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(71) Applicant (for all designated States except US): **BRISTOL-MYERS SQUIBB COMPANY** [US/US]; Route 206 and Province Line Road, Princeton, NJ 08543-4000 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **DYCKMAN, Alaric, J.** [US/US]; c/o Bristol-Myers Squibb Company, Route 206 & Province Line Road, Princeton, NJ 08543 (US). **PITTS, William, J.** [US/US]; c/o Bristol-Myers Squibb Company, Route 206 & Province Line Road, Princeton, NJ 08543 (US). **WATTERSON, Scott, Hunter** [US/US]; c/o Bristol-Myers Squibb Company, Route 206 & Province Line Road, Princeton, NJ 08543 (US).

(74) Agents: **GREENBLATT, Gary, D.** et al.; Bristol-Myers Squibb Company, P.O. Box 4000, Princeton, NJ 08543-4000 (US).

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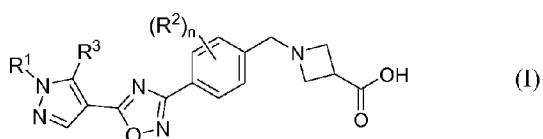
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(54) Title: PYRAZOLE-1, 2, 4 -OXADIAZOLE DERIVATIVES AS S.PHINGOSINE-1-PHOSPHATE AGONISTS



(57) Abstract: Disclosed are compounds of Formula (I) or a pharmaceutically acceptable salt thereof, wherein: n is zero or an integer selected from 1 through 4; R<sup>1</sup> is cycloalkyl, aryl, heteroaryl, or heterocyclyl, each optionally substituted with one to five substituents independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, benzyl, OR<sup>4</sup>, and/or halogen; and R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, and n are defined herein. Also disclosed are methods of using such compounds as selective agonists for G protein-coupled receptor S1P<sub>1</sub>, and pharmaceutical compositions comprising such compounds. These compounds are useful in treating, preventing, or slowing the progression of diseases or disorders in a variety of therapeutic areas, such as autoimmune diseases and vascular disease.

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## SUBSTITUTED PYRAZOLE COMPOUNDS

### DESCRIPTION

- [0001] The present invention generally relates to substituted pyrazole compounds useful as S1P<sub>1</sub> agonists. Provided herein are substituted pyrazole compounds, compositions comprising such compounds, and methods of their use. The invention further pertains to pharmaceutical compositions comprising at least one compound according to the invention that are useful for the treatment of conditions related to S1P<sub>1</sub> agonism, such as autoimmune diseases and vascular disease.
- [0002] Sphingosine-1-phosphate (S1P) has been demonstrated to induce many cellular effects, including those that result in platelet aggregation, cell proliferation, cell morphology, tumor cell invasion, endothelial cell and leukocyte chemotaxis, endothelial cell *in vitro* angiogenesis, and lymphocyte trafficking. S1P receptors are therefore good targets for a wide variety of therapeutic applications such as tumor growth inhibition, vascular disease, and autoimmune diseases. S1P signals cells in part via a set of G protein-coupled receptors named S1P<sub>1</sub> or S1P1, S1P<sub>2</sub> or S1P2, S1P<sub>3</sub> or S1P3, S1P<sub>4</sub> or S1P4, and S1P<sub>5</sub> or S1P5 (formerly called EDG-1, EDG-5, EDG-3, EDG-6, and EDG-8, respectively).
- [0003] S1P is important in the entire human body as it is also a major regulator of the vascular and immune systems. In the vascular system, S1P regulates angiogenesis, vascular stability, and permeability. In the immune system, S1P is recognized as a major regulator of trafficking of T- and B-cells. S1P interaction with its receptor S1P<sub>1</sub> is needed for the egress of immune cells from the lymphoid organs (such as thymus and lymph nodes) into the lymphatic vessels. Therefore, modulation of S1P receptors was shown to be critical for immunomodulation, and S1P receptor modulators are novel immunosuppressive agents.
- [0004] The S1P<sub>1</sub> receptor is expressed in a number of tissues. It is the predominant family member expressed on lymphocytes and plays an important role in lymphocyte trafficking. Downregulation of the S1P<sub>1</sub> receptor disrupts lymphocyte migration and homing to various tissues. This results in sequestration of the lymphocytes in lymph organs thereby decreasing the number of circulating lymphocytes that are capable of migration to the affected tissues. Thus, development

of an S1P<sub>1</sub> receptor agent that suppresses lymphocyte migration to the target sites associated with autoimmune and aberrant inflammatory processes could be efficacious in a number of autoimmune and inflammatory disease states.

**[0005]** Among the five S1P receptors, S1P<sub>1</sub> has a widespread distribution and is 5 highly abundant on endothelial cells where it works in concert with S1P<sub>3</sub> to regulate cell migration, differentiation, and barrier function. Inhibition of lymphocyte recirculation by non-selective S1P receptor modulation produces clinical immunosuppression preventing transplant rejection, but such modulation also results in transient bradycardia. Studies have shown that S1P<sub>1</sub> activity is significantly 10 correlated with depletion of circulating lymphocytes. In contrast, S1P<sub>3</sub> receptor agonism is not required for efficacy. Instead, S1P<sub>3</sub> activity plays a significant role in the observed acute toxicity of nonselective S1P receptor agonists, resulting in the undesirable cardiovascular effects, such as bradycardia and hypertension. (See, e.g., Hale et al., *Bioorg. Med. Chem. Lett.*, 14:3501 (2004); Sanna et al., *J. Biol. Chem.*, 15 279:13839 (2004); Anliker et al., *J. Biol. Chem.*, 279:20555 (2004); Mandala et al., *J. Pharmacol. Exp. Ther.*, 309:758 (2004).)

**[0006]** An example of an S1P<sub>1</sub> agonist is FTY720. This immunosuppressive compound FTY720 (JPI 1080026-A) has been shown to reduce circulating 20 lymphocytes in animals and humans, and to have disease modulating activity in animal models of organ rejection and immune disorders. The use of FTY720 in humans has been effective in reducing the rate of organ rejection in human renal transplantation and increasing the remission rates in relapsing remitting multiple sclerosis (see Brinkman et al., *J. Biol. Chem.*, 277:21453 (2002); Mandala et al., *Science*, 296:346 (2002); Fujino et al., *J. Pharmacol. Exp. Ther.*, 305:45658 (2003); 25 Brinkman et al., *Am. J. Transplant.*, 4:1019 (2004); Webb et al., *J. Neuroimmunol.*, 153:108 (2004); Morris et al., *Eur. J. Immunol.*, 35:3570 (2005); Chiba, *Pharmacology & Therapeutics*, 108:308 (2005); Kahan et al., *Transplantation*, 76:1079 (2003); and Kappos et al., *N. Engl. J. Med.*, 335:1124 (2006)). Subsequent to its discovery, it has been established that FTY720 is a prodrug, which is 30 phosphorylated *in vivo* by sphingosine kinases to a more biologically active agent that has agonist activity at the S1P<sub>1</sub>, S1P<sub>3</sub>, S1P<sub>4</sub>, and S1P<sub>5</sub> receptors. It is this activity on

the S1P family of receptors that is largely responsible for the pharmacological effects of FTY720 in animals and humans.

[0007] Clinical studies have demonstrated that treatment with FTY720 results in bradycardia in the first 24 hours of treatment (Kappos et al., *N. Engl. J. Med.*, 5 335:1124 (2006)). The observed bradycardia is commonly thought to be due to agonism at the S1P<sub>3</sub> receptor. This conclusion is based on a number of cell based and animal experiments. These include the use of S1P<sub>3</sub> knockout animals which, unlike wild type mice, do not demonstrate bradycardia following FTY720 administration and the use of S1P<sub>1</sub> selective compounds. (Hale et al., *Bioorg. Med. Chem. Lett.*, 10 14:3501 (2004); Sanna et al., *J. Biol. Chem.*, 279:13839 (2004); and Koyrakh et al., *Am. J. Transplant.*, 5:529 (2005)).

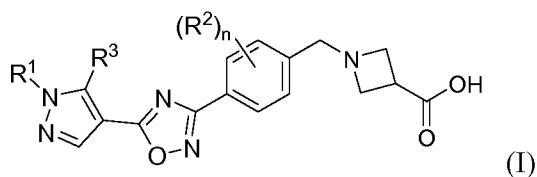
[0008] The following applications have described compounds as S1P<sub>1</sub> agonists: WO 03/061567 (U.S. Publication No. 2005/0070506), WO 03/062248 (U.S. Patent No. 7,351,725), WO 03/062252 (U.S. Publication No. 2005/0033055), WO 15 03/073986 (U.S. Patent No. 7,309,721), WO 03/105771, WO 05/058848, WO 06/047195, WO 06/100633, WO 06/115188, WO 06/131336, WO 2007/024922, WO 07/116866, WO 08/023783 (U.S. Publication No. 2008/0200535), and WO 08/074820. Also see Hale et al., *J. Med. Chem.*, 47:6662 (2004).

[0009] There still remains a need for compounds useful as S1P<sub>1</sub> agonists and yet 20 having selectivity over S1P<sub>3</sub>.

[0010] Applicants have found potent compounds that have activity as S1P<sub>1</sub> agonists. Further, applicants have found compounds that have activity as S1P<sub>1</sub> agonists and are selective over S1P<sub>3</sub>. These compounds are provided to be useful as 25 pharmaceuticals with desirable stability, bioavailability, therapeutic index, and toxicity values that are important to their drugability.

#### SUMMARY OF THE INVENTION

[0011] The present invention fills the foregoing need by providing compounds of Formula (I):



or pharmaceutically acceptable salts thereof, wherein:

n is zero or an integer selected from 1 through 4;

R<sup>1</sup> is cycloalkyl, aryl, heteroaryl, or heterocyclyl, each optionally substituted with one

5 to five substituents independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, benzyl, -OR<sup>4</sup>, and/or halogen;

each R<sup>2</sup> is independently selected from hydrogen, C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, -OR<sup>4</sup>, and/or halogen;

R<sup>3</sup> is hydrogen, alkyl, haloalkyl, halogen, -NR<sup>a</sup>R<sup>b</sup>, cycloalkyl, aryl, heteroaryl, or

10 heterocyclyl, wherein said cycloalkyl, aryl, heteroaryl, and heterocyclyl each may optionally be substituted with one to five substituents independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, -OR<sup>4</sup>, and/or halogen;

each R<sup>4</sup> is independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, and/or benzyl; and

15 R<sup>a</sup> and R<sup>b</sup> are each independently hydrogen, alkyl, and/or benzyl.

**[0012]** Also described are pharmaceutical compositions comprising a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and a pharmaceutically acceptable carrier.

20 **[0013]** Further described is a method of treating a disease or disorder associated with the activity of G protein-coupled receptor S1P<sub>1</sub>, the method comprising administering to a mammalian patient a compound of Formula (I) or a pharmaceutically acceptable salt thereof.

**[0014]** The compounds of Formula (I) and compositions comprising the 25 compounds are S1P<sub>1</sub> agonists, which are selective for S1P<sub>1</sub> activity over S1P<sub>3</sub> activity. The compounds of Formula (I) and compositions comprising said compounds may be used in treating, preventing or curing various S1P<sub>1</sub> receptor-related conditions while reducing or minimizing the side effects due to S1P<sub>3</sub> activity. Pharmaceutical compositions comprising these compounds are useful in treating,

preventing, or slowing the progression of diseases or disorders in a variety of therapeutic areas, such as autoimmune and vascular diseases.

#### DETAILED DESCRIPTION

- 5 [0015] One embodiment provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein:  
n is zero or an integer selected from 1 through 4;  
R<sup>1</sup> is C<sub>3</sub> to C<sub>8</sub> cycloalkyl, aryl, heteroaryl, or heterocyclyl, each optionally substituted with one to five substituents independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to  
10 C<sub>4</sub> haloalkyl, benzyl, -OR<sup>4</sup>, and/or halogen;  
each R<sup>2</sup> is independently selected from hydrogen, C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, -OR<sup>4</sup>, and/or halogen;  
R<sup>3</sup> is C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, halogen, -NR<sup>a</sup>R<sup>b</sup>, C<sub>3</sub> to C<sub>8</sub> cycloalkyl, aryl, heteroaryl, or heterocyclyl, wherein said cycloalkyl, aryl, heteroaryl, and  
15 heterocyclyl each may optionally be substituted with one to five substituents independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, -OR<sup>4</sup>, and/or halogen;  
each R<sup>4</sup> is independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, and/or benzyl; and  
20 R<sup>a</sup> and R<sup>b</sup> are each independently hydrogen, C<sub>1</sub> to C<sub>6</sub> alkyl, and/or benzyl.

[0016] One embodiment provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein n is zero or 1.

- [0017] One embodiment provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein n is zero.
- [0018] One embodiment provides a 2,2,2-trifluoroacetic acid salt of a compound according to Formula (I).
- [0019] One embodiment provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein R<sup>1</sup> is aryl optionally substituted with one, two, or three substituents independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, -OR<sup>4</sup>, and/or halogen. Preferably, R<sup>1</sup> is phenyl. For example, this embodiment provides compounds of Formula (I) wherein n is zero or 1. Preferably,

$R^3$  is  $C_1$  to  $C_4$  alkyl,  $C_1$  to  $C_2$  haloalkyl, halogen,  $-NH(C_1$  to  $C_4$  alkyl),  $C_3$  to  $C_6$  cycloalkyl, phenyl, or pyridinyl, wherein said cycloalkyl, phenyl, and pyridinyl each may optionally be substituted with one to three substituents independently selected from  $C_1$  to  $C_6$  alkyl,  $C_1$  to  $C_4$  haloalkyl,  $-OR^4$ , and/or halogen. Included in this

5 embodiment are compounds in which  $R^1$  is aryl, preferably phenyl, optionally substituted with one to five substituents independently selected from  $C_1$  to  $C_6$  alkyl,  $C_1$  to  $C_4$  haloalkyl, benzyl,  $-OR^4$ , and/or halogen; and  $R^3$  is haloalkyl,  $-NR^aR^b$ , cycloalkyl, heteroaryl, or heterocyclyl, wherein said cycloalkyl, heteroaryl, and heterocyclyl each may optionally be substituted with one to five substituents

10 independently selected from  $C_1$  to  $C_6$  alkyl,  $C_1$  to  $C_4$  haloalkyl,  $-OR^4$ , and/or halogen.

**[0020]** One embodiment provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein  $R^1$  is phenyl optionally substituted with one, two, or three substituents independently selected from  $C_1$  to  $C_6$  alkyl,  $C_1$  to  $C_4$  haloalkyl,  $-OR^4$ , and/or halogen;  $R^3$  is  $C_1$  to  $C_6$  alkyl,  $C_1$  to  $C_4$  haloalkyl, halogen,  $-NR^aR^b$ ,  $C_3$  to  $C_6$  cycloalkyl, phenyl, or pyridyl;  $R^4$  is  $C_1$  to  $C_4$  alkyl; and  $R^a$  and  $R^b$  are independently hydrogen and/or  $C_1$  to  $C_6$  alkyl. For example, this embodiment provides compounds of Formula (I) wherein  $n$  is zero or 1; and preferably,  $n$  is zero. Other examples of this embodiment include compounds in which  $R^3$  is methyl, ethyl, 1-propyl, cyclopentyl, trifluoromethyl, bromo, chloro, phenyl, 2-pyridyl, or  $-NHR^a$ ; and compounds in which  $R^a$  is methyl, ethyl, 1-propyl, 2-propyl, n-butyl, or isobutyl.

**[0021]** One embodiment provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein  $R^1$  is cycloalkyl, heteroaryl, or heterocyclyl, each optionally substituted with one to five substituents independently selected from  $C_1$  to  $C_6$  alkyl,  $C_1$  to  $C_4$  haloalkyl, benzyl,  $-OR^4$ , and/or halogen.

25 Suitable groups for  $R^1$  include  $C_3$  to  $C_8$  cycloalkyl, 1-ring heteroaryls having one to two heteroatoms independently selected from N, O, and S; and 1-ring heteroaryls having one to two heteroatoms independently selected from N, O, and S. Examples of suitable  $R^1$  groups include, but are not limited to, cyclopentyl, cyclohexyl, phenyl, pyridinyl, pyrimidinyl, pyrazolyl, and piperidinyl. This embodiment provides

30 compounds of Formula (I) wherein  $n$  is zero or 1; and preferably,  $n$  is zero. Other examples of this embodiment include compounds in which  $R^3$  is methyl, ethyl, 1-propyl, cyclopentyl, trifluoromethyl, bromo, chloro, phenyl, 2-pyridyl, or  $-NHR^a$ ; and

compounds in which R<sup>a</sup> is methyl, ethyl, 1-propyl, 2-propyl, n-butyl, or isobutyl.

Included in this embodiment are compounds in which R<sup>1</sup> is cycloalkyl, heteroaryl, or heterocyclyl, preferably cyclopentyl or pyridinyl, each optionally substituted with one to five substituents independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl,

5      benzyl, -OR<sup>4</sup>, and/or halogen; and R<sup>3</sup> is haloalkyl, -NR<sup>a</sup>R<sup>b</sup>, cycloalkyl, heteroaryl, or heterocyclyl, wherein said cycloalkyl, heteroaryl, and heterocyclyl each may optionally be substituted with one to five substituents independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, -OR<sup>4</sup>, and/or halogen.

**[0022]**      One embodiment provides a compound of Formula (I) or a

10     pharmaceutically acceptable salt thereof, wherein R<sup>1</sup> is cycloalkyl, optionally substituted with one to five substituents independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, benzyl, -OR<sup>4</sup>, and/or halogen. Suitable groups for R<sup>1</sup> include C<sub>3</sub> to C<sub>8</sub> cycloalkyl groups, and C<sub>3</sub> to C<sub>6</sub> cycloalkyl groups. This embodiment provides compounds of Formula (I) wherein n is zero or 1; and preferably, n is zero. Other 15     examples of this embodiment include compounds in which R<sup>3</sup> is methyl, ethyl, 1-propyl, cyclopentyl, trifluoromethyl, bromo, chloro, phenyl, 2-pyridyl, or -NHR<sup>a</sup>; and compounds in which R<sup>a</sup> is methyl, ethyl, 1-propyl, 2-propyl, n-butyl, or isobutyl.

Included in this embodiment are compounds in which R<sup>1</sup> is cycloalkyl, preferably cyclopentyl or cyclohexyl, each optionally substituted with one to five substituents

20     independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, benzyl, -OR<sup>4</sup>, and/or halogen; and R<sup>3</sup> is haloalkyl, -NR<sup>a</sup>R<sup>b</sup>, cycloalkyl, heteroaryl, or heterocyclyl, wherein said cycloalkyl may optionally be substituted with one to five substituents independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, -OR<sup>4</sup>, and/or halogen.

**[0023]**      One embodiment provides a compound of Formula (I) or a

25     pharmaceutically acceptable salt thereof, wherein R<sup>1</sup> is a heteroaryl, optionally substituted with one to five substituents independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, benzyl, -OR<sup>4</sup>, and/or halogen. Suitable groups for R<sup>1</sup> include 1- and 2-ring heteroaryls having one to four heteroatoms independently selected from N, O, and S. Other suitable groups for R<sup>1</sup> include 1- and 2-ring heteroaryls having one 30     nitrogen heteroatom and optionally one to three other heteroatoms independently selected from N, O, and S. Examples of suitable heteroaryl groups include, but are not limited to, pyrrolyl, pyrazolyl, pyrazolinyl, imidazolyl, oxazolyl, isoxazolyl,

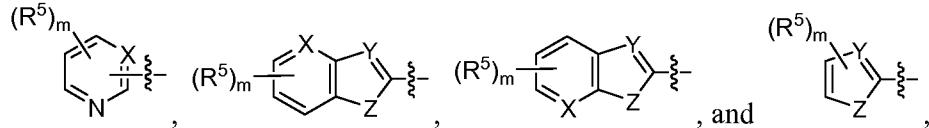
thiazolyl, thiadiazolyl, isothiazolyl, furanyl, thiophenyl, oxadiazolyl, pyridinyl, pyrazinyl, pyrimidinyl, pyridazinyl, triazinyl, tetrazolyl, and indolyl. Preferably, R<sup>1</sup> is substituted with zero to three substituents; and more preferably, zero to two substituents. Other examples of this embodiment include compounds in which R<sup>3</sup> is

5 methyl, ethyl, 1-propyl, cyclopentyl, trifluoromethyl, bromo, chloro, phenyl, 2-pyridyl, or -NHR<sup>a</sup>; and compounds in which R<sup>a</sup> is methyl, ethyl, 1-propyl, 2-propyl, n-butyl, or isobutyl. Included in this embodiment are compounds in which R<sup>1</sup> is heteroaryl, preferably a 1-ring heteroaryl such as pyridinyl, optionally substituted with one to five substituents independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, benzyl, -OR<sup>4</sup>, and/or halogen; and R<sup>3</sup> is haloalkyl, -NR<sup>a</sup>R<sup>b</sup>, cycloalkyl, heteroaryl, or heterocyclyl, wherein said cycloalkyl, heteroaryl, and heterocyclyl each may optionally be substituted with one to five substituents independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, -OR<sup>4</sup>, and/or halogen.

10

[0024] One embodiment provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein:

15 R<sup>1</sup> is selected from:



wherein:

m is zero, 1, 2, or 3;

20 X and Y are each independently nitrogen (N) and/or CR<sup>5</sup>;

Z is oxygen (O), sulfur (S), or N-R<sup>6</sup>;

each R<sup>5</sup> is independently selected from C<sub>1</sub> to C<sub>4</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, -OR<sup>4</sup>, and/or halogen;

R<sup>6</sup> is C<sub>1</sub> to C<sub>4</sub> alkyl or benzyl; and

25 each R<sup>4</sup> is independently selected from C<sub>1</sub> to C<sub>4</sub> alkyl.

This embodiment provides compounds of Formula (I) wherein n is zero or 1; and preferably, n is zero.

[0025] One embodiment provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein:

30

R<sup>1</sup> is 2-pyridyl optionally substituted with one, two, or three substituents independently selected from C<sub>1</sub> to C<sub>4</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, -OR<sup>4</sup>, and/or halogen;

R<sup>3</sup> is C<sub>1</sub> to C<sub>4</sub> alkyl, C<sub>3</sub> to C<sub>6</sub> cycloalkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, halogen, phenyl, pyridyl, or -NR<sup>a</sup>R<sup>b</sup>; R<sup>4</sup> is C<sub>1</sub> to C<sub>4</sub> alkyl; and

R<sup>a</sup> and R<sup>b</sup> are each independently hydrogen and/or C<sub>1</sub> to C<sub>4</sub> alkyl.

This embodiment provides compounds of Formula (I) wherein n is zero or 1; and preferably, n is zero.

- 10 [0026] One embodiment provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein R<sup>1</sup> is a heterocyclyl, optionally substituted with one to five substituents independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, benzyl, -OR<sup>4</sup>, and/or halogen. Suitable groups for R<sup>1</sup> include 1- and 2-ring heterocyclyls having one to three heteroatoms independently selected from N, O, and S. Other suitable groups for R<sup>1</sup> include 1- and 2-ring heterocyclyls having one nitrogen heteroatom and optionally one or two other heteroatoms independently selected from N, O, and S. Examples of suitable heterocyclyl groups include, but are not limited to, oxetanyl, azetidinyl, pyrrolidinyl, imidazolinyl, oxazolidinyl, isoxazolinyl, thiazolidinyl, isothiazolidinyl, tetrahydrofuranyl, piperidinyl, 20 piperazinyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolodinyl, 2-oxoazepinyl, azepinyl, 4-piperidonyl, tetrahydropyranyl, morpholinyl, thiomorpholinyl, thiomorpholinyl sulfoxide, thiomorpholinyl sulfone, 1,3-dioxolane, tetrahydro-1,1-dioxothienyl, and quinuclidinyl. Other examples of this embodiment include compounds in which R<sup>3</sup> is methyl, ethyl, 1-propyl, cyclopentyl, trifluoromethyl, bromo, chloro, phenyl, 2-pyridyl, or -NHR<sup>a</sup>; and compounds in which R<sup>a</sup> is methyl, ethyl, 1-propyl, 2-propyl, n-butyl, or isobutyl. Included in this embodiment are compounds in which R<sup>1</sup> is heterocyclyl, preferably 1-ring heterocyclyl, optionally substituted with one to five substituents independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, benzyl, -OR<sup>4</sup>, and/or halogen; and R<sup>3</sup> is haloalkyl, -NR<sup>a</sup>R<sup>b</sup>, 25 cycloalkyl, heteroaryl, or heterocyclyl, wherein said cycloalkyl, heteroaryl, and heterocyclyl each may optionally be substituted with one to five substituents independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, -OR<sup>4</sup>, and/or halogen.

**[0027]** One embodiment provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof, selected from the group consisting of:

- 1-(4-(5-(1-(pyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid;
- 5 1-(4-(5-(1-phenyl-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid;
- 1-(4-(5-(isopropylamino)-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid;
- 10 1-(4-(5-(ethylamino)-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid;
- 1-(4-(5-(5-methyl-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid;
- 15 1-(4-(5-(5-propyl-1-(pyridin-2-yl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid;
- 1-(4-(5-(1-(6-ethoxypyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid;
- 20 1-(4-(5-(1-cyclohexyl-5-methyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid;
- 1-(4-(5-(5-phenyl-1-(pyridin-2-yl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid;
- 25 1-(4-(5-(5-chloro-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid;
- 1-(4-(5-(isobutylamino)-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid;
- 30 1-(4-(5-(n-butylamino)-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid;
- 1-(4-(5-bromo-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid; and

1-(4-(5-(1-phenyl-5-(pyridin-3-yl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid. The compounds of this embodiment may be provided as 2,2,2-trifluoroacetic acid salts.

- 5 [0028] One embodiment provides a composition comprising a compound according to Formula (I) or a pharmaceutically acceptable salt thereof; and a pharmaceutically acceptable carrier.
- [0029] One embodiment provides a method of treating a disease or disorder associated with the activity of G protein-coupled receptor S1P<sub>1</sub>, the method
- 10 comprising administering to a mammalian patient of a compound of Formula (I) or a pharmaceutically acceptable salt thereof.

#### DEFINITIONS

- [0030] The features and advantages of the invention may be more readily understood by those of ordinary skill in the art upon reading the following detailed description. It is to be appreciated that certain features of the invention that are, for clarity reasons, described above and below in the context of separate embodiments, may also be combined to form a single embodiment. Conversely, various features of the invention that are, for brevity reasons, described in the context of a single embodiment, may also be combined so as to form sub-combinations thereof. Embodiments identified herein as exemplary or preferred are intended to be illustrative and not limiting.
- [0031] Unless specifically stated otherwise herein, references made in the singular may also include the plural. For example, "a" and "an" may refer to either one, or one or more.
- [0032] Unless otherwise indicated, any heteroatom with unsatisfied valences is assumed to have hydrogen atoms sufficient to satisfy the valences.
- [0033] The definitions set forth herein take precedence over definitions set forth in any patent, patent application, and/or patent application publication incorporated herein by reference.
- [0034] Listed below are definitions of various terms used to describe the present invention. These definitions apply to the terms as they are used throughout the

specification (unless they are otherwise limited in specific instances) either individually or as part of a larger group.

**[0035]** Throughout the specification, groups and substituents thereof may be chosen by one skilled in the field to provide stable moieties and compounds.

5 **[0036]** In accordance with a convention used in the art,



is used in structural formulas herein to depict the bond that is the point of attachment of the moiety or substituent to the core or backbone structure.

**[0037]** The terms “halo” and “halogen,” as used herein, refer to F, Cl, Br, or I.

10 **[0038]** The term “alkyl” as used herein, refers to both branched and straight-chain saturated aliphatic hydrocarbon groups containing, for example, from 1 to 12 carbon atoms, from 1 to 6 carbon atoms, and from 1 to 4 carbon atoms. Examples of alkyl groups include, but are not limited to, methyl (Me), ethyl (Et), propyl (e.g., n-propyl and i-propyl), butyl (e.g., n-butyl, i-butyl, sec-butyl, and t-butyl), and pentyl (e.g., n-pentyl, isopentyl, neopentyl), n-hexyl, 2-methylpentyl, 2-ethylbutyl, 3-methylpentyl, and 4-methylpentyl. When numbers appear in a subscript after the symbol “C”, the subscript defines with more specificity the number of carbon atoms that a particular group may contain. For example, “C<sub>1</sub>-C<sub>6</sub> alkyl” denotes straight and branched chain alkyl groups with one to six carbon atoms.

15 **[0039]** The term “haloalkyl,” as used herein, refers to an alkyl group in which one or more hydrogen atoms are replaced by halogen atom(s), the number of which can range from one up to the total number of hydrogen atoms that could otherwise exist in the parent alkyl group. Representative examples of haloalkyl groups include, but are not limited to, chloromethyl (CH<sub>2</sub>Cl-), trifluoromethyl (CF<sub>3</sub>-), and 2,2,2-trfluoroethyl (CF<sub>3</sub>CH<sub>2</sub>-). When numbers appear in a subscript after the symbol “C”, the subscript defines with more specificity the number of carbon atoms that a particular haloalkyl group may contain. For example, “C<sub>1</sub>-C<sub>4</sub> haloalkyl” denotes straight and branched chain haloalkyl groups with one to four carbon atoms.

20 **[0040]** The term “cycloalkyl,” as used herein, refers to a group derived from a non-aromatic monocyclic or polycyclic hydrocarbon molecule by removal of one hydrogen atom from a saturated ring carbon atom. Representative examples of

cycloalkyl groups include, but are not limited to, cyclopropyl, cyclopentyl, and cyclohexyl. When numbers appear in a subscript after the symbol “C”, the subscript defines with more specificity the number of carbon atoms that a particular cycloalkyl group may contain. For example, “C<sub>3</sub>-C<sub>6</sub> cycloalkyl” denotes cycloalkyl groups with three to six carbon atoms.

**[0041]** The term “alkoxy,” as used herein, refers to an alkyl group attached to the parent molecular moiety through an oxygen atom, for example, methoxy group (-OCH<sub>3</sub>).

**[0042]** The term “aryl,” as used herein, refers to a group of atoms derived from a molecule containing aromatic ring(s) by removing one hydrogen that is bonded to the aromatic ring(s). Representative examples of aryl groups include, but are not limited to, phenyl, naphthyl, indanyl, indenyl, and 1,2,3,4-tetrahydronaphth-5-yl.

**[0043]** The term “benzyl,” as used herein, refers to a methyl group in which one of the hydrogen atoms is replaced by a phenyl group.

**[0044]** The term “heteroatom” as used herein refers to oxygen, sulfur, and nitrogen.

**[0045]** The term “heterocyclyl” as used herein refers to non-aromatic 3- to 7-membered monocyclic groups, 7- to 11-membered bicyclic groups, and 10- to 15-membered tricyclic groups, in which at least one of the rings has at least one

heteroatom (O, S or N), said heteroatom containing ring preferably having 1, 2, or 3 heteroatoms independently selected from O, S, and/or N. Each ring of such a group containing a heteroatom can contain one or two oxygen or sulfur atoms and/or from one to four nitrogen atoms provided that the total number of heteroatoms in each ring is four or less, and further provided that the ring contains at least one carbon atom.

The nitrogen and sulfur atoms may optionally be oxidized and the nitrogen atoms may optionally be quaternized. The fused rings completing the bicyclic and tricyclic groups may contain only carbon atoms and may be saturated, partially saturated, or unsaturated. The heterocyclo group may be attached at any available nitrogen or carbon atom.

**[0046]** Exemplary monocyclic heterocyclyl groups include oxetanyl, azetidinyl, pyrrolidinyl, imidazolinyl, oxazolidinyl, isoxazolinyl, thiazolidinyl, isothiazolidinyl, tetrahydrofuranyl, piperidinyl, piperazinyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-

oxopyrrolodinyl, 2-oxoazepinyl, azepinyl, 4-piperidonyl, tetrahydropyranyl, morpholinyl, thiamorpholinyl, thiamorpholinyl sulfoxide, thiamorpholinyl sulfone, 1,3-dioxolane, and tetrahydro-1,1-dioxothienyl. Exemplary bicyclic heterocyclo groups include quinuclidinyl.

- 5 [0047] The term “heteroaryl” as used herein refers to aromatic 5- or 6-membered monocyclic groups, 9- or 10-membered bicyclic groups, and 11- to 14-membered tricyclic groups which have at least one heteroatom (O, S or N) in at least one of the rings, said heteroatom-containing ring preferably having 1, 2, or 3 heteroatoms independently selected from O, S, and/or N. Each ring of the heteroaryl group
- 10 containing a heteroatom can contain one or two oxygen or sulfur atoms and/or from one to four nitrogen atoms provided that the total number of heteroatoms in each ring is four or less and each ring has at least one carbon atom. The fused rings completing the bicyclic and tricyclic groups may contain only carbon atoms and may be saturated, partially saturated, or unsaturated. The nitrogen and sulfur atoms may
- 15 optionally be oxidized and the nitrogen atoms may optionally be quaternized. Heteroaryl groups which are bicyclic or tricyclic must include at least one fully aromatic ring but the other fused ring or rings may be aromatic or non-aromatic. The heteroaryl group may be attached at any available nitrogen or carbon atom of any ring.
- 20 [0048] Exemplary monocyclic heteroaryl groups include pyrrolyl, pyrazolyl, pyrazolinyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, thiadiazolyl, isothiazolyl, furanyl, thiophenyl, oxadiazolyl, pyridinyl, pyrazinyl, pyrimidinyl, pyridazinyl, triazinyl, and tetrazolyl.
- [0049] Exemplary bicyclic heteroaryl groups include indolyl, benzothiazolyl, benzodioxolyl, benzoxazolyl, benzothienyl, quinolinyl, tetrahydroisoquinolinyl, isoquinolinyl, benzimidazolyl, benzopyranyl, indolizinyl, benzofuranyl, chromonyl, coumarinyl, benzopyranyl, cinnolinyl, quinoxalinyl, indazolyl, pyrrolopyridyl, furopyridyl, dihydroisoindolyl, and tetrahydroquinolinyl.
- [0050] Exemplary tricyclic heteroaryl groups include carbazolyl, benzidolyl, phenanthrollinyl, acridinyl, phenanthridinyl, and xanthenyl.
- [0051] The phrase “pharmaceutically acceptable” is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the

scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

- [0052]** As used herein, “pharmaceutically acceptable salts” refer to derivatives of the disclosed compounds wherein the parent compound is modified by making acid or base salts thereof. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; and alkali or organic salts of acidic residues such as carboxylic acids. The pharmaceutically acceptable salts include the conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. The pharmaceutically acceptable salts of the present invention can be synthesized from the parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts are found in *Remington's Pharmaceutical Sciences*, 17th Ed., Mack Publishing Company, Easton, PA, p. 1418, (1985), the disclosure of which is hereby incorporated by reference.
- [0053]** Salt(s) of the Formula (I) compounds can be formed by, for example, reacting a Formula (I) compound with, for example, an equivalent amount of acid or base in a medium that allows the newly formed salt to, for example, either be precipitated out, or be isolated via lyophilization. Exemplary acidic salt(s) that the compounds of Formula (I) can form with inorganic and/or organic acids include, but are not limited to, for example, include acetate, ascorbate, benzoate, benzenesulfonate, bisulfate, bitartrate, acid citrate, citrate, ethanesulfonate, formate, fumarate, gentisinate, gluconate, glucuronate, glutamate, hydrochloride, hydrobromide, hydroiodide, isonicotinate, maleate, mesylate, methanesulfonate, nitrate, pantothenate, phosphate, acid phosphate, saccharate, salicylate, succinate, sulfate, tartrate, p-toluenesulfonate, trifluoroacetate, lactate, and pamoate [*i.e.*, 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)] salts. Such salts can be formed in accordance with methods known to a person of ordinary skill in the art.

- [0054]** Exemplary basic salt(s) that the compounds of Formula (I) can form with inorganic and/or organic bases include, but are not limited to, for example, ammonium salts; alkali metal salts, such as, for example, sodium, lithium and potassium salts; alkaline earth metal salts, such as, for example, calcium and magnesium salts; salts formed with organic bases, such as, for example, benzathines, dicyclohexylamines, 2-amino-2-(hydroxymethyl)propane-1,3-diol (trisamine or tris), hydrabamines (such as, for example, N,N-bis(dehydroabietyl) ethylenediamine), N-methyl-D-glucamines, N-methyl-D-glycamides, and *t*-butyl amines; salts formed with amino acids, such as, for example, arginine and lysine; and salts formed by using agents, such as, for example, lower alkyl halides (*e.g.*, methyl, ethyl, propyl, and butyl chlorides, bromides and iodides), dialkyl sulfates (*e.g.*, dimethyl, diethyl, dibutyl, and diamyl sulfates), long chain halides (*e.g.*, decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides), and aralkyl halides (*e.g.*, benzyl and phenethyl bromides) to quaternize basic nitrogen-containing groups. Such salts can be formed in accordance with methods known to a person of ordinary skill in the art.
- [0055]** In addition, compounds of Formula (I) are, subsequent to their preparation, preferably isolated and purified to obtain a composition containing an amount by weight equal to or greater than 99% of a compound of Formula (I) (“substantially pure”), which is then used or formulated as described herein. Such “substantially pure” compounds of Formula (I) are also contemplated herein as part of the present invention.
- [0056]** Any compound that can be converted *in vivo* to provide the bioactive agent (*i.e.*, the compound of Formula (I)) is a prodrug within the scope and spirit of the invention.
- [0057]** The term “prodrugs” as employed herein includes esters and carbonates formed by reacting one or more hydroxyls of compounds of Formula (I) with alkyl, alkoxy, or aryl substituted acylating agents employing procedures known to those skilled in the art to generate acetates, pivalates, methylcarbonates, benzoates, and the like.
- [0058]** Various forms of prodrugs are well known in the art and are described in:
- Wermuth, C.G. et al., *The Practice of Medicinal Chemistry*, Chapter 31, Academic Press (1996);

- b) *Design of Prodrugs*, Bundgaard, H. ed., Elsevier (1985);
- c) Bundgaard, H., Chapter 5, "Design and Application of Prodrugs," *A Textbook of Drug Design and Development*, pp. 113-191, Krosgaard-Larsen, P. et al., eds., Harwood Academic Publishers (1991); and
- 5 d) Testa, B. et al., *Hydrolysis in Drug and Prodrug Metabolism*, Wiley-VCH (2003).

[0059] In addition, compounds of the Formula (I) are, subsequent to their preparation, preferably isolated and purified to obtain a composition containing an 10 amount by weight equal to or greater than 99% Formula (I) compound ("substantially pure" compound I), which is then used or formulated as described herein. Such "substantially pure" compounds of the Formula (I) are also contemplated herein as part of the present invention.

[0060] "Stable compound" and "stable structure" are meant to indicate a 15 compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture, and formulation into an efficacious therapeutic agent. The present invention is intended to embody stable compounds.

[0061] "Therapeutically effective amount" is intended to include an amount of a 20 compound of the present invention alone or an amount of the combination of compounds claimed or an amount of a compound of the present invention in combination with other active ingredients effective to upregulate S1P<sub>1</sub>, or effective to treat or prevent vascular disease or autoimmune diseases.

[0062] As used herein, "treating" or "treatment" cover the treatment of a disease-state in a mammal, particularly in a human, and include: (a) preventing the disease-state from occurring in a mammal, in particular, when such mammal is predisposed to the disease-state but has not yet been diagnosed as having it; (b) inhibiting the disease-state, *i.e.*, arresting its development; and/or (c) relieving the disease-state, *i.e.*, causing regression of the disease state.

[0063] Compounds of the present invention may contain one or more additional 30 asymmetric carbon atoms and therefore exist in two or more stereoisomeric forms. The present invention includes all of the possible individual stereoisomers, the individual tautomeric forms thereof, together with mixtures thereof. Separation of

diastereoisomers may be achieved by conventional techniques, *e.g.*, by fractional crystallization, chromatography or HPLC of a stereoisomeric mixture of a compound of the present invention, or a suitable salt or derivative thereof. An individual enantiomer of the compound may also be prepared from a corresponding optically pure intermediate or by resolution, such as by HPLC of the corresponding racemate using a suitable chiral support or by fractional crystallization of the diastereoisomeric salts formed by reaction of the corresponding racemate with a suitable optically active acid or base, as appropriate. All stereoisomers of the compounds of the instant invention are contemplated, either in admixture or in pure or substantially pure form.

5 [0064] The compounds of the present invention is intended to include all isotopes of atoms occurring in the present compounds. Isotopes include those atoms having the same atomic number but different mass numbers. By way of general example and without limitation, isotopes of hydrogen include deuterium and tritium. Isotopes of carbon include  $^{13}\text{C}$  and  $^{14}\text{C}$ . Isotopically-labeled compounds of the invention can

10 generally be prepared by conventional techniques known to those skilled in the art or by processes analogous to those described herein, using an appropriate isotopically-labeled reagent in place of the non-labeled reagent otherwise employed.

15 [0065] Also embraced within this invention is a class of pharmaceutical compositions comprising the compound of Formula (I) or a pharmaceutically-acceptable salt thereof in association with one or more non-toxic, pharmaceutically-acceptable carriers and/or diluents and/or adjuvants (collectively referred to herein as “carrier” materials) and, if desired, other active ingredients. The compounds of Formula (I) may be administered by any suitable route, preferably in the form of a pharmaceutical composition adapted to such a route, and in a dose effective for the

20 treatment intended. The compounds and compositions of the present invention may, for example, be administered orally, mucosally, or parentally including intravascularly, intravenously, intraperitoneally, subcutaneously, intramuscularly intrasternally and infusion techniques, in dosage unit formulations containing conventional pharmaceutically acceptable carriers, adjuvants, and vehicles. For

25 example, the pharmaceutical carrier may contain a mixture of mannitol or lactose and microcrystalline cellulose. The mixture may contain additional components such as a lubricating agent, *e.g.*, magnesium stearate and a disintegrating agent such as

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crospovidone. The carrier mixture may be filled into a gelatin capsule or compressed as a tablet.

5 [0066] The pharmaceutically active compounds of this invention can be processed in accordance with conventional methods of pharmacy to produce medicinal agents for administration to patients, including humans and other mammals.

10 [0067] For oral administration, the pharmaceutical composition may be in the form of, for example, a tablet, capsule, suspension or liquid. The pharmaceutical composition is preferably made in the form of a dosage unit containing a particular amount of the active ingredient. Examples of such dosage units are tablets or capsules. For example, these may contain an amount of active ingredient from about 1 to 2000 mg, preferably from about 1 to 500 mg, more preferably from about 5 to 150 mg. A suitable daily dose for a human or other mammal may vary widely depending on the condition of the patient and other factors, but, once again, can be determined using routine methods.

15 [0068] The amounts of compounds that are administered and the dosage regimen for treating a disease condition with the compounds and/or compositions of this invention depends on a variety of factors, including the age, weight, sex and medical condition of the subject, the type of disease, the severity of the disease, the route and frequency of administration, and the particular compound employed. Thus, the 20 dosage regimen may vary widely, but can be determined routinely using standard methods. A daily dose of about 0.01 to 1500 mg/kg body weight, preferably between about 0.5 and about 50 mg/kg body weight and most preferably between about 0.1 to 20 mg/kg body weight, may be appropriate. The daily dose can be administered in one to four doses per day.

25 [0069] For therapeutic purposes, the active compounds of this invention are ordinarily combined with one or more adjuvants appropriate to the indicated route of administration. If administered orally, the compounds may be admixed with lactose, sucrose, starch powder, cellulose esters of alkanic acids, cellulose alkyl esters, talc, stearic acid, magnesium stearate, magnesium oxide, sodium and calcium salts of 30 phosphoric and sulfuric acids, gelatin, acacia gum, sodium alginate, polyvinylpyrrolidone, and/or polyvinyl alcohol, and then tableted or encapsulated for convenient administration. Such capsules or tablets may contain a controlled-release

formulation as may be provided in a dispersion of active compound in hydroxypropylmethyl cellulose.

**[0070]** The oily phase of the emulsions comprising compounds of Formula (I) may be constituted from known ingredients in a known manner. While the phase may 5 comprise merely an emulsifier, it may comprise a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier which acts as a stabilizer. It is also preferred to include both an oil and a fat. Together, the emulsifier(s) with or without stabilizer(s) make-up the so-called emulsifying wax, and the wax together with the oil 10 and fat make up the so-called emulsifying ointment base which forms the oily dispersed phase of the cream formulations. Emulsifiers and emulsion stabilizers suitable for use in the formulation of the present invention include Tween 60, Span 80, cetostearyl alcohol, myristyl alcohol, glyceryl monostearate, sodium lauryl sulfate, glyceryl distearate alone or with a wax, or other materials well known in the 15 art.

**[0071]** The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties, since the solubility of the active compound in most oils likely to be used in pharmaceutical emulsion formulations is very low. Thus, the cream should preferably be a non-greasy, non-staining and washable product with 20 suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters may be used. These may be used alone or in combination depending on 25 the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils can be used.

**[0072]** Formulations for parenteral administration may be in the form of aqueous or non-aqueous isotonic sterile injection solutions or suspensions. These solutions and suspensions may be prepared from sterile powders or granules using one or more 30 of the carriers or diluents mentioned for use in the formulations for oral administration or by using other suitable dispersing or wetting agents and suspending agents. The compounds may be dissolved in water, polyethylene glycol, propylene

glycol, ethanol, corn oil, cottonseed oil, peanut oil, sesame oil, benzyl alcohol, sodium chloride, tragacanth gum, and/or various buffers. Other adjuvants and modes of administration are well and widely known in the pharmaceutical art. The active ingredient may also be administered by injection as a composition with suitable

5 carriers including saline, dextrose, or water, or with cyclodextrin (*i.e.*, CAPTISOL®), cosolvent solubilization (*i.e.*, propylene glycol) or micellar solubilization (*i.e.*, Tween 80).

[0073] The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example as 10 a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed, including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the 15 preparation of injectables.

[0074] The pharmaceutical compositions may be subjected to conventional pharmaceutical operations such as sterilization and/or may contain conventional adjuvants, such as preservatives, stabilizers, wetting agents, emulsifiers, buffers etc. Tablets and pills can additionally be prepared with enteric coatings. Such 20 compositions may also comprise adjuvants, such as wetting, sweetening, flavoring, and perfuming agents.

[0075] Pharmaceutical compositions of this invention comprise the compound of Formula (I), or a pharmaceutically acceptable salt thereof, and optionally an additional agent selected from any pharmaceutically acceptable carrier, adjuvant and 25 vehicle. Alternate compositions of this invention comprise a compound of the Formula (I) described herein, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier, adjuvant or vehicle.

[0076] Pharmaceutically acceptable carriers, adjuvants and vehicles that may be used in the pharmaceutical compositions of this invention include, but are not limited 30 to, ion exchangers, alumina, aluminum stearate, lecithin, self-emulsifying drug delivery systems (SEDDS) such as d-alpha-tocopherol polyethyleneglycol 1000 succinate, surfactants used in pharmaceutical dosage forms such as Tweens or other

similar polymeric delivery matrices, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, 5 sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat. Cyclodextrins such as alpha-, beta-, and gamma-cyclodextrin, or chemically modified derivatives such as 10 hydroxyalkylcyclodextrins, including 2- and 3-hydroxypropyl-cyclodextrins, or other solubilized derivatives may also be advantageously used to enhance delivery of compounds of the formulae described herein.

#### UTILITY

15 [0077] The human immune system has evolved to defend the body from micro-organisms, viruses, and parasites that can cause infection, disease or death. Complex regulatory mechanisms ensure that the various cellular components of the immune system target the foreign substances or organisms, while not causing permanent or significant damage to the individual. While the initiating events are not well 20 understood at this time, in autoimmune disease states the immune system directs its inflammatory response to target organs in the afflicted individual. Different autoimmune diseases are typically characterized by the predominate or initial target organ or tissues affected; such as the joint in the case of rheumatoid arthritis, the thyroid gland in the case of Hashimoto's thyroiditis, the central nervous system in the 25 case of multiple sclerosis, the pancreas in the case of type I diabetes, and the bowel in the case of inflammatory bowel disease. Thus it has been observed that therapeutic agents which act on the immune system or certain cell types of the immune system (such as B-lymphocytes, and T lymphocytes, T cells) may have utility in more than one autoimmune disease.

30 [0078] It is well recognized in the art, including the literature references cited herein, that S1P receptors are good targets for a wide variety of therapeutic applications, including autoimmune diseases. S1P receptors make good drug targets,

because individual receptors are both tissue- and response-specific. Tissue specificity of the S1P receptors is important, because development of an agonist or antagonist selective for one receptor localizes the cellular response to tissues containing that receptor, limiting unwanted side effects. Response specificity of the S1P receptors is 5 also important because it allows for development of agonists or antagonists that initiate or suppress certain cellular responses without affecting other processes. Therefore, compounds which act on some S1P receptor family members while having diminished or no activity at other family members are desirable and are expected to provide a therapeutic effect with an improved side effect profile (*i.e.*, reduction or 10 elimination of unwanted side effects).

- 15 **[0079]** As used herein, the term “agonist” in reference to S1P<sub>1</sub> refers to an agent which exerts pharmacological effects such as decreased motility of T cells, decreased trafficking of T cells, or decreased egress of T cells from lymphoid tissues. (Rosen et al., *Trends in Immunology*, 28:102 (2007)).
- 20 **[0080]** By virtue of their S1P<sub>1</sub> activity as agonists, the compounds of the present invention are immuno-regulatory agents useful for treating or preventing autoimmune or chronic inflammatory diseases. The compounds of the present invention are useful to suppress the immune system in instances where immuno-suppression is in order, such as in bone marrow, organ or transplant rejection, autoimmune and chronic inflammatory diseases, including systemic lupus erythematosis, chronic rheumatoid arthritis, type I diabetes mellitus, inflammatory bowel disease, biliary cirrhosis, uveitis, multiple sclerosis, Crohn’s disease, ulcerative colitis, bullous pemphigoid, sarcoidosis, psoriasis, autoimmune myositis, Wegener’s granulomatosis, ichthyosis, Graves’ ophthalmopathy, and asthma.
- 25 **[0081]** More particularly, the compounds of the present invention are useful to treat or prevent a disease or disorder selected from the group consisting of: transplantation of organs or tissue, graft-versus-host diseases brought about by transplantation, autoimmune syndromes including rheumatoid arthritis, systemic lupus erythematosus, Hashimoto’s thyroiditis, multiple sclerosis, myasthenia gravis, 30 type I diabetes, uveitis, posterior uveitis, allergic encephalomyelitis, glomerulonephritis, post-infectious autoimmune diseases including rheumatic fever and post-infectious glomerulonephritis, inflammatory and hyperproliferative skin

diseases, psoriasis, atopic dermatitis, contact dermatitis, eczematous dermatitis, seborrhoeic dermatitis, lichen planus, pemphigus, bullous pemphigoid, epidermolysis bullosa, urticaria, angioedemas, vasculitis, erythema, cutaneous eosinophilia, lupus erythematosus, acne, alopecia areata, keratoconjunctivitis, vernal conjunctivitis,

5 uveitis associated with Behcet's disease, keratitis, herpetic keratitis, conical cornea, dystrophia epithelialis cornea, corneal leukoma, ocular pemphigus, Mooren's ulcer, scleritis, Graves' ophthalmopathy, Vogt-Koyanagi-Harada syndrome, sarcoidosis, pollen allergies, reversible obstructive airway disease, bronchial asthma, allergic asthma, intrinsic asthma, extrinsic asthma, dust asthma, chronic or inveterate asthma,

10 late asthma and airway hyper-responsiveness, bronchitis, gastric ulcers, vascular damage caused by ischemic diseases and thrombosis, ischemic bowel diseases, inflammatory bowel diseases, necrotizing enterocolitis, intestinal lesions associated with thermal burns, coeliac diseases, proctitis, eosinophilic gastroenteritis, mastocytosis, Crohn's disease, ulcerative colitis, migraine, rhinitis, eczema,

15 interstitial nephritis, Goodpasture's syndrome, hemolytic-uremic syndrome, diabetic nephropathy, multiple myositis, Guillain-Barre syndrome, Meniere's disease, polyneuritis, multiple neuritis, mononeuritis, radiculopathy, hyperthyroidism, Basedow's disease, pure red cell aplasia, aplastic anemia, hypoplastic anemia, idiopathic thrombocytopenic purpura, autoimmune hemolytic anemia,

20 agranulocytosis, pernicious anemia, megaloblastic anemia, anerythroplasia, osteoporosis, sarcoidosis, fibroid lung, idiopathic interstitial pneumonia, dermatomyositis, leukoderma vulgaris, ichthyosis vulgaris, photoallergic sensitivity, cutaneous T cell lymphoma, arteriosclerosis, atherosclerosis, aortitis syndrome, polyarteritis nodosa, myocarditis, scleroderma, Wegener's granuloma, Sjögren's

25 syndrome, adiposis, eosinophilic fascitis, lesions of gingiva, periodontium, alveolar bone, substantia ossea dentis, glomerulonephritis, male pattern alopecia or alopecia senilis by preventing epilation or providing hair germination and/or promoting hair generation and hair growth, muscular dystrophy, pyoderrna and Sezary's syndrome, Addison's disease, ischemia-reperfusion injury of organs which occurs upon

30 preservation, transplantation or ischemic disease, endotoxin-shock, pseudomembranous colitis, colitis caused by drug or radiation, ischemic acute renal insufficiency, chronic renal insufficiency, toxinosis caused by lung-oxygen or drugs,

lung cancer, pulmonary emphysema, cataracta, siderosis, retinitis pigmentosa, senile macular degeneration, vitreal scarring, corneal alkali burn, dermatitis erythema multiforme, linear IgA ballous dermatitis and cement dermatitis, gingivitis, periodontitis, sepsis, pancreatitis, diseases caused by environmental pollution, aging,

5 carcinogenesis, metastasis of carcinoma and hypobaropathy, disease caused by histamine or leukotriene-C<sub>4</sub> release, Behcet's disease, autoimmune hepatitis, primary biliary cirrhosis, sclerosing cholangitis, partial liver resection, acute liver necrosis, necrosis caused by toxin, viral hepatitis, shock, or anoxia, B-virus hepatitis, non-A/non-B hepatitis, cirrhosis, alcoholic cirrhosis, hepatic failure, fulminant hepatic failure, late-onset hepatic failure, "acute-on-chronic" liver failure, augmentation of chemotherapeutic effect, cytomegalovirus infection, HCMV infection, AIDS, cancer, senile dementia, trauma, and chronic bacterial infection.

10 [0082] Also embodied within the present invention is a method of preventing or treating resistance to transplantation or transplantation rejection of organs or tissues in a mammalian patient in need thereof, which comprises administering a compound of Formula (I) or a pharmaceutically acceptable salt thereof. A therapeutically effective amount for preventing or treating resistance to transplantation or transplantation rejection may be administered.

15 [0083] A method of suppressing the immune system in a mammalian patient in need thereof, which comprises administering to the patient a compound of Formula (I) or a pharmaceutically acceptable salt thereof, is yet another embodiment. A therapeutically effective amount for suppressing the immune system may be administered.

20 [0084] Most particularly, the method described herein encompasses a method of treating or preventing bone marrow or organ transplant rejection which is comprised of administering to a mammalian patient in need of such treatment or prevention a compound of Formula (I) or a pharmaceutically acceptable salt thereof. A therapeutically effective amount for treating or preventing bone marrow or organ transplant rejection may be administered.

25 [0085] One embodiment provides a method for treating autoimmune and/or inflammatory diseases, comprising administering to a mammal in need thereof at least one compound of Formula (I) or a pharmaceutically acceptable salt thereof. Another

embodiment provides the compounds of Formula (I) or pharmaceutically acceptable salts thereof, for use in therapy for the treatment of autoimmune and/or inflammatory diseases. In another embodiment, provided is the use of the compounds of Formula (I) or pharmaceutically acceptable salts thereof, for the manufacture of a medicament for the treatment or prophylaxis of autoimmune and/or inflammatory disease. A therapeutically effective amount may be employed in these embodiments. Preferably, in these embodiments, the autoimmune and inflammatory diseases are selected from multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease (including Crohn's disease and ulcerative colitis), psoriasis, and as an agent to prevent the rejection of transplanted organs.

**[0086]** In another embodiment, a method for treating vascular disease is provided comprising administering to a mammal in need thereof at least one compound of Formula (I) or a pharmaceutically acceptable salt thereof. Another embodiment provides the compounds of Formula (I) or pharmaceutically acceptable salts thereof, for use in therapy for the treatment of vascular disease. In another embodiment, provided is the use of the compounds of Formula (I) or pharmaceutically acceptable salts thereof, for the manufacture of a medicament for treatment of vascular disease. A therapeutically effective amount may be employed in these embodiments. Preferably, in these embodiments, the vascular disease is selected from atherosclerosis and ischemia reperfusion injury.

**[0087]** One embodiment provides a method of treating a disease or disorder associated with the activity of G protein-coupled receptor S1P<sub>1</sub>, the method comprising administering to a mammalian patient of a compound of Formula (I) or a pharmaceutically acceptable salt thereof. The method of the present embodiment includes administration of a therapeutically effective amount of a compound of Formula (I) or a pharmaceutically effective salt thereof.

**[0088]** The methods of treating S1P<sub>1</sub>-associated conditions may comprise administering compounds of Formula (I) alone or in combination with each other and/or other suitable therapeutic agents useful in treating such conditions.

Accordingly, "therapeutically effective amount" is also intended to include an amount of the combination of compounds claimed that is effective to act as an agonist at the S1P<sub>1</sub> receptor. The combination of compounds is preferably a synergistic

combination. Synergy, as described, for example, by Chou et al., *Adv. Enzyme Regul.*, 22:27-55 (1984), occurs when the effect of the compounds when administered in combination is greater than the additive effect of the compounds when administered alone as a single agent. In general, a synergistic effect is most clearly

5 demonstrated at sub-optimal concentrations of the compounds. Synergy can be in terms of lower cytotoxicity, increased efficacy, or some other beneficial effect of the combination compared with the individual components.

**[0089]** Exemplary of such other therapeutic agents include corticosteroids or glucocorticoids such as dexamethasone, methylprednisolone, prednisolone, and

10 prednisone; PDE4 inhibitors such as rolipram, cilomilast, roflumilast, and oglemilast; cytokine-suppressive anti-inflammatory drugs (CSAIDs) and inhibitors of p38 kinase, 4-substituted imidazo [1,2-A]quinoxalines as disclosed in U.S. Patent No. 4,200,750; antibodies or fusion proteins directed to cell surface molecules such as CD2, CD3,

CD4, CD8, CD20 such as RITUXAN®, CD25, CD30, CD40, CD69, CD80 (B7.1),

15 CD86 (B7.2), CD90, CTLA, for example abatacept (ORENCIA®), or their ligands including CD154 (GP39, or CD40L); antibodies to, fusion proteins, or soluble receptors of human cytokines or growth factors, for example, TNF such as,

infliximab (REMICADE®), etanercept (ENBREL®), adalimumab (HUMIRA®), LT, IL-1 such as anakinra (KINERET®) (an IL-1 receptor antagonist), IL-2, IL-4, IL-5, IL-

20 6, such as CNTO 328 (a chimeric anti-IL-6 antibody), IL-7, IL-8, IL-12, IL-15, IL-16, IL-17, IL-21, IL-23 such as Ustekinumab (a human anti-IL-12/23 monoclonal antibody), and interferons such as interferon beta 1a (AVOREX®, REBIF®), interferon beta 1b (BETASERON®); integrin receptor antagonists such as TYSABRI®; polymeric agents such as glatiramer acetate (COPAXONE®); sulfasalazine, mesalamine,

25 hydroxychloroquine, non-steroidal antiinflammatory drugs (NSAIDs) such as

salicylates including aspirin, salsalate, and magnesium salicylate, and non-salicylates such as, ibuprofen, naproxen, meloxicam, celecoxib and rofecoxib; antiviral agents such as abacavir; antiproliferative agents such as methotrexate, mercaptopurine, leflunomide, cyclosporine, mycophenolate, FK506 (tacrolimus, PROGRAF®);

30 cytotoxic drugs such as azathioprine and cyclophosphamide; nuclear translocation inhibitors, such as deoxyspergualin (DSG); gold containing products such as

auronofin; penicillamine, and rapamycin (sirolimus or RAPAMUNE®) or derivatives thereof.

[0090] The above other therapeutic agents, when employed in combination with the compounds of the present invention, may be used, for example, in those amounts indicated in the Physicians' Desk Reference (PDR) or as otherwise determined by one of ordinary skill in the art. In the methods of the present invention, such other therapeutic agent(s) may be administered prior to, simultaneously with, or following the administration of the inventive compounds.

10

#### METHODS OF PREPARATION

[0091] The compounds of the present invention can be prepared in a number of ways well known to one skilled in the art of organic synthesis. The compounds of the present invention can be synthesized using the methods described below, together with synthetic methods known in the art of synthetic organic chemistry, or variations thereon as appreciated by those skilled in the art. Preferred methods include, but are not limited to, those described below. All references cited herein are hereby incorporated in their entirety by reference.

[0092] The compounds of this invention may be prepared using the reactions and techniques described in this section. The reactions are performed in solvents appropriate to the reagents and materials employed and are suitable for the transformations being effected. Also, in the description of the synthetic methods described below, it is to be understood that all proposed reaction conditions, including choice of solvent, reaction atmosphere, reaction temperature, duration of the experiment and work up procedures, are chosen to be the conditions standard for that reaction, which should be readily recognized by one skilled in the art. It is understood by one skilled in the art of organic synthesis that the functionality present on various portions of the molecule must be compatible with the reagents and reactions proposed. Such restrictions to the substituents that are compatible with the reaction conditions will be readily apparent to one skilled in the art and alternate methods must then be used. This will sometimes require a judgment to modify the order of the synthetic steps or to select one particular process scheme over another in order to obtain a desired compound of the invention. It will also be recognized that

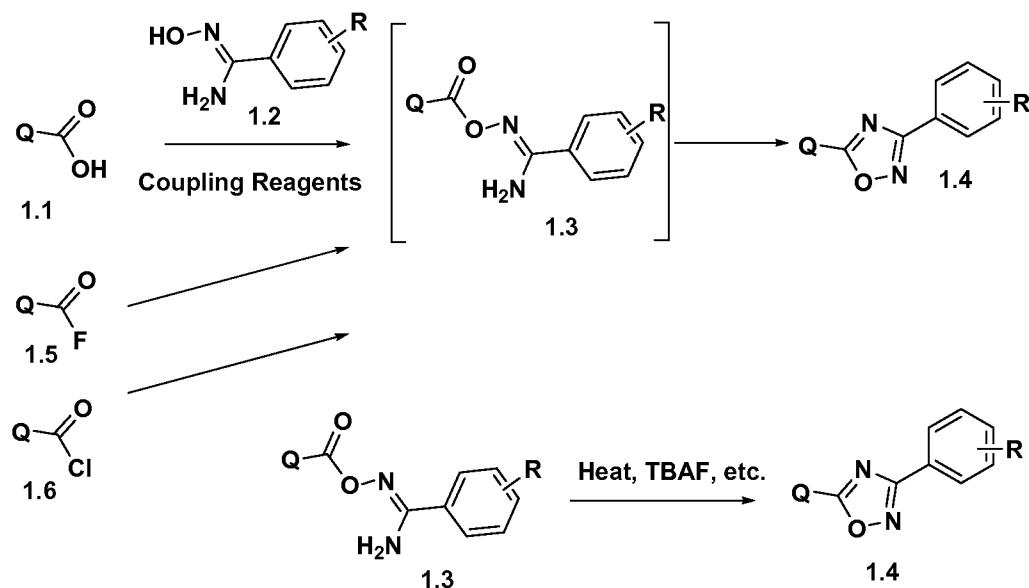
another major consideration in the planning of any synthetic route in this field is the judicious choice of the protecting group used for protection of the reactive functional groups present in the compounds described in this invention. An authoritative account describing the many alternatives to the trained practitioner is Greene et al.

5     *(Protective Groups in Organic Synthesis, Third Edition, Wiley and Sons (1999)).*

[0093]     Compounds of Formula (I) may be prepared by reference to the methods illustrated in the following Schemes. As shown therein the end product is a compound having the same structural formula as Formula (I). It will be understood that any compound of Formula (I) may be produced by the schemes by the suitable 10 selection of reagents with appropriate substitution. Solvents, temperatures, pressures, and other reaction conditions may readily be selected by one of ordinary skill in the art. Starting materials are commercially available or readily prepared by one of ordinary skill in the art. Constituents of compounds are as defined herein or elsewhere in the specification.

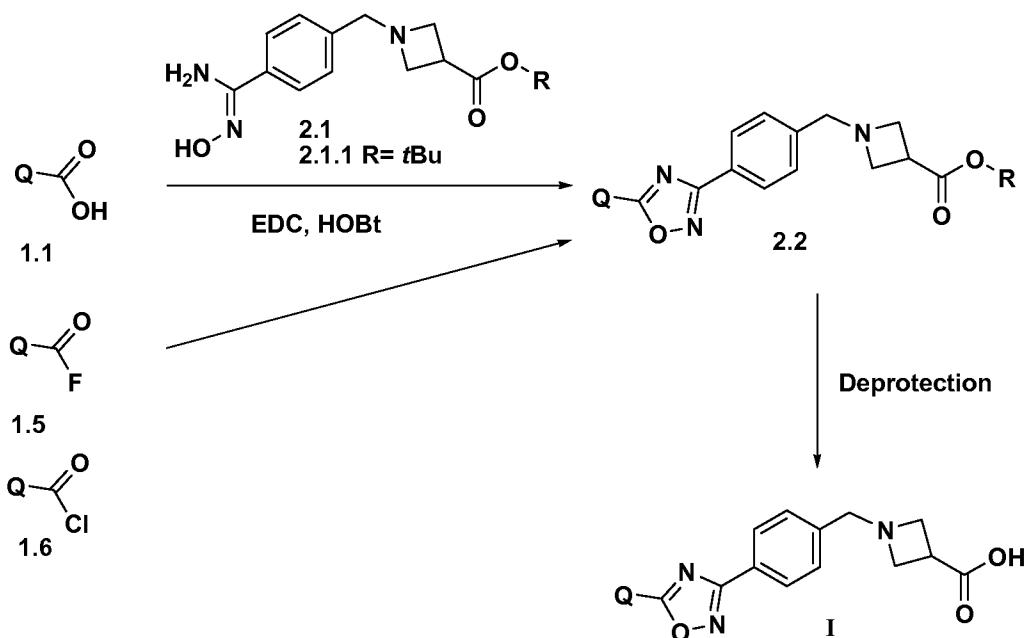
[0094]     As shown in Scheme 1, the oxadiazole compounds of the present invention (1.4) may be prepared through the reaction of carboxylic acids (1.1) with N'-hydroxybenzimidamides (1.2) with a variety of coupling reagents (e.g., EDC, HOBr, BOP, BOP-Cl). Alternatively, the N'-hydroxybenzimidamides may be reacted with acid fluoride (1.5) or acid chloride compounds (1.6). In each case, the initially 20 formed N'-acyloxybenzimidamides (1.3) may spontaneously convert to the oxadiazoles under the reaction conditions. In cases where the N'-acyloxybenzimidamide (1.3) does not cyclize spontaneously, it may be isolated and subjected to reaction conditions to effect the cyclodehydration to 1.4. Such conditions may include heating (either conventional or microwave), or treatment with 25 fluoride source (such as tetrabutyl ammonium fluoride).

Scheme 1



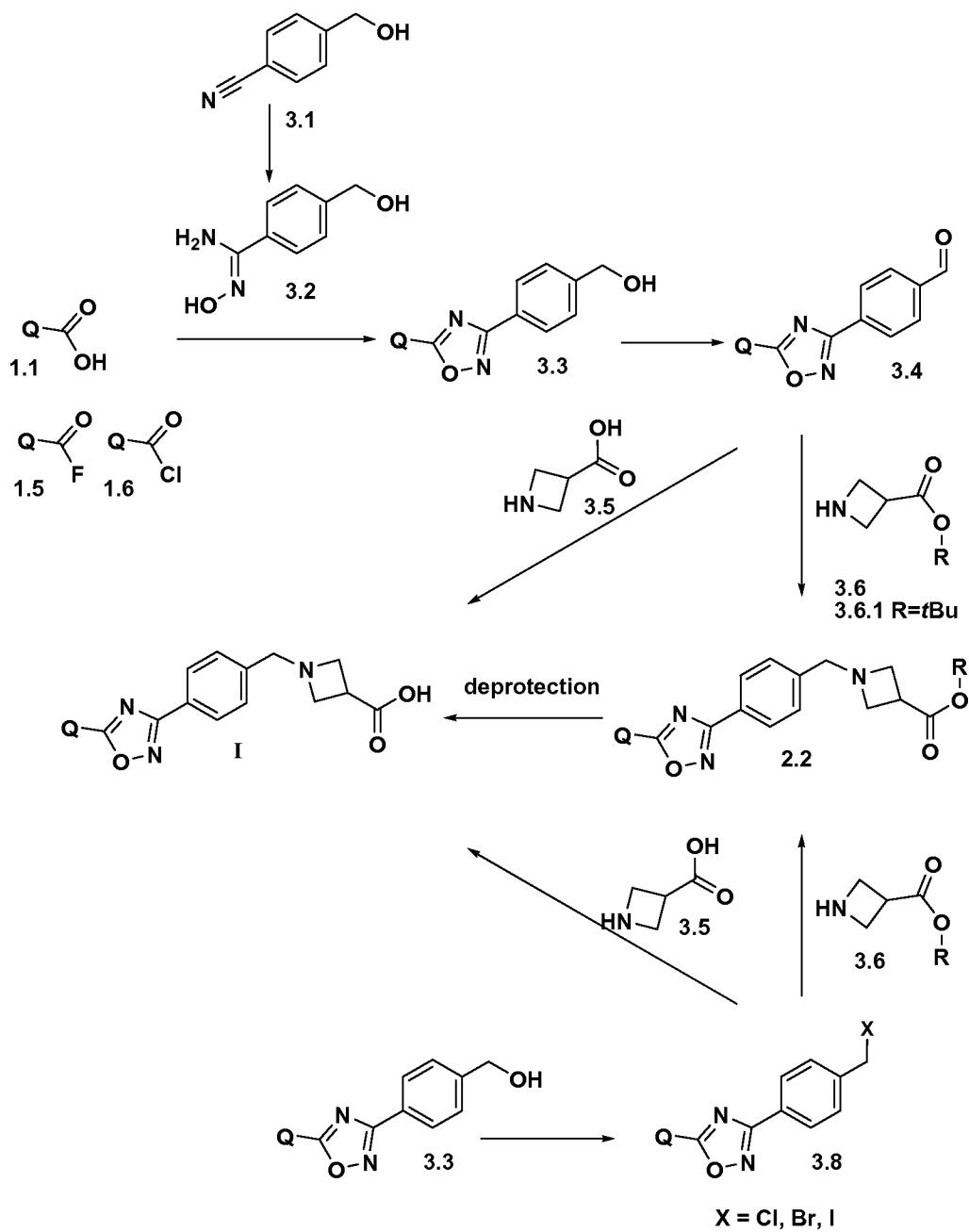
[0095] Compounds of formula (I) may be prepared through the reaction of acids 5 (1.1) acid fluorides (1.5) or acid chlorides (1.6) with N-hydroxyamidine derivatives 2.1 such as (Z)-*tert*-butyl 1-(4-(N'-hydroxycarbamimidoyl)benzyl)azetidine-3-carboxylate (2.1.1) via means described above to produce compounds of structure 2.2. Deprotection of the ester derivatives (2.2) (by treatment with an acid, for example 10 trifluoroacetic acid, in the case of *t*-butyl ester derivatives) provides compounds of formula (I).

Scheme 2



[0096] Alternatively, compounds of formula (I) may also be produced as described in Scheme 3. The reaction of acids (1.1) acid fluorides (1.5) or acid chlorides (1.6) with (Z)-N'-hydroxy-4-(hydroxymethyl)benzimidamide (3.2) via means described above can produce compounds of structure 3.3 which, after oxidation to the corresponding aldehyde (3.4), can undergo reductive amination with azetidine-3-carboxylic acid (3.5) or azetidine-3-carboxylate esters (3.6) to provide compounds of formula (I) or 2.2 respectively. Compound 2.2 may be converted to a compound of formula (I) as described above. Hydroxymethyl compounds (3.3) may be converted to benzyl halide derivatives (3.8). Reaction of 3.8 with azetidine-3-carboxylic acid (3.5) or azetidine-3-carboxylate esters (3.6) provides compounds of formula (I) or 2.2 respectively.

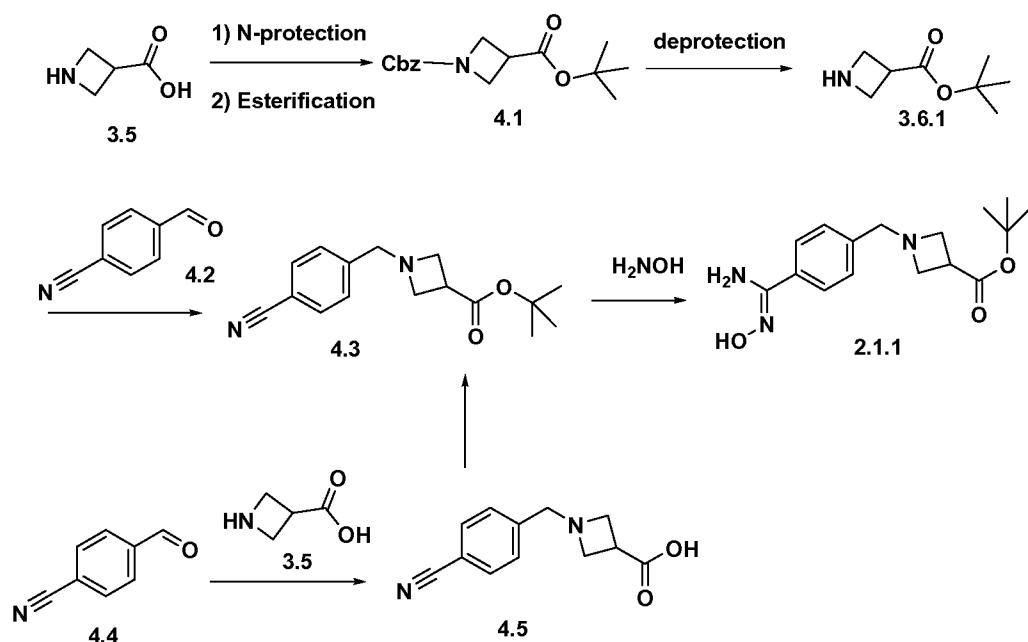
**Scheme 3**



[0097] *tert*-Butyl azetidine-3-carboxylate (**3.6.1**) may be prepared from azetidine-3-carboxylic acid (**3.5**) via protection of the amine (for example with the CBZ group) followed by esterification of the acid with *tert*-butyl alcohol in the presence of a coupling reagent (for example CDI) and then removal of the amine protecting group. (Z)-*tert*-butyl 1-(4-(N'-hydroxycarbamimidoyl)benzyl)azetidine-3-carboxylate (**2.1.1**)

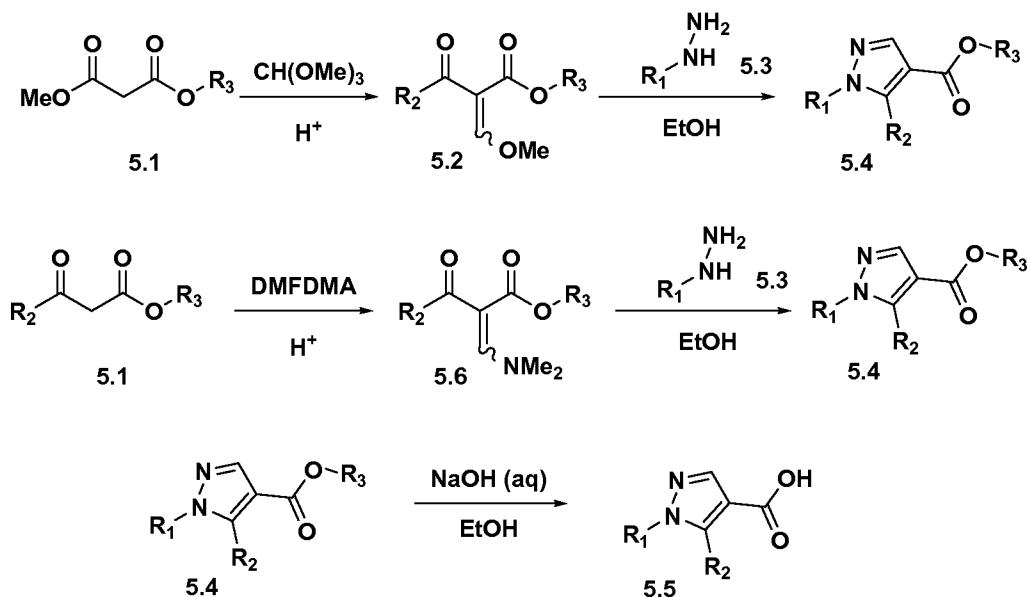
is available from the reaction of *tert*-butyl azetidine-3-carboxylate (**3.6.1**) with 4-formylbenzonitrile (**4.2**) under reducing conditions to give **4.3**, which is then reacted with hydroxylamine. Alternatively, compound **4.3** may be prepared by esterification of **4.5**, which is obtained from the reaction of azetidine-3-carboxylic acid (**3.5**) with 5 4-formylbenzonitrile (**4.2**) under reducing conditions.

Scheme 4



10 [0098] The carboxylic acid fragments (**1.1**) of the present invention may be prepared by a variety of methods, including those illustrated in Scheme 5. Acetoacetate ester compounds (**5.1**) can be converted to activated methylene derivatives (**5.2** or **5.6**) for example through reaction with triethyl orthoformate or *N,N*-dimethylformamide-dimethylacetal (DMF-DMA) respectively in the presence of 15 a catalytic amount of acid (such as para-toluenesulfonic acid). Compounds **5.2** or **5.6** can then be reacted with mono-substituted hydrazines in a variety of solvents (polar, non-polar, protic, aprotic) in the presence or absence of additional base as required to afford pyrazole esters (**5.4**). Compounds **5.4** can be isolated, or directly hydrolyzed to the corresponding pyrazole acids (**5.5**).

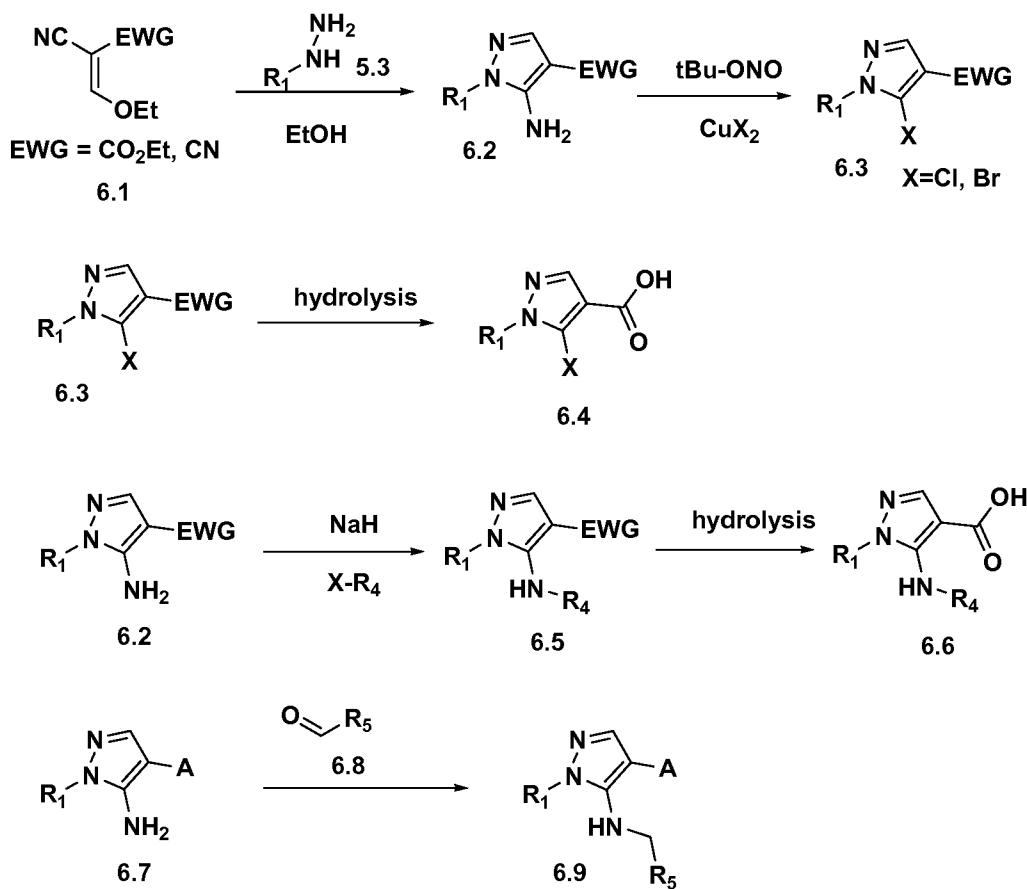
Scheme 5



[0099] Pyrazoles bearing halo, amino, or alkylamino substitution at C5 can be prepared according to Scheme 5. Reaction of compounds **6.1** (such as 2-ethoxymethylene-malononitrile or 2-cyano-3-ethoxy-acrylic acid ethyl ester) with mono-substituted hydrazines gives C5-amino-pyrazoles **6.2**, where the C4 position is substituted by a nitrile, or ester. Conversion of the C5-amino group to the C5-halo group can be accomplished through reaction with tBuONO and copper (II) bromide or copper (II) chloride. Hydrolysis of the ester or nitrile to the carboxylic acid (**6.4**) can take place under standard conditions (basic or acidic).

[00100] Aminopyrazoles **6.2** can be alkylated through the reaction with alkyl halides (such as ethyl bromide) in the presence of a suitable base (such as NaH). Hydrolysis of the ester or nitrile leads to the C5-alkylamino substituted pyrazole carboxylic acids (**6.6**). Aminopyrazoles (**6.7**) may also be modified through reductive amination with carbonyl compounds (**6.8**).

Scheme 6



ABBREVIATIONS

5	Ac	acetyl
	AcOH	acetic acid
	AIBN	azobisisobutyronitrile
	aq.	aqueous
	CDI	carbonyldiimidazole
10	CEM	CEM Corp., PO Box 200, 3100 Smith Farm Rd, Matthews NC 28106
	Biotage	Biotage AB, Kungsgatan 76 SE-753 18 Uppsala, Sweden
	Bn	benzyl
	Boc	<i>tert</i> -butoxycarbonyl

	BOP	benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium-hexafluorophosphate
	BOP-Cl	bis(2-oxooxazolidin-3-yl)phosphinic chloride
	Bu	butyl
5	DIPEA	diisopropylethylamine (Hunig's Base)
	DCE	1,2-dichloroethane
	DMAP	N,N-Dimethylpyridine-4-amine
	DMA	N,N-Dimethylacetamide
	DMF	dimethylformamide
10	DMF-DMA	N,N-Dimethylformamide dimethylacetal
	DMSO	dimethylsulfoxide
	EDC, EDCI	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
	EtOAc	ethyl acetate
	Et	ethyl
15	Ether	diethyl ether
	EtOH	ethanol
	H	hydrogen
	h, hr	hours
	<i>i</i>	<i>iso</i>
20	Isco	Teledyne Isco, Inc. 4700 Superior St., Lincoln NE 68504
	Hex.	hexanes
	HMPA	hexamethylphosphoramide
	HOAc	acetic acid
	HOEt	1H-benzo[d][1,2,3]triazol-1-ol
25	HPLC	high pressure liquid chromatography
	LC	liquid chromatography
	Me	methyl
	MeOH	methanol
	MeOD	deuterated methanol (CD <sub>3</sub> OD)
30	min.	minutes
	M <sup>+1</sup>	(M+H) <sup>+</sup>
	MS	mass spectrometry

	<i>n</i>	<i>normal</i>
	Pd/C	palladium on carbon
	pet. ether	petroleum ether
	Ph	phenyl
5	Pr	propyl
	Prep. HPLC	reverse phase preparative HPLC
	PSI	pounds per square inch
	Ret Time	retention time
	rt or RT	room temperature
10	sat.	saturated
	<i>t</i>	<i>tertiary</i>
	TFA	trifluoroacetic acid
	THF	tetrahydrofuran
	TLC	thin layer chromatography
15	TMS	trimethylsilyl
	Phenomenex	Phenomenex, Macclesfield, Cheshire, UK
	YMC	YMC, Inc, Wilmington, NC 20403

## EXAMPLES

- 20 [00101] The following Examples illustrate the particular and preferred embodiments of the present invention and do not limit the scope of the present invention. Chemical abbreviations and symbols as well as scientific abbreviations and symbols have their usual and customary meanings unless otherwise specified. Additional abbreviations employed in the Examples and elsewhere in this application 25 are defined above. Common intermediates are generally useful for the preparation of more than one Example and are identified sequentially (e.g., Intermediate 1, Intermediate 2, etc. and are abbreviated as Int.1, Int.2, etc. In some instances the preparation of common intermediates may require multiple steps to be prepared. Each step is identified by the common intermediate and the step (e.g., Int.1-A, Int.1-B, and so forth. Compounds of the Examples are identified by the example and step 30 in which they were prepared (e.g., “1-A” denotes the Example 1, step A), or by the example only where the compound is the title compound of the example (for example,

“1” denotes the title compound of Example 1). In some instances alternate preparations of intermediates or Examples are described. Frequently chemists skilled in the art of synthesis may devise alternative preparations which may be desirable based on one or more considerations such as shorter reaction time, less expensive

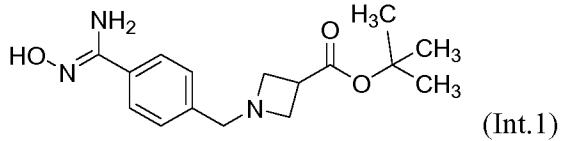
5 starting materials, ease of operation, amenable to catalysis, avoidance of toxic reagents, accessibility of specialized instrumentation, and decreased number of linear steps, etc. The intent of describing alternative preparations is to further enable the preparation of the Examples of this invention.

[00102] Those experiments specifying that they were performed in a microwave 10 oven were conducted in a SmithSynthesizer oven manufactured by Personal Chemistry or a Discover microwave oven manufactured by CEM corporation. The microwave ovens generate a temperature which can be selected to be between 60-250°C. The microwave ovens automatically monitor the pressure which is between 0-300 PSI. Reaction hold times and temperature set points are reported.

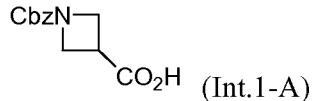
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Preparation of Intermediate 1 (Int.1)

(Z)-*tert*-Butyl 1-(4-(N'-hydroxycarbamimidoyl)-benzyl)azetidine-3-carboxylate



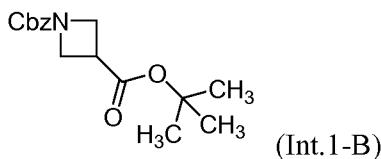
20 Int.1-A. 1-(Benzylloxycarbonyl)azetidine-3-carboxylic acid



[00103] To a solution of azetidine-3-carboxylic acid (88 g, 0.871 mol) and sodium bicarbonate (161 g, 1.92 mol) in water (1.75 L) at room temperature was added a solution of benzyl 2,5-dioxopyrrolidin-1-ylcarbonate (239 g, 0.959 mol) in 25 tetrahydrofuran (3.5 L). The reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure, and the aqueous layer was washed with ethyl acetate (2 x 500 mL). The aqueous layer was acidified with a 1.0 N aqueous solution of hydrochloric acid and was then extracted with ethyl acetate (3 x 750 mL). The organic layer was washed with water, followed by brine, and dried

over anhydrous sodium sulfate. Concentration under reduced pressure afforded 1-(benzyloxycarbonyl)azetidine-3-carboxylic acid as colorless oil (202 g, 99% yield). The compound had an HPLC retention time = 2.27 min. – Column: YMC COMBISCREEN® ODS-A 4.6 x 50 mm (4 min.); Solvent A = 10% MeOH, 90% H<sub>2</sub>O, 0.1% TFA; Solvent B = 90% MeOH, 10% H<sub>2</sub>O, 0.1% TFA. LC/MS M<sup>+1</sup> = 236.15. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ ppm 3.39 – 3.49 (m, 1H), 4.22 (d, J=7.28 Hz, 4H), 5.11 (s, 2H), and 7.29 – 7.39 (m, 5H).

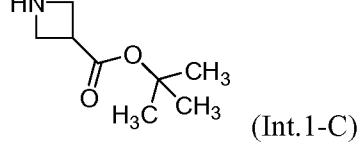
Int.1-B. 1-Benzyl 3-*tert*-butyl azetidine-1,3-dicarboxylate



[00104] To a solution of 1-(benzyloxycarbonyl)azetidine-3-carboxylic acid (200 g, 0.851 mol) in dichloromethane (6.0 L) at 0°C was added *t*-butanol (158 g, 2.13 mol), DMAP (52.0 g, 0.425 mol), and EDCI (163 g, 0.853 mol). The reaction was stirred at room temperature overnight. The reaction mixture was concentrated and the residue 15 was dissolved in ethyl acetate. The organic layer washed with 10% aqueous citric acid, 10 % aqueous sodium bicarbonate solution and brine. Drying over anhydrous sodium sulfate and concentration under reduced pressure afforded 1-benzyl-3-*tert* butyl-azetidine-1,3-dicarboxylate (200 g, 81% yield) as a colorless oil. The compound had an HPLC retention time = 3.27 min. – Column: YMC

20 COMBISCREEN® ODS-A 4.6 x 50 mm (4 min.); Solvent A = 10% MeOH, 90% H<sub>2</sub>O, 0.1% TFA; Solvent B = 90% MeOH, 10% H<sub>2</sub>O, 0.1% TFA. LC/MS M<sup>+1</sup> = 292.15. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ ppm 1.46 (s, 9H), 3.24 – 3.33 (m, 1H), 4.14 (d, J=7.53 Hz, 4H), 5.10 (s, 2H), and 7.30 – 7.39 (m, 5H).

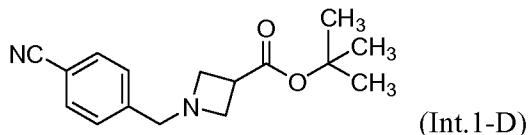
25 Int.1-C. *tert*-Butyl azetidine-3-carboxylate



[00105] A mixture of 1-benzyl-3-*tert*-butyl-azetidine-1,3-dicarboxylate (140 g, 0.480 mol) and 10 % palladium on carbon (28.0 g) in ethyl acetate (1.40 L) was placed in an autoclave under 3.0kg/cm<sup>2</sup> of hydrogen pressure overnight. The reaction mixture was filtered through CELITE®, and the CELITE® bed was washed with 5 ethyl acetate. Acetic acid (28.9 g, 0.480 mol) was added to the filtrate and it was concentrated under reduced pressure maintaining the temperature below 50°C to give *tert*-butyl azetidine-3-carboxylate acetic acid salt (96 g, 92% yield) as a colorless oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ ppm 1.47 (s, 9H), 2.02 (s, 3H), 3.52 – 3.63 (m, 1H), and 4.00 – 4.10 (m, 4H).

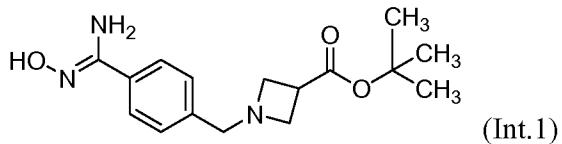
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Int.1-D. *tert*-Butyl 1-(4-cyanobenzyl)azetidine-3-carboxylate

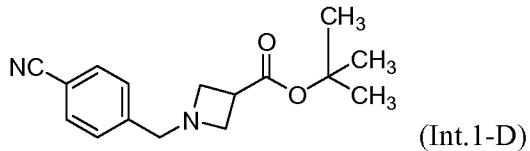


[00106] To a solution of *tert*-butyl azetidine-3-carboxylate acetic acid salt (92.0 g, 0.423 mol) in methanol (1.0 L) at room temperature was added 4-formylbenzonitrile 15 (50.8 g, 0.381 mol). The reaction mixture was cooled to 0°C, and sodium cyanoborohydride (28.8 g, 0.458 mol) was added portion-wise (caution: potential cyanide generation). The reaction mixture was allowed to warm to room temperature and stirring continued overnight. After the reaction mixture was concentrated under reduced pressure the residue was diluted with 10% aqueous sodium bicarbonate 20 solution and extracted with ethyl acetate. The organic layer was collected, washed with brine and dried over anhydrous sodium sulfate. Concentration under reduced pressure followed by purification by silica gel chromatography using 20% ethyl acetate in petroleum ether afforded *tert*-butyl 1-(4-cyanobenzyl)azetidine-3-carboxylate (89%) (After chromatography, Int.1-D contained a small amount of 4-25 hydroxymethylbenzonitrile but was taken forward to the next step without further purification). LC/MS M<sup>+1</sup> = 273.18. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ ppm 1.46 (s, 9H), 3.22 – 3.31 (m, 3H), 3.48 – 3.56 (m, 2H), 3.66 (s, 2H), 7.39 (d, *J*=8.28 Hz, 2H), and 7.60 (d, *J*=8.28 Hz, 2H).

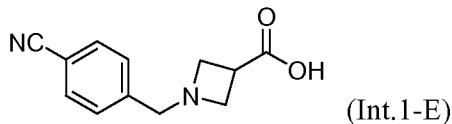
Int.1. Preparation of (Z)-*tert*-butyl 1-(4-(N'-hydroxycarbamimidoyl)benzyl)azetidine-3-carboxylate

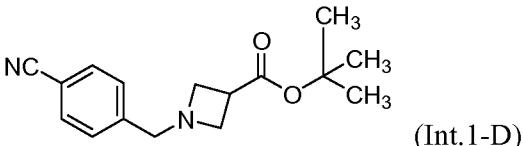


- [00107] To *tert*-butyl-1-(4-cynaobenzyl)azetidine-3-carboxylate (89.0 g, 0.326 mol) in *tert*-butanol (1.30 L) was added sodium bicarbonate (109.8 g, 1.31 mol) and hydroxylamine hydrochloride (45.5 g, 0.654 mol). The reaction was heated at reflux for 7 h and then cooled to room temperature and stirred overnight. The reaction mixture was diluted with water and extracted with ethyl acetate. The organic layer was collected, washed with water, washed with brine, then dried over anhydrous sodium sulfate. Concentration followed by purification by silica gel chromatography using 2.5 % methanol in chloroform containing 0.2% triethylamine as eluent afforded (Z)-*tert*-butyl 1-(4-(N'-hydroxycarbamimidoyl)benzyl)azetidine-3-carboxylate (64 g, 0.210 mol, 55% yield over 2 steps). The compound had an HPLC retention time = 7.03 min. – Column: XBridge Phenyl 150x4.6 mm 3.5u, SC/749. 1mL/min. Solvent A = 5% MeCN, 95% H<sub>2</sub>O, 0.05% TFA; Solvent B = 95% MeCN, 5% H<sub>2</sub>O, 0.05% TFA. Time/%B: 0min/0%, 15min/50%, 18min/100%, 20min/100%. LC/MS M<sup>+1</sup> = 306.2. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ ppm 1.45 (s, 9H), 3.23 – 3.30 (m, 3H), 3.49 – 3.57 (m, 2H), 3.63 (s, 2H), 4.85 (s, 2H), 7.31 (d, *J*=8.28 Hz, 2H), and 7.57 (d, *J*=8.28 Hz, 2H).
- 20 Alternative Preparation of Int.1-D: *tert*-Butyl 1-(4-cyanobenzyl)azetidine-3-carboxylate



Int. 1-E. 1-(4-Cyanobenzyl)azetidine-3-carboxylic acid



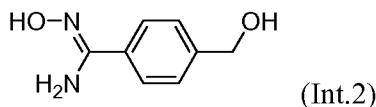
- [00108] A mixture of 4-formylbenzonitrile (2.88 g, 22.0 mmol), azetidine-3-carboxylic acid (2.02 g, 20 mmol), and acetic acid (1.15 mL, 20.0 mmol) in dichloromethane (20 mL) and methanol (80 mL) was stirred at room temperature for 1 h. Sodium triacetoxyborohydride (6.78 g, 32.0 mmol) was added and stirring was continued at room temperature for 18 hr. The volatiles were removed under reduced pressure, and the residue was partitioned between water (50 mL) and diethyl ether (50 mL). The aqueous layer was collected, washed with diethyl ether (50 mL), and concentrated. The residue was dissolved in water (20 mL) and loaded onto a 2.5 x 20 cm HP-20 column [Preparation of HP-20 Gel: ~400 ml of dry, unused MCI CHP-20 Gel (75-150 micron) was swelled in methanol for 24 hrs. The gel was filtered and rinsed with 1 liter of methanol. It was then transferred to a bottle for storage under methanol. Immediately before use, the desired amount of gel was rinsed thoroughly with 20 volumes of water]. The column was eluted with 240 mL of water and 400 mL of methanol. The product containing fractions were concentrated and co-evaporated from ethanol and ethyl acetate/heptane to afford 1-(4-cyanobenzyl)azetidine-3-carboxylic acid (3.25 g, 15.0 mmol, 75 % yield) as a white solid. MS:(M+H) = 217.18. <sup>1</sup>H NMR (400 MHz, MeOD) δ ppm 3.39 (m, 1H), 4.08 (m, 4H), 4.32 (s, 2H), 7.63 (d, *J*= 8.3 Hz, 2H), and 7.82 (d, *J*= 8.3 Hz, 2H).
- 10 Int.1-Alt.D2. *tert*-Butyl 1-(4-cyanobenzyl)azetidine-3-carboxylate
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- 30
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- [00109] To a mixture of 1-(4-cyanobenzyl)azetidine-3-carboxylic acid (3.25 g, 15.0 mmol), DMAP (1.84 g, 15.0 mmol), and *tert*-butanol (14.1 mL, 150 mmol) in dichloroethane (150 mL) was added EDCI (4.32 g, 22.5 mmol), and the reaction mixture was allowed to stir over the weekend. The volatiles were removed under reduced pressure, and the residue was partitioned between ethyl acetate (250 mL) and a saturated aqueous solution of sodium bicarbonate (250 mL). The organic layer was washed with water (250 mL), washed with brine (100 mL), and dried over anhydrous magnesium sulfate. Concentration under reduced pressure afforded a light yellow oil which was chromatographed on a 5 x 15 cm silica gel column, eluting with a 0-40%

ethyl acetate/hexane gradient to give *tert*-butyl 1-(4-cyanobenzyl)azetidine-3-carboxylate (3.5 g, 12.9 mmol, 86 % yield) as a colorless liquid. HPLC retention time = 1.38 minutes – Column: YMC-Combi 4.6 x 50 mm S-5 ODS column) eluting with 10-90% aqueous methanol + 0.2% phosphoric acid over a 4 minute gradient.

5 MS:(M+H) = 273.18.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 1.46 (s, 9H), 3.26 (m, 3H), 3.52 (m, 2H), 3.66 (s, 2H), 7.39 (d,  $J$ = 8.3 Hz, 2H), and 7.60 (d,  $J$ = 8.3 Hz, 2H).

#### Preparation of Intermediate 2 (Int.2)

##### N'-Hydroxy-4-(hydroxymethyl)benzimidamide



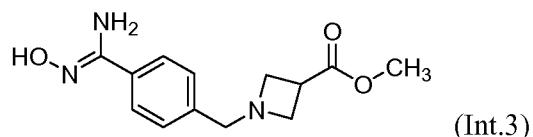
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[00110] A solution of 4-(hydroxymethyl)benzonitrile (22.43g, 0.1685g), hydroxylamine hydrochloride (18.67g, 0.2689 mol) and  $\text{NaHCO}_3$  (45.22g, 0.538 mol) in methanol (250 mL) was heated to reflux overnight. Upon cooling, the heterogeneous mixture was filtered and the solids were washed with additional methanol. The filtrate was evaporated to dryness to afford an off-white solid. The solids were suspended in ~75 mL hot methanol, then cooled to room temperature and filtered. The filtrate was evaporated to afford N'-hydroxy-4-(hydroxymethyl)benzimidamide (28.5g) as a white solid. The material was used without further purification. The compound had an HPLC retention time = 0.232 min. - Column: CHROMOLITH® SpeedROD 4.6 x 50 mm (4 min.); Solvent A = 10% MeOH, 90%  $\text{H}_2\text{O}$ , 0.2%  $\text{H}_3\text{PO}_4$ ; Solvent B = 90% MeOH, 10%  $\text{H}_2\text{O}$ , 0.2%  $\text{H}_3\text{PO}_4$ . LC/MS  $\text{M}^{+1}$  = 167.3

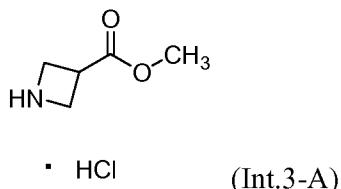
#### Preparation of Intermediate 3 (Int.3)

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##### (Z)-Methyl 1-(4-(N'-hydroxycarbamimidoyl)benzyl)azetidine-3-carboxylate

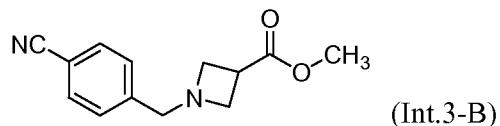


Int.3-A. Methyl azetidine-3-carboxylate, HCl



[00111] To methanol (5 mL), pre-cooled to 0°C, was added acetyl chloride (0.852 mL, 11.99 mmol). After stirring 5 minutes at 0°C, the reaction mixture was allowed to warm to RT. At this time, azetidine-3-carboxylic acid (404 mg, 4.00 mmol) was 5 added and the reaction mixture was refluxed for 2.5 hr. After cooling to rt, the volatiles were removed *in vacuo* and the residue was dried to afford methyl azetidine-3-carboxylate, HCl (600 mg, 3.96 mmol) as a white solid. <sup>1</sup>H NMR (400 MHz, methanol-d<sub>4</sub>) δ ppm 3.77 (m, 1H) 3.81 (s, 3H) 4.22-4.33 (m, 4H).

10 Int.3-B. Methyl 1-(4-cyanobenzyl)azetidine-3-carboxylate:



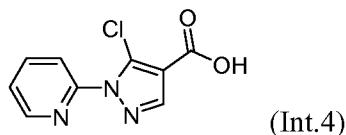
[00112] A mixture of 4-formylbenzonitrile (510 mg, 3.89 mmol), methyl azetidine-3-carboxylate (590 mg, 3.89 mmol), acetic acid (0.245 mL, 4.28 mmol) and ~1 gm of powdered 4 Angstrom molecular sieves in DCE (15 mL) was stirred briskly at RT for 15 1 hr. At this time, sodium triacetoxyborohydride (907 mg, 4.28 mmol) was added and the reaction mixture was stirred at RT for 2 hr. After filtering through a sintered glass funnel, the filtrate was concentrated to a residue that was partitioned between EtOAc (50 mL) and saturated sodium bicarbonate solution (50 mL). The organic layer was washed with brine (50 mL), dried (MgSO<sub>4</sub>) and concentrated to afford a colorless oil 20 that was chromatographed on a 12 gm Isco silica gel cartridge, eluting with a 0-100%EtOAc/Hex gradient. The essentially pure fractions were concentrated to afford methyl 1-(4-cyanobenzyl)azetidine-3-carboxylate (765 mg, 3.32 mmol). HPLC retention time = 0.49 minutes [broad peak] (PHENOMENEX® Luna 4.6 x 30 mm S-5 ODS column) eluting with 10-90% aqueous methanol + 0.1% TFA over a 2 minute 25 gradient. MS:(M+H)= 231.23. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ ppm 3.32-3.36 (m, 3H) 3.49-3.58 (m, 2H) 3.67 (s, 2H) 3.72 (s, 3H), 7.39 (d, *J*= 8.6 Hz, 2H) 7.60 (d, *J*= 8.3 Hz, 2H).

Int.3-C. (Z)-Methyl 1-(4-(N'-hydroxycarbamimidoyl)benzyl)azetidine-3-carboxylate [00113] A mixture of methyl 1-(4-cyanobenzyl)azetidine-3-carboxylate (760 mg, 3.30 mmol), hydroxylamine hydrochloride (459 mg, 6.60 mmol) and sodium 5 bicarbonate (1109 mg, 13.20 mmol) in MeOH (4.9 mL) was heated to reflux for 2.5 hr. After cooling to rt, the reaction mixture was filtered and the filtrate was concentrated to a residue that was partitioned between EtOAc (75 mL) and water (50 mL). The aqueous layer was extracted with EtOAc (75 mL) and the combined organic layers were dried ( $\text{MgSO}_4$ ) and concentrated to afford (Z)-methyl 1-(4-(N'-hydroxycarbamimidoyl)benzyl)azetidine-3-carboxylate (470 mg, 1.785 mmol) as a colorless foam. MS:  $(\text{M}+\text{H})=264.28$ .  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 3.30-3.39 (m, 3H) 3.54 (m, 2H) 3.64 (s, 2H) 3.71 (s, 3H) 4.86 (brs, 2H) 7.31 (d,  $J=8.3$  Hz, 2H) 10 7.57 (d,  $J=8.3$  Hz, 2H).

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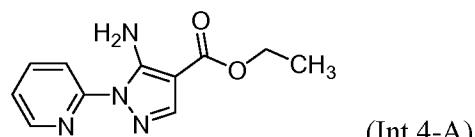
## Preparation of Intermediate 4 (Int.4)

5-Chloro-1-(pyridin-2-yl)-1H-pyrazole-4-carboxylic acid



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## Int.4-A. Ethyl 5-amino-1-(pyridin-2-yl)-1H-pyrazole-4-carboxylate

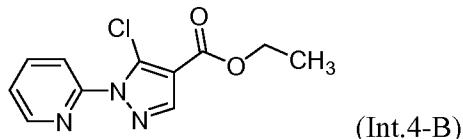


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[00114] A mixture of 2-hydrazinopyridine (4.84 g, 44.3 mmol), ethyl (ethoxymethylene) cyanoacetate (5 g, 29.6 mmol) and  $\text{Et}_3\text{N}$  (12.36 mL, 89 mmol) which was heated to 80 °C for 30 minutes. The reaction mixture was concentrated and purified on silica gel column with  $\text{CH}_2\text{Cl}_2$ /EtOAc to yield ethyl 5-amino-1-(pyridin-2-yl)-1H-pyrazole-4-carboxylate (5.97g, 25.7 mmol) HPLC rt=2.856 min. HPLC: Column: YMC S5 COMBISCREEN® 4.6x50 mm; Gradient time: 4 min; Flow rate = 4 mL/min; Solvent A = 10% MeOH - 90% Water - 0.2%  $\text{H}_3\text{PO}_4$ ; Solvent B = 90% MeOH - 10% water - 0.2%  $\text{H}_3\text{PO}_4$ ; Start % B = 0; Final % B = 100. LC-MS:

$M^{+1} = 233.1$ .  $^1H$  NMR (500 MHz, methanol-d<sub>3</sub>)  $\delta$  ppm 8.44 (1 H, ddd,  $J=4.95, 1.92, 0.82$  Hz), 7.92 - 7.96 (1 H, m), 7.87 - 7.90 (1 H, m), 7.74 (1 H, s), 7.27 (1 H, ddd,  $J=7.22, 5.02, 1.24$  Hz), 4.29 (2 H, q,  $J=7.15$  Hz), 1.35 (3 H, t,  $J=7.15$  Hz).

5 Int.4-B. Ethyl 5-chloro-1-(pyridin-2-yl)-1H-pyrazole-4-carboxylate

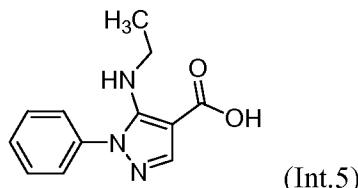


- [00115] Method 1: To a solution of ethyl 5-amino-1-(pyridin-2-yl)-1H-pyrazole-4-carboxylate (200 mg, 0.861 mmol) and copper (II) chloride (116 mg, 0.861 mmol) in 1,2-dichloroethane (8 mL) was added *tert*-butyl nitrite (0.154 mL, 1.292 mmol). The reaction mixture was stirred at rt for 16 hrs. The reaction mixture was diluted with 30 mL of dichloromethane and was washed with 10 mL of 2M NH<sub>4</sub>OH solution, 10 mL of brine and dried over Na<sub>2</sub>SO<sub>4</sub>. Filtration and concentration yielded a crude product which was purified on silica gel column eluting with hexanes/EtOAc to afford ethyl 5-chloro-1-(pyridin-2-yl)-1H-pyrazole-4-carboxylate.
- [00116] Method 2: A solution of ethyl 5-amino-1-(pyridin-2-yl)-1H-pyrazole-4-carboxylate (200 mg, 0.861 mmol) and 1-chloropyrrolidine-2,5-dione (172 mg, 1.292 mmol) in dichloromethane (8 mL) was added *tert*-butyl nitrite (0.154 mL, 1.292 mmol). The reaction mixture was stirred at rt for 30 minutes, after which time it was concentrated to yield a crude product which was purified on a silica gel column eluting with hexanes/EtOAc to yield ethyl 5-chloro-1-(pyridin-2-yl)-1H-pyrazole-4-carboxylate. The material from Method 1 and Method 2 were combined (106 mg, 0.42 mmol). HPLC ret. time = 2.50 min. HPLC: Column: YMC S5 COMBISCREEN® 4.6x50 mm; Gradient time: 4 min; Flow rate = 4 mL/min; Solvent A = 10% MeOH - 90% Water - 0.2% H<sub>3</sub>PO<sub>4</sub>; Solvent B = 90% MeOH - 10% water - 0.2% H<sub>3</sub>PO<sub>4</sub>; Start % B = 0; Final % B = 100. LC-MS:  $M^{+1} = 252.1$ .  $^1H$  NMR (400 MHz, MeOD)  $\delta$  ppm 8.61 (1 H, dd,  $J=4.89, 1.13$  Hz), 8.16 (1 H, s), 8.09 (1 H, td,  $J=7.78, 1.76$  Hz), 7.74 (1 H, d,  $J=8.03$  Hz), 7.57 (1 H, ddd,  $J=7.53, 4.77, 1.00$  Hz), 4.36 (2 H, q,  $J=7.28$  Hz), 1.38 (3 H, t,  $J=7.15$  Hz).
- 30 Int.4-C. 5-Chloro-1-(pyridin-2-yl)-1H-pyrazole-4-carboxylic acid

[00117] A solution of ethyl 5-chloro-1-(pyridin-2-yl)-1H-pyrazole-4-carboxylate (106 mg, 0.421 mmol) and NaOH 1N solution (.632 mL, 0.632 mmol) in EtOH (3.5 mL) was heated to 100 °C under microwave irradiation for 15 minutes. The reaction mixture was concentrated *in vacuo* and the residue was diluted with 5 mL of water 5 which was then acidified with 1N HCl solution until approximately pH 3. After stirring for 5 minutes, the solid was collected as 5-chloro-1-(pyridin-2-yl)-1H-pyrazole-4-carboxylic acid (39.6mg, 0.177 mmol). HPLC ret. time=1.67 min. HPLC: Column: YMC S5 COMBISCREEN® 4.6X50 mm; Gradient time: 4 min; Flow rate = 4 mL/min; Solvent A = 10% MeOH - 90% Water - 0.2% H<sub>3</sub>PO<sub>4</sub>; Solvent B = 90% 10 MeOH - 10% water - 0.2% H<sub>3</sub>PO<sub>4</sub>; Start % B = 0; Final % B = 100. LC-MS: LCMS: M<sup>+1</sup> = 224.1.

Preparation of Intermediate 5 (Int.5)

5-(Ethylamino)-1-phenyl-1H-pyrazole-4-carboxylic acid

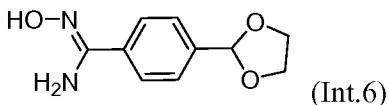


[00118] To a solution of ethyl 5-amino-1-phenyl-1H-pyrazole-4-carboxylate (10 g, 43 mmol) in DMF (100 mL) at 0°C was slowly added sodium hydride (60% dispersion, 1.9 g, 47.6 mmol). After the evolution of gas had ceased, bromoethane (3.55mL, 47.6 mmol) was added dropwise. The reaction was stirred at 0°C for 2 20 hour, then gradually warmed to RT. Water was added to the reaction, resulting in the formation of a precipitate which was collected by filtration and used directly in the next step. The material thus obtained was dissolved in EtOH (50 mL) along with 3N NaOH (38 mL) and the solution was heated to reflux overnight. The reaction was cooled to 0 °C and the pH was slowly adjusted to between 3-4 using 1N HCl. The 25 resulting white precipitate was collected by filtration and recrystallized from acetonitrile to afford 5-(ethylamino)-1-phenyl-1H-pyrazole-4-carboxylic acid (6.6 g, 28.6 mmol). The compound had an HPLC retention time = 2.32 min. CHROMOLITH® SpeedROD 4.6 x 50 mm (4 min.); Solvent A = 10% MeOH, 90% H<sub>2</sub>O, 0.2% H<sub>3</sub>PO<sub>4</sub>; Solvent B = 90% MeOH, 10% H<sub>2</sub>O, 0.2% H<sub>3</sub>PO<sub>4</sub>. LC/MS M<sup>+1</sup> =

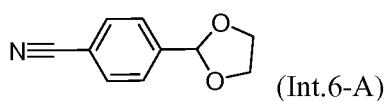
232.3.  $^1\text{H}$  NMR (500 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 0.89 (t,  $J$ =7.2 Hz, 3H), 2.77 (m, 2H), 6.14 (br. s, 1H), 7.43 (m, 1H), 7.52 (m, 4H), 7.72 (s 1H).

Preparation of Intermediate 6 (Int.6)

5 (Z)-4-(1,3-Dioxolan-2-yl)-N'-hydroxybenzimidamide



Int.6-A. 4-(1,3-dioxolan-2-yl)benzonitrile



10 [00119] A solution of 4-formylbenzonitrile (10g, 76.3 mmol), ethylene glycol (18.95g, 305 mmol) and *p*-toluene sulfonic acid (7 mg, 0.003 mmol) in toluene (100 mL) was heated to reflux for 9 hours. During this time a Dean-Stark trap was used to remove water formed during the reaction. To the reaction was added aq. Na<sub>2</sub>CO<sub>3</sub> (150 mL). The organic layer was washed twice with water (100 mL), then dried over Na<sub>2</sub>SO<sub>4</sub>. The solvent was removed under vacuum to provide 4-(1,3-dioxolan-2-yl)benzonitrile (12 g, 68 mmol). The compound had an HPLC retention time = 4.24 min. – Column: HYPERSIL® BDS C18 (4.6 x 50 mm) Flow rate = 0.8 mL/min. Solvent A =, 0.1% TFA in water; Solvent B = acetonitrile. 5%-100% B (0-6min), 100% B (6-9 min), 100%-5% B (9-11 min).

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Int.6-B. (Z)-4-(1,3-Dioxolan-2-yl)-N'-hydroxybenzimidamide

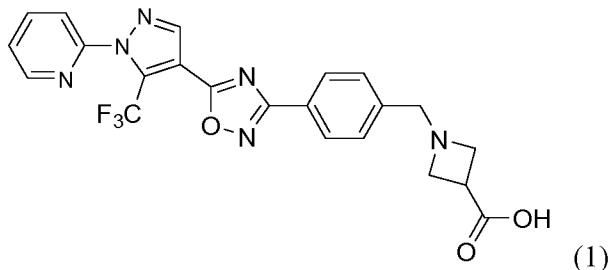
[00120] A mixture of 4-(1,3-dioxolan-2-yl)benzonitrile (16.4 g, 93.6 mmol), hydroxylamine hydrochloride (9.76 g, 140 mmol) and sodium bicarbonate (15.7 g, 187 mmol) in MeOH (100 mL) was heated to reflux for 4 hr. After cooling to RT, the reaction mixture was filtered and the filtrate was concentrated to provide solids that were collected by filtration and washed with cold methanol to provide (Z)-4-(1,3-dioxolan-2-yl)-N'-hydroxybenzimidamide (16g, 76.8 mmol). The compound had an HPLC retention time = 0.38 min. – CHROMOLITH® SpeedROD 4.6 x 30 mm. flow = 5mL/min (2 min. gradient); Solvent A = 10% MeOH, 90% H<sub>2</sub>O, 0.1% TFA; Solvent B = 90% MeOH, 10% H<sub>2</sub>O, 0.1% TFA. LC/MS M<sup>+1</sup> = 209.2

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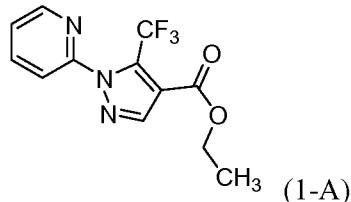
30

Example 1

1-(4-(5-(1-(Pyridin-2-yl)-5-(trifluoromethyl)isoxazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid



1-A. Ethyl 1-(pyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxylate

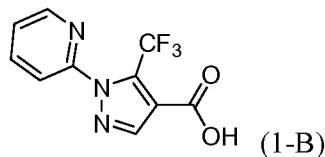


[00121] A solution of 2- hydrazinopyridine (5 g, 45.8 mmol) in THF (200 mL) under nitrogen was cooled in a dry ice / isopropanol bath to -34°C. A solution of (Z)-ethyl 2-(ethoxymethylene)-4,4,4-trifluoro-3-oxobutanoate (11 g, 45.8 mmol) in THF (73 mL) was added in a slow stream over 25 minutes, with nitrogen flush. During the addition, the internal reaction temperature averaged -34°C, and never exceeded -31°C. After completion of the addition, stirring at -33.5°C was continued for ~40 min. Additional (Z)-ethyl 2-(ethoxymethylene)-4,4,4-trifluoro-3-oxobutanoate (0.89 mL, 0.10 eq) was added and stirring at -30°C was continued for 11 min. EtOAc (270 mL) was added along with sat. aq. NaHCO<sub>3</sub> solution (250 mL) and brine (20 mL). The layers were separated and the organic layer was washed with sat. aq. NaHCO<sub>3</sub> solution, brine, then dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated under vacuum to yield an orange viscous oil. Flash chromatography on silica gel yielded ethyl 1-(pyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxylate (10.62 g) as a yellow solid. The compound had an HPLC retention time = 2.5 min. – Column: YMC COMBISCREEN® ODS-A 4.6 x 50 mm (4 min.); Solvent A = 10% MeOH, 90%

H<sub>2</sub>O, 0.1% TFA; Solvent B = 90% MeOH, 10% H<sub>2</sub>O, 0.1% TFA. LC/MS M<sup>+</sup> = 286.1.

1-B. 1-(Pyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxylic acid

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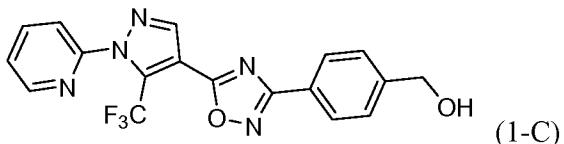


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[00122] To a solution of ethyl 1-(pyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxylate (8.7g, 30.5 mmol) in ethanol (2 mL) was added 3 N NaOH (30.5 mL, 92 mmol). After the reaction was stirred at room temperature for 1 hour, the solvents were evaporated and 3N aq. HCl was added. The resulting solids were collected by filtration to afford 1-(pyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxylic acid (5.58g, 21.7 mmol). The compound had an HPLC retention time = 2.0 min. –  
Column: PHENOMENEX® Luna 5u C18 4.6 x 30 mm (2 min.); Solvent A = 10% MeOH, 90% H<sub>2</sub>O, 0.1% TFA; Solvent B = 90% MeOH, 10% H<sub>2</sub>O, 0.1% TFA.  
LC/MS M<sup>+</sup> = 258.1.

15

1-C. (4-(5-(1-(Pyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)phenyl)methanol



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[00123] To a solution of 1-(pyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxylic acid (2.57g, 9.99 mmol) in DMF (30mL) was added EDC (2.395 g, 12.49 mmol), HOBr (1.913 g, 12.49 mmol) and Hunig's Base (5.24 mL, 30.0 mmol). The reaction was stirred for 40 min. at room temperature, then N-hydroxy-4-(hydroxymethyl)benzimidamide, Int.2, (2.076 g, 12.49 mmol) was added. The reaction mixture was stirred at room temperature for 7h, then additional EDC (2.395 g, 12.49 mmol), HOBr (1.913 g, 12.49 mmol) and Hunig's Base (5.24 mL, 30.0 mmol) were added. The reaction was heated to 60 °C for 2h, then at 110 °C for 10min. 1N aq. HCl was added to adjust the pH to 1, then 30 mL of water was added. The resulting precipitated product was collected by filtration and washed with water

to afford (4-(5-(1-(pyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)phenyl)methanol (2.28g, 5.83 mmol, 58%) as a light yellow solid.

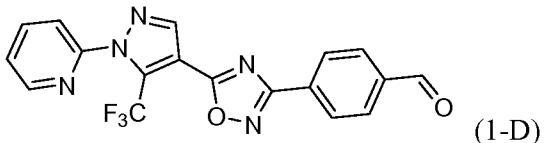
The compound had an HPLC retention time = 3.37 min. – Column:

CHROMOLITH® SpeedROD 4.6 x 50 mm (4 min.); Solvent A = 10% MeOH, 90%

5 H<sub>2</sub>O, 0.2% H<sub>3</sub>PO<sub>4</sub>; Solvent B = 90% MeOH, 10% H<sub>2</sub>O, 0.2% H<sub>3</sub>PO<sub>4</sub>. LC/MS M<sup>+1</sup> = 388.0 <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 4.61 (d, *J* = 6 Hz, 2H), 5.39 (t, *J* = 6 Hz, 1H), 7.55 (d, *J* = 8.4 Hz, 2H), 7.70 (ddd, *J* = 0.9 Hz, 4.8 Hz, 7.5 Hz, 1H), 7.90 (d, *J* = 7.9 Hz, 1H), 8.06 (d, *J* = 8.4 Hz, 2H), 8.20 (dt, *J* = 2.2 Hz, 7.5 Hz, 1H), 8.67 (dd, *J* = 1.3 Hz, 4.8 Hz, 1 H), 8.73 (s, 1H).

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1-D. 4-(5-(1-(Pyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzaldehyde



[00124] 300 mL of DCM was cooled to -78°C, then oxalyl chloride (0.475 mL,

15 5.42 mmol) was added and the mixture was stirred at -78 °C for 10 min. DMSO (0.586 mL, 8.26 mmol) was added and the mixture was stirred at -78 °C for 15min. A solution of (4-(5-(1-(pyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)phenyl)methanol (1 g, 2.58 mmol) in 60 mL of DCM was added dropwise over 10 minutes into the reaction mixture, with the SM flask being rinsed 20 with another 10 mL of DCM which was also added into the reaction dropwise. The reaction was kept at -78 °C for 40min, then Hunig's Base (3.61 mL, 20.66 mmol) was added over 10 minutes. Stirring at -78 °C continued for 10min, then the dry ice bath was removed and the reaction flask gradually warmed to room temperature. A 0.5N KHSO<sub>4</sub> solution (70 mL) was added and the mixture was extracted twice with DCM (50mL). The organics were combined, dried with Na<sub>2</sub>SO<sub>4</sub>, and concentrated. Diethyl ether (100mL) was added to the product, with the mixture being stirred overnight, then filtered and the solids washed with diethyl ether to afford 4-(5-(1-(pyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzaldehyde (700 mg, 1.74 mmol). The compound had an HPLC retention time = 3.69 min. – Column:

25 CHROMOLITH® SpeedROD 4.6 x 50 mm (4 min.); Solvent A = 10% MeOH, 90%

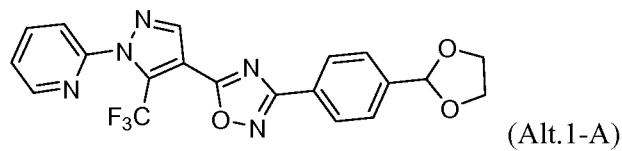
H<sub>2</sub>O, 0.2% H<sub>3</sub>PO<sub>4</sub>; Solvent B = 90% MeOH, 10% H<sub>2</sub>O, 0.2% H<sub>3</sub>PO<sub>4</sub>. LC/MS M<sup>+1</sup> = 386.01. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 7.69 (ddd, *J* = 0.8 Hz, 5.0 Hz, 7.4 Hz, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 8.15 (d, *J* = 8.3 Hz, 2H), 8.20 (dt, *J* = 1.9 Hz, 7.7 Hz, 1H), 8.32 (d, *J* = 8.3 Hz, 2H), 8.67 (dd, *J* = 1.9 Hz, 4.7 Hz, 1 H), 8.76 (s, 1H), 5 10.13 (s, 1H)

1-E. Preparation of 1-(4-(5-(1-(pyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid

[00125] To a solution of 4-(5-(1-(pyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzaldehyde (1.0 g, 2.60 mmol) in DCM (10 mL) was added acetic acid (15 mL) and azetidine-3-carboxylic acid (1.5 eq, 3.90 mmol). The mixture was stirred at RT for 40min., then a suspension of sodium triacetoxyborohydride (1eq, 2.60 mmol) in DCM (30 mL) was added dropwise over 1.5 h. The volatiles were removed *in vacuo*, water (200mL) was added and the resulting solid was collected by filtration. Ethyl acetate (40 mL) was added to the crude product and after sonication for 30min, the solids were collected by filtration and washed additional ethyl acetate to afford 1-(4-(5-(1-(pyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl) azetidine-3-carboxylic acid (777 mg, 1.65 mmol) as a white solid. The compound had an HPLC retention time = 2.55 min. Column: CHROMOLITH® SpeedROD 4.6 x 50 mm (4 min.); Solvent A = 10% MeOH, 90% H<sub>2</sub>O, 0.2% H<sub>3</sub>PO<sub>4</sub>; Solvent B = 90% MeOH, 10% H<sub>2</sub>O, 0.2% H<sub>3</sub>PO<sub>4</sub>. LC/MS M<sup>+1</sup> = 471.05. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 3.21-3.25 (m, 3H), 3.40-3.44 (m, 2H), 3.64 (s, 2H), 7.51 (d, *J* = 8.3 Hz, 2H), 7.69 (ddd, *J* = 0.8 Hz, 4.7 Hz, 8.3 Hz, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 8.03 (d, *J* = 8.3 Hz, 2H), 8.20 (dt, *J* = 1.9 Hz, 7.7 Hz, 1H), 8.66 (dd, *J* = 1.1 Hz, 4.1 Hz, 1 H) 8.73 (s, 1H).

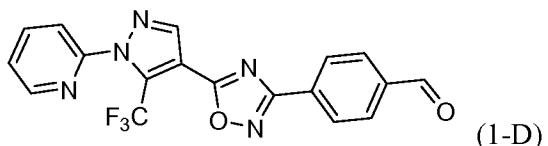
Alternative Preparation of Example 1 (Alt.1)

30 Alt.1-A. 3-(4-(1,3-Dioxolan-2-yl)phenyl)-5-(1-(pyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazole



- [00126] To a solution of 1-(pyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxylic acid (31.46 g, 122 mmol) in DMF (200 mL) was added EDCI (23.34 g, 134 mmol) and HOBr (16.53 g, 122 mmol). After the reaction was stirred for 30 min.
- 5 at room temperature 4-(1,3-dioxolan-2-yl)benzonitrile, Int.6-A, (16.53 g, 122 mmol) was added. The reaction mixture was stirred at 140 °C for 4 hr. The reaction mixture was concentrated under reduced pressure to remove the volatiles. The residue was partitioned between water and ethyl acetate and the organic layer was washed with aq. HCl solution, NaHCO<sub>3</sub> solution, water, and brine before being dried over Na<sub>2</sub>SO<sub>4</sub>
- 10 and concentrated to provide 3-(4-(1,3-dioxolan-2-yl)phenyl)-5-(1-(pyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazole (25 g, 58 mmol). The compound had an HPLC retention time = 6.15 min. – Column: HYPERSIL® BDS C18 (4.6 x 50 mm) Flow rate = 0.8 mL/min. Solvent A =, 0.1% TFA in water; Solvent B = acetonitrile. 5%-100% B (0-6min), 100% B (6-9 min), 100%-5% B (9-11 min).
- 15 LC/MS M<sup>+1</sup> = 430.0.

Alternate Preparation of 1-D. 4-(5-(1-(Pyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzaldehyde



- 20 [00127] A solution of 3-(4-(1,3-dioxolan-2-yl)phenyl)-5-(1-(pyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazole (25 g, 58.2 mmol) and con. HCl (500 mL) in THF/water (500 mL/500 mL) was stirred at RT for 3 hours. Upon completion of the reaction, as monitored by TLC, the reaction mixture was diluted with water and extracted with ethyl acetate. The organic layer was washed with water and brine and then dried and concentrated under reduced pressure to afford 4-(5-(1-(pyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzaldehyde (19 g, 49 mmol). The compound had an HPLC retention time = 1.90 min. – CHROMOLITH® SpeedROD 4.6 x 30 mm. flow = 5mL/min (2 min).

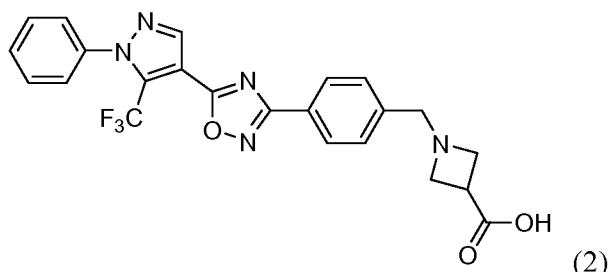
gradient); Solvent A = 10% MeOH, 90% H<sub>2</sub>O, 0.1% TFA; Solvent B = 90% MeOH, 10% H<sub>2</sub>O, 0.1% TFA. LC/MS M<sup>+1</sup> = 386.2.

Alternate Preparation of Example 1. 1-(4-(5-(1-(Pyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid

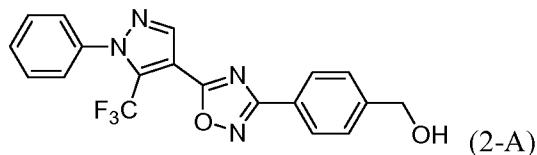
[00128] A solution of 4-(5-(1-(pyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzaldehyde (21 g, 54.5 mmol) and azetidine-3-carboxylic acid (6.61 g, 65.4 mmol) in ethanol (750 mL) and acetic acid (20 mL) was stirred at RT for 30 min. The reaction was cooled to 0°C and a solution of sodium cyanoborohydride (1.17 g, 27.3 mmol) in ethanol was added slowly. The reaction was warmed to RT and stirred overnight. The reaction mixture was concentrated under reduced pressure and the residue was diluted with water. The solids were collected by filtration and washed with water. The product was purified by triturating once each with DCM:methanol (98:2), DCM:methanol (96:4) and ethyl acetate to afford 1-(4-(5-(1-(pyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid (16g, 34 mmol). The compound had an HPLC retention time = 8.48 min. – Column: XbridgeA 4.6 x 150 mm Flow rate = 1.0 mL/min. Buffer = 0.05% TFA in water pH2.5. Solvent A = Buffer: Acetonitrile (95:5), Solvent B = Acetonitrile:Buffer (95:5). 0-100% B over 12 minutes.. LC/MS M<sup>+1</sup> = 471.2.

## Example 2

1-(4-(5-(1-Phenyl-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid



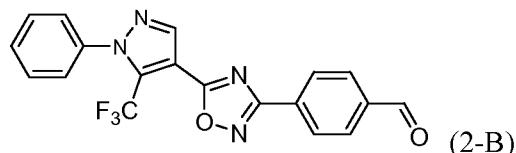
2-A. (4-(5-(1-Phenyl-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)phenyl)methanol



[00129] 1-Phenyl-5-(trifluoromethyl)-1H-pyrazole-4-carboxylic acid (2g, 7.81 mmol), HOBT (1.315 g, 8.59 mmol) and EDC (1.646 g, 8.59 mmol) were dissolved in DMF (18 mL) under nitrogen at room temperature. The resulting clear, pale yellow solution was stirred for 20 minutes, then N-hydroxy-4-(hydroxymethyl)benzimidamide, Int.2, (1.297 g, 7.81 mmol) was added in several portions. The reaction was placed in an oil bath preheated to 140°C for 10 hours. The reaction was cooled to room temperature, poured into water (150 mL), stirred vigorously and placed in a sonicator bath for 1 hour. The solids were collected by filtration, washed with water, air dried and then placed under vacuum to give (4-(5-(1-phenyl-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)phenyl)methanol (2.5g, 6.47 mmol) as a very light yellow solid. The compound had an HPLC retention time = 3.76 min. – Column: CHROMOLITH® SpeedROD 4.6 x 50 mm (4 min.); Solvent A = 10% MeOH, 90% H<sub>2</sub>O, 0.2% H<sub>3</sub>PO<sub>4</sub>; Solvent B = 90% MeOH, 10% H<sub>2</sub>O, 0.2% H<sub>3</sub>PO<sub>4</sub>. LC/MS M<sup>+</sup> = 387.1 <sup>1</sup>H NMR (500 MHz, MeOD) δ ppm 4.70 (s, 2H), 7.53-7.57 (m, 4 H), 7.57-7.62 (m, 1H), 7.61 (d, *J* = 6.4 Hz, 2H), 8.12 (d, *J* = 8.2 Hz, 2H), 8.48 (s, 1H).

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2-B. 4-(5-(1-Phenyl-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzaldehyde



[00130] A 50 mL round bottom flask was flame dried and cooled under a stream of dry nitrogen. DCM (25 mL) followed by oxalyl chloride (6.79 mL, 13.59 mmol) (2M in DCM) were added to the flask and it was cooled in an acetone/dry ice bath. Dimethyl sulfoxide (1.47 mL, 20.71 mmol) was added dropwise. After 10 minutes,

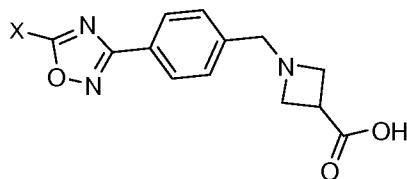
(4-(5-(1-phenyl-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)phenyl)methanol (2.5 g, 6.47 mmol) was added in small portions over 5-10 minutes providing a pale yellow slurry. After stirring for 5 minutes, DIPEA (9.27 mL, 53.1 mmol) was added dropwise. The cloudy mixture began to clear up, then 5 became cloudy again with the color fading from pale yellow to off-white during the course of the addition. After 5 minutes, the cooling bath was removed and the reaction was allowed to slowly warm to room temperature. After stirring at room temperature for 3 hours the solvents were evaporated and the residue was partitioned between EtOAc (100 mL) and 0.5N KHSO<sub>4</sub> (70 mL). The EtOAc phase was washed 10 with 50 mL each 1N HCl, water, sat. aq. NaHCO<sub>3</sub>, water and brine, then dried over MgSO<sub>4</sub>, filtered and evaporated. The solid was slurried in hexanes (with ~5% EtOAc) with sonication and heating, then cooled to room temperature and filtered. The solids were washed with hexanes, air dried and placed under vacuum to afford 4-(5-(1-phenyl-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzaldehyde 15 (1.55g). The compound had an HPLC retention time = 2.04 min. – Column: PHENOMENEX® Luna 5u C18 4.6 x 30 mm (2 min.); Solvent A = 10% MeOH, 90% H<sub>2</sub>O, 0.1% TFA; Solvent B = 90% MeOH, 10% H<sub>2</sub>O, 0.1% TFA. LC/MS M<sup>+1</sup> = 385.17.

20 2-C. Preparation of 1-(4-(5-(1-phenyl-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid  
[00131] To a solution of 4-(5-(1-phenyl-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzaldehyde (700 mg, 1.821 mmol) in MeOH (12 mL) and 1,2-dichloroethane (3 mL) was added 0.1mL of acetic acid and azetidine-3-carboxylic acid (203 mg, 2.0 mmol). The mixture was allowed to stir at room temperature for 40 min. and then sodium triacetoxyborohydride (618 mg, 2.91 mmol) was added. The mixture was allowed to stir at room temperature for 5 hours, then was filtered to afford a white solid that was washed with water (1mL) and MeOH (3 x 1mL). The solid was dried under vacuum to give 1-(4-(5-(1-phenyl-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid (480mg, 1.02 mmol). The compound had an HPLC retention time = 6.91 min. – Column: Low pH Col-1: Sunfire C18 3.5um, 4.6x150mm; Start % B = 10; 12 Min. 100%; 15 Min.

100%; Flow Rate = 2 mL/min; Wavelength1 = 220; Wavelength2 = 254; Solvent Pair = TFA - MeCN/H<sub>2</sub>O; Solvent A = 0.05% TFA in H<sub>2</sub>O:MeCN (95:5); Solvent B = 0.05% TFA in H<sub>2</sub>O:MeCN (5:95); Column 1 = LOW pH - Parallel HPLC; At 220nm LC/MS M<sup>+1</sup> = 470.1 <sup>1</sup>H NMR (500 MHz, MeOD) δ ppm 3.41 (dt, J= 8.8 Hz, 7.7 Hz, 5 1H), 4.12-4.18 (m, 4H), 4.38 (s, 2H), 7.57-7.66 (m, 7H), 8.23 (d, J= 8.3 Hz, 2H), 8.50 (s, 1H).

Table 1

[00132] The compounds in Table 1 were prepared in a manner similar to the above 10 Examples, by reaction of the corresponding pyrazole carboxylic acid with Int.2 and elaborated as described for 1-C. The requisite pyrazole carboxylic acids were either commercially available (as in the case of Ex. 5, Ex. 6, and Ex. 11), or were prepared by condensation similar to that described for the preparation of 1-A, from the corresponding hydrazines (all commercially available) with the product of the 15 condensation of the requisite beta keto ester and DMF dimethyl acetal, (*tert*-butyl 3-cyclopentyl-3-oxopropanoate in the case of Ex. 7, ethyl 2-acetoacetate, in the case of Ex. 9, ethyl 3-oxo-3-phenylpropanoate in the case of Ex. 10. The pyrazole carboxylate for Ex. 3 was prepared in a similar manner to that described for Int.5, substituting isopropyl amine for ethylamine. The pyrazole carboxylate for Ex. 8 was 20 prepared by the condensation of 2-fluoro-6-hydrazinylpyridine with (Z)-ethyl 2-(ethoxymethylene)-4,4,4-trifluoro-3-oxobutanoate.



Ex. No.	X	MW	HPLC ret. time (min.)	HPLC method	MS (M <sup>+1</sup> )

Ex. No.	X	MW	HPLC ret. time (min.)	HPLC method	MS ( $M^{+1}$ )
3		458.51	6.81	C	459.3
4		444.49	7.46	C	445.3
5		415.44	2.81	A	416.2
6		444.49	6.635	C	445.4
7		470.52	2.74	B	471.2
8		514.26	3.21	B	515.2
9		421.49	6.76	C	422.3
10		478.5	1.57	D	478.9
11		435.86	6.51	C	436.2

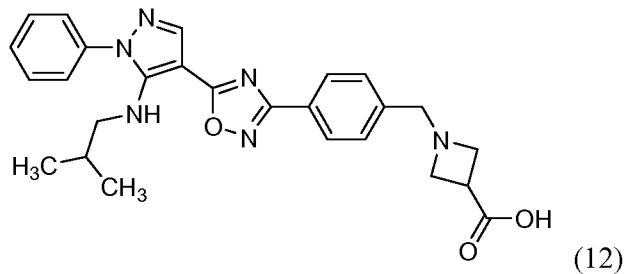
HPLC Method	HPLC Conditions
A	CHROMOLITH® SpeedROD 4.6 x 50 mm (4 min.); Solvent A = 10% MeOH, 90% H <sub>2</sub> O, 0.2% H <sub>3</sub> PO <sub>4</sub> ; Solvent B = 90% MeOH, 10% H <sub>2</sub> O, 0.2% H <sub>3</sub> PO <sub>4</sub> .
B	Column: YMC COMBISCREEN® ODS-A 4.6 x 50 mm (4 min.); Solvent A = 10% MeOH, 90% H <sub>2</sub> O, 0.2% H <sub>3</sub> PO <sub>4</sub> ; Solvent B = 90% MeOH, 10% H <sub>2</sub> O, 0.2% H <sub>3</sub> PO <sub>4</sub>
C	Low pH Col-1: Sunfire C18 3.5um, 4.6x150mm; Start % B = 10; 12 Min. 100%; 15 Min. 100%; Flow Rate = 2 mL/min; Wavelength1 = 220; Wavelength2 = 254; Solvent Pair = TFA - MeCN/H <sub>2</sub> O; Solvent A = 0.05% TFA in H <sub>2</sub> O:MeCN (95:5); Solvent B = 0.05% TFA in H <sub>2</sub> O:MeCN (5:95); Column 1 = LOW pH - Parallel HPLC; At 220nm
D	Column: XbridgeA 4.6 x 50 mm (4 min.); Solvent A = 5% acetonitrile 95% H <sub>2</sub> O, 10 mM NH <sub>4</sub> OAc; Solvent B = 95% acetonitrile 5% H <sub>2</sub> O, 10 mM NH <sub>4</sub> OAc;

## Example 12

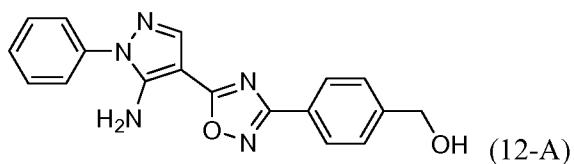
1-(4-(5-(Isobutylamino)-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-

5

yl)benzyl)azetidine-3-carboxylic acid

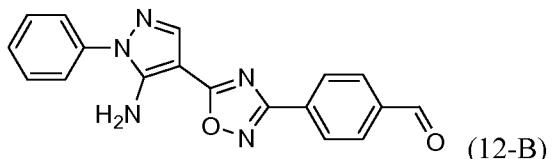


12-A. (4-(5-Amino-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)phenyl)methanol



[00133] 5-Amino-1-phenyl-1H-pyrazole-4-carboxylic acid (1.8 g, 8.86 mmol), HOBr (1.357 g, 8.86 mmol) and EDCI (1.698 g, 8.86 mmol) were dissolved in DMF (12 mL) under nitrogen at room temperature. The resulting clear, pale yellow solution was stirred for 30 minutes then N-hydroxy-4-(hydroxymethyl)benzimidamide (1.472 g, 8.86 mmol) was added in several portions. The reaction was placed in an oil bath set to 130°C. After 4 hours, the solution was cooled to RT and then added to rapidly stirring water (50mL). The initial precipitate was stirred and sonicated to give a yellow solid which was collected by filtration, washed with water, air dried and then dried under vacuum to afford (4-(5-(5-amino-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)phenyl)methanol (1.5g 4.05 mmol). The compound had an HPLC retention time = 3.19 min Column: CHROMOLITH® SpeedROD 4.6 x 50 mm (4 min.); Solvent A = 10% MeOH, 90% H<sub>2</sub>O, 0.2% H<sub>3</sub>PO<sub>4</sub>; Solvent B = 90% MeOH, 10% H<sub>2</sub>O, 0.2% H<sub>3</sub>PO<sub>4</sub>. LC/MS M<sup>+1</sup> = 334.2 <sup>1</sup>H NMR (500 MHz, MeOD) δ ppm 3.34 (s, 2H), 7.53-7.57 (m, 4 H), 7.57-7.62 (m, 1H), 7.61 (d, *J* = 6.4 Hz, 2H), 8.12 (d, *J* = 8.2 Hz, 2H), 8.48 (s, 1H).

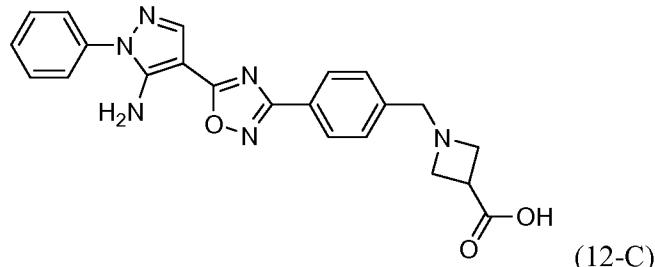
12-B. 4-(5-(5-Amino-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzaldehyde



[00134] A 50 mL RB flask was flame dried and cooled under a stream of dry nitrogen. DCM (10 mL) followed by oxalyl chloride (2M in DCM, 0.882 mL, 1.764 mmol) was added to the flask and it was cooled in an acetone/dry ice bath. DMSO (0.191 mL, 2.69 mmol) was added dropwise. After 10 minutes, (4-(5-(5-amino-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)phenyl)methanol (280 mg, 0.840 mmol) was added in small portions over 1-2 minutes providing a pale yellow slurry. After stirring for 2-3 minutes, DIPEA (1.203 mL, 6.89 mmol) was added dropwise. The cloudy mixture began to clear up, then became cloudy again with the color fading

from pale yellow to off-white during the course of the addition. After 5 minutes, the cooling bath was removed and the reaction was allowed to slowly warm to room temperature. After stirring at RT for 2 hours, the solvents were evaporated and the residue partitioned between EtOAc (60 mL) and 0.5N KHSO<sub>4</sub> (25 mL). The EtOAc phase was washed with 25 mL each 1N HCl, water, sat. aq. NaHCO<sub>3</sub>, water and brine, then dried over MgSO<sub>4</sub>, filtered and evaporated. The solid was slurried in hexanes (with ~10% EtOAc) with sonication and heating then cooled to RT and filtered. The solids were washed with hexanes, air dried, then placed under vacuum to afford 4-(5-(5-amino-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzaldehyde (250 mg, 0.664 mmol) which was used without further purification. The compound had an HPLC retention time = 1.77 min. – Column: (PHENOMENEX® Luna 4.6 x 30 mm S-5 ODS column) eluting with 10-90% aqueous methanol + 0.1% TFA over a 2 minute gradient. LC/MS M<sup>+1</sup> = 334.2

15 12-C. 1-(4-(5-(5-Amino-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid



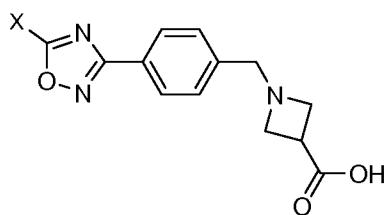
[00135] To a solution of 4-(5-(5-amino-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzaldehyde (100 mg, 0.302 mmol) in MeOH (6 mL) and 1,2-dichloroethane (2 mL) was added 0.1mL of acetic acid and azetidine-3-carboxylic acid (45.8 mg, 0.453 mmol), the mixture was allowed to stir at RT for 40 min. and then sodium triacetoxyborohydride (64.0 mg, 0.302 mmol) was added in several portions. The reaction was stirred at RT overnight then the solvents were removed *in vacuo* and the residue was purified by preparative HPLC to afford 1-(4-(5-(5-amino-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid, trifluoroacetic acid salt (95mg 0.18 mmol). The compound had an HPLC retention time = 5.73 min. – Column: Low pH Col-1: Sunfire C18 3.5um, 4.6x150mm; Start %

B = 10; 12 Min. 100%; 15 Min. 100%; Flow Rate = 2 mL/min; Wavelength1 = 220; Wavelength2 = 254; Solvent Pair = TFA - MeCN/H<sub>2</sub>O; Solvent A = 0.05% TFA in H<sub>2</sub>O:MeCN (95:5); Solvent B = 0.05% TFA in H<sub>2</sub>O:MeCN (5:95); Column 1 = LOW pH - Parallel HPLC; At 220nm LC/MS M<sup>+1</sup> = 417.2. <sup>1</sup>H NMR (500 MHz, MeOD) δ 5 ppm 3.63 (m, 1H), 4.26 (m, 4H), 4.40 (s, 2H), 7.40 (m, 1H), 7.46 (m, 4H), 7.51 (d, J=8.3 Hz, 2H), 7.92 (s, 1H), 8.05 (d, J=8.3Hz, 2H).

12. Preparation of 1-(4-(5-(isobutylamino)-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid, 2,2,2-trifluoroacetic acid salt  
10 [00136] To a solution of 1-(4-(5-(5-amino-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid (20 mg, 0.048 mmol) in acetonitrile (1.5 mL) was added isobutyraldehyde (10.39 mg, 0.144 mmol), TFA (0.011 mL, 0.144 mmol) and triethylsilane (0.023 mL, 0.144 mmol). The reaction was stirred at 55°C overnight. Purification by preparative HPLC afforded 1-(4-(5-(isobutylamino)-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid, 2,2,2-trifluoroacetic acid salt (9 mg, 0.015 mmol). The compound had an HPLC retention time = 7.17 min. – Column: Low pH Col-1; Sunfire C18 3.5um, 4.6x150mm; Start % B = 10; 12 Min. 100%; 15 Min. 100%; Flow Rate = 2 mL/min; Wavelength1 = 220; Wavelength2 = 254; Solvent Pair = TFA - MeCN/H<sub>2</sub>O; 15 Solvent A = 0.05% TFA in H<sub>2</sub>O:MeCN (95:5); Solvent B = 0.05% TFA in H<sub>2</sub>O:MeCN (5:95); Column 1 = LOW pH - Parallel HPLC; At 220nm LC/MS M<sup>+1</sup> = 473.3. <sup>1</sup>H NMR (500 MHz, MeOD) δ ppm 0.71 (dt, 6H, J=6.6 Hz), 1.49-1.61 (m, 1H), 2.67 (d, 2H, J=6.6Hz), 3.63-3.69 (m, 1H), 4.25-4.31 (m, 4H), 4.42 (s, 2 H), 7.34-7.41 (m, 1H), 7.44-7.49 (m, 4H), 7.51 (d, 2H, J=8.3Hz), 7.88 (s, 1H), 8.10 (d, 2H, 20 J=8.3Hz).  
25

Table 2

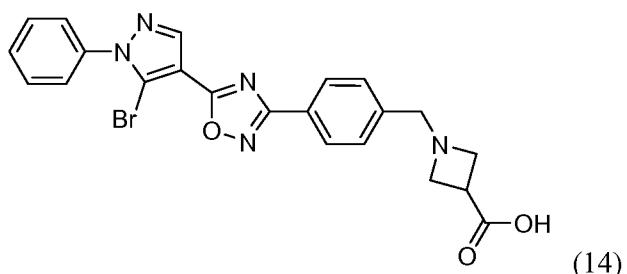
[00137] The compound in Table 2 was prepared in a manner similar to the above Example.



Ex. No.	X	MW	HPLC ret. time (min.)	HPLC methods	MS ( $M^{+1}$ )
13		472.5	7.2	D	473.3

Example 14

5 1-(4-(5-(5-Bromo-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid, 2,2,2-trifluoroacetic acid salt

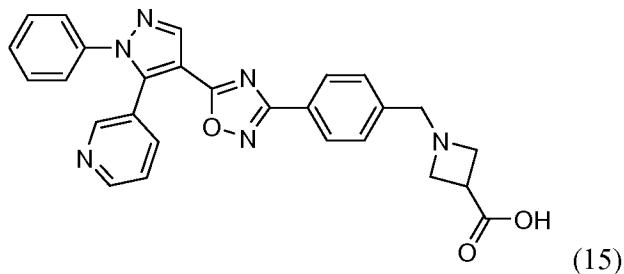


[00138] To a solution of 1-(4-(5-(5-amino-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid (30 mg, 0.072 mmol) and copper (II) bromide (24.14 mg, 0.108 mmol) in acetonitrile (2 mL) at 0°C was added *tert*-10 butyl nitrite (0.013 mL, 0.108 mmol) dropwise. The mixture was allowed to warm to RT slowly and then stirred at RT for 3 hours. The solvents were removed *in vacuo* and the residue was purified by preparative HPLC to afford 1-(4-(5-(5-bromo-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid, 2,2,2-trifluoroacetic acid salt (9.9 mg, 0.017 mmol). The compound had an HPLC 15 retention time = 6.52 min. – Column: Low pH Col-1: Sunfire C18 3.5um, 4.6x150mm; Start % B = 10; 12 Min. 100%; 15 Min. 100%; Flow Rate = 2 mL/min;

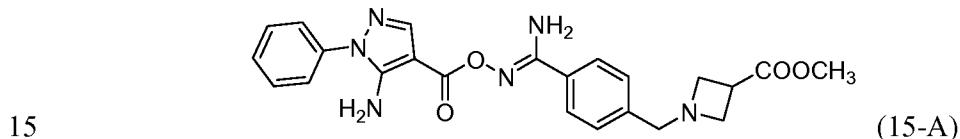
Wavelength1 = 220; Wavelength2 = 254; Solvent Pair = TFA - MeCN/H<sub>2</sub>O; Solvent A = 0.05% TFA in H<sub>2</sub>O:MeCN (95:5); Solvent B = 0.05% TFA in H<sub>2</sub>O:MeCN (5:95); Column 1 = LOW pH - Parallel HPLC; At 220nm LC/MS M<sup>+1</sup> = 480.1, 482.1 <sup>1</sup>H NMR (500 MHz, MeOD) δ ppm 3.68-3.75 (m, 1H), 4.34-4.40 (m, 4H), 5 4.61 (s, 2H), 7.57-7.63 (m, 5H), 7.66 (d, 2H, J=8.3Hz), 8.25 (d, 2H, J=8.3Hz), 8.45 (s, 1H)

### Example 15

10 1-(4-(5-(1-Phenyl-5-(pyridin-3-yl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid, 2,2,2-trifluoroacetic acid salt



15-A. (Z)-Methyl 1-(4-(N'-(5-amino-1-phenyl-1H-pyrazole-4-carboxyloxy) carbamimidoyl)benzyl)azetidine-3-carboxylate

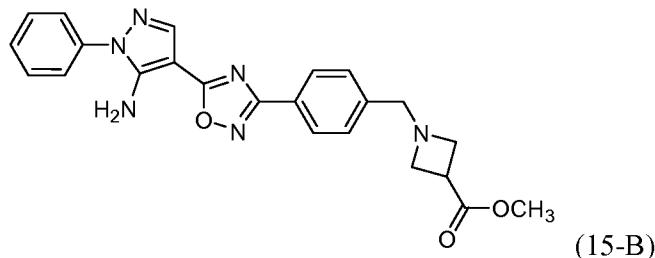


[00139] A mixture of 5-amino-1-phenyl-1H-pyrazole-4-carboxylic acid (90 mg, 0.441 mmol), (Z)-methyl 1-(4-(N'-hydroxycarbamimidoyl)benzyl)azetidine-3-carboxylate (116 mg, 0.441 mmol), EDC (84 mg, 0.441 mmol) and HOBT (67.5 mg, 0.441 mmol) in DMF (1 mL) was stirred at RT for 1 hr. At this time, Et<sub>3</sub>N (0.061 mL, 0.441 mmol) was added and stirring was continued at RT for 3 days. The mixture was then heated to 50°C for 10 minutes. The reaction mixture was partitioned between EtOAc (30 mL) and saturated sodium bicarbonate solution (30 mL). The organic layer was washed with saturated sodium bicarbonate solution (30 mL), water (30 mL) and brine (30 mL). After drying (MgSO<sub>4</sub>) and filtration, the 20 organic layer was concentrated to afford (Z)-methyl 1-(4-(N'-(5-amino-1-phenyl-1H- 25

pyrazole-4-carbonyloxy)carbamimidoyl)benzyl)azetidine-3-carboxylate (103 mg, 0.230 mmol) as a white solid. HPLC retention time = 1.05 minutes (PHENOMENEX® Luna 4.6 x 30 mm S-5 ODS column) eluting with 10-90% aqueous methanol + 0.1% TFA over a 2 minute gradient. MS:(M+H)= 449.3.

5

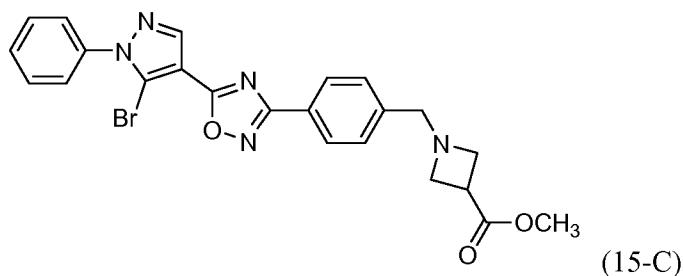
15-B. Methyl 1-(4-(5-(5-amino-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylate



(15-B)

[00140] To a suspension of (Z)-methyl 1-(4-(N'-(5-amino-1-phenyl-1H-pyrazole-4-carbonyloxy)carbamimidoyl)benzyl)azetidine-3-carboxylate (102 mg, 0.227 mmol) in EtOH (3 mL) was added sodium acetate (37.3 mg, 0.455 mmol) as a solution in water (1 mL). The mixture was heated to 90°C for 3 hr. The reaction mixture was transferred to a microwave vial and was then heated to 120°C for 30 minutes. The reaction mixture was partitioned between EtOAc (30 mL) and water (30 mL). The aqueous layer was extracted with EtOAc (15 mL) and the combined organic layers were dried (MgSO<sub>4</sub>) and concentrated to afford an oil that was chromatographed on a 12 gm Isco silica gel cartridge, eluting with a 0-100%EtOAc/hexane gradient. The pure fractions were concentrated to afford methyl 1-(4-(5-(5-amino-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylate (70 mg, 0.163 mmol) as a colorless oil. HPLC retention time = 1.35 (ethyl ester-1.42) minutes (PHENOMENEX® Luna 4.6 x 30 mm S-5 ODS column) eluting with 10-90% aqueous methanol + 0.1% TFA over a 2 minute gradient. MS:(M+H)= 431.23 (ethyl ester- 445.25). (The product is a mixture of methyl ester and ethyl ester due to partial transesterification during the reaction. The mixture was carried on through the sequence.)

15-C. Preparation of methyl 1-(4-(5-(5-bromo-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylate:



**[00141]** To a solution of methyl 1-(4-(5-(5-amino-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylate (69 mg, 0.160 mmol) and copper (II) bromide (53.7 mg, 0.240 mmol) in acetonitrile (3 mL) at 0°C was added *tert*-butyl nitrite (0.029 mL, 0.240 mmol). The reaction mixture was allowed to warm to RT and stir for 1 hr. The reaction mixture was then partitioned between EtOAc (30 mL) and saturated NH<sub>4</sub>Cl solution:conc. NH<sub>4</sub>OH, 1:1 (30 mL). The organic layer was washed with saturated NH<sub>4</sub>Cl solution:conc. NH<sub>4</sub>OH, 1:1 (30 mL) and saturated NH<sub>4</sub>Cl solution (30 mL). After drying (MgSO<sub>4</sub>) and filtration, the organic layer was 5 concentrated to afford methyl 1-(4-(5-(5-bromo-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylate (48 mg, 0.097 mmol) as an orange oil. HPLC retention time = 1.53 (ethyl ester-1.58) minutes (PHENOMENEX® Luna 4.6 x 30 mm S-5 ODS column) eluting with 10-90% aqueous methanol + 0.1% TFA over a 2 minute gradient. MS:(M+H)= 494/496.11 (ethyl ester- 508/510.11). (Note: This 10 product is a mixture of methyl and ethyl ester).

15. Preparation of 1-(4-(5-(1-phenyl-5-(pyridin-3-yl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid, 2,2,2-trifluoroacetic acid salt

**[00142]** A mixture of methyl 1-(4-(5-(5-bromo-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylate (47 mg, 0.095 mmol), pyridin-3-ylboronic acid (12.86 mg, 0.105 mmol), tricyclohexylphosphine (2.67 mg, 9.51 µmol), tris(dibenzylideneacetone)dipalladium(0) (3.48 mg, 3.80 µmol) and potassium phosphate, tribasic, 2.0M (0.081 mL, 0.162 mmol) in dioxane (1 mL) was heated to 100°C under nitrogen for 8 hr. After cooling to rt, the reaction mixture was 20 partitioned between EtOAc (30 mL) and water (20 mL). The organic layer was dried and concentrated to a yellow oil. This oil was dissolved in MeOH (1 mL) and 1N NaOH (0.2 mL) was added. After stirring 1 hr at rt, 0.2 mL of 1N HCl was added and 25

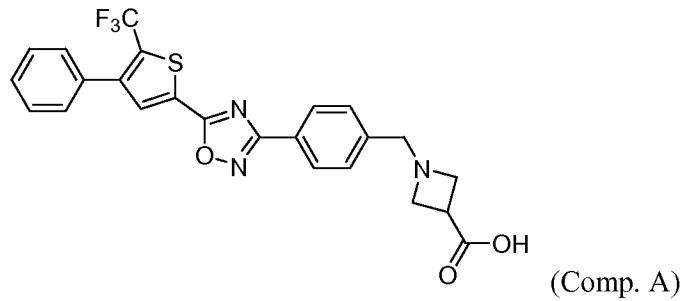
the reaction mixture was concentrated to dryness. The residue was suspended in water and the pH was adjusted to ~4 resulting in a suspension. The suspension was filtered and the filtrate was determined to contain desired product. After concentrating the filtrate, the residue was dissolved in MeOH and was subjected to 5 preparative HPLC (YMC 20 x 100 mm S-10 ODS column; 10-90% aqueous methanol + 0.1%TFA over a 10 minute gradient). The pure fraction was concentrated and dried overnight to afford 1-(4-(5-(1-phenyl-5-(pyridin-3-yl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid, 2,2,2-trifluoroacetic acid salt (5.3 mg, 8.94  $\mu$ mol) as a white solid. HPLC retention time = 2.31 minutes (YMC-Combi 10 4.6 x 50 mm S-5 ODS column) eluting with 10-90% aqueous methanol + 0.2% phosphoric acid over a 4 minute gradient. MS:(M+H)= 479.30.  $^1$ H NMR (400 MHz, MeOD)  $\delta$  ppm 3.73 (m, 1H) 4.38 (m, 4H) 4.51 (s, 2H) 7.40 (m, 2H) 7.46 (m, 2H) 7.56 (m, 2H) 7.62 (d,  $J$ = 8.0 Hz, 2H) 7.96 (m, 1H) 8.12 (d,  $J$ = 8.1 Hz, 2H) 8.56 (s, 1H), 8.68 (m, 1H) 8.74 (s, 1H).

15

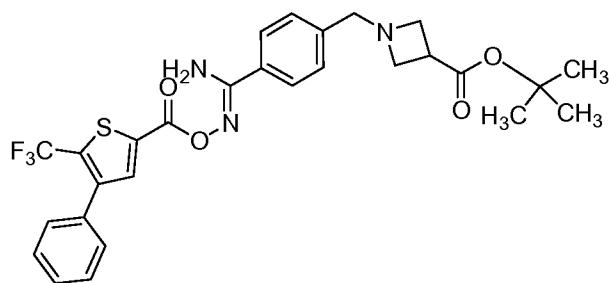
## Comparative Compound A (Comp. A)

Comp A-1. 1-(4-(5-(4-Phenyl-5-(trifluoromethyl)thiophen-2-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid

20 [00143] A comparative compound (Comp. A) was prepared for evaluation. This compound is Example 54 from WO 2003/062252 which has also been described in Hale et al., *J. Med. Chem.*, 6662 (2004).



25 Comp. A-1. (Z)-*tert*-Butyl 1-(4-(N'-(4-phenyl-5-(trifluoromethyl)thiophene-2-carbonyloxy)-carbamimidoyl)benzyl)azetidine-3-carboxylate

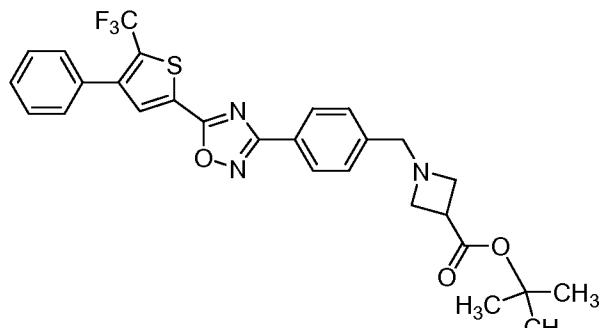


(Comp. A-1)

[00144] A mixture of 4-phenyl-5-(trifluoromethyl)thiophene-2-carboxylic acid (408 mg, 1.50 mmol), (Z)-*tert*-butyl 1-(4-(N'-hydroxycarbamimidoyl)benzyl)azetidine-3-carboxylate (Int.1, 458 mg, 1.50 mmol), 5 HOBT (345 mg, 2.250 mmol), Hunig's Base (1.05 mL, 6.00 mmol), and EDC (431 mg, 2.25 mmol) in N,N-dimethylformamide (7.5 mL) was stirred at room temperature for 18 h. The reaction mixture was partitioned between ethyl acetate (120 mL) and a saturated aqueous solution of sodium bicarbonate (60 mL). The organic layer was washed with water (2 x 120 mL), washed with brine (120 mL), and dried over anhydrous magnesium sulfate. Concentration under reduced pressure afforded (Z)-*tert*-butyl 1-(4-(N'-(4-phenyl-5-(trifluoromethyl)thiophene-2-carbonyloxy)carbamimidoyl)benzyl)azetidine-3-carboxylate (744 mg, 1.33 mmol, 89% yield) as a light peach colored solid that was used in the next step without further purification. HPLC: ret. time = 3.26 minutes (YMC Combi S-5 4.6 x 50 mm 10 ODS column) eluting with 10-90% aqueous methanol + 0.2% phosphoric acid over a 4 minute gradient. MS: (M+H) = 560.25.

15

Comp. A-2. *tert*-Butyl 1-(4-(5-(4-phenyl-5-(trifluoromethyl)thiophen-2-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylate



(Comp. A-2)

**[00145]** To a solution of (*Z*)-*tert*-butyl 1-(4-(N'-(4-phenyl-5-(trifluoromethyl)thiophene-2-carbonyloxy)carbamimidoyl)benzyl)azetidine-3-carboxylate (744 mg, 1.33 mmol) in acetonitrile (30 mL) was added a 1M solution of TBAF in tetrahydrofuran (3.99 mL, 3.99 mmol), and the reaction mixture was 5 allowed to stir at room temperature for 3 days. The volatiles were removed under reduced pressure, and the residue was chromatographed on a 5 x 12 cm silica gel column, eluting with a 0-50% EtOAc/Hex gradient. The essentially pure fractions containing product were concentrated to afford *tert*-butyl 1-(4-(5-(4-phenyl-5-(trifluoromethyl)thiophen-2-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylate 10 (521 mg, 0.962 mmol, 72.4 % yield) as a colorless solid. HPLC ret. time = 3.63 min. (YMC Combi S-5 4.6 x 50 mm ODS column) eluting with 10-90% aqueous methanol + 0.2% phosphoric acid over a 4 minute gradient. MS: (M+H)= 542.22. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ ppm 1.46 (s, 9H), 3.25 - 3.31 (m, 3H), 3.52 - 3.58 (m, 2H), 3.69 (s, 2H), 7.43 (d, *J*=8.25 Hz, 2H), 7.47 (m, 5H), 7.91 (s, d, *J*=1.65 Hz, 1H), and 8.09 15 (d, *J*=8.25 Hz, 2 H).

Comp. A. Preparation of 1-(4-(5-(4-phenyl-5-(trifluoromethyl)thiophen-2-yl)-1,2,4-oxadiazol-3-yl)benzyl)-azetidine-3-carboxylic acid

**[00146]** A solution of *tert*-butyl 1-(4-(5-(4-phenyl-5-(trifluoromethyl)thiophen-2-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylate (518 mg, 0.956 mmol) in trifluoroacetic acid (15 mL) was allowed to stand at room temperature for 1.5 h. The volatiles were removed under reduced pressure, and the residue was co-evaporated from ethyl acetate/hexanes. (2 x 10 mL). The residue was suspended in water (10 mL) and the pH was adjusted to ~11 with 1N aqueous sodium hydroxide. To this 25 solution was added sufficient 1N aqueous hydrochloric acid to adjust the pH ~4.5. The resulting suspension was stirred at room temperature overnight. The white suspension was filtered through a medium porosity sintered glass filter, and the filter cake was washed thoroughly with water. The solid was dried, the white powder was suspended in methanol, and the suspension was sonicated until it was uniform. The 30 methanol was removed under reduced pressure, and the procedure was repeated twice more to afford a white solid that was stirred as a suspension in methanol (~30 mL) overnight. Vacuum filtration and drying afforded 1-(4-(5-(4-phenyl-5-

(trifluoromethyl)thiophen-2-yl)-1,2,4-oxadiazol-3-yl)benzyl)-azetidine-3-carboxylic acid (345 mg, 0.707 mmol, 74% yield) as a white powder. HPLC ret. time = 3.45 min. (YMC Combi S-5 4.6 x 50 mm ODS column) eluting with 10-90% aqueous methanol + 0.2% phosphoric acid over a 4 minute gradient. MS: (M+H) = 486.12.

5       $^1\text{H}$  NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 3.23 (s, 3H), 3.39 - 3.45 (m, 2H), 3.64 (s, 2H), 7.50 (d, *J*=8.28 Hz, 2H), 7.58 (m, 5H), 8.02 (d, *J*=8.28 Hz, 2H), and 8.26 (d, *J*=1.25 Hz, 1H). Elemental Analysis: Calc. for C<sub>24</sub>H<sub>18</sub>N<sub>3</sub>O<sub>3</sub>SF<sub>3</sub> • 0.1H<sub>2</sub>O: C 59.22, H 3.76, N 8.63, S 6.59, F 11.71; Found: C<sub>24</sub>H<sub>18</sub>N<sub>3</sub>O<sub>3</sub>SF<sub>3</sub> • 0.1H<sub>2</sub>O: C 59.06, H 3.45, N 8.60, S 6.61, F 11.42; KF (Found)= 0.25% water.

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## BIOLOGICAL ASSAYS

### S1P<sub>1</sub> Binding Assay

[00147]    Membranes were prepared from CHO cells expressing human S1P<sub>1</sub>. Cells were dissociated in buffer containing 20 mM HEPES, pH 7.5, 50 mM NaCl, 2 mM EDTA and Protease Inhibitor cocktail (Roche), and disrupted on ice using the Polytron homogenizer. The homogenate was centrifuged at 20,000 rpm (48,000g) and the supernatant was discarded. The membrane pellets were resuspended in buffer containing 50 mM HEPES, pH 7.5, 100 mM NaCl, 1 mM MgCl<sub>2</sub>, 2 mM EDTA and stored in aliquots at -80 °C after protein concentration determination.

15      [00148]    Membranes (2  $\mu$ g/well) and 0.03 nM final concentration of <sup>33</sup>P-S1P ligand (1 mCi/ml, American Radiolabeled Chemicals) were added to the compound plates. Binding was performed for 60 min at room temperature, terminated by collecting the membranes onto GF/B filter plates, and radioactivity was measured by TOPCOUNT®. The competition data of the test compounds over a range of 20 concentrations was plotted as percentage inhibition of radioligand specific binding. The IC<sub>50</sub> is defined as the concentration of competing ligand needed to reduce specific binding by 50%.

25      [00149]    Compounds of the present invention and Comparative Compound A were tested in the S1P<sub>1</sub> binding assay described hereinabove and the results rounded to two digits, shown in Table A were obtained. In some instances, the value is an average of multiple experiments where N is the number of experiments conducted that provided satisfactory dose response curves. When more than one batch of an example was

tested, the value presented is from the batch which allowed a comparison of GTP $\gamma$ S S1P<sub>1</sub> and GTP $\gamma$ S S1P<sub>3</sub> in Method A (Data shown in Table B). Data was not averaged across different batches of an example compound.

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Table A

Ex.	S1P1 B IC <sub>50</sub> (nM)	N
1	0.40	2
2	0.30	286
3	0.48	2
4	1.1	2
5	30	6
6	0.12	1
7	0.19	1
8	0.85	1
9	ND	0
10	1.1	1
11	ND	0
12	1.7	2
13	3.1	2
14	0.98	1
15	5.4	1
Comp. A	2.5	1

Method A: Receptor [<sup>35</sup>S] GTP $\gamma$ S Binding Assays

- [00150] Compounds were loaded in a 384 FALCON® v-bottom plate (0.5  $\mu$ l/well in a 3-fold dilution). Membranes prepared from S1P<sub>1</sub>/CHO cells or EDG3-Ga15-bla 10 HEK293T cells were added to the compound plate (40  $\mu$ l/well, final protein 3  $\mu$ g/well) with MULTIDROP®. [<sup>35</sup>S]GTP (1250 Ci/mmol, Perkin Elmer) was diluted in assay buffer: 20 mM HEPES, pH7.5, 10 mM MgCl<sub>2</sub>, 150 mM NaCl, 1 mM EGTA, 1 mM DTT, 10  $\mu$ M GDP, 0.1% fatty acid free BSA, and 10  $\mu$ g/ml Saponin to 0.4 nM.

40  $\mu$ l of the [ $^{35}$ S] GTP solution was added to the compound plate with a final concentration of 0.2 nM. The reaction was kept at room temperature for 45 min. At the end of incubation, all the mixtures in the compound plate were transferred to a 384 well FB filter plates via GPCR robot system. The filter plate was washed with 5 water 4 times by using the modified manifold Embla plate washer and dried at 60°C for 45 min. 30  $\mu$ l of MicroScint 20 scintillation fluid was added to each well for counting at Packard TOPCOUNT®. EC<sub>50</sub> is defined as the agonist concentration that corresponds to 50% of the Ymax (maximal response) obtained for each individual compound tested.

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Method B: Receptor [35S] GTP $\gamma$ S SPA Binding Assays

[00151] Membranes prepared from S1P<sub>1</sub> or S1P<sub>3</sub> transfected CHO cells (2  $\mu$ g protein) were incubated in 96-well white plates (CORNING® 3693) with test compounds diluted in DMSO, in 50 $\mu$ l of reaction mixture containing 7.5  $\mu$ l WGA-15 PVT beads (20 mg/ml), and 5  $\mu$ M GDP, 20 mM HEPES pH 7.4, 100 mM NaCl, 1 mM EDTA, 5 mM MgCl<sub>2</sub>, 10  $\mu$ g /ml saponin, 0.1% BSA, and 1  $\mu$ M leupeptin. The assay was initiated with the addition of 25  $\mu$ l of 0.2 nM [ $^{35}$ S]-GTP $\gamma$ S (1250 Ci/mmol; NEN) in assay buffer. After 90 min incubation at room temperature, spin the plate at 1000rpm for 5min. The bound radionuclides were measured at TOPCOUNT®, expressed as % response relative to S1P (1  $\mu$ M) activation. Data was analyzed using 20 the four parameter logistic equation in Excel. The four parameters in the equation, Y=A+((B-A)/(1+((EC<sub>50</sub>/X) $^D$ ))), are described as following: A is the Y value (agonist activity) at the bottom plateau; B is the Y value at the top plateau; EC<sub>50</sub> is the X value (agonist concentration) when the response is halfway between bottom and 25 top; D is Hill coefficient. Curves were not generated for compounds having Ymax values were below 50%.

[00152] Compounds of the present invention and Comparative Compound A were tested in the Receptor [35S] GTP $\gamma$ S Binding Assays (Method A) and Receptor [35S] GTP $\gamma$ S SPA Binding Assays (Method B) described hereinabove and the results 30 rounded to two digits, shown in Table B were obtained. In some instances, the value is an average of multiple experiments where N is the number of experiments conducted that provided satisfactory dose response curves. When more than one

batch of an example was tested, the value presented is from a batch which allowed a comparison of GTP $\gamma$ S S1P<sub>1</sub> and GTP $\gamma$ S S1P<sub>3</sub> in which the most number of experiments were performed. Preferably, the same batch was examined in Method A and Method B. Data was not averaged across different batches of an example 5 compound.

Table B

Ex.	Method A				Method B			
	GTP $\gamma$ S S1P <sub>1</sub> EC <sub>50</sub> (nM)	N	GTP $\gamma$ S S1P <sub>3</sub> EC <sub>50</sub> (nM)	N	GTP $\gamma$ S S1P <sub>1</sub> EC <sub>50</sub> (nM)	N	GTP $\gamma$ S S1P <sub>3</sub> EC <sub>50</sub> (nM)	N
1	2.9	2	6,700	2	0.50	31	4,400	9
2	1.9	71	NC	0	0.17	12	2,100	3
3	ND	0	ND	0	0.15	1	1,700	1
4	ND	0	ND	0	0.19	1	400	2
5	260	3	13,000	2	17	6	NC	0
6	4.2	2	2,900	2	NC	0	1,300	1
7	ND	0	ND	0	0.087	1	220	1
8	22	1	5,900	1	NC	0	NC	0
9	ND	0	ND	0	1.3	1	710	2
10	ND	0	ND	0	0.42	1	1,100	1
11	ND	0	ND	0	1.9	2	2,000	1
12	ND	0	ND	0	0.28	3	9,800	1
13	ND	0	ND	0	0.17	2	51	51
14	NC	0	ND	0	2.1	2	7,100	1
15	52	1	3,600	1	2.2	3	4,100	2
Comp. A	84	1	97	1	0.25	10	20	3

ND = not determined.

NC = a satisfactory dose response curve was not obtained.

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[00153] The ratios of the GTP $\gamma$ S S1P<sub>3</sub> EC<sub>50</sub> values to the GTP $\gamma$ S S1P<sub>1</sub> EC<sub>50</sub> values, calculated from the data in Table B, are shown in Table C.

Table C

Ex.	GTP $\gamma$ S S1P <sub>3</sub> / S1P <sub>1</sub> (Method A)	GTP $\gamma$ S S1P <sub>3</sub> / S1P <sub>1</sub> (Method B)
1	2,300	8,800
2	ND	12,000
3	ND	11,000
4	ND	2,100
5	50	ND
6	690	ND
7	ND	2,500
8	268	ND
9	ND	550
10	ND	2,600
11	ND	1,100
12	ND	35,000
13	ND	300
14	ND	3,400
15	69	1,900
Comp. A	1.2	78

[00154] In Table C, a larger value for the ratio of the GTP $\gamma$ S S1P<sub>3</sub> EC<sub>50</sub> value to the GTP $\gamma$ S S1P<sub>1</sub> EC<sub>50</sub> value indicates greater selectivity of S1P<sub>1</sub> activity over S1P<sub>3</sub> activity.

[00155] The compounds of the present invention, as exemplified by Examples 1 to 15, show the surprising advantage as agonists of S1P<sub>1</sub> and are selective over S1P<sub>3</sub>, as compared to Comparative Compound A. Exemplified compounds of the invention reported in Table C had selectivity ratios in the range of 50 to 2,300, while in contrast, Comparative Compound A had a selectivity ratio of 1.2, as measured by Method A. Exemplified compounds of the invention reported in Table C had

selectivity ratios in the range of 300 to 35,000, while in contrast, Comparative Compound A had a selectivity ratio of 78, as measured by Method B.

**[00156]** The compounds of the present invention possess activity as agonists of S1P<sub>1</sub> and are selective over S1P<sub>3</sub>, and thus may be used in treating, preventing, or 5 curing various S1P<sub>1</sub> receptor-related conditions while reducing or minimizing the side effects due to S1P<sub>3</sub> activity. The surprising selectivity of the compounds of the present invention indicate their potential use in treating, preventing, or curing autoimmune and inflammatory diseases such as multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, or psoriasis, while reducing or minimizing 10 possible cardiovascular side effects such as bradycardia and hypertension. Other potential uses of the compounds of the present invention include minimizing or reducing rejection of transplanted organs, while reducing or minimizing side effects due to S1P<sub>3</sub> activity.

15 Blood Lymphocyte Reduction Assay (BLR) in Rodents

**[00157]** Lewis rats or BALB/c mice were dosed orally with test article (as a solution or suspension in the vehicle) or vehicle alone (polyethylene glycol 300, “PEG300”). Blood was drawn at 4hr and 24h by retro-orbital bleeding. Blood lymphocyte counts were determined on an ADVIA 120 Hematology Analyzer 20 (Siemens Healthcare Diagnostics). The results were measured as a reduction in the percentage of circulating lymphocytes as compared to the vehicle treated group at the 4hr and 24 hr measurement. The results represent the average results of all animals within each treatment group (n = 3-4).

**[00158]** Compounds of the present invention were tested in the Blood Lymphocyte 25 Reduction assay (BLR) described hereinabove and the results are shown in Table D for rats and Table E for mice.

Table D

Dose (mg/kg)	Example 1 % reduction in lymphocytes at 4 hr.	Example 1 % reduction in lymphocytes at 24 hr.	Example 2 % reduction in lymphocytes at 4 hr.	Example 2 % reduction in lymphocytes at 24 hr.
0.04	49%	6%	64%	8%
0.2	77%	15%	76%	55%
1.0	86%	61%	76%	79%
3.0	81%	75%	NT	NT

Table E

Dose (mg/kg)	Example 1 % reduction in lymphocytes at 4 hr.	Example 1 % reduction in lymphocytes at 24 hr.	Example 2 % reduction in lymphocytes at 4 hr.	Example 2 % reduction in lymphocytes at 24 hr.
0.04	NT	NT	68%	5%
0.2	NT	NT	76%	64%
0.3	74%	30%	NT	NT
1.0	85%	68%	80%	82%
3.0	85%	76%	73%	89%
10.0	84%	88%	NT	NT

## Rat Adjuvant Induced Arthritis Assay (AA)

- 5 [00159] Male Lewis rats (150-175 g; Harlan, n=8 treatment group) were immunized at the base of the tail with 100  $\mu$ l of 10 mg/ml freshly ground *Mycobacterium butyricum* (Difco Laboratories) in incomplete Freund's adjuvant (sigma). Animals were dosed once daily with the test article (as a solution or suspension in the vehicle) or vehicle alone (polyethylene glycol 300, "PEG300")
- 10 starting from the day of immunization. The volumes of their hind paws were measured in a water displacement plethysmometer (Ugo Basile, Italy). The baseline paw measurements were taken before onset of the disease (between day 7 to day 10). The paw measurements were then taken three times a week until the end of the study

on day 20 to 21. All procedures involving animals were reviewed and approved by the Institutional Animal Care Use Committee.

[00160] Compounds of the present invention were tested in the Rat Adjuvant Induced Arthritis assay described hereinabove and the results are shown in Table F 5 and Table G.

Table F

Group	Paw Swelling (mL) on Day 20	
Vehicle	Mean	2.11
	SD	0.44
Example 1 (1.0 mg/kg)	Mean	2.36
	SD	0.63
Example 1 (3.0 mg/kg)	Mean	1.45
	SD	0.99
Example 1 (6.0 mg/kg)	Mean	0.17
	SD	0.29
Example 1 (10.0 mg/kg)	Mean	0.12
	SD	0.10

Table G

Group	Paw Swelling (mL) on Day 21	
Vehicle	Mean	1.58
	SD	0.63
Example 2 (0.2 mg/kg)	Mean	02.12
	SD	0.33
Example 2 (1.0 mg/kg)	Mean	0.91
	SD	0.73
Example 2 (3.0 mg/kg)	Mean	0.19
	SD	0.29

Group	Paw Swelling (mL) on Day 21	
Example 2 (10.0 mg/kg)	Mean	0.10
	SD	0.20

**[00161]** In the rat adjuvant-induced arthritis model, an animal model for rheumatoid arthritis, Example 1 and Example 2 inhibit disease progression as measured by paw swelling in the Lewis rat using a prophylactic oral dosing regimen.

5

Mouse Experimental Autoimmune Encephalomyelitis Assay (EAE)

**[00162]** Mice (C57BL/6 female, 6-8 weeks of age, Charles River, n=10-12 treatment group) were immunized subcutaneously with 150 µg MOG<sub>35-55</sub> emulsified 1:1 with incomplete Freund's adjuvant (sigma) supplemented with 150 µg 10 *Mycobacterium tuberculosis* H37RA (Difco Laboratories). 400 ng of pertussis toxin (CalBiochem) was injected intraperitoneally on the day of immunization and two days later. Animals were dosed once daily with the test article (as a solution or suspension in the vehicle) or vehicle alone (polyethylene glycol 300, "PEG300") starting from 1 day after immunization. Clinical scoring and body weight were taken 15 3 times per week until the end of the study on day 24. Clinical scoring system: 0.5: partial tail weakness; 1: limp tail or waddling gait with tail tonicity; 1.5: waddling gait with partial tail weakness; 2: waddling gait with limp tail (ataxia); 2.5: ataxia with partial limb paralysis; 3: full paralysis of one limb; 3.5: full paralysis of one limbs with partial paralysis of a second limb; 4: full paralysis of two limbs; 4.5: 20 moribund; 5: death. A compound of the present invention was tested in the Mouse Experimental Autoimmune Encephalomyelitis assay described hereinabove and the results are shown in Table H.

Table H

Group	Clinical Score on Day 24	
Vehicle	Mean	2.63
	SD	1.51

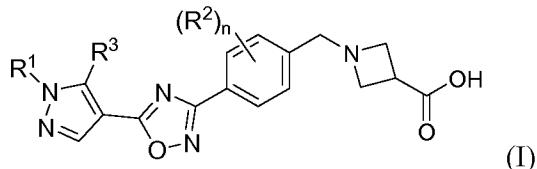
Group	Clinical Score on Day 24	
Example 1 (1.0 mg/kg)	Mean	0.32
	SD	0.51
Example 1 (3.0 mg/kg)	Mean	0.77
	SD	1.01
Example 1 (10.0 mg/kg)	Mean	0.30
	SD	0.63

[00163] In the mouse experimental autoimmune encephalomyelitis (EAE) model, an animal model for multiple sclerosis, Example 1 inhibits disease progression as determined by the clinical scores in C57Bl/6 mice using a prophylactic oral dosing 5 regimen.

CLAIMS

WHAT IS CLAIMED IS:

5 1. A compound of Formula (I):



or a pharmaceutically acceptable salt thereof, wherein:

n is zero or an integer selected from 1 through 4;

R<sup>1</sup> is cycloalkyl, aryl, heteroaryl, or heterocyclyl, each optionally substituted with one

10 to five substituents independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, benzyl, -OR<sup>4</sup>, and/or halogen;

each R<sup>2</sup> is independently selected from hydrogen, C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, -OR<sup>4</sup>, and/or halogen;

R<sup>3</sup> is hydrogen, alkyl, haloalkyl, halogen, -NR<sup>a</sup>R<sup>b</sup>, cycloalkyl, aryl, heteroaryl, or

15 heterocyclyl, wherein said cycloalkyl, aryl, heteroaryl, and heterocyclyl each may optionally be substituted with one to five substituents independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, -OR<sup>4</sup>, and/or halogen;

each R<sup>4</sup> is independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, and/or benzyl; and

20 R<sup>a</sup> and R<sup>b</sup> are each independently hydrogen, alkyl, and/or benzyl.

2. The compound of claim 1 or a pharmaceutically acceptable salt thereof, wherein

R<sup>1</sup> is aryl optionally substituted with one, two, or three substituents independently

25 selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, -OR<sup>4</sup>, and/or halogen.

3. The compound of claim 2 or a pharmaceutically acceptable salt thereof, wherein

n is zero;

R<sup>1</sup> is phenyl optionally substituted with one, two, or three substituents independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, -OR<sup>4</sup>, and/or halogen;

R<sup>3</sup> is C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, halogen, -NR<sup>a</sup>R<sup>b</sup>, C<sub>3</sub> to C<sub>6</sub> cycloalkyl, phenyl, or pyridyl;

5 R<sup>4</sup> is C<sub>1</sub> to C<sub>4</sub> alkyl; and

R<sup>a</sup> and R<sup>b</sup> are independently hydrogen and/or C<sub>1</sub> to C<sub>6</sub> alkyl.

4. The compound of claim 3 or a pharmaceutically acceptable salt thereof, wherein:

10 R<sup>3</sup> is methyl, ethyl, 1-propyl, cyclopentyl, trifluoromethyl, bromo, chloro, phenyl, 2-pyridyl, or -NHR<sup>a</sup>; and

R<sup>a</sup> is methyl, ethyl, 1-propyl, 2-propyl, n-butyl, or isobutyl.

5. The compound of claim 1 or a pharmaceutically acceptable salt thereof, wherein

15 R<sup>1</sup> is cycloalkyl, heteroaryl, or heterocyclyl, each optionally substituted with one to five substituents independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, benzyl, -OR<sup>4</sup>, and/or halogen.

20 6. The compound of claim 5 or a pharmaceutically acceptable salt thereof, wherein:

n is zero;

R<sup>3</sup> is alkyl, haloalkyl, halogen, -NR<sup>a</sup>R<sup>b</sup>, cycloalkyl, aryl, heteroaryl, or heterocyclyl, wherein said cycloalkyl, aryl, heteroaryl, and heterocyclyl each may

25 optionally be substituted with one to five substituents independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, -OR<sup>4</sup>, and/or halogen;

each R<sup>4</sup> is independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, and/or benzyl; and

R<sup>a</sup> and R<sup>b</sup> are each independently hydrogen, alkyl, and/or benzyl.

30

7. The compound of claim 6 or a pharmaceutically acceptable salt thereof, wherein:

R<sup>1</sup> is pyridyl or cyclohexyl, optionally substituted with one to five substituents independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, benzyl, -OR<sup>4</sup>, and/or halogen.

5 8. The compound of claim 5 or a pharmaceutically acceptable salt thereof, wherein:

R<sup>1</sup> is C<sub>3</sub> to C<sub>6</sub> cycloalkyl optionally substituted with one, two, or three substituents independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, -OR<sup>4</sup>, and/or halogen; and

10 R<sup>4</sup> is C<sub>1</sub> to C<sub>6</sub> alkyl.

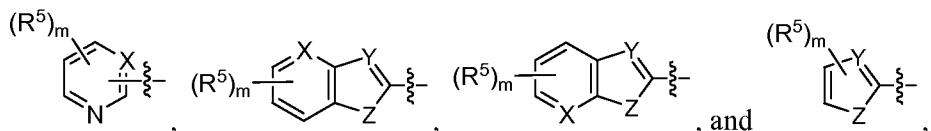
9. The compound of claim 5 or a pharmaceutically acceptable salt thereof, wherein:

R<sup>1</sup> is heteroaryl optionally substituted with one, two, or three substituents

15 independently selected from C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, benzyl, -OR<sup>4</sup>, and/or halogen.

10. The compound of claim 1 or a pharmaceutically acceptable salt thereof, wherein:

20 R<sup>1</sup> is selected from:



wherein:

m is zero, 1, 2, or 3;

X and Y are each independently N and/or CR<sup>5</sup>;

25 Z is O, S, or N-R<sup>6</sup>;

each R<sup>5</sup> is independently selected from C<sub>1</sub> to C<sub>4</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, -OR<sup>4</sup>, and/or halogen;

R<sup>6</sup> is C<sub>1</sub> to C<sub>4</sub> alkyl or benzyl; and

each R<sup>4</sup> is independently selected from C<sub>1</sub> to C<sub>4</sub> alkyl.

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11. The compound of claim 10 or a pharmaceutically acceptable salt thereof, wherein:

R<sup>1</sup> is 2-pyridyl optionally substituted with one, two, or three substituents

independently selected from C<sub>1</sub> to C<sub>4</sub> alkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, -OR<sup>4</sup>, and/or 5 halogen;

R<sup>3</sup> is C<sub>1</sub> to C<sub>4</sub> alkyl, C<sub>3</sub> to C<sub>6</sub> cycloalkyl, C<sub>1</sub> to C<sub>4</sub> haloalkyl, halogen, phenyl, pyridyl, or -NR<sup>a</sup>R<sup>b</sup>;

R<sup>4</sup> is C<sub>1</sub> to C<sub>4</sub> alkyl; and

R<sup>a</sup> and R<sup>b</sup> are each independently hydrogen and/or C<sub>1</sub> to C<sub>4</sub> alkyl.

10

12. The compound of claim 1 or a pharmaceutically acceptable salt thereof, selected from the group consisting of:

1-(4-(5-(1-(pyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid;

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1-(4-(5-(1-phenyl-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid (2);

1-(4-(5-(isopropylamino)-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid;

20

1-(4-(5-(ethylamino)-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid;

1-(4-(5-methyl-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid;

1-(4-(5-(5-n-propyl-1-(pyridin-2-yl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid;

25

1-(4-(5-(5-cyclopentyl-1-(pyridin-2-yl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid;

1-(4-(5-(1-(6-ethoxypyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid;

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1-(4-(5-(1-cyclohexyl-5-methyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid;

1-(4-(5-(5-phenyl-1-(pyridin-2-yl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid;

- 1-(4-(5-(5-chloro-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid;
- 1-(4-(5-(isobutylamino)-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid;
- 5 1-(4-(5-(n-butylamino)-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid;
- 1-(4-(5-bromo-1-phenyl-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid; and
- 1-(4-(5-(1-phenyl-5-(pyridin-3-yl)-1H-pyrazol-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid.
- 10

13. A composition comprising a compound according to claim 1 or a pharmaceutically acceptable salt thereof; and a pharmaceutically acceptable carrier.

15 14. Use of a compound according to claim 1 or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of autoimmune disease or chronic inflammatory disease.

15. A compound according claim 1 or a pharmaceutically acceptable salt  
20 thereof, for use in therapy in treating autoimmune disease or chronic inflammatory disease.

## PATENT COOPERATION TREATY

## PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference  11161-PCT	<b>FOR FURTHER ACTION</b> see Form PCT/ISA/220 as well as, where applicable, item 5 below.	
International application No.  PCT/US2010/021696	International filing date (day/month/year)  22/01/2010	(Earliest) Priority Date (day/month/year)  23/01/2009
Applicant  BRISTOL-MYERS SQUIBB COMPANY		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of:

- the international application in the language in which it was filed  
 a translation of the international application into \_\_\_\_\_, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))

b.  This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6bis(a)).

c.  With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2.  **Certain claims were found unsearchable** (See Box No. II)

3.  **Unity of invention is lacking** (see Box No III)

4. With regard to the **title**,

- the text is approved as submitted by the applicant  
 the text has been established by this Authority to read as follows:

PYRAZOLE-1, 2, 4-OXADIAZOLE DERIVATIVES AS SPHINGOSINE-1-PHOSPHATE AGONISTS

5. With regard to the **abstract**,

- the text is approved as submitted by the applicant  
 the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. \_\_\_\_\_

- as suggested by the applicant  
 as selected by this Authority, because the applicant failed to suggest a figure  
 as selected by this Authority, because this figure better characterizes the invention

b.  none of the figures is to be published with the abstract

# INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2010/021696

**A. CLASSIFICATION OF SUBJECT MATTER**  
INV. C07D413/14 A61K31/4245 A61P37/00

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)  
C07D

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

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

EPO-Internal, WPI Data, BEILSTEIN Data, CHEM ABS Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 03/062252 A1 (MERCK & CO INC [US]; BUGIANESI ROBERT L [US]; DOHERTY GEORGE A [US]; G) 31 July 2003 (2003-07-31) page 77 - page 87; claims; examples	1-15
A	WO 2008/141731 A2 (BAYER HEALTHCARE AG [DE]; BAYER SCHERING PHARMA AG [DE]; BOYER STEPHEN) 27 November 2008 (2008-11-27) page 178, line 1 - page 180, column 20; claims; example 169	1-15

Further documents are listed in the continuation of Box C.

See patent family annex.

\* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

26 February 2010

Date of mailing of the international search report

05/03/2010

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040,  
Fax: (+31-70) 340-3016

Authorized officer

Gavriliu, Daniela

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No  
PCT/US2010/021696

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 03062252	A1	31-07-2003	AT CA EP JP	441654 T 2472715 A1 1470137 A1 2005515259 T
				15-09-2009 31-07-2003 27-10-2004 26-05-2005
WO 2008141731	A2	27-11-2008	AR AU CA EP PA UY	066617 A1 2008253311 A1 2687306 A1 2148877 A2 8780201 A1 31080 A1
				02-09-2009 27-11-2008 27-11-2008 03-02-2010 09-02-2009 05-01-2009