, 14H).

Example 25I. *tert*-Butyl 2(S)-(2-(4-cyclohexylpiperazinylcarbonyl)methyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetate. The imine prepared from 0.3 g (0.88 mmol) of L-aspartic acid  $\alpha$ -*t*-butyl ester  $\gamma$ -(4-cyclohexyl)piperazinamide and cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (Example 1) to give 464 mg (80%) of Example 25I as a white solid after flash column chromatography purification (50:50 hexanes/ethyl acetate). Example 25I exhibited an <sup>1</sup>H NMR spectrum consistent with the assigned structure.

Example 25J. *tert*-Butyl 3(R)-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-3-methyl-4(R)-(styr-2-yl)azetidin-2-on-1-yl]-3-[3-(3-trifluoromethyl)phenylmethylaminocarbonyl]propanoate. The imine prepared from 0.307 g (0.89 mmol) of D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide (Example 20) and cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl)propanoyl chloride (Example 1E) to give 120 mg (20%) after flash column chromatography purification (hexanes 70% / EtOAc 30%); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.25 (s, 3H), 1.38 (s, 9H); 3.09 (dd, J=3.0 Hz, J=18.0 Hz, 1H); 3.33 (dd, J=12.5 Hz, J=18.0 Hz, 1H); 4.01 (dd, J=3.0 Hz, J=11.5 Hz, 1H); 4.04 (dd, J=3.5 Hz, J=8.8 Hz, 1H); 4.42 (d, J=9.0 Hz, 1H); 4.45-4.51 (m, 3H); 4.61-4.66 (m, 1H); 4.75 (dd, J=3.5 Hz, J=8.5 Hz, 1H); 6.23 (dd, J=9.0 Hz, J=15.5 Hz, 1H); 6.78 (d, J=15.5 Hz, 1H); 7.23-7.53 (m, 13H); 7.64 (s, 1H).

Example 25K. 2(R)-(tert-Butoxycarbonylmethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(prop-1-enyl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide. The imine prepared from 0.289 g (0.83 mmol) of D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide and crotonaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (Example 1) to give 381 mg (76%) of Example 25K after flash column chromatography purification (99:1 CH<sub>2</sub>Cl<sub>2</sub>/MeOH); <sup>1</sup>H NMR

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(CDCl<sub>3</sub>) δ 1.36 (s, 9H), 1.69 (dd, J=2 Hz, J=6.5 Hz, 3H); 3.08 (dd, J = 3.3 Hz, J = 17.8 Hz, 1H); 3.18 (dd, J = 11 Hz, J = 17.5 Hz, 1H); 3.94 (dd, J = 3.5 Hz, J = 11 Hz, 1H); 4.12 (d, J=5 Hz, 1H); 4.15 (dd, J = 7 Hz, J = 8 Hz, 1H); 4.35 (dd, J = 4.8 Hz, J=9.8Hz, 1H); 4.44 (dd, J=6 Hz, J=15 Hz, 1H); 4.61 (dd, J=6 Hz, J=15 Hz, 1H); 4.67-4.75 (m, 2H); 5.52-5.58 (m, 1H); 5.92-6.00 (m, 1H); 7.33-7.60 (m, 9H); 8.47-8.50 (m, 1H).

Example 25O. Methyl 2(S)-(tert-Butoxycarbonyl ethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetate. The imine prepared from 433 mg (1.99 mmol) of L-glutamic acid  $\gamma$ -*t*-butyl ester  $\alpha$ -methyl ester and cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (Example 1) to give 682 mg (64%) of Example 25O after flash column chromatography purification (70:30 hexanes/ethyl acetate); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.32 (s, 9H); 2.10-2.26 (m, 1H); 2.30-2.41 (m, 3H); 3.66 (s, 3H); 3.95-3.99 (m, 1H); 4.16 (dd, J=7.5 Hz, J=9 Hz, 1H); 4.38 (dd, J=5 Hz, J=9 Hz, 1H); 4.55 (d, J= 5 Hz 1H); 4.61 (t, J= 9 Hz, 1H); 4.86 (dd, J=7.5 Hz, J=9 Hz, 1H); 6.00 (dd, J=9 Hz, J=16 Hz, 1H); 6.60 (d, J=16 Hz, 1H); 7.26-7.43 (m, 10H).

Example 25M. *tert*-Butyl 2(S)-(methoxycarbonyl ethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetate. The imine prepared from 428 mg (1.97 mmol) of L-glutamic acid  $\gamma$ -*t*-butyl ester  $\alpha$ -methyl ester and cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (Example 1) to give 864 mg (82%) of Example 25M after flash column chromatography purification (70:30 hexanes/ethyl acetate); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.40 (s, 9H); 2.12-2.27 (m, 1H); 2.32-2.55 (m, 3H); 3.50 (s, 3H); 3.72 (dd, J=4.6 Hz, J=10.4 Hz, 1H); 4.12-4.17 (m, 1H); 4.34 (dd, J=5 Hz, J=9 Hz, 1H); 4.50 (d, J= 5 Hz, 1H); 4.60 (t, J= 8.9 Hz, 1H); 4.81-4.86 (m, 1H); 6.06 (dd, J=9 Hz, J=16 Hz, 1H); 6.59 (d, J=16 Hz, 1H); 7.25-7.42 (m, 10H).

Example 25P. Methyl 2(S)-(tert-Butoxycarbonylmethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetate. The imine prepared from 424 mg (2.09 mmol) of L-aspartic acid  $\gamma$ -*t*-butyl ester  $\alpha$ -methyl ester and cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (Example 1) to give 923 mg (85%) of Example 25P after after recrystallization from CH<sub>2</sub>Cl<sub>2</sub>/hexanes; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.41 (s, 9H); 2.77 (dd, J=7.5 Hz, J=16.5 Hz, 1H); 3.00 (dd, J=7 Hz, J=16.5 Hz, 1H); 4.16 (dd, J=7. 5Hz, J=9 Hz, 1H); 4.41-48 (m, 2H); 4.55 (d, J= 5 Hz, 1H); 4.60 (t, J= 8.8 Hz, 1H); 4.86 (dd, J=7.5 Hz, J=9 Hz, 1H); 5.93 (dd, J=9.5 Hz, J=15.5 Hz, 1H); 6.61 (d, J=15.5 Hz, 1H); 7.25-7.43 (m, 10H).

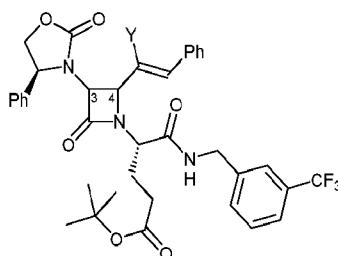
Example 25L. 2(R)-(tert-Butoxycarbonylmethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[(R)-1-(3-

trifluoromethylphenyl)ethyl]amide. The imine prepared from 160 mg (0.44 mmol) of D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -[(R)-1-(3-trifluoromethylphenyl)ethyl]amide and cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (Example 1) to give 166 mg (55%) of Example 25L after flash column chromatography purification (70:30 hexanes/EtOAc). Example 25L exhibited an  $^1$ H NMR spectrum consistent with the assigned structure.

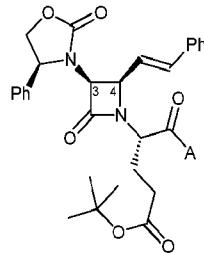
Example 25N. 2(R)-(tert-Butoxycarbonylmethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[(S)-1-(3-trifluoromethylphenyl)ethyl]amide. The imine prepared from 120 mg (0.22 mmol) of D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -[(S)-1-(3-trifluoromethylphenyl)ethyl]amide and cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (Example 1) to give 75 mg (50%) of Example 25N after flash column chromatography purification (70:30 hexanes/EtOAc). Example 25N exhibited an  $^1$ H NMR spectrum consistent with the assigned structure.

Example 25Q. Methyl 2(R)-(2-(3-trifluoromethylbenzyl)aminocarbonyl)ethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetate. The imine prepared from 517 mg (1.62 mmol) of D-glutamic acid  $\alpha$ -methyl ester  $\gamma$ -(3-trifluoromethyl)benzylamide and cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (Example 1) to give 527 mg (51%) of Example 25Q after flash column chromatography purification (50:50 hexanes/ EtOAc). Example 25Q exhibited an  $^1$ H NMR spectrum consistent with the assigned structure.

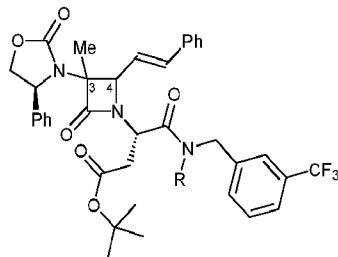
The following compounds were prepared according to the processes described herein:



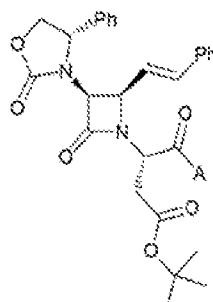
Example	Y	C(3)-C(4) Stereochemistry
25R	F	(3S,4R)
25S	F	not determined
25T	Br	not determined
25U	Br	not determined



Example	A
25V	(R)-1,2,3,4-tetrahydro-1-naphthylamide
25W	1-phenyl-cyclopentylamide

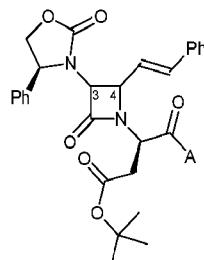


Example	C(3)-C(4) Stereochemistry	R
25X	(3S)-cis	Me
25Y	not determined	H



5

Example	A
25Z	1-phenyl-cyclopentylamino
25AA	(R)-1-phenylethyl-amino



Example	C(3)-C(4) Stereochemistry	A	A'
25AB	(3S,4R)	$\alpha,\alpha$ -dimethylbenzylamino	t-butyl ester
25AC	not determined	N-methyl-3-CF3-benzylamino	t-butyl ester
25AD	not determined	(R)- $\alpha$ -methylbenzylamino	t-butyl ester
25AE	(3S,4R)	(R)- $\alpha$ ,N-dimethylbenzylamino	t-butyl ester

Example 25AF. *t*-Butyl 2(S)-(2-(3-trifluoromethylbenzyl)aminocarbonyl)ethyl-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetate.

Example 26. General procedure for hydrolysis of a *tert*-butyl ester. A solution of *tert*-butyl ester derivative in formic acid, typically 1 g in 10 mL, is stirred at ambient 5 temperature until no more ester is detected by thin layer chromatography (dichloromethane 95% / methanol 5%), a typical reaction time being around 3 hours. The formic acid is evaporated under reduced pressure; the resulting solid residue is partitioned between dichloromethane and saturated aqueous sodium bicarbonate. The organic layer is evaporated to give an off-white solid that may be used directly for further reactions, or recrystallized from an 10 appropriate solvent system if desired.

Examples 27-34AE were prepared from the appropriate *tert*-butyl ester according to the procedure used in Example 26.

Example 27. 2(R,S)-(Carboxy)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide. Example 18 (0.30 15 g, 0.46 mmol) was hydrolyzed to give 0.27 g (quantitative yield) of Example 27 as an off-white solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  4.17-5.28 (m, 9H); 6.21-6.29 (m, 1H), 6.68-6.82 (m, 1H); 7.05-7.75 (m, 13H); 9.12-9.18 (m, 1H).

Example 28. 2(S)-(Carboxymethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide. Example 19 20 (1.72 g, 2.59 mmol) was hydrolyzed to give 1.57 g (quantitative yield) of Example 28 as an off-white solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  2.61 (dd,  $J=9.3$  Hz,  $J=16.6$  Hz, 1H); 3.09-3.14 (m, 1H); 4.10-4.13 (m, 1H); 4.30 (d,  $J=4.5$  Hz, 1H); 4.39-4.85 (m, 6H); 6.20 (dd,  $J=9.6$  Hz,  $J=15.7$  Hz, 1H); 6.69 (d,  $J=15.8$  Hz, 1H); 7.12-7.15 (m, 2H); 7.26-7.50 (m, 11H); 7.61 (s, 1H); 8.41-8.45 (m, 1H).

Example 28A. 2(S)-(Carboxymethyl)-2-[3(R)-(4(R)-phenyloxazolidin-2-on-3-yl)-4(S)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide. Example 19A (41 mg, 0.06 mmol) was hydrolyzed to give 38 mg (quantitative yield) of Example 28A as an off-white solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  2.26 (d,  $J=7$  Hz, 1H); 4.03 (t,  $J=7$  Hz, 1H); 4.16 (t,  $J=8$  Hz, 1H); 4.26 (d,  $J=4.3$  Hz, 1H); 4.46 (dd,  $J=5.7$  Hz,  $J=15.1$ , 1H); 4.53-4.75 (m, 5H); 6.25 (dd, 30  $J=9.5$  Hz,  $J=15.7$  Hz, 1H); 6.77 (d,  $J=15.7$  Hz, 1H); 7.28-7.53 (m, 13H); 7.64 (s, 1H); 8.65-8.69 (m, 1H).

Example 29. 2(S)-(Carboxyethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide. Example 20 (4.97 g, 7.34 mmol) was hydrolyzed to give 4.43 g (97%) of Example 29 as an off-white solid;

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.92-2.03 (m, 1H); 2.37-2.51 (m, 3H); 4.13-4.19 (m, 1H); 3.32 (d, J=4.9 Hz, 1H); 4.35-4.39 (m, 1H); 4.44 (dd, J=5.9 Hz, J=14.9 Hz, 1H); 4.50-4.57 (m, 2H); 4.61-4.67 (m, 1H); 4.70-4.76 (m, 1H); 6.24 (dd, J=9.6 Hz, J=15.8 Hz, 1H); 6.70 (d, J=15.8 Hz, 1H); 7.18-7.47 (m, 14H).

5 Example 30. 2(S)-(Carboxymethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[4-(2-phenylethyl)]piperazinamide. Example 21 (1.88 g, 2.78 mmol) was hydrolyzed to give 1.02 g (60%) of Example 30 as an off-white solid; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.63 (dd, J=6.0 Hz, J=16.5 Hz, 1H); 2.75-2.85 (m, 1H); 3.00 (dd, J=8.2 Hz, J=16.6 Hz, 1H); 3.13-3.26 (m, 4H); 3.37-3.56 (m, 4H); 3.86-4.00 (m, 1H); 4.05-4.11 (m, 1H); 4.24 (d, J=5.0 Hz, 1H); 4.46-4.66 (m, 1H); 4.65-4.70 (m, 1H); 5.10-5.15 (m, 1H); 6.14 (dd, J=9.3 Hz, J=15.9 Hz, 1H); 6.71 (d, J=15.9 Hz, 1H); 7.22-7.41 (m, 15H); 12.02 (s, 1H).

10 Example 31. 2(S)-(Carboxyethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[4-(2-phenylethyl)]piperazinamide. Example 22 (0.383 g, 0.55 mmol) was hydrolyzed to give 0.352 g (quantitative yield) of Example 31 as an off-white solid; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.93-2.01 (m, 1H); 2.07-2.36 (m, 6H); 2.82-2.90 (m, 1H); 3.00-3.20 (m, 4H); 3.36-3.54 (m, 4H); 3.74-3.82 (m, 1H); 4.06-4.11 (m, 1H); 4.29 (d, J=4.9 Hz, 1H); 4.33-4.46 (m, 2H); 4.50-4.58 (m, 2H); 4.67-4.72 (m, 1H); 4.95-5.00 (m, 1H); 6.18 (dd, J=9.2 Hz, J=16.0 Hz, 1H); 6.67 (d, J=15.9 Hz, 1H); 7.19-7.42 (m, 15H); 8.80 (brs, 1H).

15 Example 32. 2(R)-(Carboxymethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide. Example 23 (1.51 g, 2.27 mmol) was hydrolyzed to give 1.38 g (quantitative yield) of Example 32 as an off-white solid.

20 Example 32A. 2(R)-(Carboxymethyl)-2-[3(R)-(4(R)-phenyloxazolidin-2-on-3-yl)-4(S)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide. Example 23A (550 mg, 0.83 mmol) was hydrolyzed to give 479 mg (95%) of Example 32A as an off-white solid. Example 32A exhibited an <sup>1</sup>H NMR spectrum consistent with the assigned structure.

25 Example 33. 2(R)-(Carboxyethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide. Example 24 (0.604 g, 0.89 mmol) was hydrolyzed to give 0.554 g (quantitative yield) of Example 33 as an off-white solid.

30 Example 34. 2(S)-(Carboxyethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(4-cyclohexyl)piperazinamide. Example 25 (0.537 g, 0.80 mmol) was hydrolyzed to give 0.492 g (quantitative yield) of Example 34 as an

off-white solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.09-1.17 (m, 1H); 1.22-1.33 (m, 2H); 1.40-1.47 (m, 2H); 1.63-1.67 (m, 1H); 1.85-1.90 (m, 2H); 1.95-2.00 (m, 1H); 2.05-2.15 (m, 3H); 2.20-2.24 (m, 1H); 2.30-2.36 (m, 1H); 2.85-2.93 (m, 1H); 3.25-3.33 (m, 1H); 3.36-3.46 (m, 2H); 3.81-3.87 (m, 1H); 4.08 (t,  $J=8.3$  Hz, 1H); 4.28 (d,  $J=5.0$  Hz, 1H); 4.33-4.56 (m, 4H); 4.70 (t,  $J=8.3$  Hz, 1H); 4.83-4.91 (m, 1H); 6.17 (dd,  $J=9.1$  Hz,  $J=15.9$  Hz, 1H); 6.67 (d,  $J=15.9$  Hz, 1H); 7.25-7.44 (m, 10H); 8.22 (brs, 1H).

Example 34A. 2(S)-(2-(4-Cyclohexylpiperazinylcarbonyl)ethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid. Example 25A (0.787 g, 1.28 mmol) was hydrolyzed to give 0.665 g (92%) of Example 34A as an off-white solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.05-1.13 (m, 1H); 1.20-1.40 (m, 5H); 1.60-1.64 (m, 1H); 1.79-1.83 (m, 2H); 2.00-2.05 (m, 2H); 2.22-2.44 (m, 3H); 2.67-2.71 (m, 1H); 2.93-3.01 (m, 4H); 3.14-3.18 (m, 1H); 3.38-3.42 (m, 1H); 3.48-3.52 (m, 1H); 3.64-3.69 (m, 1H); 4.06-4.14 (m, 2H); 4.34-4.43 (m, 2H); 4.56 (t,  $J=8.8$  Hz, 1H); 4.73 (t,  $J=8.4$  Hz, 1H); 6.15 (dd,  $J=9.1$  Hz,  $J=16.0$  Hz, 1H); 6.65 (d,  $J=16.0$  Hz, 1H); 7.25-7.42 (m, 10H).

Example 34B. 2(R)-(Carboxymethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(2-fluoro-3-trifluoromethylbenzyl)carboxamide. Example 25B (0.26 g, 0.38 mmol) was hydrolyzed to give 0.238 g (quantitative yield) of Example 34B as an off-white solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  3.27 (d,  $J=7.2$  Hz, 1H); 4.06 (t,  $J=7.2$  Hz, 1H); 4.15 (t,  $J=8.1$  Hz, 1H); 4.27 (d,  $J=4.8$  Hz, 1H); 4.56-4.76 (m, 5H); 6.34 (dd,  $J=9.5$  Hz,  $J=15.7$  Hz, 1H); 6.80 (d,  $J=15.7$  Hz, 1H); 7.06 (t,  $J=7.7$  Hz, 1H); 7.31-7.54 (m, 12H); 8.58 (t,  $J=5.9$  Hz, 1H).

Example 34C. 2(R)-(Carboxymethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[(S)- $\alpha$ -methylbenzyl]amide. Example 25C (0.215 g, 0.35 mmol) was hydrolyzed to give 0.195 g (quantitative yield) of Example 34C as an off-white solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.56 (d,  $J=7.0$  Hz, 1H); 3.10 (dd,  $J=4.5$  Hz,  $J=17.9$  Hz, 1H); 3.18 (dd,  $J=9.8$  Hz,  $J=17.9$  Hz, 1H); 4.00 (dd,  $J=4.5$  Hz,  $J=9.7$  Hz, 1H); 4.14 (t,  $J=8.2$  Hz, 1H); 4.26 (d,  $J=4.7$  Hz, 1H); 5.02-5.09 (m, 1H); 6.41 (dd,  $J=9.4$  Hz,  $J=15.8$  Hz, 1H); 6.78 (d,  $J=15.8$  Hz, 1H); 7.18 (t,  $J=7.3$  Hz, 1H); 7.26-7.43 (m, 12H); 8.29 (d,  $J=8.2$  Hz, 1H).

Example 34D. 2(R)-(Carboxymethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[(R)- $\alpha$ -methylbenzyl]amide. Example 25D (0.22 g, 0.35 mmol) was hydrolyzed to give 0.20 g (quantitative yield) of Example 34D as an off-white solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.59 (d,  $J=7.0$  Hz, 1H); 3.25 (d,  $J=7.0$  Hz, 2H); 3.92 (t,  $J=7.3$  Hz, 1H); 4.15 (t,  $J=8.3$  Hz, 1H); 4.26 (d,  $J=5.0$  Hz, 1H); 4.52 (dd,  $J=4.8$  Hz,  $J=9.3$  Hz, 1H); 4.65 (t,  $J=8.8$  Hz, 1H); 4.72 (t,  $J=8.3$  Hz, 1H); 5.07-5.28 (m, 1H); 6.29 (dd,  $J=9.5$  Hz,

Example 34E. 2(R)-(Carboxymethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-methyl-N-(3-trifluoromethylbenzyl)amide.

Example 25E (0.253 g, 0.37 mmol) was hydrolyzed to give 0.232 g (quantitative yield) of

5 Example 34E as an off-white solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  3.07-3.15 (m, 4H); 4.13 (t,  $J$ =8.2 Hz, 1H); 4.30 (d,  $J$ =4.9 Hz, 1H); 4.46-4.78 (m, 5H); 5.23 (dd,  $J$ =4.6 Hz,  $J$ =9.7 Hz, 1H); 6.20 (dd,  $J$ =9.4 Hz,  $J$ =15.9 Hz, 1H); 6.73 (d,  $J$ =15.9 Hz, 1H); 7.25-7.43 (m, 15H).

Example 34F. 2(S)-(Carboxyethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-chlorostyryl-2-yl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide.

10 Example 25F (0.707 g, 0.99 mmol) was hydrolyzed to give 0.648 g (99%) of Example 34F as an off-white solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  2.22-2.28 (m, 2H); 2.49-2.64 (m, 2H); 4.09 (t,  $J$ =8.0 Hz, 1H); 4.25-4.62 (m, 6H); 4.87 (t,  $J$ =8.0 Hz, 1H); 6.88 (s, 1H); 7.25-7.66 (m, 15H).

Example 34G. 2(R)-(Carboxymethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2'-methoxystyryl-2-yl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide.

15 Example 25G (0.268 g, 0.39 mmol) was hydrolyzed to give 0.242 g (98%) of Example 34G as an off-white solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  3.26 (d,  $J$ =7.1 Hz, 1H); 3.79 (s, 3H); 4.14 (t,  $J$ =8.2 Hz, 1H); 4.25 (d,  $J$ =4.5 Hz, 1H); 4.51 (dd,  $J$ =5.9 Hz,  $J$ =15.5 Hz, 1H); 4.53-4.66 (m, 4H); 6.36 (dd,  $J$ =9.4 Hz,  $J$ =15.8 Hz, 1H); 8.88 (t,  $J$ =8.2 Hz, 1H); 6.70 (d,  $J$ =15.8 Hz, 1H); 7.18 (d,  $J$ =6.5 Hz, 1H); 7.25-7.48 (m, 10H); 7.48 (s, 1H); 8.66-8.69 (m, 1H).

20 Example 34H. (2R)-(Benzylloxymethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid. Example 25H (0.16 g, 0.28 mmol) was hydrolyzed to give 0.144 g (quantitative yield) of Example 34H as an off-white solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  3.65 (dd,  $J$ =4.0 Hz,  $J$ =9.5 Hz, 1H); 3.82 (dd,  $J$ =5.5 Hz,  $J$ =9.5 Hz, 1H); 4.11 (dd,  $J$ =7.8 Hz,  $J$ =8.8 Hz, 1H); 4.33 (s, 2H); 4.50 (d,  $J$ =5.0 Hz, 1H); 4.57 (t,  $J$ =9.0 Hz, 1H); 4.67 (dd,  $J$ =4.0 Hz,  $J$ =5.0 Hz, 1H); 4.69 (dd,  $J$ =5.0 Hz,  $J$ =9.5 Hz, 1H); 4.75 (t,  $J$ =8.0 Hz, 1H); 6.17 (dd,  $J$ =9.3 Hz,  $J$ =15.8 Hz, 1H); 6.55 (d,  $J$ =16.0 Hz, 1H); 7.09-7.12 (m, 2H); 7.19-7.42 (m, 13H).

Example 34I. 2(S)-(2-(4-Cyclohexylpiperazinylcarbonyl)methyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid. Example 25I (737 mg, 1.12 mmol) was hydrolyzed to give 640 mg (95%) of Example 34I as an off-white solid.

30 Example 34I exhibited an  $^1\text{H}$  NMR spectrum consistent with the assigned structure.

Example 34J. 3(R)-[3(S)-(4(S)-Phenyloxazolidin-2-on-3-yl)-3-methyl-4(R)-(styryl-2-yl)azetidin-2-on-1-yl]-3-[(3-trifluoromethyl)phenylmethylaminocarbonyl]propanoic acid. Using the general method of Example 26, 120 mg (0.18 mmol) of Example 25J was hydrolyzed to give 108 mg (98%) of Example 34J as an off-white solid;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$

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1.22 (s, 3H); 3.25 (dd, J=3.5 Hz, J=18.0 Hz, 1H); 3.36 (dd, J=10.8 Hz, J=18.2 Hz, 1H); 4.01 (dd, J=4.0 Hz, J=10.5 Hz, 1H); 4.05 (dd, J=3.8 Hz, J=8.8 Hz, 1H); 4.33 (d, J=9.0 Hz, 1H); 4.44-4.51 (m, 3H); 4.61-4.66 (m, 1H); 4.73 (dd, J=3.8 Hz, J=8.8 Hz, 1H); 6.19 (dd, J=9.0 Hz, J=16.0 Hz, 1H); 6.74 (d, J=16.0 Hz, 1H); 7.22-7.54 (m, 13H); 7.65 (s, 1H).

5 Example 34K. 2(R)-(Carboxymethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(propen-1-yl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide. Using the general method of Example 26, 160 mg (0.27 mmol) of Example 25K was hydrolyzed to give 131 mg (90%) of Example 34K as an off-white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.69 (dd, J=1 Hz, J=6.5 Hz, 3H); 3.23 (d, J = 7 Hz, 1H); 3.93 (t, J= 7.3Hz, 1H); 4.14-4.20 (m, 3H); 4.29 (dd, J = 5 Hz, J = 9.5 Hz, 1H); 4.43 (dd, J = 6 Hz, J = 15 Hz, 1H); 4.61 (dd, J=6.5 Hz, J=15 Hz, 1H); 4.66 -4.74 (m, 2H); 5.50-5.55 (m, 1H); 5.90-5.98 (m, 1H); 7.32-7.60 (m, 9H); 8.60-8.64 (m, 1H).

10 Example 34L. 2(R)-(Carboxymethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[(R)-1-(3-trifluoromethylphenyl)ethyl]amide. 15 Example 25L (166 mg, 0.24 mmol) was hydrolyzed to give 152 mg (quantitative yield) of Example 34L as an off-white solid; and exhibited an <sup>1</sup>H NMR spectrum consistent with the assigned structure.

20 Example 34M. 2(S)-(Methoxycarbonylethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid. Example 25M (875 mg, 1.64 mmol) was hydrolyzed to give 757 mg (97%) of Example 34M as an off-white solid, and exhibited an <sup>1</sup>H NMR spectrum consistent with the assigned structure.

25 Example 34N. 2(R)-(Carboxymethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[(S)-1-(3-trifluoromethylphenyl)ethyl]amide. Example 25N (38.5 mg, 0.057 mmol) was hydrolyzed to give 35 mg (quantitative yield) of Example 34N as an off-white solid, and exhibited an <sup>1</sup>H NMR spectrum consistent with the assigned structure.

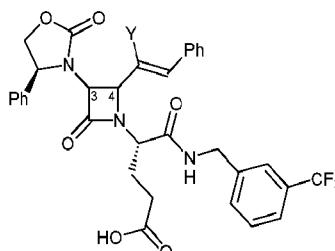
30 Example 34O. 2(S)-(tert-Butoxycarbonylethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid. Example 25O (97 mg, 0.18 mmol) was dissolved in methanol/tetrahydrofuran (2.5 mL/2 mL) and reacted with lithium hydroxide (0.85 mL of a 0.85M solution in water; 0.72 mmol) for 6 hours at room temperature. The reaction was diluted with 15 mL dichloromethane and aqueous hydrochloric acid (1M) was added until the pH of the aqueous layer reached 5 (as measured by standard pH paper). The organic layer was then separated and evaporated to dryness to give 84 mg (89%) of Example 34O as an off-white solid, and exhibited an <sup>1</sup>H NMR spectrum consistent with the assigned structure.

Example 34P. 2(S)-(tert-Butoxycarbonyl)ethyl-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid. Example 25P (200 mg, 0.39 mmol) was hydrolyzed according to the method used for Example 34O to give 155 mg (88%) of Example 34P as an off-white solid; and exhibited an <sup>1</sup>H NMR spectrum consistent with the assigned structure.

Example 34Q. 2(R)-(2-(3-trifluoromethylbenzyl)amino-1-ylcarbonyl)ethyl-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid. Example 25Q (150 mg, 0.24 mmol) was hydrolyzed according to the method used for Example 34O to give 143 mg (97%) of Example 34Q as an off-white solid, and exhibited an <sup>1</sup>H NMR spectrum consistent with the assigned structure.

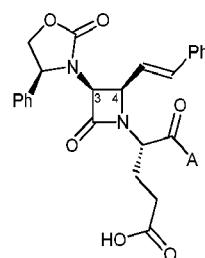
Example 34R. 2(R)-(tert-Butoxycarbonylmethyl)-2-[3(RS)-2-thienylmethyl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide. The imine prepared from 290 mg (0.84 mmol) of D-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide and cinnamaldehyde was combined with 2-thiophene-acetyl chloride to give 42 mg (8%) of Example 34R after flash column chromatography purification (70:30 hexanes/ethyl acetate), and exhibited an <sup>1</sup>H NMR spectrum consistent with the assigned structure.

The following compounds were prepared according to the processes described herein:

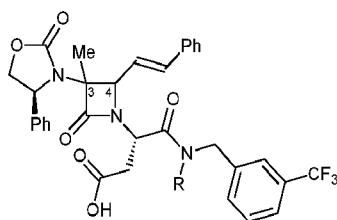


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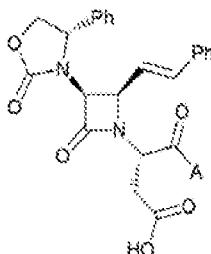
Example	Y	C(3)-C(4) Stereochemistry
34S	F	(3S,4R)
34T	F	not determined
34U	Br	not determined



Example	A
34V	(R)-1,2,3,4-tetrahydro-1-naphthylamide
34W	1-phenyl-cyclopentylamide

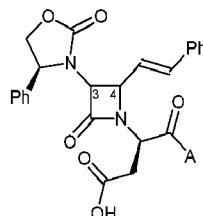


Example	C(3)-C(4) Stereochemistry	R
34X	(3S,4R)	Me
34Y	not determined	H



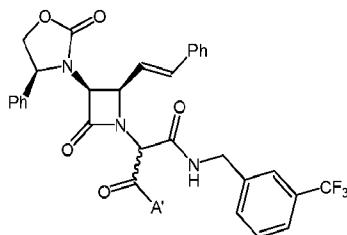
Example	A
34Z	1-phenyl-cyclopent-1-ylamino
34AA	(R)-1-phenylethyl-amino

5



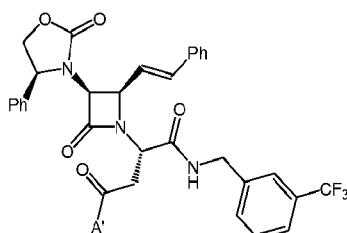
Example	C(3)-C(4) Stereochemistry	A
34AB	(3S,4R)	$\alpha,\alpha$ -dimethylbenzylamino
34AC	not determined	N-methyl-3-CF <sub>3</sub> -benzylamino
34AD	not determined	(R)- $\alpha$ -methylbenzylamino
34AE	(3S,4R)	(R)- $\alpha$ ,N-dimethylbenzylamino

Examples 36-42A, shown in the following table, were prepared using the procedure of Example 6, except that N-benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate was replaced with Example 27, and 3-(trifluoromethyl)benzyl amine was replaced with the appropriate amine; all listed Examples exhibited an <sup>1</sup>H NMR spectrum consistent with the assigned structure.



Example	A'
36	2-(piperidinyl)ethylamino
37	4-(piperidinyl)piperidinyl
38	4-(2-phenylethyl)piperazinyl
39	1-benzylpiperidin-4-ylamino
40	4-butylpiperazinyl
41	4-isopropylpiperazinyl
42	4-cyclohexylpiperazinyl
42A	4-[2-(piperidinyl)ethyl]piperidinyl

Examples 43-86A, shown in the following table, were prepared using the procedure of Example 6, except that N-benzyloxy carbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate was replaced with Example 28, and 3-(trifluoromethyl)benzyl amine was replaced with the appropriate amine; all listed Examples exhibited an  $^1\text{H}$  NMR spectrum consistent with the assigned structure.



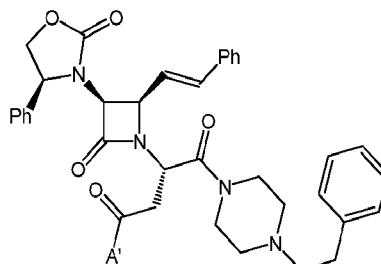
Example	A'
43	2-(piperidinyl)ethylamino
44	4-(piperidinyl)piperidinyl
45	4-(phenylethyl)piperazinyl
46	fur-2-ylmethylamino
47	4-(pyrrolidinyl)piperazinyl
48	4-(3-trifluoromethylphenyl)piperazinyl
49	4-(benzyloxycarbonyl)piperazinyl
50	4-[2-(2-hydroxyethoxy)ethyl]piperazinyl
51	4-benzylpiperazinyl
52	4-(3,4-methylenedioxybenzyl)piperazinyl
53	4-phenylpiperazinyl
54	4-(3-phenylprop-2-enyl)piperazinyl
55	4-ethylpiperazinyl
56	2-(dimethylamino)ethylamino
57	4-(pyrrolidinylcarbonylmethyl)piperazinyl
58	4-(1-methylpiperidin-4-yl)piperazinyl
59	4-butylpiperazinyl
60	4-isopropylpiperazinyl
61	4-pyridylmethylamino
62	3-(dimethylamino)propylamino
63	1-benzylpiperidin-4-ylamino
64	N-benzyl-2-(dimethylamino)ethylamino
65	3-pyridylmethylamino
66	4-(cyclohexyl)piperazinyl
67	4-(2-cyclohexylethyl)piperazinyl
68	4-[2-(morpholin-4-yl)ethyl]piperazinyl
69	4-(4- <i>tert</i> -butylbenzyl)piperazinyl
70	4-[2-(piperidinyl)ethyl]piperazinyl
71	4-[3-(piperidinyl)propyl]piperazinyl
72	4-[2-(N,N-dipropylamino)ethyl]piperazinyl
73	4-[3-(N,N-diethylamino)propyl]piperazinyl

Example	A'
74	4-[2-(dimethylamino)ethyl]piperazinyl
75	4-[3-(pyrrolidinyl)propyl]piperazinyl
76	4-(cyclohexylmethyl)piperazinyl
77	4-cyclopentylpiperazinyl
78	4-[2-(pyrrolidinyl)ethyl]piperazinyl
79	4-[2-(thien-2-yl)ethyl]piperazinyl
80	4-(3-phenylpropyl)piperazinyl
81	4-[2-(N,N-diethylamino)ethyl]piperazinyl
82	4-benzylhomopiperazinyl
83	4-(bisphenylmethyl)piperazinyl
84	3-(4-methylpiperazinyl)propylamino
85	(+)-3(S)-1-benzylpyrrolidin-3-ylamino
86	2-pyridylmethylamino
86A	4-[2-(piperidinyl)ethyl]piperidinyl
86B	1-benzylpiperidin-4-ylamino N-oxide

Example 86B. Example 63 (44 mg, 0.06 mmol) was dissolved in 4 mL dichloromethane and reacted with 3-chloroperoxybenzoic acid (12 mg, 0.07 mmol) until the reaction was complete as assessed by TLC (dichloromethane 94%/methanol 6%, UV detection).

5 The reaction was quenched with aqueous sodium sulfite, the dichloromethane layer was washed with 5% aqueous sodium bicarbonate and distilled water. Evaporation of the dichloromethane layer afforded Example 86B as an off-white solid (35 mg, 78%), and exhibited an <sup>1</sup>H NMR spectrum consistent with the assigned structure.

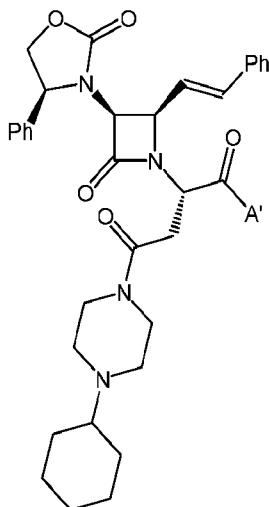
10 Examples 121-132, shown in The following table, were prepared using the procedure of Example 6, except that N-benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate was replaced with Example 30, and 3-(trifluoromethyl)benzyl amine was replaced with the appropriate amine; all listed Examples exhibited an <sup>1</sup>H NMR spectrum consistent with the assigned structure.



Example	A'
121	3-trifluoromethylbenzylamino
122	morpholin-4-ylamino
123	2-(dimethylamino)ethylamino
124	3-(dimethylamino)propylamino
125	cyclohexylamino
126	piperidinyl
127	2-methoxyethylamino
128	isopropylamino
129	isobutylamino
130	ethylamino
131	dimethylamino

Example	A'
132	methylamino

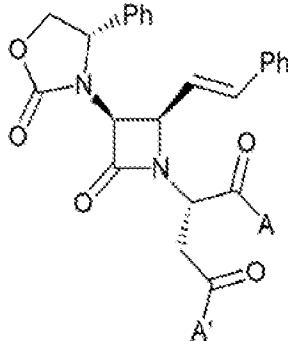
Examples 132A-132B, shown in the following table, were prepared using the procedure of Example 6, except that N-benzyloxy carbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate was replaced with Example 34I, and 3-(trifluoromethyl)benzyl amine was replaced with the appropriate amine; all listed Examples exhibited an  $^1\text{H}$  NMR spectrum consistent with the assigned structure.



Example	A'
132A	(2,3-dichlorobenzyl)amino
132B	1-phenylcyclohexylamino

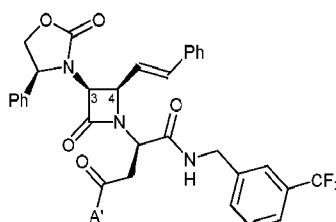
Example 132C 2(S)-(tert-Butoxycarbonylmethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl] acetic acid N-(4-cyclohexyl)piperazinamide. Example 132C was prepared using the procedure of Example 6, except that N-benzyloxy carbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate was replaced with Example 34P, and 3-(trifluoromethyl)benzyl amine was replaced with 1-cyclohexyl-piperazine. Example 132C exhibited an  $^1\text{H}$  NMR spectrum consistent with the assigned structure.

The compounds shown in the following table were prepared according to the processes described herein.



Example	A	A'
132D	1-phenyl-cyclopent-1-ylamino	4-(piperidinyl)piperidinyl
132E	1-phenyl-cyclopent-1-ylamino	1-benzylpiperidin-4-ylamino
132F	(R)-1-phenylethy-1-amino	4-(piperidinyl)piperidinyl

Examples 133-134G, shown in the following table, were prepared using the procedure of Example 6, except that N-benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate was replaced with Example 32, and 3-(trifluoromethyl)benzyl amine was replaced with the appropriate amine; all listed Examples exhibited an  $^1\text{H}$  NMR spectrum consistent with the assigned structure.



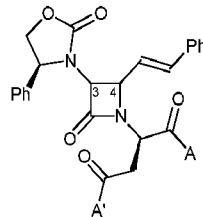
Example	A'
133	4-(piperidinyl)piperidinyl
134	4-(2-phenylethyl)piperazinyl
134A	4-[2-(piperidinyl)ethyl]piperidinyl
134B	4-(pyrrolidinyl)piperazinyl
134C	1-benzylpiperidin-4-ylamino
134D	(pyridin-3-ylmethyl)amino
134E	3-(dimethylamino)propylamino
134F	3-(S)-(1-benzylpyrrolidin-3-yl)amino
134G	4-[(piperidinyl)methyl]piperidinyl
134H	4-(piperidinyl)piperidinyl N-oxide

Example 134H. Example 134H was prepared using the procedure of Example 86B, except that Example 133 was replaced with Example 110. Example 134H was obtained as an off-white solid (48 mg, 94%), and exhibited an  $^1\text{H}$  NMR spectrum consistent with the assigned structure.

Example 134I. 2(R)-[[4-(Piperidinyl)piperidinyl]carboxymethyl]-2-[3(S)-(4(R)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide. Example 134I was prepared using the procedure of Example 6,

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 except that N-benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate was replaced with Example 32A, and 3-(trifluoromethyl)benzyl amine was replaced with 4-(piperidinyl)piperidine, and exhibited an  $^1\text{H}$  NMR spectrum consistent with the assigned structure.

5 The compounds shown in the following table were prepared according to the processes described herein.



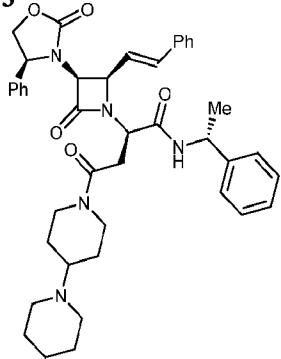
Example	C(3)-C(4) Stereochemistry	A	A'
134J	(3S,4R)	$\alpha,\alpha$ -dimethylbenzylamino	4-(piperidinyl)piperidinyl
134K	(3S,4R)	$\alpha,\alpha$ -dimethylbenzylamino	1-benzylpiperidin-4-ylamino
134L	not determined	N-methyl-3-CF <sub>3</sub> -benzylamino	4-(piperidinyl)piperidinyl
134M	(3S,4R)	N-methyl-3-CF <sub>3</sub> -benzylamino	3-(pyrrolidinyl)piperidinyl
134N	not determined	(R)- $\alpha$ -methylbenzylamino	4-(piperidinyl)piperidinyl
134O	(3S,4R)	(R)- $\alpha$ ,N-dimethylbenzylamino	4-(piperidinyl)piperidinyl

10 Example 222. 2(R)-[[4-(Piperidinyl)piperidinyl]carbonylmethyl]-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(2-fluoro-3-trifluoromethylbenzyl)carboxamide. Example 222 was prepared using the procedure of Example 6, except that N-benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate was replaced with Example 34B, and 3-(trifluoromethyl)benzyl amine was replaced with 4-(piperidinyl)piperidine; Example 222 exhibited an  $^1\text{H}$  NMR spectrum consistent with the assigned structure.

15

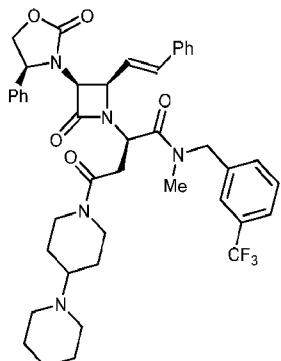
15 Example 223. 2(R)-[[4-(Piperidinyl)piperidinyl]carbonylmethyl]-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[(S)- $\alpha$ -methylbenzyl]amide. Example 223 was prepared using the procedure of Example 6, except that N-benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate was replaced with Example 20 34C, and 3-(trifluoromethyl)benzyl amine was replaced with 4-(piperidinyl)piperidine; Example 223 exhibited an  $^1\text{H}$  NMR spectrum consistent with the assigned structure.

Example 224.



2(R)-[[4-(Piperidinyl)piperidinyl]carbonylmethyl]-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[(R)- $\alpha$ -methylbenzyl]amide. Example 224 was prepared using the procedure of Example 6, except that N-benzyloxy carbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate was replaced with Example 34D, and 3-(trifluoromethyl)benzyl amine was replaced with 4-(piperidinyl)piperidine; Example 223 exhibited an  $^1$ H NMR spectrum consistent with the assigned structure.

Example 225.



10 2(R)-[[4-(Piperidinyl)piperidinyl]carbonylmethyl]-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-methyl-N-(3-trifluoromethylbenzyl)amide. Example 225 was prepared using the procedure of Example 6, except that N-benzyloxy carbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate was replaced with Example 34E, and 3-(trifluoromethyl)benzyl amine was replaced with 4-(piperidinyl)piperidine; Example 223 exhibited an  $^1$ H NMR spectrum consistent with the assigned structure; Calc'd for C<sub>43</sub>H<sub>48</sub>F<sub>3</sub>N<sub>5</sub>O<sub>5</sub>: C, 66.91; H, 6.27; N, 9.07; found. C, 66.68; H, 6.25; N, 9.01.

15 Example 225 Hydrochloride salt. Example 225 (212.5 mg) was dissolved in 30 mL dry Et<sub>2</sub>O. Dry HCl gas was bubbled through this solution resulting in the rapid formation of an off-white precipitate. HCl addition was discontinued when no more precipitate was observed forming (ca. 5 minutes). The solid was isolated by suction filtration, washed twice with 15 mL of dry Et<sub>2</sub>O and dried to 213.5 mg (96% yield) of an off-white solid; Calc'd for C<sub>43</sub>H<sub>49</sub>ClF<sub>3</sub>N<sub>5</sub>O<sub>5</sub>: C, 63.89; H, 6.11; N, 8.66; Cl, 4.39; found. C, 63.41; H, 5.85; N, 8.60; Cl,

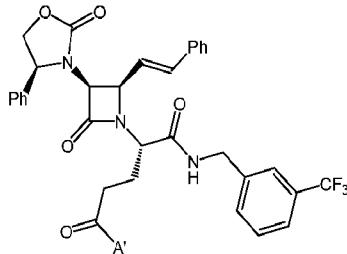
Example 225A. 2(R)-[[4-[2-(piperidinyl)ethyl]piperidinyl]carbonylmethyl]-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[(S)- $\alpha$ -methylbenzyl]amide. Example 225A was prepared using the procedure of Example 6, except that N-benzyloxy carbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate was replaced with Example 34C, and 3-(trifluoromethyl)benzyl amine was replaced with 4-[2-(piperidinyl)ethyl]piperidine. Example 225A exhibited an  $^1$ H NMR spectrum consistent with the assigned structure.

Example 225B. 2(R)-[[4-[2-(piperidinyl)ethyl]piperidinyl]carbonylmethyl]-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[(R)- $\alpha$ -methylbenzyl]amide. Example 225B was prepared using the procedure of Example 6, except that N-benzyloxy carbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate was replaced with Example 34D, and 3-(trifluoromethyl)benzyl amine was replaced with 4-[2-(piperidinyl)ethyl]piperidine. Example 225B exhibited an  $^1$ H NMR spectrum consistent with the assigned structure.

Example 225C. 2(R)-[[4-(Piperidinyl)piperidinyl]carbonylmethyl]-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[(R)-1-(3-trifluoromethylphenyl)ethyl]amide. Example 225C was prepared using the procedure of Example 6, except that N-benzyloxy carbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate was replaced with Example 34L, and 3-(trifluoromethyl)benzyl amine was replaced with 4-(piperidinyl)piperidine. Example 225C exhibited an  $^1$ H NMR spectrum consistent with the assigned structure.

Example 225D. 2(R)-[[4-(Piperidinyl)piperidinyl]carbonylmethyl]-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[(S)-1-(3-trifluoromethylphenyl)ethyl]amide. Example 225D was prepared using the procedure of Example 6, except that N-benzyloxy carbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate was replaced with Example 34N, and 3-(trifluoromethyl)benzyl amine was replaced with 4-(piperidinyl)piperidine. Example 225D exhibited an  $^1$ H NMR spectrum consistent with the assigned structure.

Examples 87-120E, shown in the following table, were prepared using the procedure of Example 6, except that N-benzyloxy carbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate was replaced with Example 29, and 3-(trifluoromethyl)benzyl amine was replaced with the appropriate amine; all listed Examples exhibited an  $^1$ H NMR spectrum consistent with the assigned structure.



Example	A'
87	2-(piperidinyl)ethylamino
88	4-(piperidinyl)piperidinyl
89	2-(pyrid-2-yl)ethylamino
90	morpholin-4-ylamino
91	4-(pyrrolidinyl)piperazinyl
92	4-(3-trifluorophenyl)piperazinyl
93	4-(benzyl oxy carbonyl)piperazinyl
94	4-[2-(2-hydroxyethoxy)ethyl]piperazinyl
95	4-benzylpiperazinyl
96	4-(3,4-methylenedioxybenzyl)piperazinyl
97	4-phenylpiperazinyl
98	4-(3-phenylprop-2-enyl)piperazinyl
99	4-ethylpiperazinyl
100	2-(dimethylamino)ethylamino
101	4-(pyrrolidinyl carbonylmethyl)piperazinyl
102	4-(1-methylpiperidin-4-yl)piperazinyl
103	4-butylpiperazinyl
104	4-isopropylpiperazinyl
105	4-pyridylmethylethylamino
106	3-(dimethylamino)propylamino
107	1-benzylpiperidin-4-ylamino
108	N-benzyl-2-(dimethylamino)ethylamino
109	3-pyridylmethylethylamino
110	4-cyclohexylpiperazinyl
111	4-(2-cyclohexylethyl)piperazinyl
112	4-[2-(morpholin-4-yl)ethyl]piperazinyl
113	4-(4- <i>tert</i> -butylbenzyl)piperazinyl
114	4-[2-(piperidinyl)ethyl]piperazinyl
115	4-[3-(piperidinyl)propyl]piperazinyl
116	4-[2-(diisopropylamino)ethyl]piperazinyl
117	4-[3-(diethylamino)propyl]piperazinyl
118	4-(2-dimethylaminoethyl)piperazinyl
119	4-[3-(pyrrolidinyl)propyl]piperazinyl
120	4-(cyclohexylmethyl)piperazinyl
120A	4-[2-(piperidinyl)ethyl]piperidinyl
120B	4-propyl-piperazinyl
120C	4-[N-(isopropyl)acetamid-2-yl]piperazinyl
120D	3-benzyl-hexahydro-(1H)-1,3-diazepinyl
120E	4-(piperidinylmethyl)piperidinyl
120F	4-cyclohexylpiperazinyl N-oxide
120G	methoxy
120H	4-cyclohexylpiperazinyl

Example 120F. Example 120F was prepared using the procedure of Example 86B, except that Example 63 was replaced with Example 110 to give an off-white solid (54.5 mg, 98%). Example 120F exhibited an <sup>1</sup>H NMR spectrum consistent with the assigned

Example 120G. 2(S)-(Methoxycarbonylethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide.

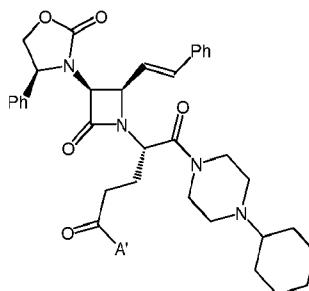
Example 120G was prepared using the procedure of Example 6, except that N-

5 benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate was replaced with Example 34M, and exhibited an  $^1$ H NMR spectrum consistent with the assigned structure.

Example 35. 2(S)-[4-(2-phenylethyl)piperazinyl-carbonylethyl]-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide. Using the procedure of Example 6, except that N-

10 benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate was replaced with the carboxylic acid of Example 29 and 3-(trifluoromethyl)benzyl amine was replaced with 4-(2-phenylethyl)piperazine, the title compound was prepared;  $^1$ H NMR (CDCl<sub>3</sub>)  $\delta$  2.21-2.23 (m, 1H); 2.25-2.45 (m, 6H); 2.52-2.63 (m, 3H); 2.72-2.82 (m, 2H); 3.42-3.48 (m, 2H); 3.52-3.58 (m, 1H); 4.13-4.18 (m, 1H); 4.26 (dd, J=5.1 Hz, J=8.3 Hz, 1H); 4.29 (d, J=5.0 Hz, 1H); 4.44 (dd, J=6.0 Hz, J=15.0 Hz, 1H); 4.54 (dd, J=6.2 Hz, J=14.9 Hz, 1H); 4.61-4.68 (m, 2H); 4.70-4.75 (m, 1H); 6.27 (dd, J=9.6 Hz, J=15.8 Hz, 1H); 6.73 (d, J=15.8 Hz, 1H); 7.16-7.60 (m, 19H); 8.07-8.12 (m, 1H); FAB<sup>+</sup> (M+H)<sup>+</sup>/z 794; Elemental Analysis calculated for C<sub>45</sub>H<sub>46</sub>F<sub>3</sub>N<sub>5</sub>O<sub>5</sub>: C, 68.08; H, 5.84; N, 8.82; found: C, 67.94; H, 5.90; N, 8.64.

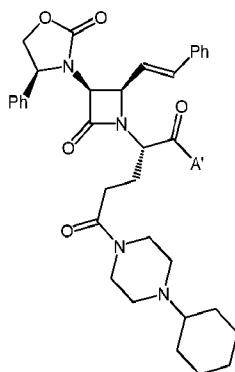
Examples 141-171, shown in the following table, were prepared using the procedure of Example 6, except that N-benzyloxycarbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate was replaced with Example 34, and 3-(trifluoromethyl)benzyl amine was replaced with the appropriate amine; all listed Examples exhibited an  $^1$ H NMR spectrum consistent with the assigned structure.



Example	A'
141	benzylamino
142	(2-methylbenzyl)amino
143	(3-methylbenzyl)amino
144	(4-methylbenzyl)amino
145	( $\alpha$ -methylbenzyl)amino
146	N-benzyl-N-methylamino
147	N-benzyl-N-( <i>t</i> -butyl)amino
148	N-benzyl-N-butylamino
149	(3,5-dimethylbenzyl)amino

Example	A'
150	(2-phenylethyl)amino
151	dimethylamino
152	(3-trifluoromethoxybenzyl)amino
153	(3,4-dichlorobenzyl)amino
154	(3,5-dichlorobenzyl)amino
155	(2,5-dichlorobenzyl)amino
156	(2,3-dichlorobenzyl)amino
157	(2-fluoro-5-trifluoromethylbenzyl)amino
158	(4-fluoro-3-trifluoromethylbenzyl)amino
159	(3-fluoro-5-trifluoromethylbenzyl)amino
160	(2-fluoro-3-trifluoromethylbenzyl)amino
161	(4-chloro-3-trifluoromethylbenzyl)amino
162	indan-1-ylamino
163	4-(2-hydroxybenzimidazol-1-yl)-piperidinyl
164	3(S)-(tert-butylaminocarbonyl)-1,2,3,4-tetrahydroisoquinolin-2-yl
165	(3,3-dimethylbutyl)amino
166	4-hydroxy-4-phenylpiperidinyl
167	(cyclohexylmethyl)amino
168	(2-phenoxyethyl)amino
169	3,4-methylenedioxybenzylamino
170	4-benzylpiperidinyl
171	(3-trifluoromethylphenyl)amino

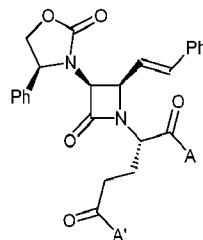
Examples 172-221R, shown in the following table, were prepared using the procedure of Example 6, except that N-benzyloxy carbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate was replaced with Example 34A, and 3-(trifluoromethyl)benzyl amine was replaced with the appropriate amine; all listed Examples exhibited an  $^1\text{H}$  NMR spectrum consistent with the assigned structure.



Example	A'
172	(3-trifluoromethoxybenzyl)amino
173	(3,4-dichlorobenzyl)amino
174	(3,5-dichlorobenzyl)amino
175	(2,5-dichlorobenzyl)amino
176	(2,3-dichlorobenzyl)amino
177	(2-fluoro-5-trifluoromethylbenzyl)amino
178	(4-fluoro-3-trifluoromethylbenzyl)amino
179	(3-fluoro-5-trifluoromethylbenzyl)amino
180	(2-fluoro-3-trifluoromethylbenzyl)amino
181	(4-chloro-3-trifluoromethylbenzyl)amino
182	(2-trifluoromethylbenzyl)amino
183	(3-methoxybenzyl)amino

Example	A'
184	(3-fluorobenzyl)amino
185	(3,5-difluorobenzyl)amino
186	(3-chloro-4-fluorobenzyl)amino
187	(3-chlorobenzyl)amino
188	[3,5-bis(trifluoromethyl)benzyl]amino
189	(3-nitrobenzyl)amino
190	(3-bromobenzyl)amino
191	benzylamino
192	(2-methylbenzyl)amino
193	(3-methylbenzyl)amino
194	(4-methylbenzyl)amino
195	( $\alpha$ -methylbenzyl)amino
196	(N-methylbenzyl)amino
197	(N- <i>tert</i> -butylbenzyl)amino
198	(N-butylbenzyl)amino
199	(3,5-dimethylbenzyl)amino
200	(2-phenylethyl)amino
201	(3,5-dimethoxybenzyl)amino
202	(1 <i>R</i> )-(3-methoxyphenyl)ethylamino
203	(1 <i>S</i> )-(3-methoxyphenyl)ethylamino
204	( $\alpha,\alpha$ -dimethylbenzyl)amino
205	N-methyl-N-(3-trifluoromethylbenzyl)amino
206	[(S)- $\alpha$ -methylbenzyl]amino
207	(1-phenylcycloprop-1-yl)amino
208	(pyridin-2-ylmethyl)amino
209	(pyridin-3-ylmethyl)amino
210	(pyridin-4-ylmethyl)amino
211	(fur-2-ylmethyl)amino
212	[(5-methylfur-2-yl)methyl]amino
213	(thien-2-ylmethyl)amino
214	[(S)-1,2,3,4-tetrahydro-1-naphth-1-yl]amino
215	[(R)-1,2,3,4-tetrahydro-1-naphth-1-yl]amino
216	(indan-1-yl)amino
217	(1-phenylcyclopent-1-yl)amino
218	( $\alpha,\alpha$ -dimethyl-3,5-dimethoxybenzyl)amino
219	(2,5-dimethoxybenzyl)amino
220	(2-methoxybenzyl)amino
221	( $\alpha,\alpha$ ,2-trimethylbenzyl)amino
221A	N-methyl-3-Me-benzylamide
221B	N-methyl-2,3-Cl-benzylamide
221C	N-methyl-3-Cl-benzylamide
221D	N-methyl-3-Br-benzylamide
221E	N-methyl-3,5-Cl-benzylamide
221F	(R)-1-(3-trifluorophenyl)ethylamide
221G	1-phenyl-cyclohexylamide
221H	1-(2-fluorophenyl)-cyclopentylamide
221I	1-(4-fluorophenyl)-cyclopentylamide
221J	4-CF <sub>3</sub> -benzylamide
221K	$\alpha$ -phenyl-benzylamide
221L	3-phenyl-benzylamide
221M	dibenzylamide
221N	1-naphthalene-methylamide
221O	1,2,3,4-tetrahydro-isoquinolinamide
221P	indan-2-ylamino
221Q	$\alpha$ -(2-OH-ethyl)benzylamide
221R	(S)-indan-1-ylamino

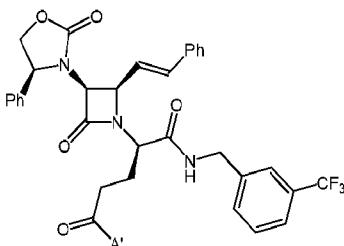
The compounds shown in the following table were prepared according to the processes described herein.



Example	A	A'
221S	(R)-1-indanylamino	4-cyclohexylpiperazinyl
221T	( <i>αR</i> )- <i>α</i> -( <i>t</i> -butoxycarbonylmethyl)benzylamino	4-cyclohexylpiperazinyl
221U	(R)-1,2,3,4-tetrahydro-1-naphthylamino	4-(2-morpholinoethyl)-piperazinyl
221V	(R)-1,2,3,4-tetrahydro-1-naphthylamino	2-dimethylaminoethylamino
221W	(R)-1,2,3,4-tetrahydro-1-naphthylamino	4-(2-phenylethyl)-homopiperazinyl
221X	(R)-1,2,3,4-tetrahydro-1-naphthylamino	2-(1-piperidyl)ethylamino
221Y	(R)-1,2,3,4-tetrahydro-1-naphthylamino	(S)-2-(1-pyrrolidinylmethyl)pyrrolidinyl
221Z	(R)-1,2,3,4-tetrahydro-1-naphthylamino	2-(1-pyrrolidinyl)ethylamino
221AA	(R)-1,2,3,4-tetrahydro-1-naphthylamino	4-(1-piperidyl)piperidinyl
221AB	3-CF <sub>3</sub> -benzylamino	4-n-butylpiperazinyl
221AC	3-CF <sub>3</sub> -benzylamino	4-ethylpiperazinyl
221AD	(R)-1,2,3,4-tetrahydro-1-naphthylamino	(R)-1-benzylpyrrolidin-3-ylamino
221AE	(R)-1,2,3,4-tetrahydro-1-naphthylamino	quinuclidin-3-ylamino
221AF	(R)-1,2,3,4-tetrahydro-1-naphthylamino	4-methylhomopiperazinyl
221AG	(R)-1,2,3,4-tetrahydro-1-naphthylamino	2-pyrrolylphenylamino
221AH	(R)-1,2,3,4-tetrahydro-1-naphthylamino	morpholin-4-ylethylamino
221AI	(R)-1,2,3,4-tetrahydro-1-naphthylamino	(S)-1-ethylpyrrolidin-2-ylaminomethyl
221AJ	(R)-1,2,3,4-tetrahydro-1-naphthylamino	(R)-1-ethylpyrrolidin-2-ylaminomethyl
221AK	(R)-1,2,3,4-tetrahydro-1-naphthylamino	(S)-1-butoxycarbonylpyrrolidin-3-ylamino
221AL	(R)-1,2,3,4-tetrahydro-1-naphthylamino	quinolin-3-ylamino
221AM	1-(3-fluorophenyl)-cyclopentylamino	4-cyclohexylpiperazinyl
221AN	1-(4-chlorophenyl)-cyclopropylamino	4-cyclohexylpiperazinyl
221AO	1-(4-methoxyphenyl)-cyclopropylamino	4-cyclohexylpiperazinyl
221AP	1-(4-methylphenyl)-cyclopropylamino	4-cyclohexylpiperazinyl
221AQ	1-(4-chlorophenyl)-cyclopentylamino	4-cyclohexylpiperazinyl
221AS	1-(4-methylphenyl)-cyclopentylamino	4-cyclohexylpiperazinyl
221AT	(R)-1,2,3,4-tetrahydro-1-naphthylamino	3-(4-chlorophenyl)isoxazolin-5-ylamino
221AU	1-phenylcyclopentylamino	4-(1-pyrrolidyl)piperidinyl
221AV	indolinyl	4-cyclohexylpiperazinyl
221AW	5-indanylamino	4-cyclohexylpiperazinyl
221AX	1-phenylcyclopentylamino	4-[3-((R)-Boc-amino)-1-pyrrolidyl)piperidinyl
221AY	4-indanylamino	4-cyclohexylpiperazinyl
221AZ	1-phenylcyclopentylamino	(3 <i>R</i> )-4-(3-chloroammoniumpyrrolidinyl)piperidinyl
221BA	(R)-1,2,3,4-tetrahydro-1-naphthylamino	4-(2-fluorophenyl)piperazinyl
221BB	(R)-1,2,3,4-tetrahydro-1-naphthylamino	4-(3-chlorophenyl)piperazinyl

221BC	(R)-1,2,3,4-tetrahydro-1-naphthylamino	4-(4-fluorophenyl)piperazinyl
221BD	(R)-1,2,3,4-tetrahydro-1-naphthylamino	4-ethylpiperazinyl
221BE	(R)-1,2,3,4-tetrahydro-1-naphthylamino	4-phenylpiperazinyl
221BF	(R)-1,2,3,4-tetrahydro-1-naphthylamino	4-benzylpiperazinyl
221BG	(R)-1,2,3,4-tetrahydro-1-naphthylamino	4-methylpiperazinyl
221BH	(R)-1,2,3,4-tetrahydro-1-naphthylamino	4-(2-methoxyphenyl)piperazinyl
221BI	(R)-1,2,3,4-tetrahydro-1-naphthylamino	4-(3-OH-n-propyl)piperazinyl
221BJ	(R)-1,2,3,4-tetrahydro-1-naphthylamino	4-(4-hydroxyphenyl)piperazinyl

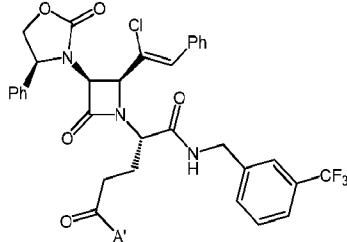
Examples 135-140, shown in the following table, were prepared using the procedure of Example 6, except that N-benzyloxy carbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate was replaced with Example 33, and 3-(trifluoromethyl)benzyl amine was replaced with the appropriate amine; all listed Examples exhibited an  $^1\text{H}$  NMR spectrum consistent with the assigned structure.



Example	A'
135	4-(piperidinyl)piperidinyl
136	4-(2-phenylethyl)piperazinyl
137	4-butylpiperazinyl
138	4-isopropylpiperazinyl
139	4-cyclohexylpiperazinyl
140	4-(cyclohexylmethyl)piperazinyl

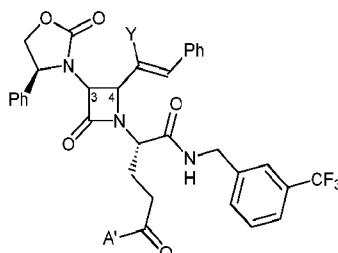
Example 140A. 2(R)-(2-(3-trifluoromethylbenzyl)amino-1-ylcarbonyl)ethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(4-cyclohexyl)piperazinamide. Example 140A was prepared using the procedure of Example 6, except that N-benzyloxy carbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate was replaced with Example 34Q, and 3-(trifluoromethyl)benzyl amine was replaced with 1-cyclohexylpiperazine, and exhibited an  $^1\text{H}$  NMR spectrum consistent with the assigned structure.

Examples 226-230C, shown in the following table, were prepared using the procedure of Example 6, except that N-benzyloxy carbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate was replaced with Example 34F, and 3-(trifluoromethyl)benzyl amine was replaced with the appropriate amine; all listed Examples exhibited an  $^1\text{H}$  NMR spectrum consistent with the assigned structure.



Example	A'
226	4-cyclohexylpiperazinyl
227	4-(pyrrolidinyl)piperazinyl
227A	4-[2-(2-hydroxyethoxy)ethyl]piperazinyl
227B	4-benzylpiperazinyl
227C	4-(3,4-methylenedioxybenzyl)piperazinyl
228	4-ethylpiperazinyl
229	4-n-butylpiperazinyl
230	4-isopropylpiperazinyl
230A	1-benzylpiperidin-4-ylamino
230B	4-(2-cyclohexylethyl)piperazinyl
230C	4-[2-(morpholin-4-yl)ethyl]piperazinyl

The following compounds were prepared according to the processes described herein:



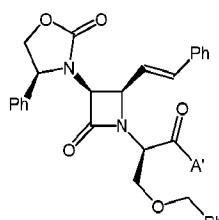
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Example	Y	C(3)-C(4) Stereochemistry	A'
230D	F	not determined	4-n-butylpiperazinyl
230E	F	not determined	(R)-1-benzylpyrrolidin-3-amino
230F	F	not determined	quinuclidin-3-ylamino
230G	F	(3S,4R)	(S)-1-benzylpyrrolidin-3-amino
230H	Cl	not determined	(R)-1-benzylpyrrolidin-3-amino
230I	Cl	(3S,4R)	(R)-1-benzylpyrrolidin-3-amino
230J	Cl	(3S,4R)	(S)-1-benzylpyrrolidin-3-amino
230K	Cl	not determined	(S)-1-benzylpyrrolidin-3-amino
230L	Br	not determined	4-n-butylpiperazinyl
230M	Br	not determined	4-ethylpiperazinyl

Example 86C. 2(S)-[[4-(Piperidinyl)piperidinyl]carbonylmethyl]-2-[3(S)-(4(R)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide. Example 86C was prepared using the procedure of Example 6, except that N-benzyloxy carbonyl-D-aspartic acid  $\beta$ -t-butyl ester monohydrate was replaced with Example 28A, and 3-(trifluoromethyl)benzyl amine was replaced with 4-(piperidinyl)piperidine, and exhibited an  $^1\text{H}$  NMR spectrum consistent with the assigned structure.

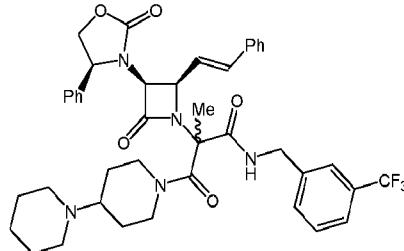
Example 231. 2(R)-[[4-(Piperidinyl)piperidinyl]carbonylmethyl]-2-[3(S)-4(S)-phenyloxazolidin-2-on-3-yl]-4(R)-(2'-methoxysty-2-yl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide. Example 231 was prepared using the procedure of Example 6, except that N-benzyloxy carbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate was replaced with Example 34G, and 3-(trifluoromethyl)benzyl amine was replaced with 4-(piperidinyl)piperidine, and exhibited an  $^1$ H NMR spectrum consistent with the assigned structure.

Examples 232-233A, shown in the following table, were prepared using the procedure of Example 6, except that N-benzyloxy carbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate was replaced with Example 34H, and 3-(trifluoromethyl)benzyl amine was replaced with the appropriate amine; all listed Examples exhibited an  $^1$ H NMR spectrum consistent with the assigned structure.



Example	A'	$\alpha$
232	4-(piperidinyl)piperidinyl	D
232A	(3-trifluoromethylbenzyl)amino	D
232B	4-(3-trifluoromethylphenyl)piperazinyl	D or L
232C	4-(3-trifluoromethylphenyl)piperazinyl	D or L
232D	4-cyclohexylpiperazinyl	DL
232E	4-(piperidinylmethyl)piperidinyl	D
233	4-[2-(piperidinyl)ethyl]piperidinyl	D
233A	4-[1-(piperidyl)methyl]piperidinamide	D

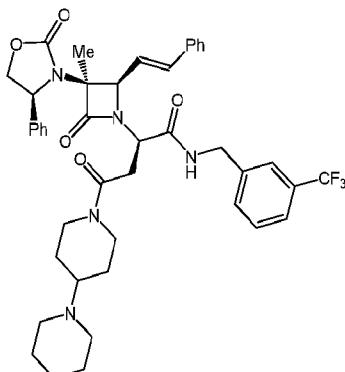
Example 234. (2RS)-[4-(piperidinyl)piperidinylcarbonyl]-2-methyl-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide.



Example 37 (50 mg, 0.067 mmol) in tetrahydrofuran (4 mL) was treated sequentially with sodium hydride (4 mg, 0.168 mmol) and methyl iodide (6  $\mu$ L, 0.094 mmol) at -78 °C. The resulting mixture was slowly warmed to ambient temperature, and evaporated. The resulting residue was partitioned between dichloromethane and water, and the organic layer

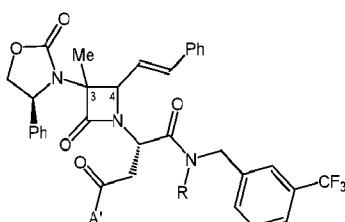
WO 2019/055913 PCT/US2018/051331  
 was evaporated. The resulting residue was purified by silica gel chromatography (95:5 chloroform/methanol) to give 28 mg (55%) of the title compound as an off-white solid; MS (ES<sup>+</sup>): m/z=757 (M<sup>+</sup>).

Example 234A. 4-(Piperidinyl)-piperidinyl 3(R)-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-3-methyl-4(R)-(styr-2-yl)azetidin-2-on-1-yl]-3-[3-(trifluoromethyl)phenylmethylaminocarbonyl]propanoic acid.



Using the procedure of Example 6, except that N-benzyloxy carbonyl-D-aspartic acid  $\beta$ -t-butyl ester monohydrate was replaced with the carboxylic acid of Example 34J and 3-(trifluoromethyl)benzyl amine was replaced with 4-(piperidinyl)piperidine, the title compound was prepared in quantitative yield; MS (m+H)<sup>+</sup> 772.

The compounds shown in the following table were prepared according to the processes described herein.



C(3)-C(4) Stereochemistry	R	A'
(3S,4R)	H	4-(piperidinyl)piperidinyl
(3S,4R)	Me	4-(piperidinyl)piperidinyl
not determined	H	4-(piperidinyl)piperidinyl

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Example 235. 2(S)-[[1-Benzylpiperidin-4-yl)amino]carbonylmethyl]-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-phenyleth-1-yl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide. Example 235 was prepared using the procedure of Example 8, except that N-benzyloxy carbonyl-L-aspartic acid  $\beta$ -t-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide was replaced with Example 63 (50 mg, 0.064 mmol) to give 40 mg (80%) of Example 235 as an off-white solid; Example 235 exhibited an <sup>1</sup>H NMR spectrum consistent with the assigned structure.

Example 236. (2S)-[(4-cyclohexylpiperazinyl)carbonylethyl]-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-phenyleth-1-yl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide. Example 236 was prepared using the procedure of Example 8, except that N-benzyloxy carbonyl-L-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide was replaced with Example 110 (50 mg, 0.065 mmol) to give 42 mg (84%) of Example 236 as an off-white solid; Example 236 exhibited an  $^1$ H NMR spectrum consistent with the assigned structure.

Example 236A. (2S)-[(4-cyclohexylpiperazinyl)carbonylethyl]-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-phenyleth-1-yl)azetidin-2-on-1-yl]acetic acid N-[(R)-1,2,3,4-tetrahydronaphth-1-yl]amide. Example 236A was prepared using the procedure of Example 8, except that N-benzyloxy carbonyl-L-aspartic acid  $\beta$ -*t*-butyl ester  $\alpha$ -(3-trifluoromethyl)benzylamide was replaced with Example 215 (76 mg, 0.10 mmol) to give 69 mg (90%) of Example 236A as an off white solid. Example 236A exhibited an  $^1$ H NMR spectrum consistent with the assigned structure.

Example 237. 2(R)-[4-(Piperidinyl)piperidinyl]carbonylmethyl]-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(propen-1-yl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide. Example 237 was prepared using the procedure of Example 6, except that N-benzyloxy carbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate was replaced with Example 34K, and 3-(trifluoromethyl)benzyl amine was replaced with 4-(piperidinyl)piperidine. Example 237 exhibited an  $^1$ H NMR spectrum consistent with the assigned structure.

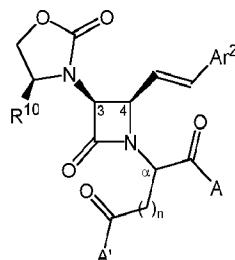
Example 238. (2S)-(Benzylthiomethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[4-[2-(piperid-1-yl)ethyl]piperidin-1-yl]amide. This Example was prepared using the procedure of Example 6, except that N-benzyloxy carbonyl-D-aspartic acid  $\beta$ -*t*-butyl ester monohydrate was replaced with the corresponding benzyl protected cysteine analog, and 3-(trifluoromethyl)benzyl amine was replaced with 4-[2-(piperid-1-yl)ethyl]piperidine.

Step 1. N-*t*Butyloxycarbonyl-(S)-(benzyl)-D-cysteine-[4-(2-(1-piperidyl)ethyl)]piperidinenamide. N-*t*Butyloxycarbonyl-(S)-Benzyl-N-(*t*butyloxycarbonyl)-D-cysteine (0.289 g, 0.93 mmole) and 4-[2-(1-piperidyl)ethyl]piperidine (0.192 g, 0.98 mmole) in dichloromethane (20 mL) gave 0.454 g (quantitative yield) of Example X as an off-white solid.  $^1$ H NMR ( $CDCl_3$ )  $\delta$  0.89-1.15 (m, 2H); 1.39-1.44 (m, 16H); 1.54-1.61 (m, 4H); 1.62-1.71 (m, 1H); 2.21-2.35 (m, 5H); 2.49-2.58 (m, 2H); 2.66-2.74 (m, 1H); 2.79-2.97 (m, 1H); 3.67-3.76 (m, 3H); 4.48-4.51 (m, 1H); 4.72-4.75 (m, 1H); 5.41-5.44 (m, 1H); 7.19-7.34 (m, 5H).

Step 2. (S)-(benzyl)-D-cysteine-[4-(2-(1-piperidyl)ethyl)]piperidinenamide, dihydrochloride. N-*t*Butyloxycarbonyl-(S)-(benzyl)-D-cysteine-[4-(2-(1-piperidyl)ethyl)]piperidinenamide (0.453 g, 0.93 mmole) was reacted overnight with acetyl chloride (0.78 mL, 13.80 mmole) in anhydrous methanol (15 mL). The title compound was obtained as an off-white solid by evaporating the reaction mixture to dryness (0.417 g, 97%).  
<sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 0.94-1.29 (m, 2H); 1.49-1.57 (m, 1H); 1.62-1.95 (m, 10H); 2.65-2.80 (m, 2H); 2.81-2.97 (m, 4H); 3.01-3.14 (m, 2H); 3.50-3.60 (m, 3H); 3.81-3.92 (m, 2H); 4.41-4.47 (m, 2H); 7.25-7.44 (m, 5H).

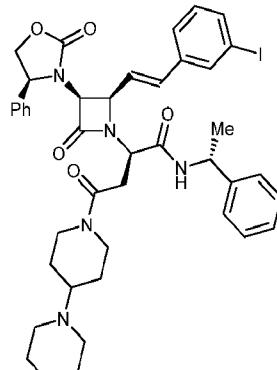
Step 3. Using the general procedures described herein, the imine prepared from (S)-(benzyl)-D-cysteine-[4-(2-(1-piperidyl)ethyl)]piperidinenamide, dihydrochloride (0.417 g, 0.90 mmole) and cinnamaldehyde, in the presence of triethylamine (0.26 mL, 1.87 mmole), was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (Example 1) to give 0.484 g (76%) of Example 238 as an off-white solid after recrystallization from dichloromethane/hexanes. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.89-1.06 (m, 2H); 1.40-1.44 (m, 5H); 1.57-1.67 (m, 6H); 2.25-2.43 (m, 6H); 2.45-2.59 (m, 2H); 2.71-2.88 (m, 2H); 3.55-3.70 (m, 3H); 4.11-4.17 (m, 1H); 4.37-4.47 (m, 2H); 4.54-4.61 (m, 1H); 4.64-4.69 (m, 1H); 4.76-4.84 (m, 2H); 6.05-6.19 (m, 1H); 6.66-6.71 (m, 1H); 7.12-7.40 (m, 15H).

The following compounds are described



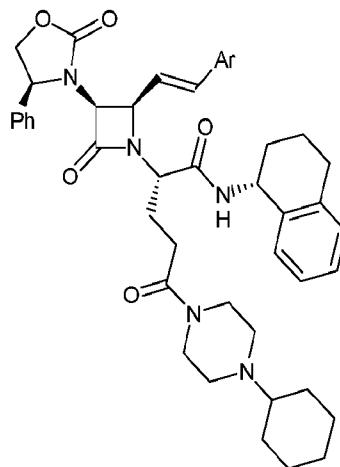
Example	R <sup>10</sup>	Ar <sup>2</sup>	n	α	A	A'
239	Ph	Ph	2	L	1-Ph-cyclopentylamino	4-ethylpiperazin-1-yl
240	Ph	Ph	2	L	1-Ph-cyclopentylamino	4-benzylpiperazin-1-yl
241	Ph	Ph	2	L	(R)-1,2,3,4-tetrahydronaphth-1-ylamino	4-cyclopentylpiperazin-1-yl
242	Ph	3-MeO-Ph	2	L	(R)-1,2,3,4-tetrahydronaphth-1-ylamino	4-cyclohexylpiperazin-1-yl
243	Ph	3-Cl-Ph	2	L	(R)-1,2,3,4-tetrahydronaphth-1-ylamino	4-cyclohexylpiperazin-1-yl
244	Ph	3-Cl-Ph	2	L	1-phenyl-cyclopent-1-ylamino	4-cyclohexylpiperazin-1-yl
245	Ph	3-F-Ph	2	L	(R)-1,2,3,4-tetrahydronaphth-1-ylamino	4-cyclohexylpiperazin-1-yl
246	Ph	3-CF <sub>3</sub> -Ph	2	L	(R)-1,2,3,4-tetrahydronaphth-1-ylamino	4-cyclohexylpiperazin-1-yl
247	Ph	3-Cl-Ph	1	D	N-methyl-3-CF <sub>3</sub> -benzylamino	4-(1-piperidyl)piperidin-1-yl
248	Ph	3-CN-Ph	2	L	(R)-1,2,3,4-tetrahydronaphth-1-ylamino	4-cyclohexylpiperazin-1-yl
249	Ph	3-NO <sub>2</sub> -Ph	2	L	(R)-1,2,3,4-tetrahydronaphth-1-ylamino	4-cyclohexylpiperazin-1-yl

250	Ph	2-Cl-Ph	2	L	(R)-1,2,3,4-tetrahydronaphth-1-ylamino	4-cyclohexylpiperazin-1-yl
251	3-Cl-Ph	3-Cl-Ph	2	L	(R)-1,2,3,4-tetrahydronaphth-1-ylamino	4-cyclohexylpiperazin-1-yl
252	Ph	3,5-Cl <sub>2</sub> -Ph	2	L	(R)-1,2,3,4-tetrahydronaphth-1-ylamino	4-cyclohexylpiperazin-1-yl
253	Ph	Ph	1	L	(S)-1-Ph-ethylamino	4-(1-piperidyl)piperidin-1-yl
256	3-Cl-Ph	Ph	1	D	(R)-1-Ph-ethylamino	4-(1-piperidyl)piperidin-1-yl



Example 266 (AVN576)

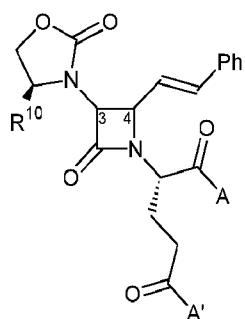
The following compounds are described



Example	Ar
257	benzothiophen-7-yl
254	fur-2-yl
255	thien-2-yl

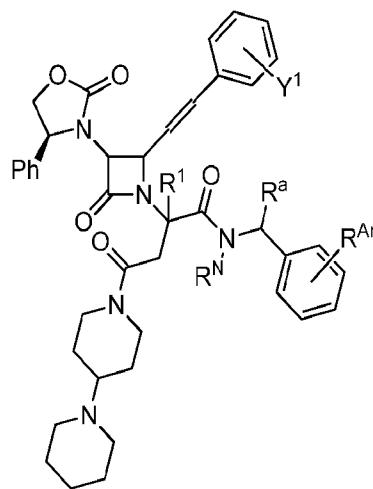
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The following compounds are described



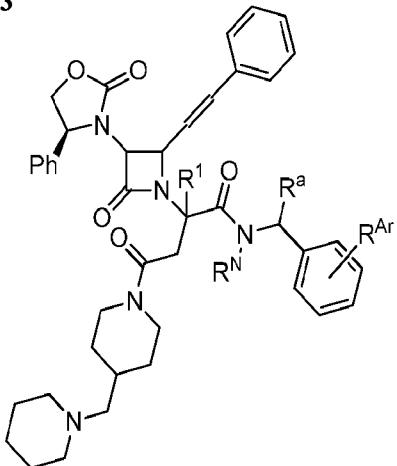
Example	R <sup>10</sup>	Stereo-chemistry	A	A'
258	Ph	(3S,4R)	(R)-1,2,3,4-tetrahydronaphth-1-ylamino	4-cycloheptylpiperazin-1-yl
259	Ph	(3S,4R)	(R)-1,2,3,4-tetrahydronaphth-1-ylamino	4-(tetrahydrothiopyran-4-yl)piperazin-1-yl
260	Ph	(3R,4S)	3-CF <sub>3</sub> -benzylamino	4-cyclohexylpiperazin-1-yl
261	Ph	(3S,4R)	4-phenylpiperazin-1-yl	3-F-5-CF <sub>3</sub> -benzylamino
262	Ph	(3S,4R)	4-(2-cyclohexylethyl)piperazin-1-yl	3-F-5-CF <sub>3</sub> -benzylamino
263	Ph	(3S,4R)	4-(pyrid-2-yl)piperazin-1-yl	3-F-5-CF <sub>3</sub> -benzylamino
264	Ph	(3S,4R)	4-(2-thien-2-ylethyl)piperazin-1-yl	3-F-5-CF <sub>3</sub> -benzylamino
265	3-Cl-Ph	(3S,4R)	(R)- $\alpha$ -methylbenzylamino	4-cyclohexylpiperazin-1-yl

The following compounds are described



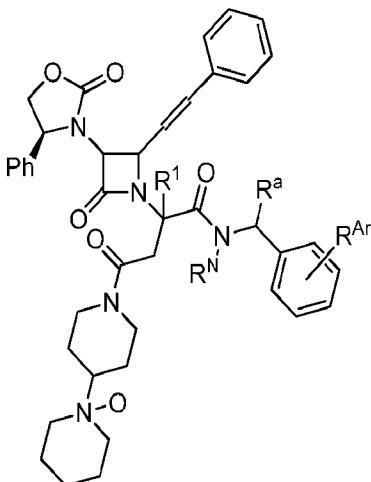
Example	Y <sup>1</sup>	R <sup>N</sup>	R <sup>a</sup>	R <sup>Ar</sup>
559	3-Cl	H	(R)-Me	H
594	4-OH	H	(R)-Me	H
597	3-NO <sub>2</sub>	H	(R)-Me	H
600	3-NH <sub>2</sub>	H	(R)-Me	H
606	3-Br	H	(R)-Me	H
633	3-F	H	(R)-Me	H
778	3-Me	H	(R)-Me	H
623	H	H	(R)-CF <sub>3</sub>	H
626	H	H	(S)-CF <sub>3</sub>	H
682	H	H	H	2-Br
677	H	H	H	2-F
617	3-Br	Me	H	3-CF <sub>3</sub>

The following compounds are described



Example	R <sup>N</sup>	R <sup>a</sup>	R <sup>Ar</sup>
599	Me	H	3-CF <sub>3</sub>
601	H	(R)-Me	H

The following compounds are described



Example	R <sup>N</sup>	R <sup>a</sup>	R <sup>Ar</sup>
670	Me	H	3-CF <sub>3</sub>
672	H	(R)-Me	H

5

The following table illustrates selected compounds further characterized by mass spectral analysis using FAB<sup>+</sup> to observe the corresponding (M+H)<sup>+</sup> parent ion.

Example	(m+H) <sup>+</sup> /z
37	744
38	766
39	766
40	718
41	704
42	744
42A	772
44	758
63	780
85	766
86A	786
86C	758

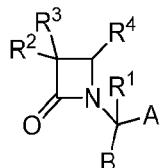
Example	(m+H) <sup>+</sup> /z
187	738
188	840
189	749
190	782
191	704
192	718
193	718
199	732
200	718
201	764
202	748
203	748

Example	(m+H) <sup>+/z</sup>
88	772
91	759
95	780
96	824
104	732
110	772
111	800
112	803
120	786
120A	800
120B	732
120E	788
132B	758
133	758
134A	786
134C	780
134H	772
136	794
137	746
138	732
139	772
174	772
175	772
176	772
177	790
179	790
180	790
182	772
183	734
184	722
185	740
186	756

Example	(m+H) <sup>+/z</sup>
205	786
206	718
207	730
208	705
209	705
210	705
211	694
212	708
213	710
214	744
215	744
216	7530
217	758
218	792
219	764
220	734
221	746
222	776
224	704
225	772
226	806
227	792
228	752
229	780
230	766
231	788
232	663
233	691
234	758
235	782
236	774

## WHAT IS CLAIMED IS:

1. A method for treating a brain injury in a host animal, the method comprising administering a composition comprising one or more selective vasopressin V1a receptor antagonists to the host animal, wherein one or more of the antagonists are selected from compounds of the formula:



and pharmaceutically acceptable salts thereof, wherein

A is a carboxylic acid, an ester, or an amide;

B is a carboxylic acid, an ester, or an amide; or B is an alcohol or thiol, or a derivative thereof;

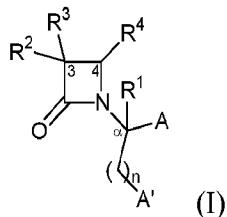
R<sup>1</sup> is hydrogen or C<sub>1</sub>-C<sub>6</sub> alkyl;

R<sup>2</sup> is hydrogen, alkyl, alkenyl, alkynyl, alkoxy, alkylthio, halo, haloalkyl, cyano, formyl, alkylcarbonyl, or a substituent selected from the group consisting of -CO<sub>2</sub>R<sup>8</sup>, -CONR<sup>8</sup>R<sup>8'</sup>, and -NR<sup>8</sup>(COR<sup>9</sup>); where R<sup>8</sup> and R<sup>8'</sup> are each independently selected from hydrogen, alkyl, cycloalkyl, optionally substituted aryl, or optionally substituted arylalkyl; or R<sup>8</sup> and R<sup>8'</sup> are taken together with the attached nitrogen atom to form a heterocyclyl group; and where R<sup>9</sup> is selected from hydrogen, alkyl, cycloalkyl, alkoxyalkyl, optionally substituted aryl, optionally substituted arylalkyl, optionally substituted heteroaryl, optionally substituted heteroarylalkyl, and R<sup>8</sup>R<sup>8'</sup>N-(C<sub>1</sub>-C<sub>4</sub> alkyl);

R<sup>3</sup> is an amino, amido, acylamido, or ureido group, which is optionally substituted; or R<sup>3</sup> is a nitrogen-containing heterocyclyl group attached at a nitrogen atom; and

R<sup>4</sup> is alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, alkylcarbonyl, optionally substituted aryl, optionally substituted arylalkyl, optionally substituted arylhaloalkyl, optionally substituted arylalkoxyalkyl, optionally substituted arylalkenyl, optionally substituted arylhaloalkenyl, or optionally substituted arylalkynyl.

2. The method of claim 1 wherein one or more of the antagonists are selected from compounds of the formula:



and pharmaceutically acceptable salts thereof, wherein

A and A' are each independently selected from  $-\text{CO}_2\text{H}$ , or an ester or amide derivative thereof;

n is an integer selected from 0 to about 3;

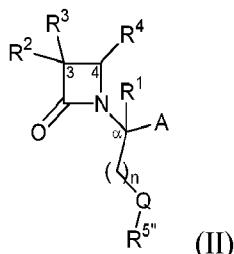
$\text{R}^1$  is hydrogen or  $\text{C}_1\text{-C}_6$  alkyl;

$\text{R}^2$  is hydrogen, alkyl, alkenyl, alkynyl, alkoxy, alkylthio, halo, haloalkyl, cyano, formyl, alkylcarbonyl, or a substituent selected from the group consisting of  $-\text{CO}_2\text{R}^8$ ,  $-\text{CONR}^8\text{R}^{8'}$ , and  $-\text{NR}^8(\text{COR}^9)$ ; where  $\text{R}^8$  and  $\text{R}^{8'}$  are each independently selected from hydrogen, alkyl, cycloalkyl, optionally substituted aryl, or optionally substituted arylalkyl; or  $\text{R}^8$  and  $\text{R}^{8'}$  are taken together with the attached nitrogen atom to form an heterocycle; and where  $\text{R}^9$  is selected from hydrogen, alkyl, cycloalkyl, alkoxyalkyl, optionally substituted aryl, optionally substituted arylalkyl, optionally substituted heteroaryl, optionally substituted heteroarylalkyl, and  $\text{R}^8\text{R}^{8'}\text{N}-(\text{C}_1\text{-C}_4$  alkyl);

$\text{R}^3$  is an amino, amido, acylamido, or ureido group, which is optionally substituted; or  $\text{R}^3$  is a nitrogen-containing heterocyclyl group attached at a nitrogen atom; and

$\text{R}^4$  is alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, alkylcarbonyl, optionally substituted aryl, optionally substituted arylalkyl, optionally substituted arylhaloalkyl, optionally substituted arylalkoxyalkyl, optionally substituted arylalkenyl, optionally substituted arylhaloalkenyl, or optionally substituted arylalkynyl.

3. The method of claim 1 wherein one or more of the antagonists are selected from compounds of the formula:



and pharmaceutically acceptable salts thereof, wherein

A is  $-\text{CO}_2\text{H}$ , or an ester or amide derivative thereof;

Q is oxygen; or Q is sulfur or disulfide, or an oxidized derivative thereof;

n is an integer from 1 to 3;

$\text{R}^1$ ,  $\text{R}^2$ ,  $\text{R}^3$ , and  $\text{R}^4$  are as defined in formula I; and

$\text{R}^5''$  is selected from hydrogen, alkyl, cycloalkyl, alkoxyalkyl, optionally substituted arylalkyl, optionally substituted heterocyclyl or optionally substituted heterocyclalkyl, and optionally substituted aminoalkyl.

4. The method of claim 2 wherein one or both of A and A' is an independently selected monosubstituted amido of the formula  $\text{C}(\text{O})\text{NHX}-$ , where X is selected

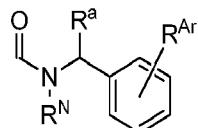
5. The method of claim 2 wherein one or both of A and A' is an independently selected disubstituted amido of the formula C(O)NR<sup>14</sup>X-, where R<sup>14</sup> is selected from hydroxy, alkyl, alkoxy carbonyl, and benzyl; and X is selected from alkyl, cycloalkyl, alkoxyalkyl, optionally substituted aryl, optionally substituted arylalkyl, heterocyclyl, heterocyclyl-(C<sub>1</sub>-C<sub>4</sub> alkyl), R<sup>6</sup>R<sup>7</sup>N-, and R<sup>6</sup>R<sup>7</sup>N-(C<sub>2</sub>-C<sub>4</sub> alkyl), where each heterocyclyl is independently selected.

6. The method of claim 2 wherein one or both of A and A' is an amide of an independently selected optionally substituted nitrogen-containing heterocycle attached at a nitrogen.

7. The method of claim 6 wherein the nitrogen-containing heterocycle is independently selected from pyrrolidinyl, pyrrolidinonyl, piperidinyl, piperidinonyl, piperazinyl, homopiperazinyl, triazolidinyl, triazinyl, oxazolidinyl, isoxazolidinyl, thiazolidinyl, isothiazolidinyl, 1,2-oxazinyl, 1,3-oxazinyl, morpholinyl, oxadiazolidinyl, thiadiazolidinyl, and 1,2,3,4-tetrahydroisoquinolin-2-yl; each of which is optionally substituted.

8. The method of claim 7 wherein the optional substitutions are independently selected from R<sup>10</sup>, R<sup>12</sup>, R<sup>6</sup>R<sup>7</sup>N-, and R<sup>6</sup>R<sup>7</sup>N-(C<sub>1</sub>-C<sub>4</sub> alkyl).

9. The method of claim 2 wherein A is of the formula

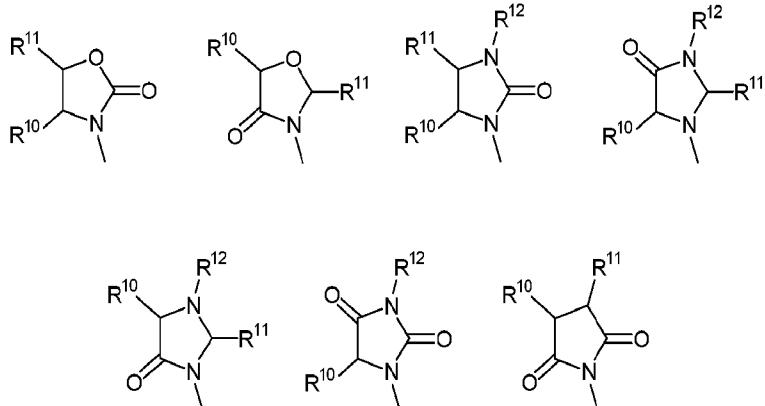


where R<sup>N</sup> is hydrogen or optionally substituted alkyl, or an amide prodrug forming group; R<sup>a</sup> is hydrogen or optionally substituted alkyl; and R<sup>Ar</sup> is hydrogen or one or more aryl substituents.

10. The method of claim 2 wherein one or both of A and A' is an amide of a substituted piperidine or piperazine of the formula:

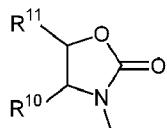

11. The method of claim 3 wherein Q is oxygen or sulfur.

12. The method of claim 3 wherein R<sup>5</sup>" is optionally substituted arylalkyl.
13. The method of claim 2 or 3 wherein n is 1 or 2.
14. The method of claim 2 or 3 wherein R<sup>2</sup> is hydrogen or alkyl.
15. The method of claim 2 or 3 wherein R<sup>1</sup> is hydrogen.
16. The method of claim 2 or 3 wherein R<sup>3</sup> is of the formulae:



wherein R<sup>10</sup>, R<sup>11</sup>, and R<sup>12</sup> are as defined herein.

17. The method of claim 2 or 3 wherein R<sup>3</sup> is of the formula:



wherein R<sup>10</sup> and R<sup>11</sup> are as defined herein.

18. The method of claim 2 or 3 wherein R<sup>4</sup> is of the formulae:



wherein Y is an electron withdrawing group, and Y<sup>1</sup> is hydrogen or one or more aryl substituents.

19. The method of claim 1 wherein at least one compound is selected from the group consisting of AVN228, AVN246, AVN251, AVN296, and AVN576, and combinations thereof.

20. The method of any one of claims 1 to 12 or 19 wherein the brain injury is traumatic brain injury (TBI), including blast TBI, repeated mild TBI (rmTBI), cerebral edema, chronic traumatic encephalopathy (CTE), subarachnoid hemorrhage, stroke, ischemic stroke, concussion, and falls.

21. A pharmaceutical composition adapted for treating a brain injury in a host animal, the composition comprising one or more compounds of any one of claims 1 to 12 or 19, and optionally, one or more carriers, diluents, or adjuvants, or a combination thereof.

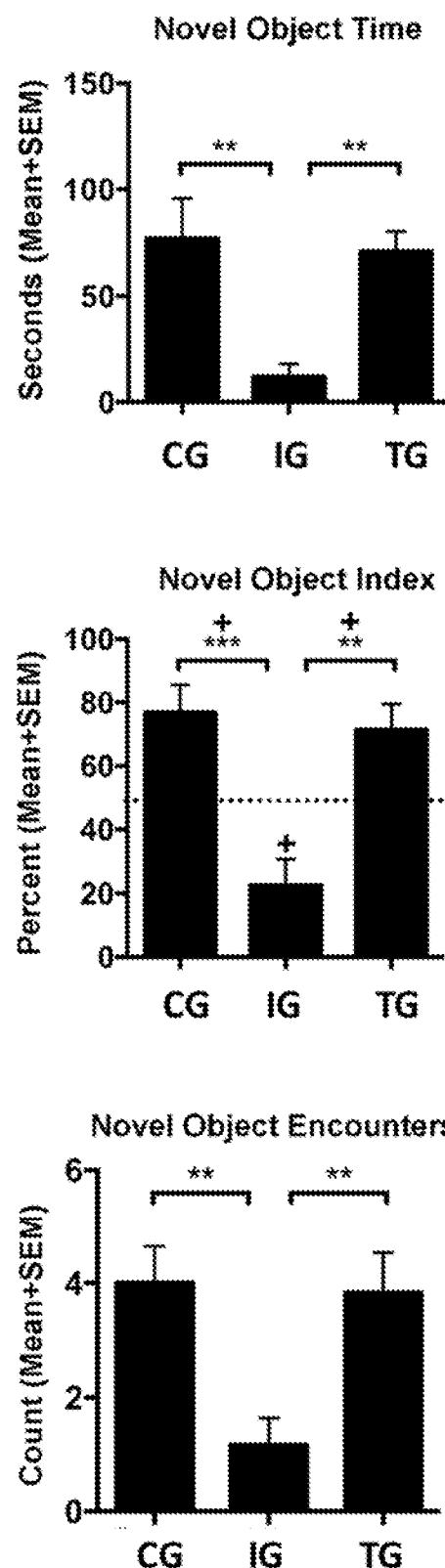


FIG. 1

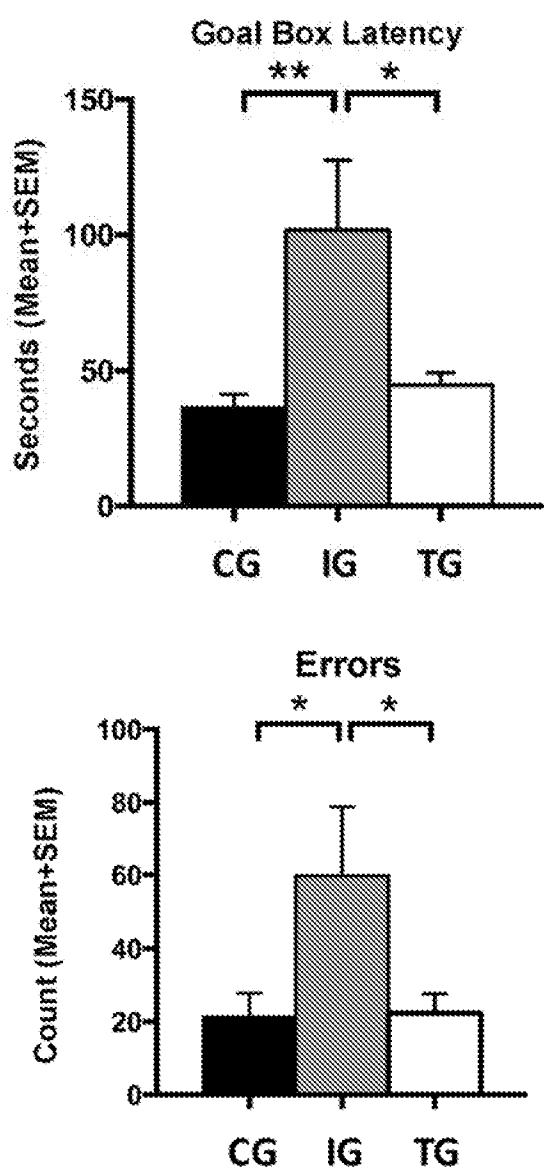


FIG. 2A

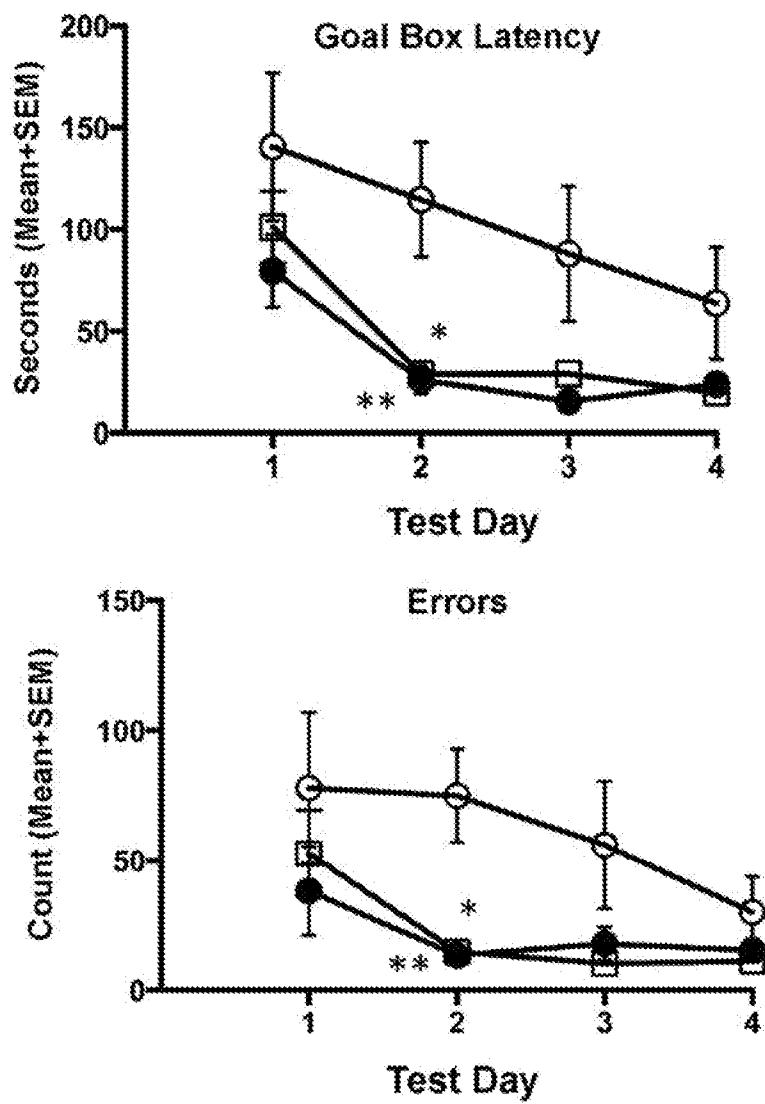


FIG. 2B

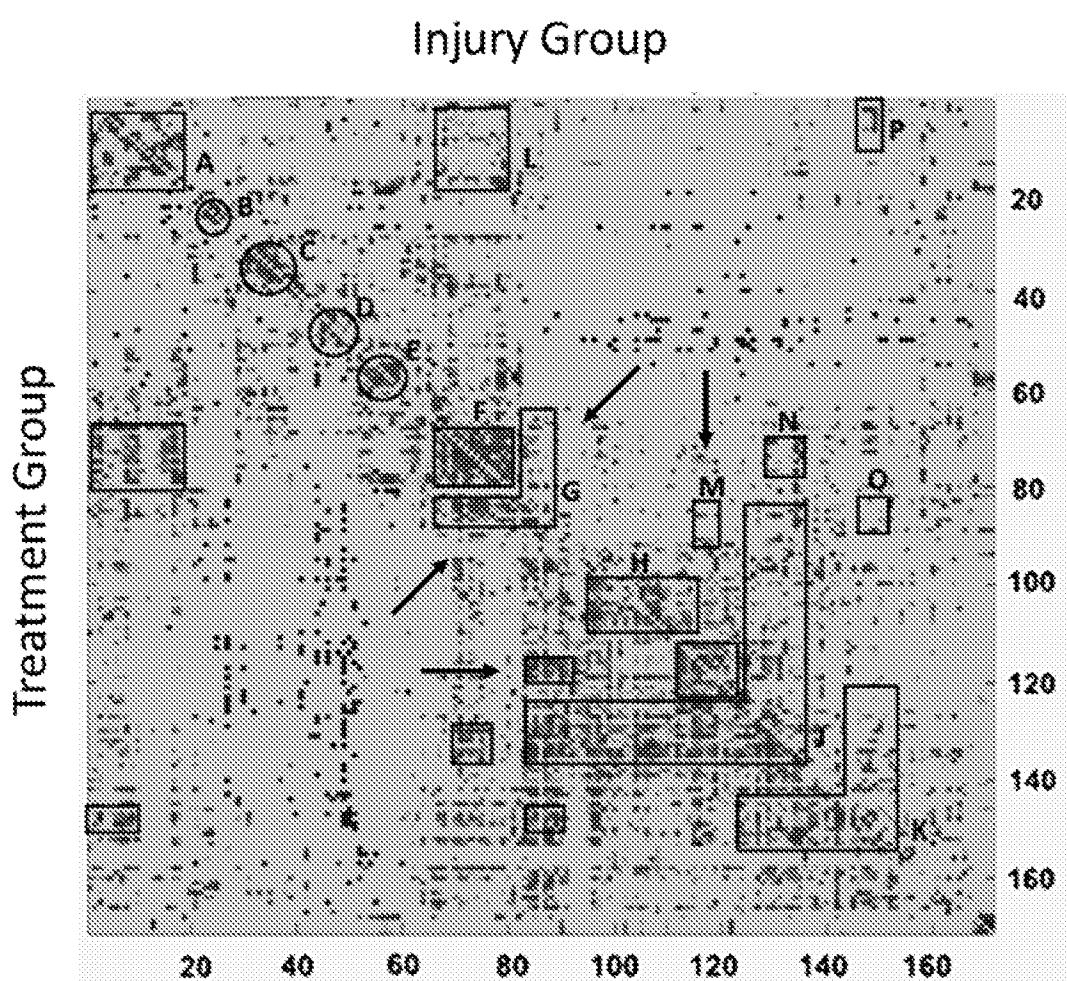


FIG. 3

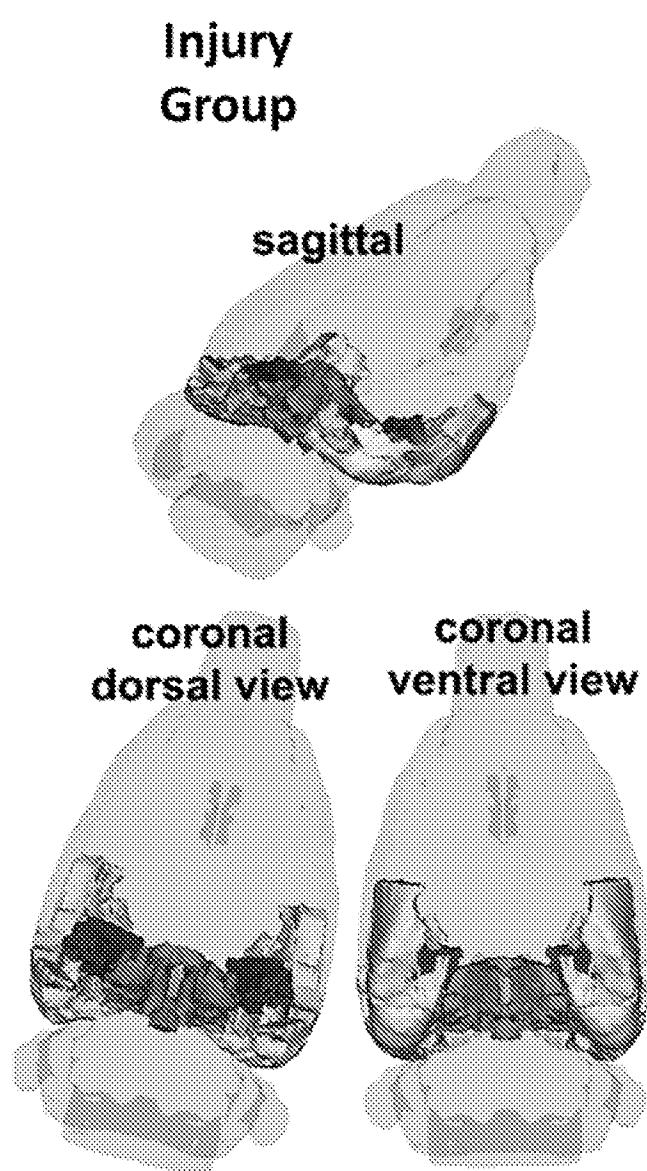


FIG. 4A

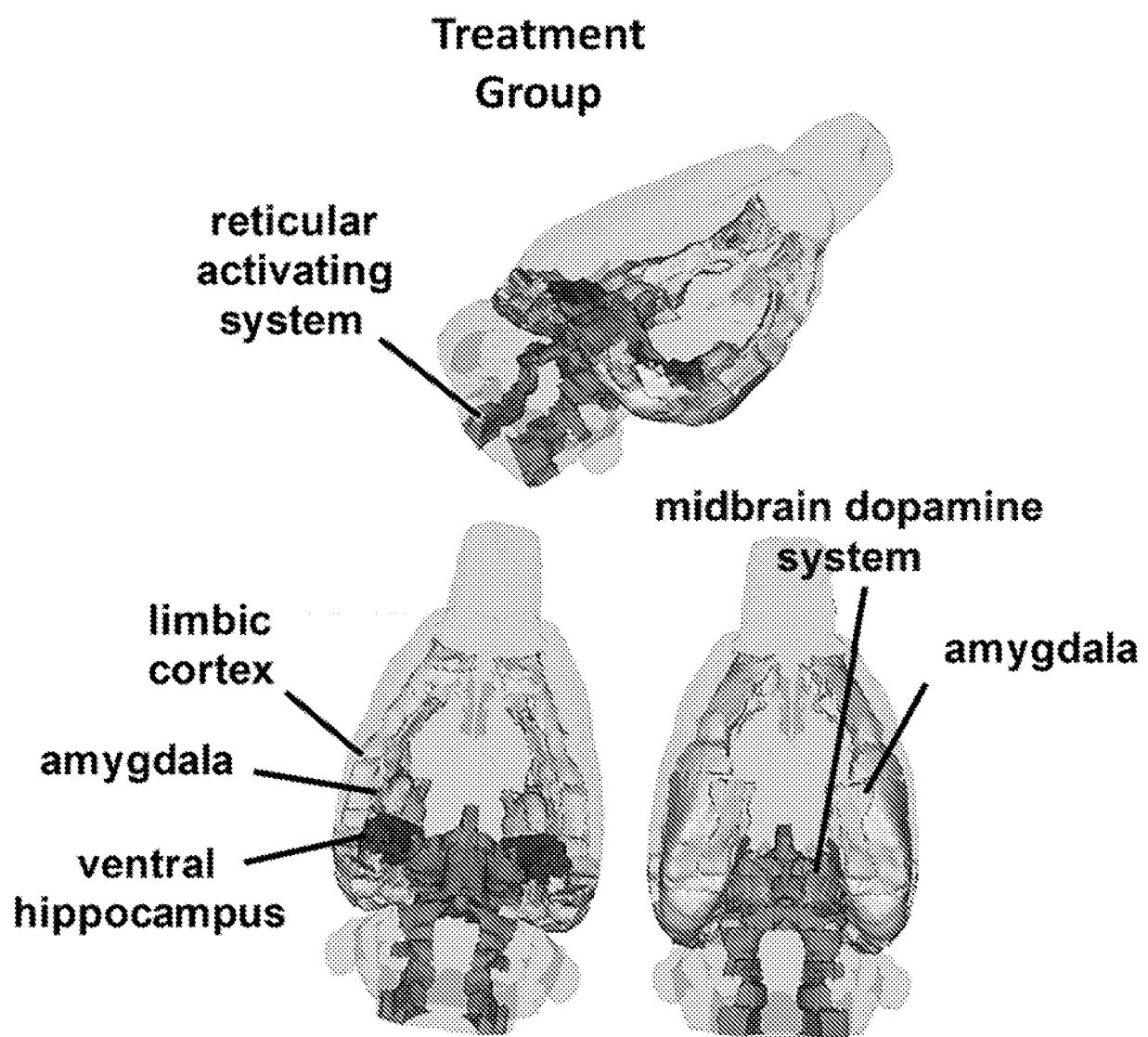


FIG. 4B

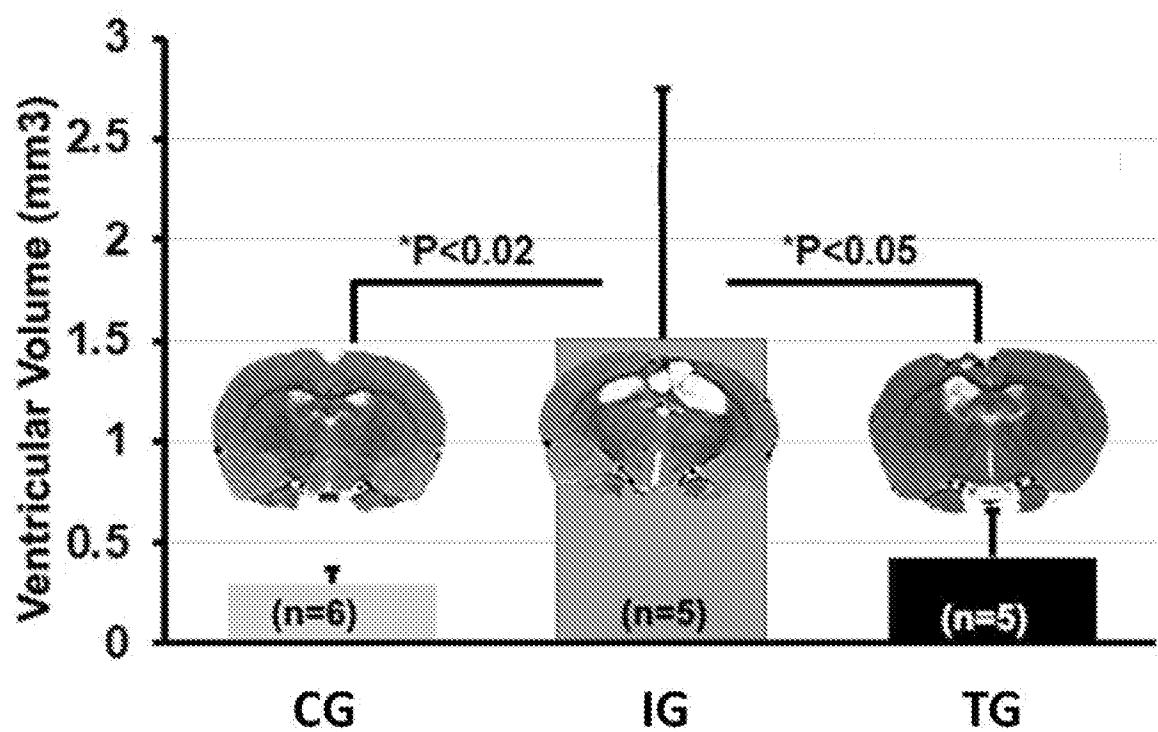


FIG. 5

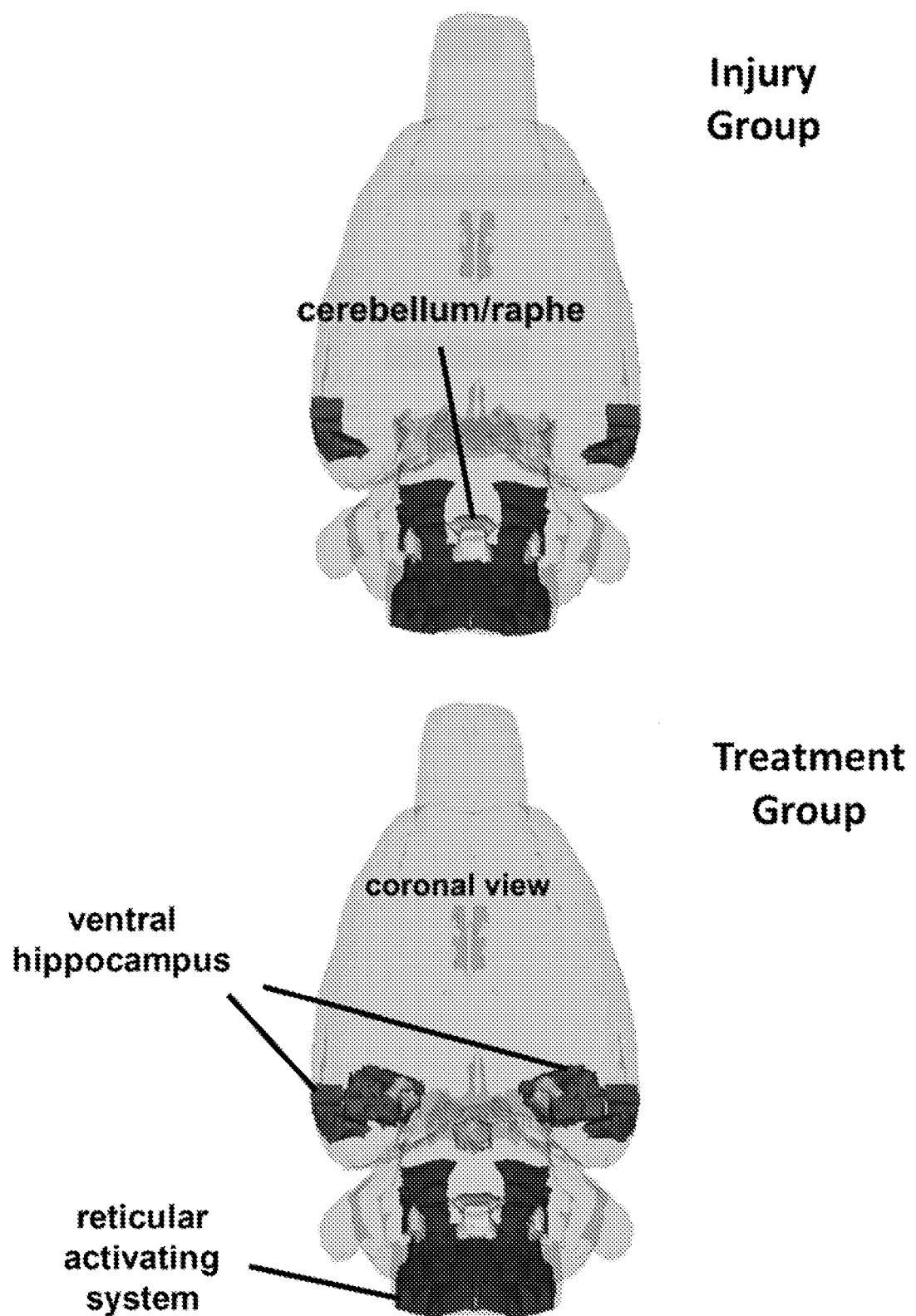


FIG. 6

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/51331

**A. CLASSIFICATION OF SUBJECT MATTER**  
 IPC(8) - C07D 229/02 (2018.01)  
 CPC - C07D 207/16, C07D 211/62, C07D 213/74

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)

See Search History Document

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

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

See Search History Document

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2017/0174670 A1 (Azevan Pharmaceuticals, Inc) 22 June 2017 (22.06.2017); para [0020], [0031], [0356], [0375], [0379]	1-21
Y	WO 2014/127350 A1 (THE SCRIPPS RESEARCH INSTITUTE) 21 August 2014 (21.08.2014); pg. 6, ln 1-6, 31-32, pg. 163, ln 23	1-21
A	WO 2003/031407 A2 (SERENIX PHARMACEUTICALS LLC) 17 April 2003 (17.04.2003); entire document	1-21

Further documents are listed in the continuation of Box C.

See patent family annex.

\* Special categories of cited documents:

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 "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  
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Date of the actual completion of the international search

09 November 2018

Date of mailing of the international search report

30 NOV 2018

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