PREPARATION AND THERAPEUTIC APPLICATIONS OF (2S,3R)-N-2-((3-PYRIDINYL)METHYL)-1-AZABICYCLO[2.2.2]OCT-3-YL)-3,5-DIFLUOROBENZAMIDE

Field of the Invention

The present invention relates to compounds that bind to and modulate the activity of neuronal nicotinic acetylcholine receptors, to processes for preparing these compounds, to pharmaceutical compositions containing these compounds, and to methods of using these compounds for treating a wide variety of conditions and disorders, including those associated with dysfunction of the central nervous system (CNS).

Background of the Invention

The therapeutic potential of compounds that target neuronal nicotinic receptors (NNRs), also known as nicotinic acetylcholine receptors (nAChRs), has been the subject of several reviews (see, for example, Breining et al., *Ann. Rep. Med. Chem.* **40**: 3 (2005), Hogg and Bertrand, *Curr. Drug Targets: CNS Neurol. Disord.* **3**: 123 (2004), Suto and Zacharias, *Expert Opin. Ther. Targets* **8**: 61 (2004), Dani et al., *Bioorg. Med. Chem. Lett.* **14**: 1837 (2004), Bencherif and Schmitt, *Curr. Drug Targets: CNS Neurol. Disord.* **1**: 349 (2002)). Among the kinds of indications for which NNR ligands have been proposed as therapies are cognitive disorders, including Alzheimer's disease, attention deficit disorder, and schizophrenia (Newhouse et al., *Curr. Opin. Pharmacol.* **4**: 36 (2004), Levin and Rezvani, *Curr. Drug Targets: CNS Neurol. Disord.* **1**: 423 (2002), Graham et al., *Curr. Drug Targets: CNS Neurol. Disord.* **1**: 387 (2002), Ripoll et al., *Curr. Med. Res. Opin.* **20(7)**: 1057 (2004), and McEvoy and Allen, *Curr. Drug Targets: CNS Neurol. Disord.* **1**: 433 (2002)); pain and inflammation (Decker et al., *Curr. Top. Med. Chem.* **4(3)**: 369 (2004), Vincler, *Expert Opin. Invest. Drugs* **14(10)**: 1191 (2005), Jain, *Curr. Opin. Inv. Drugs* **5**: 76 (2004), Miao et al., *Neuroscience* **123**: 777 (2004)); depression and anxiety (Shytle et al., *Mol. Psychiatry* **7**: 525 (2002), Damaj et al., *Mol. Pharmacol.* **66**: 675 (2004), Shytle et al., *Depress. Anxiety* **16**: 89 (2002)); neurodegeneration (O'Neill et al., *Curr. Drug Targets: CNS Neurol. Disord.* **1**: 399 (2002), Takata et al., *J. Pharmacol. Exp. Ther.* **306**: 772 (2003), Marrero et al., *J. Pharmacol. Exp. Ther.* **309**: 16 (2004)); Parkinson's disease (Jonnala and Buccafusco, *J. Neurosci. Res.* **66**: 565 (2001)); addiction (Dwoskin and Crooks, *Biochem. Pharmacol.* **63**: 89 (2002), Coe et al., *Bioorg. Med. Chem. Lett.* **15(22)**: 4889 (2005)); obesity (Li et al., *Curr. Top. Med. Chem.* **3**: 899

(2003)); and Tourette's syndrome (Sacco et al., *J. Psychopharmacol.* **18(4)**: 457 (2004), Young et al., *Clin. Ther.* **23(4)**: 532 (2001)).

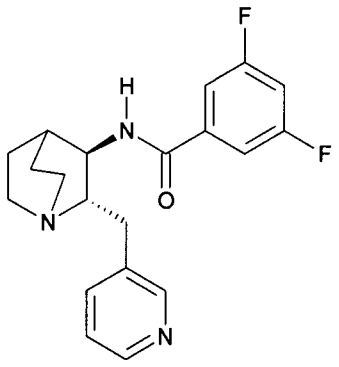
There exists a heterogeneous distribution of nAChR subtypes in both the central and peripheral nervous systems. For instance, the nAChR subtypes which are predominant in vertebrate brain are $\alpha4\beta2$, $\alpha7$, and $\alpha3\beta2$, whereas those which predominate at the autonomic ganglia are $\alpha3\beta4$ and those of neuromuscular junction are $\alpha1\beta1\delta\gamma$ and $\alpha1\beta1\delta\varepsilon$ (see Dwoskin et al., *Exp. Opin. Ther. Patents* **10**: 1561 (2000) and Holliday et al. *J. Med. Chem.* **40(26)**, 4169 (1997)).

A limitation of some nicotinic compounds is that they are associated with various undesirable side effects due to non-specific binding to multiple nAChR subtypes. For example, binding to and stimulation of muscle and ganglionic nAChR subtypes can lead to side effects which can limit the utility of a particular nicotinic binding compound as a therapeutic agent.

The compounds of the present invention exhibit a high degree of specific binding to the $\alpha7$ nAChR subtype and low affinity for the $\alpha4\beta2$ subtype as well as ganglionic and muscle nAChR subtypes. Thus, these compounds can serve as therapeutic modulators of $\alpha7$ nAChRs in patients in need of such treatment, without producing side effects caused by non-specific nAChR subtype binding.

Summary of the Invention

The present invention includes (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide (Formula I) or a pharmaceutically acceptable salt thereof.



Formula I

The compound of the present invention binds with high affinity to NNRs of the $\alpha7$ subtype and exhibit selectivity for this subtype over the $\alpha4\beta2$ NNR subtype,

as well as over ganglion and muscle subtypes.

The present invention includes pharmaceutical compositions comprising the compound of the present invention or a pharmaceutically acceptable salt thereof. The pharmaceutical compositions of the present invention can be used for treating or preventing a wide variety of conditions or disorders, including those disorders characterized by dysfunction of nicotinic cholinergic neurotransmission or the degeneration of the nicotinic cholinergic neurons.

The present invention includes a method for treating or preventing disorders and dysfunctions, such as CNS disorders and dysfunctions, inflammation, inflammatory response associated with bacterial and/or viral infection, pain, metabolic syndrome, autoimmune disorders, or other disorders described in further detail herein. The present invention includes a method for modulating neovascularization. The methods involve administering to a subject a therapeutically effective amount of a compound of the present invention, including a salt thereof, or a pharmaceutical composition that includes such compounds. Additionally, the present invention includes compounds that have utility as diagnostic agents and in receptor binding studies as described herein.

The foregoing and other aspects of the present invention are explained in further detail in the detailed description and examples set forth below.

Brief Description of the Figures

Figure 1 depicts novel object recognition (NOR) vs. dose for (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide or pharmaceutically acceptable salt thereof. A statistically significant effect was observed for doses as low as 0.1 mg/kg.

Figure 2 depicts the data used for the determination of the minimum effective dose for novel object recognition (NOR) upon administration of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide or pharmaceutically acceptable salt thereof. A statistically significant effect was observed for doses as low as 0.03 mg/kg.

Figure 3 depicts novel object recognition (NOR) vs. time following the 3rd administration of 0.1 mg/kg (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide or a pharmaceutically acceptable salt thereof. A statistically significant effect was observed for doses out to 6 h after dosing.

Figure 4 depicts novel object recognition (NOR) vs. time following the 3rd administration of 0.3 mg/kg (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide or a pharmaceutically acceptable salt thereof. A statistically significant effect was observed for doses out to 18 h after dosing.

Figure 5 depicts a dose response for each of Compound A and Compound B with α7 nicotinic receptors.

Figure 6 depicts the electrophysiological response to co-application of each of Compound A and Compound B with acetylcholine (Ach).

Figures 7A, 7B, and **7C** depict electrophysiological response for interaction of Compound A with Ach, regarding activation of the nicotinic α7 receptor.

Figures 8A, 8B, and **8C** depict electrophysiological response for interaction of Compound B with Ach, regarding activation of the nicotinic α7 receptor.

Figure 9 is an x-ray diffraction pattern for Compound A mono-hydrochloride salt.

Figure 10 is a crystal structure for Compound A mono-hydrochloride salt.

Figure 11 is an x-ray diffraction pattern for Compound A hemi-galactarate salt.

Figure 12 illustrates an overlay of six (6) different x-ray diffraction patterns for salts from the salt screen for Compound A.

Figure 13 illustrates the results of assessment of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide in CFA-induced thermal hyperalgesia. Test substance, morphine, and vehicle were each administered subcutaneously to groups of 8 SD rats 24 hours after CFA injection. The thermal hyperalgesia was performed prior to CFA injection (pre-CFA). before treatment, and 1 hour after SC injection. One-way ANOVA followed by the Dunnett's test was applied to compare between the treatment groups and the vehicle controlled group. Differences are considered significant at the *$P<0.05$ level.

Figure 14 illustrates the results of Von Frey assessment indicating that (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide is effective in reducing diabetic neuropathy pain at doses of 1 mg/kg and 10mg/kg compared to the Vehicle treated group.

Figure 15 illustrates comparison weight gain as significantly lower in the (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide-treated obese ("db-Test Article") mice. Notably, animals that were co-administered MLA with (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide failed to show the reduced weight gain exhibited by the obese rats administered (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide alone.

Figure 16 illustrates average food consumption was significantly lower in the (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide-treated obese mice ("db-Test Article") than in the obese controls. The food consumption of the lean mice was unaffected by (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide ("Db-Test Article"). Animals that were co-administered MLA with (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide failed to show the reduced daily average food consumption exhibited by the obese rats administered (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide alone.

Figure 17 illustrates that (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide significantly inhibited fasting plasma glucose levels in obese mice ("db-Test Article"). However, this effect was not reversed by co-administration with MLA.

Figure 18 illustrates that (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide significantly inhibited glycosylated HbA1c levels in obese mice ("db-Test Article"). The reduction in glycosylated HbA1c by (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide was attenuated by co-administration of MLA.

Figure 19 illustrates that (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide significantly reduced the pro-inflammatory cytokine TNF alpha in obese mice ("db-Test Article"). These effects were inhibited by co-administration of the alpha7 antagonist MLA.

Figure 20 illustrates that (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide resulted in significantly lower triglyceride levels in obese mice ("db-Test Article") compared with vehicle-treated controls ("db"). The reduction in triglycerides by (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide was not attenuated by co-administration of MLA.

Figure 21 illustrates the effect of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide on % changes in Penh response to methacholine challenge in ovalbumin-sensitized mice. (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide and vehicle were administered subcutaneously bid or given intratracheally qd for 6 consecutive days from day 21 to day 25 at 30 min before OVA challenge and the last dosing was administrated at 30 min before MCh provocation on day 26. The Penh values were determined. One-way ANOVA followed by Dunnett's test was applied for comparison between the OVA immunized vehicle and other treatment groups. *P<0.05 vs. OVA-vehicle control.

Figure 22 illustrates the effect of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide on white blood cell counts and differential cell counts in ovalbumin sensitized mice. (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide and vehicle were administered subcutaneously bid or were given intratracheally qd for 6 consecutive days from day 21 to day 25 at 30 minutes before OVA challenge and the last dosing was administrated at 30 minutes before bronchoalveolar lavage fluid harvest on day 26. The total white blood cell count and differential cell counts were determined. One-way ANOVA followed by Dunnett's test was applied for comparison between the OVA immunized vehicle and other treatment groups. *P<0.05 vs. OVA-vehicle control.

Figure 23 illustrates the effect of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide on % white blood cell count and differential cell counts in ovalbumin sensitized mice. (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide and vehicle were administered subcutaneously bid or were given intratracheally qd for 6 consecutive days from day 21 to day 25 at 30 minutes before OVA challenge and the last dosing was administrated at 30 minutes before bronchoalveolar lavage fluid harvest on day 26. The total white blood cell count and differential cell counts were determined. One-way ANOVA followed by Dunnett's test was applied for comparison between the OVA immunized vehicle and other treatment groups. *P<0.05 vs. OVA-vehicle control.

Detailed Description

Definitions

The following definitions are meant to clarify, but not limit, the terms defined. If a particular term used herein is not specifically defined, such term should not be considered indefinite. Rather, terms are used within their accepted meanings.

As used herein, the term "compound(s)" may be used to mean the free base form, or alternatively, a salt form of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide, depending on the context, which will be readily apparent. Those skilled in the art will be able to distinguish the difference.

For ease of reference, (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide (Formula I) or a pharmaceutically acceptable salt thereof is also referred to as Compound A. Additionally, a structural analog is used herein for comparative purposes. (2S,3R)-N-2-((3-Pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-4-fluorobenzamide or a pharmaceutically acceptable salt thereof is referred to as Compound B. Compound B is a single isomer of a racemic mixture as published in WO 04/76449, herein incorporated by reference.

As used herein, the term "pharmaceutically acceptable" refers to carrier(s), diluent(s), excipient(s) or salt forms of the compound of the present invention that are compatible with the other ingredients of the formulation and not deleterious to the recipient of the pharmaceutical composition.

As used herein, the term "pharmaceutical composition" refers to a compound of the present invention optionally admixed with one or more pharmaceutically acceptable carriers, diluents, or exipients. Pharmaceutical compositions preferably exhibit a degree of stability to environmental conditions so as to make them suitable for manufacturing and commercialization purposes.

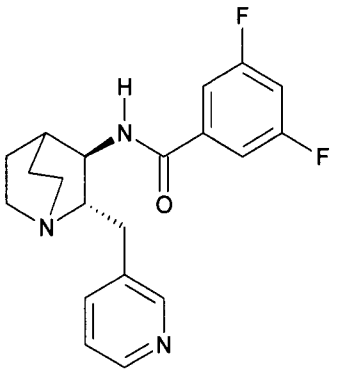
As used herein, the terms "effective amount", "therapeutically effective amount", "therapeutic amount," or "effective dose" refer to an amount of the compound of the present invention sufficient to elicit the desired pharmacological or therapeutic effects, thus resulting in effective prevention or treatment of a disorder. Prevention of the disorder may be manifested by delaying or preventing the progression of the disorder, as well as the onset of the symptoms associated with the disorder. Treatment of the disorder may be manifested by a decrease or elimination of symptoms, inhibition or reversal of the progression of the disorder,

as well as any other contribution to the well being of the patient.

As will be discussed in more detail below and with reference to Figures 1 2, 3, and 4, a statistically significant effect is observed for doses of the compound of Formula I, or a pharmaceutically acceptable salt thereof, as low as 0.03 μM/kg, including effects observed out to 18 hours after dosing. The effective dose can vary, depending upon factors such as the condition of the patient, the severity of the symptoms of the disorder, and the manner in which the pharmaceutical composition is administered. Thus, as used herein, the effective dose may be less than 100 mg, preferably less than 50 mg, more preferably less than 10 mg, and most preferably less than 1mg. These effective doses typically represent the amount administered as a single dose, or as one or more doses administered over a 24 hours period.

Compounds

One aspect of the present invention includes a compound (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide (Formula I) or a pharmaceutically acceptable salt thereof.



Formula I

In one embodiment, the compound is substantially free of one or more of (2R,3S)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide, (2R,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide, and (2S,3S)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide.

In one embodiment, the is an acid addition salt, wherein the acid is selected from hydrochloric acid, methanesulfonic acid, maleic acid, phosphoric acid, 1-hydroxy-2-naphthoic acid, malonic acid, L-tartaric acid, fumaric acid, citric acid, L-malic acid, R-mandelic acid, S-mandelic acid, succinic acid, 4-acetamidobenzoic acid, adipic acid, galactaric acid, di-p-toluoyl-D-tartaric acid,

oxalic acid, D-glucuronic acid, 4-hydroxybenzoic acid, 4-methoxybenzoic acid, (1S)-(+)-10-camphorsulfonic acid, (1R,3S)-(+)-camphoric acid, and p-toluenesulfonic acid, or a hydrate or solvate thereof. In a further embodiment, the molar ratio of acid to (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide is 1:2 or 1:1.

Another aspect of the present invention includes a compound selected from:

(2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide mono-hydrochloride or a hydrate or solvate thereof;

(2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide mono-phosphate or a hydrate or solvate thereof;

(2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide mono-4-hydroxybenzoate or a hydrate or solvate thereof; and

(2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide hemi-4-hydroxybenzoate or a hydrate or solvate thereof.

Another aspect of the present invention includes a compound, (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide or a pharmaceutically acceptable salt thereof containing less than 25%, preferably containing less than 15%, preferably containing less than 5%, preferably containing less than 2%, preferably containing containing less than 1% of (2R,3R)-, (2S,3S)-, or (2R,3S)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide, either individually or in combination, by weight.

Another aspect of the present invention includes a compound (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide (Formula I) or a pharmaceutically acceptable salt thereof which is substantially crystalline. Another aspect includes a polymorphic form of a compound (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide hydrochloride characterized by an x-ray diffraction pattern comprising one or more peaks within ± 0.5 degrees 2θ of the following peaks:

2θ
8.4
8.8
11.9
13.2
15.2

16.0
17.6
18.4
18.9
19.9
20.1
21.3
23.1
25.4
26.2

Another aspect of the present invention is a polymorphic form of a compound (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide hydrochloride characterized by an x-ray powder diffraction pattern that substantially corresponds to Figure 9.

Another aspect of the present invention includes use of a compound of the present invention, in the manufacture of a medicament for the treatment or prevention of an α7-mediated disease or dysfunction. Another aspect of the present invention includes a method for treating or preventing an α7-mediated disease or dysfunction, comprising administering a therapeutically effective amount of a compound of the present invention. Another aspect of the present invention includes a compound of the present invention for use in treating or preventing an α7-mediated disease or dysfunction. In one embodiment, the disease or dysfunction is selected from the group consisting of:

i) pain, including one or more of acute, neurologic, inflammatory, neuropathic, chronic pain, severe chronic pain, post-operative pain, pain associated with cancer, angina, renal or biliary colic, menstruation, migraine, gout, arthritis, rheumatoid disease, teno-synovitis, vasculitis, trigeminal or herpetic neuralgia, diabetic neuropathy pain, causalgia, low back pain, deafferentation syndromes, and brachial plexus avulsion;

ii) metabolic syndrome, weight gain, type I diabetes mellitus, type II diabetes mellitus, or diabetic neuropathy;

iii) inflammation, including one or more of psoriasis, asthma, atherosclerosis, idiopathic pulmonary fibrosis, chronic and acute inflammation, psoriasis, endotoxemia, gout, acute pseudogout, acute gouty arthritis, arthritis,

rheumatoid arthritis, osteoarthritis, allograft rejection, chronic transplant rejection, asthma, atherosclerosis, mononuclear-phagocyte dependent lung injury, atopic dermatitis, chronic obstructive pulmonary disease, adult respiratory distress syndrome, acute chest syndrome in sickle cell disease, inflammatory bowel disease, Crohn's disease, ulcerative colitis, acute cholangitis, aphteous stomatitis, pouchitis, glomerulonephritis, lupus nephritis, thrombosis, and graft vs. host reaction; and

iv) cognition, including one or more of age-associated memory impairment, mild cognitive impairment, pre-senile dementia, early onset Alzheimer's disease, senile dementia, dementia of the Alzheimer's type, mild to moderate dementia of the Alzheimer's type, Lewy body dementia, vascular dementia, Alzheimer's disease, stroke, AIDS dementia complex, attention deficit disorder, attention deficit hyperactivity disorder, dyslexia, schizophrenia, schizophreniform disorder, schizoaffective disorder, cognitive deficits in schizophrenia, and cognitive dysfunction in schizophrenia.

Another aspect of the present invention includes a pharmaceutical composition comprising a compound of the present invention and one or more pharmaceutically acceptable carrier.

Another aspect of the present invention includes a method of enhancing acetylcholine-induced current comprising administering an effective amount of a compound of the present invention.

Another embodiment of the present invention includes (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide or a pharmaceutically acceptable salt thereof with reference to any one of the Examples.

Another embodiment of the present invention includes (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide or a pharmaceutically acceptable salt thereof for use as an active therapeutic substance.

Another embodiment of the present invention includes a method of modulating NNR in a subject in need thereof through the administration of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide or a pharmaceutically acceptable salt thereof.

The scope of the present invention includes combinations of aspects and embodiments.

Unless otherwise stated, structures depicted herein are also meant to

include compounds which differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structure except for the replacement of a hydrogen atom by deuterium or tritium, or the replacement of a carbon atom by ^{13}C or ^{14}C, or the replacement of a nitrogen atom by ^{15}N, or the replacement of an oxygen atom with ^{17}O or ^{18}O are within the scope of the invention. Such isotopically labeled compounds are useful as research or diagnostic tools.

The present invention includes a salt or solvate of the (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide, including combinations thereof, such as a solvate of a salt. The compounds of the present invention may exist in solvated, for example hydrated, as well as unsolvated forms, and the present invention encompasses all such forms.

Typically, but not absolutely, the salts of the present invention are pharmaceutically acceptable salts. Salts encompassed within the term "pharmaceutically acceptable salts" refer to non-toxic salts of the compounds of this invention.

Examples of suitable pharmaceutically acceptable salts include inorganic acid addition salts such as chloride, bromide, sulfate, phosphate, and nitrate; organic acid addition salts such as acetate, galactarate, propionate, succinate, lactate, glycolate, malate, tartrate, citrate, maleate, fumarate, methanesulfonate, p-toluenesulfonate, and ascorbate; salts with acidic amino acid such as aspartate and glutamate; alkali metal salts such as sodium salt and potassium salt; alkaline earth metal salts such as magnesium salt and calcium salt; ammonium salt; organic basic salts such as trimethylamine salt, triethylamine salt, pyridine salt, picoline salt, dicyclohexylamine salt, and N,N'-dibenzylethylenediamine salt; and salts with basic amino acid such as lysine salt and arginine salt. The salts may be in some cases hydrates or ethanol solvates.

As noted herein, the present invention includes specific compounds, which are identified herein with particularity. The compounds of this invention may be made by a variety of methods, including well-known standard synthetic methods. Illustrative general synthetic methods are set out below and then specific compounds of the invention are prepared in the working Examples.

In all of the examples described below, protecting groups for sensitive or reactive groups are employed where necessary in accordance with general principles of synthetic chemistry. Protecting groups are manipulated according to standard methods of organic synthesis (see, for example, T. W. Green and P. G.

M. Wuts, *Protecting Groups in Organic Synthesis, 3rd Edition*, John Wiley & Sons, New York (1999)). These groups are removed at a convenient stage of the compound synthesis using methods that are readily apparent to those skilled in the art. The selection of processes as well as the reaction conditions and order of their execution shall be consistent with the preparation of compounds of the present invention.

The present invention also provides a method for the synthesis of compounds useful as intermediates in the preparation of compounds of the present invention along with methods for their preparation.

The compounds can be prepared according to the following methods using readily available starting materials and reagents. In these reactions, variants may be employed which are themselves known to those of ordinary skill in this art, but are not mentioned in greater detail.

Salt Forms

One aspect of the present invention relates to novel salt forms of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide.

(2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide in the free base form is a solid with limited water solubility. However, the free base will react with both inorganic and organic acids to make certain acid addition salts that have physical properties that are advantageous for the preparation of pharmaceutical compositions such as crystallinity, water solubility, and stability toward chemical degradation. Typically, these salt forms are pharmaceutically acceptable salts.

The present invention includes pharmaceutically acceptable salts of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide. Examples of suitable pharmaceutically acceptable salts include inorganic acid addition salts such as chloride, bromide, sulfate, phosphate, and nitrate; organic acid addition salts such as acetate, galactarate, propionate, succinate, lactate, glycolate, malate, tartrate, citrate, maleate, fumarate, methanesulfonate, p-toluenesulfonate, and ascorbate; salts with acidic amino acid such as aspartate and glutamate; alkali metal salts such as sodium salt and potassium salt; alkaline earth metal salts such as magnesium salt and calcium salt; ammonium salt; organic basic salts such as trimethylamine salt, triethylamine salt, pyridine salt, picoline salt, dicyclohexylamine salt, and N,N'-dibenzylethylenediamine salt; and salts with basic amino acid such as lysine salt

and arginine salt. The salts may be in some cases hydrates or solvates, such as ethanol solvates.

One aspect of the present invention includes acid addition salts of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide wherein the acid is selected from hydrochloric acid, methanesulfonic acid, maleic acid, phosphoric acid, 1-hydroxy-2-naphthoic acid, malonic acid, L-tartaric acid, fumaric acid, citric acid, L-malic acid, R-mandelic acid, S-mandelic acid, succinic acid, 4-acetamidobenzoic acid, adipic acid, galactaric acid, di-p-toluoyl-D-tartaric acid, oxalic acid, D-glucuronic acid, 4-hydroxybenzoic acid, 4-methoxybenzoic acid, (1S)-(+)-10-camphorsulfonic acid, (1R,3S)-(+)-camphoric acid, and p-toluenesulfonic acid. The present invention also includes hydrates and solvates of these salt forms.

The stoichiometry of the salts comprising the present invention can vary. For example, it is typical that the molar ratio of acid to (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide is 1:2 or 1:1, but other ratios, such as 3:1, 1:3, 2:3, 3:2 and 2:1, are possible.

In one embodiment of the present invention, the salt has a stoichiometry of acid to of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide of 1:2. In another embodiment, the salt has a stoichiometry of acid of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide of 1:1.

As herein noted, depending upon the manner by which the salts described herein are formed, the salts can have crystal structures that occlude solvents that are present during salt formation. Thus, the salts can occur as hydrates and other solvates of varying stoichiometry of solvent relative to (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide.

Another embodiment of the present invention includes (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide or a hydrate or solvate thereof.

Another embodiment of the present invention includes (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide mono-hydrochloride or a hydrate or solvate thereof.

Another embodiment of the present invention (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide mono-phosphate or a hydrate or solvate thereof.

Another embodiment of the present invention includes (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide mono-4-hydroxybenzoate or a hydrate or solvate thereof.

Another embodiment of the present invention includes (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide hemi-4-hydroxybenzoate or a hydrate or solvate thereof.

A further aspect of the present invention includes processes for the preparation of the salts. The precise conditions under which the salts are formed may be empirically determined. The salts may be obtained by crystallization under controlled conditions.

One embodiment of the present invention includes a method for the preparation of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide or a pharmaceutically acceptable salt thereof containing less than 25%, preferably less than 15%, more preferably less than 5%, even more preferably less than 2%, and most preferably less than 1% of (2R,3R)-, (2S,3S)-, or (2R,3S)- N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide by weight either individually or in combination.

The method for preparing the salt forms can vary. The preparation (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide salt forms typically involves:

(i) mixing the free base, or a solution of the free base of suitably pure (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide in a suitable solvent, with any of the acids in pure form or as a solution of any of the acids in a suitable solvent, typically 0.5 to 1 equivalents of the acid;

(ii) (a) cooling the resulting salt solution if necessary to cause precipitation;

or

(ii) (b) adding a suitable anti-solvent to cause precipitation;

or

(ii) (c) evaporating the first solvent and adding and new solvent and repeating either steps (ii) (a) or step (ii) (b);

and

(iii) filtering and collecting the salt.

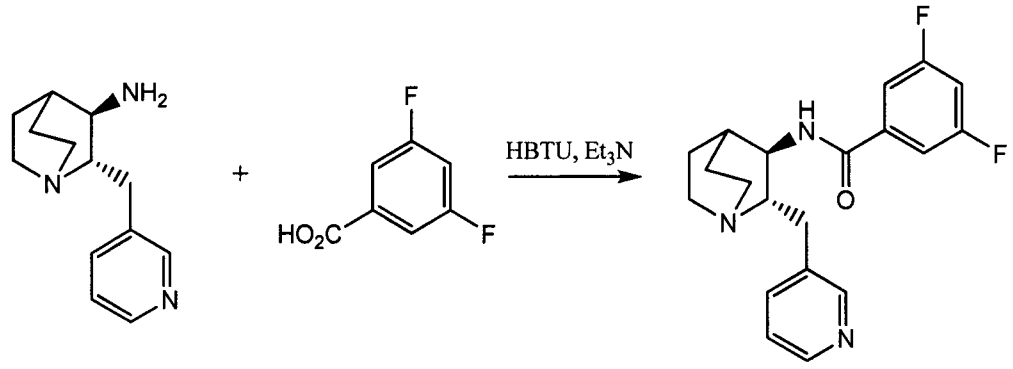
The stoichiometry, solvent mix, solute concentration, and temperature employed can vary. Representative solvents that can be used to prepare or recrystallize the salt forms include, without limitation, ethanol, methanol, propanol,

isopropyl alcohol, isopropyl acetate, acetone, ethyl acetate, toluene, water, methyl ethyl ketone, methyl isobutyl ketone, tert-butyl methyl ether, tetrahydrofuran, dichloromethane, n-heptane, and acetonitrile.

Several of these salts demonstrate stability sufficient to establish their promise in the production of pharmaceutical preparations. Such stability can be demonstrated in a variety of ways. Propensity to gain and release atmospheric moisture can be assessed by dynamic vapor sorption (DVS).

General Synthetic Methods

A synthesis of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide is achieved by O-(benzotriazol-1-yl)-N,N,N,1-tetramethyluronium hexafluorophosphate (HBTU) mediated coupling of (2S,3R)-3-amino-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]octane (obtained as described in PCT/US08/71872, herein incorporated by reference with regard to such synthesis) and 3,5-difluorobenzoic acid as illustrated in Scheme 1.



Scheme 1

The synthesis of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide can be similarly achieved by the use of other agents to activate the carboxylic acid. For example, the use of activating agents such as N,N'-dicyclohexylcarbodiimide (DCC), (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP), (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP), O-(benzotriazol-1-yl)-N,N,N',N'-bis(tetramethylene)uronium hexafluorophosphate (HBPyU), O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU), O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (TBTU), and (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) (EDCI) with 1-hydroxybenzotriazole (HOBt), as well as those described in, for example, Kiso and Yajima, Peptides, pp 39-91, Academic Press, San Diego, CA (1995), are well known to those skilled in the art.

Methods of Treatment

The compounds of the present invention have the ability to selectively bind to and modulate the activity of α7 NNRs. Consequently, these compounds can be used for the prevention or treatment of various conditions or disorders for which other types of nicotinic compounds have been proposed or are shown to be useful as therapeutics, such as CNS disorders, inflammation, inflammatory response associated with bacterial and/or viral infection, pain, metabolic syndrome, autoimmune disorders or other disorders described in further detail herein. These compounds can be used for modulating neovascularization and as diagnostic agents in receptor binding studies (*in vitro* and *in vivo*). Such therapeutic and other teachings are described, for example, in Williams et al., *Drug News Perspec.* **7(4)**: 205 (1994), Arneric et al., *CNS Drug Rev.* **1(1)**: 1-26 (1995), Arneric et al., *Exp. Opin. Invest. Drugs* **5(1)**: 79-100 (1996), Bencherif et al., *J. Pharmacol. Exp. Ther.* **279**: 1413 (1996), Lippiello et al., *J. Pharmacol. Exp. Ther.* **279**: 1422 (1996), Damaj et al., *J. Pharmacol. Exp. Ther.* **291**: 390 (1999); Chiari et al., *Anesthesiology* **91**: 1447 (1999), Lavand'homme and Eisenbach, *Anesthesiology* **91**: 1455 (1999), Holladay et al., *J. Med. Chem.* **40(28)**: 4169-94 (1997), Bannon et al., *Science* **279**: 77 (1998), PCT WO 94/08992, PCT WO 96/31475, PCT WO 96/40682, and U.S. Patent Nos. 5,583,140 to Bencherif et al., 5,597,919 to Dull et al., 5,604,231 to Smith et al. and 5,852,041 to Cosford et al., and other references previously listed herein.

CNS Disorders

The compounds and their pharmaceutical compositions are useful in the treatment or prevention of a variety of CNS disorders, including neurodegenerative disorders, neuropsychiatric disorders, neurologic disorders, and addictions. The compounds and their pharmaceutical compositions can be used to treat or prevent cognitive deficits and dysfunctions, age-related and otherwise; attentional disorders and dementias, including those due to infectious agents or metabolic disturbances; to provide neuroprotection; to treat convulsions and multiple cerebral infarcts; to treat mood disorders, compulsions and addictive behaviors; to provide analgesia; to control inflammation, such as mediated by cytokines and nuclear factor kappa B; to treat inflammatory disorders; to provide pain relief; and to treat infections, as anti-infectious agents for treating bacterial, fungal, and viral infections. Among the disorders, diseases and conditions that the compounds and pharmaceutical compositions of the present invention can be used to treat or prevent are: age-associated memory impairment (AAMI), mild

cognitive impairment (MCI), age-related cognitive decline (ARCD), pre-senile dementia, early onset Alzheimer's disease, senile dementia, dementia of the Alzheimer's type, Alzheimer's disease, cognitive impairment no dementia (CIND), Lewy body dementia, HIV-dementia, AIDS dementia complex, vascular dementia, Down syndrome, head trauma, traumatic brain injury (TBI), dementia pugilistica, Creutzfeld-Jacob Disease and prion diseases, stroke, ischemia, attention deficit disorder, attention deficit hyperactivity disorder, dyslexia, schizophrenia, schizophreniform disorder, schizoaffective disorder, cognitive dysfunction in schizophrenia, cognitive deficits in schizophrenia, Parkinsonism including Parkinson's disease, postencephalitic parkinsonism, parkinsonism-dementia of Gaum, frontotemporal dementia Parkinson's Type (FTDP), Pick's disease, Niemann-Pick's Disease, Huntington's Disease, Huntington's chorea, tardive dyskinesia, hyperkinesia, progressive supranuclear palsy, progressive supranuclear paresis, restless leg syndrome, Creutzfeld-Jakob disease, multiple sclerosis, amyotrophic lateral sclerosis (ALS), motor neuron diseases (MND), multiple system atrophy (MSA), corticobasal degeneration, Guillain-Barré Syndrome (GBS), and chronic inflammatory demyelinating polyneuropathy (CIDP), epilepsy, autosomal dominant nocturnal frontal lobe epilepsy, mania, anxiety, depression, premenstrual dysphoria, panic disorders, bulimia, anorexia, narcolepsy, excessive daytime sleepiness, bipolar disorders, generalized anxiety disorder, obsessive compulsive disorder, rage outbursts, oppositional defiant disorder, Tourette's syndrome, autism, drug and alcohol addiction, tobacco addiction, obesity, cachexia, psoriasis, lupus, acute cholangitis, aphthous stomatitis, ulcers, asthma, ulcerative colitis, inflammatory bowel disease, Crohn's disease, spastic dystonia, diarrhea, constipation, pouchitis, viral pneumonitis, arthritis (including rheumatoid arthritis and osteoarthritis), endotoxaemia, sepsis, atherosclerosis, idiopathic pulmonary fibrosis, acute pain, chronic pain, neuropathies, urinary incontinence, diabetes and neoplasias.

Cognitive impairments or dysfunctions may be associated with psychiatric disorders or conditions, such as schizophrenia and other psychotic disorders, including but not limited to psychotic disorder, schizophreniform disorder, schizoaffective disorder, delusional disorder, brief psychotic disorder, shared psychotic disorder, and psychotic disorders due to a general medical conditions, dementias and other cognitive disorders, including but not limited to mild cognitive impairment, pre-senile dementia, Alzheimer's disease, senile dementia, dementia of the Alzheimer's type, age-related memory impairment, Lewy body dementia,

vascular dementia, AIDS dementia complex, dyslexia, Parkinsonism including Parkinson's disease, cognitive impairment and dementia of Parkinson's Disease, cognitive impairment of multiple sclerosis, cognitive impairment caused by traumatic brain injury, dementias due to other general medical conditions, anxiety disorders, including but not limited to panic disorder without agoraphobia, panic disorder with agoraphobia, agoraphobia without history of panic disorder, specific phobia, social phobia, obsessive-compulsive disorder, post-traumatic stress disorder, acute stress disorder, generalized anxiety disorder and generalized anxiety disorder due to a general medical condition, mood disorders, including but not limited to major depressive disorder, dysthymic disorder, bipolar depression, bipolar mania, bipolar I disorder, depression associated with manic, depressive or mixed episodes, bipolar II disorder, cyclothymic disorder, and mood disorders due to general medical conditions, sleep disorders, including but not limited to dyssomnia disorders, primary insomnia, primary hypersomnia, narcolepsy, parasomnia disorders, nightmare disorder, sleep terror disorder and sleepwalking disorder, mental retardation, learning disorders, motor skills disorders, communication disorders, pervasive developmental disorders, attention-deficit and disruptive behavior disorders, attention deficit disorder, attention deficit hyperactivity disorder, feeding and eating disorders of infancy, childhood, or adults, tic disorders, elimination disorders, substance-related disorders, including but not limited to substance dependence, substance abuse, substance intoxication, substance withdrawal, alcohol-related disorders, amphetamine or amphetamine-like-related disorders, caffeine-related disorders, cannabis-related disorders, cocaine-related disorders, hallucinogen-related disorders, inhalant-related disorders, nicotine-related disorders, opioid-related disorders, phencyclidine or phencyclidine-like-related disorders, and sedative-, hypnotic- or anxiolytic-related disorders, personality disorders, including but not limited to obsessive-compulsive personality disorder and impulse-control disorders.

Cognitive performance may be assessed with a validated cognitive scale, such as, for example, the cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-cog). One measure of the effectiveness of the compounds of the present invention in improving cognition may include measuring a patient's degree of change according to such a scale.

The above conditions and disorders are discussed in further detail, for example, in the American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision, Washington, DC,

American Psychiatric Association, 2000. This Manual may also be referred to for greater detail on the symptoms and diagnostic features associated with substance use, abuse, and dependence.

Inflammation

The nervous system, primarily through the vagus nerve, is known to regulate the magnitude of the innate immune response by inhibiting the release of macrophage tumor necrosis factor (TNF). This physiological mechanism is known as the "cholinergic anti-inflammatory pathway" (see, for example, Tracey, "The inflammatory reflex," *Nature* **420**: 853-9 (2002)). Excessive inflammation and tumor necrosis factor synthesis cause morbidity and even mortality in a variety of diseases. These diseases include, but are not limited to, endotoxemia, rheumatoid arthritis, osteoarthritis, psoriasis, asthma, atherosclerosis, idiopathic pulmonary fibrosis, and inflammatory bowel disease.

Inflammatory conditions that can be treated or prevented by administering the compounds described herein include, but are not limited to, chronic and acute inflammation, psoriasis, endotoxemia, gout, acute pseudogout, acute gouty arthritis, arthritis, rheumatoid arthritis, osteoarthritis, allograft rejection, chronic transplant rejection, asthma, atherosclerosis, mononuclear-phagocyte dependent lung injury, idiopathic pulmonary fibrosis, atopic dermatitis, chronic obstructive pulmonary disease, adult respiratory distress syndrome, acute chest syndrome in sickle cell disease, inflammatory bowel disease, Crohn's disease, ulcerative colitis, acute cholangitis, aphteous stomatitis, pouchitis, glomerulonephritis, lupus nephritis, thrombosis, and graft vs. host reaction.

Inflammatory Response Associated with Bacterial and/or Viral Infection

Many bacterial and/or viral infections are associated with side effects brought on by the formation of toxins, and the body's natural response to the bacteria or virus and/or the toxins. The body's response to infection often involves generating a significant amount of TNF and/or other cytokines. The over-expression of these cytokines can result in significant injury, such as septic shock (when the bacteria is sepsis), endotoxic shock, urosepsis and toxic shock syndrome.

Cytokine expression is mediated by NNRs, and can be inhibited by administering agonists or partial agonists of these receptors. Those compounds described herein that are agonists or partial agonists of these receptors can therefore be used to minimize the inflammatory response associated with bacterial infection, as well as viral and fungal infections. Examples of such

bacterial infections include anthrax, botulism, and sepsis. Some of these compounds may also have antimicrobial properties.

These compounds can also be used as adjunct therapy in combination with existing therapies to manage bacterial, viral and fungal infections, such as antibiotics, antivirals and antifungals. Antitoxins can also be used to bind to toxins produced by the infectious agents and allow the bound toxins to pass through the body without generating an inflammatory response. Examples of antitoxins are disclosed, for example, in U.S. Patent No. 6,310,043 to Bundle *et al.* Other agents effective against bacterial and other toxins can be effective and their therapeutic effect can be complemented by co-administration with the compounds described herein.

Pain

The compounds can be administered to treat and/or prevent pain, including acute, neurologic, inflammatory, neuropathic and chronic pain. The analgesic activity of compounds described herein can be demonstrated in models of persistent inflammatory pain and of neuropathic pain, performed as described in U.S. Published Patent Application No. 20010056084 A1 (Allgeier *et al.*) (*e.g.*, mechanical hyperalgesia in the complete Freund's adjuvant rat model of inflammatory pain and mechanical hyperalgesia in the mouse partial sciatic nerve ligation model of neuropathic pain).

The analgesic effect is suitable for treating pain of various genesis or etiology, in particular in treating inflammatory pain and associated hyperalgesia, neuropathic pain and associated hyperalgesia, chronic pain (*e.g.*, severe chronic pain, post-operative pain and pain associated with various conditions including cancer, angina, renal or biliary colic, menstruation, migraine and gout). Inflammatory pain may be of diverse genesis, including arthritis and rheumatoid disease, teno-synovitis and vasculitis. Neuropathic pain includes trigeminal or herpetic neuralgia, diabetic neuropathy pain, causalgia, low back pain and deafferentation syndromes such as brachial plexus avulsion.

Neovascularization

The α7 NNR is associated with neovascularization. Inhibition of neovascularization, for example, by administering antagonists (or at certain dosages, partial agonists) of the α7 NNR can treat or prevent conditions characterized by undesirable neovascularization or angiogenesis. Such conditions can include those characterized by inflammatory angiogenesis and/or ischemia-induced angiogenesis. Neovascularization associated with tumor

growth can also be inhibited by administering those compounds described herein that function as antagonists or partial agonists of α7 NNR.

Specific antagonism of α7 NNR-specific activity reduces the angiogenic response to inflammation, ischemia, and neoplasia. Guidance regarding appropriate animal model systems for evaluating the compounds described herein can be found, for example, in Heeschen, C. *et al.*, "A novel angiogenic pathway mediated by non-neuronal nicotinic acetylcholine receptors," *J. Clin. Invest.* **110(4)**:527-36 (2002).

Representative tumor types that can be treated using the compounds described herein include NSCLC, ovarian cancer, pancreatic cancer, breast carcinoma, colon carcinoma, rectum carcinoma, lung carcinoma, oropharynx carcinoma, hypopharynx carcinoma, esophagus carcinoma, stomach carcinoma, pancreas carcinoma, liver carcinoma, gallbladder carcinoma, bile duct carcinoma, small intestine carcinoma, urinary tract carcinoma, kidney carcinoma, bladder carcinoma, urothelium carcinoma, female genital tract carcinoma, cervix carcinoma, uterus carcinoma, ovarian carcinoma, choriocarcinoma, gestational trophoblastic disease, male genital tract carcinoma, prostate carcinoma, seminal vesicles carcinoma, testes carcinoma, germ cell tumors, endocrine gland carcinoma, thyroid carcinoma, adrenal carcinoma, pituitary gland carcinoma, skin carcinoma, hemangiomas, melanomas, sarcomas, bone and soft tissue sarcoma, Kaposi's sarcoma, tumors of the brain, tumors of the nerves, tumors of the eyes, tumors of the meninges, astrocytomas, gliomas, glioblastomas, retinoblastomas, neuromas, neuroblastomas, Schwannomas, meningiomas, solid tumors arising from hematopoietic malignancies (such as leukemias, chloromas, plasmacytomas and the plaques and tumors of mycosis fungoides and cutaneous T-cell lymphoma/leukemia), and solid tumors arising from lymphomas.

The compounds can also be administered in conjunction with other forms of anti-cancer treatment, including co-administration with antineoplastic antitumor agents such as cis-platin, adriamycin, daunomycin, and the like, and/or anti-VEGF (vascular endothelial growth factor) agents, as such are known in the art.

The compounds can be administered in such a manner that they are targeted to the tumor site. For example, the compounds can be administered in microspheres, microparticles or liposomes conjugated to various antibodies that direct the microparticles to the tumor. Additionally, the compounds can be present in microspheres, microparticles or liposomes that are appropriately sized to pass through the arteries and veins, but lodge in capillary beds surrounding

tumors and administer the compounds locally to the tumor. Such drug delivery devices are known in the art.

Other Disorders

In addition to treating CNS disorders, inflammation, and undesirable neovascularization, and pain, the compounds of the present invention can be also used to prevent or treat certain other conditions, diseases, and disorders in which NNRs play a role. Examples include autoimmune disorders such as Lupus, disorders associated with cytokine release, cachexia secondary to infection *(e.g.*, as occurs in AIDS, AIDS related complex and neoplasia), obesity, pemphitis, urinary incontinence, retinal diseases, infenctious diseases, myasthenia, Eaton-Lambert syndrome, hypertension, osteoporosis, vasoconstriction, vasodilatation, cardiac arrhythmias, type I diabetes, bulimia, anorexia as well as those indications set forth in published PCT application WO 98/25619. The compounds of this invention can also be administered to treat convulsions such as those that are symptomatic of epilepsy, and to treat conditions such as syphillis and Creutzfeld-Jakob disease.

Diagnostic Uses

The compounds can be used in diagnostic compositions, such as probes, particularly when they are modified to include appropriate labels. The probes can be used, for example, to determine the relative number and/or function of specific receptors, particularly the α7 receptor subtype. For this purpose the compounds of the present invention most preferably are labeled with a radioactive isotopic moiety such as ^{11}C, ^{18}F, ^{76}Br, ^{123}I or ^{125}I.

The administered compounds can be detected using known detection methods appropriate for the label used. Examples of detection methods include position emission topography (PET) and single-photon emission computed tomography (SPECT). The radiolabels described above are useful in PET (*e.g.*, ^{11}C, ^{18}F or ^{76}Br) and SPECT (*e.g.*, ^{123}I) imaging, with half-lives of about 20.4 minutes for ^{11}C, about 109 minutes for ^{18}F, about 13 hours for ^{123}I, and about 16 hours for ^{76}Br. A high specific activity is desired to visualize the selected receptor subtypes at non-saturating concentrations. The administered doses typically are below the toxic range and provide high contrast images. The compounds are expected to be capable of administration in non-toxic levels. Determination of dose is carried out in a manner known to one skilled in the art of radiolabel imaging. See, for example, U.S. Patent No. 5,969,144 to London *et al.*

The compounds can be administered using known techniques. See, for example, U.S. Patent No. 5,969,144 to London *et al.* The compounds can be administered in formulation compositions that incorporate other ingredients, such as those types of ingredients that are useful in formulating a diagnostic composition. Compounds useful in accordance with carrying out the present invention most preferably are employed in forms of high purity. See, U.S. Patent No. 5,853,696 to Elmalch *et al.*

After the compounds are administered to a subject (*e.g.*, a human subject), the presence of that compound within the subject can be imaged and quantified by appropriate techniques in order to indicate the presence, quantity, and functionality of selected NNR subtypes. In addition to humans, the compounds can also be administered to animals, such as mice, rats, dogs, and monkeys. SPECT and PET imaging can be carried out using any appropriate technique and apparatus. See Villemagne *et al.*, In: Arneric *et al.* (Eds.) *Neuronal Nicotinic Receptors: Pharmacology and Therapeutic Opportunities, 235-250 (1998)* and U.S. Patent No. 5,853,696 to Elmalch *et al.*

The radiolabeled compounds bind with high affinity to selective NNR subtypes (*e.g.*, α7) and preferably exhibit negligible non-specific binding to other nicotinic cholinergic receptor subtypes (*e.g.*, α4β2 and those receptor subtypes associated with muscle and ganglia). As such, the compounds can be used as agents for noninvasive imaging of nicotinic cholinergic receptor subtypes within the body of a subject, particularly within the brain for diagnosis associated with a variety of CNS diseases and disorders.

In one aspect, the diagnostic compositions can be used in a method to diagnose disease in a subject, such as a human patient. The method involves administering to that patient a detectably labeled compound as described herein, and detecting the binding of that compound to selected NNR subtypes (*e.g.*, α7 receptor subtypes). Those skilled in the art of using diagnostic tools, such as PET and SPECT, can use the radiolabeled compounds described herein to diagnose a wide variety of conditions and disorders, including conditions and disorders associated with dysfunction of the central and autonomic nervous systems. Such disorders include a wide variety of CNS diseases and disorders, including Alzheimer's disease, Parkinson's disease, and schizophrenia. These and other representative diseases and disorders that can be evaluated include those that are set forth in U.S. Patent No. 5,952,339 to Bencherif *et al.*

In another aspect, the diagnostic compositions can be used in a method to monitor selective nicotinic receptor subtypes of a subject, such as a human patient. The method involves administering a detectably labeled compound as described herein to that patient and detecting the binding of that compound to selected nicotinic receptor subtypes namely, the α7 receptor subtype.

Receptor Binding

The compounds of this invention can be used as reference ligands in binding assays for compounds which bind to NNR subtypes, particularly the α7 receptor subtype. For this purpose the compounds of this invention are preferably labeled with a radioactive isotopic moiety such as ^{3}H, or ^{14}C. Examples of such binding assays are described in detail below.

Pharmaceutical Compositions

Although it is possible to administer the compound of the present invention in the form of a bulk active chemical, it is preferred to administer the compound in the form of a pharmaceutical composition or formulation. Thus, one aspect the present invention includes pharmaceutical compositions comprising the compound of the present invention and one or more pharmaceutically acceptable carriers, diluents, or excipients. Another aspect of the invention provides a process for the preparation of a pharmaceutical composition including admixing the compound of the present invention with one or more pharmaceutically acceptable carriers, diluents or excipients.

The manner in which the compound of the present invention is administered can vary. The compound of the present invention is preferably administered orally. Preferred pharmaceutical compositions for oral administration include tablets, capsules, caplets, syrups, solutions, and suspensions. The pharmaceutical compositions of the present invention may be provided in modified release dosage forms such as time-release tablet and capsule formulations.

The pharmaceutical compositions can also be administered via injection, namely, intravenously, intramuscularly, subcutaneously, intraperitoneally, intraarterially, intrathecally, and intracerebroventricularly. Intravenous administration is a preferred method of injection. Suitable carriers for injection are well known to those of skill in the art and include 5% dextrose solutions, saline, and phosphate buffered saline.

The formulations may also be administered using other means, for example, rectal administration. Formulations useful for rectal administration, such

as suppositories, are well known to those of skill in the art. The compounds can also be administered by inhalation, for example, in the form of an aerosol; topically, such as, in lotion form; transdermally, such as, using a transdermal patch (for example, by using technology that is commercially available from Novartis and Alza Corporation), by powder injection, or by buccal, sublingual, or intranasal absorption.

Pharmaceutical compositions may be formulated in unit dose form, or in multiple or subunit doses

The administration of the pharmaceutical compositions described herein can be intermittent, or at a gradual, continuous, constant or controlled rate. The pharmaceutical compositions may be administered to a warm-blooded animal, for example, a mammal such as a mouse, rat, cat, rabbit, dog, pig, cow, or monkey; but advantageously is administered to a human being. In addition, the time of day and the number of times per day that the pharmaceutical composition is administered can vary.

The compound of the present invention may be used in the treatment of a variety of disorders and conditions and, as such, may be used in combination with a variety of other suitable therapeutic agents useful in the treatment or prophylaxis of those disorders or conditions. Thus, one embodiment of the present invention includes the administration of the compound of the present invention in combination with other therapeutic compounds. For example, the compound of the present invention can be used in combination with other NNR ligands (such as varenicline), antioxidants (such as free radical scavenging agents), antibacterial agents (such as penicillin antibiotics), antiviral agents (such as nucleoside analogs, like zidovudine and acyclovir), anticoagulants (such as warfarin), anti-inflammatory agents (such as NSAIDs), anti-pyretics, analgesics, anesthetics (such as used in surgery), acetylcholinesterase inhibitors (such as donepezil and galantamine), antipsychotics (such as haloperidol, clozapine, olanzapine, and quetiapine), immuno-suppressants (such as cyclosporin and methotrexate), neuroprotective agents, steroids (such as steroid hormones), corticosteroids (such as dexamethasone, predisone, and hydrocortisone), vitamins, minerals, nutraceuticals, anti-depressants (such as imipramine, fluoxetine, paroxetine, escitalopram, sertraline, venlafaxine, and duloxetine), anxiolytics (such as alprazolam and buspirone), anticonvulsants (such as phenytoin and gabapentin), vasodilators (such as prazosin and sildenafil), mood stabilizers (such as valproate and aripiprazole), anti-cancer drugs (such as anti-

proliferatives), antihypertensive agents (such as atenolol, clonidine, amlopidine, verapamil, and olmesartan), laxatives, stool softeners, diuretics (such as furosemide), anti-spasmotics (such as dicyclomine), anti-dyskinetic agents, and anti-ulcer medications (such as esomeprazole). Such a combination of pharmaceutically active agents may be administered together or separately and, when administered separately, administration may occur simultaneously or sequentially, in any order. The amounts of the compounds or agents and the relative timings of administration will be selected in order to achieve the desired therapeutic effect. The administration in combination of a compound of the the present invention with other treatment agents may be in combination by administration concomitantly in: (1) a unitary pharmaceutical composition including both compounds; or (2) separate pharmaceutical compositions each including one of the compounds. Alternatively, the combination may be administered separately in a sequential manner wherein one treatment agent is administered first and the other second. Such sequential administration may be close in time or remote in time.

Another aspect of the present invention includes combination therapy comprising administering to the subject a therapeutically or prophylactically effective amount of the compound of the present invention and one or more other therapy including chemotherapy, radiation therapy, gene therapy, or immunotherapy.

Examples

The following examples are provided to illustrate the present invention, and should not be construed as limiting thereof. In these examples, all parts and percentages are by weight, unless otherwise noted.

Nuclear Magnetic Resonance (NMR) Spectrometry

NMR spectra were collected on either a Varian Unity 300 MHz instrument or a Bruker 400MHz instrument equipped with an auto-sampler and controlled by a DRX400 console. Automated experiments were acquired using ICONNMR v4.0.4 (build 1) running with Topspin v 1.3 (patch level 8) using the standard Bruker loaded experiments. For non-routine spectroscopy, data were acquired through the use of Topspin alone.

Melting Point

A Fisher-Johns hot stage melting point apparatus was used, at a setting corresponding to a heating rate of about 5 °C per min.

Differential Scanning Calorimetry (DSC)

Dsc data were collected on a Mettler DSC 823e equipped with a 50 position auto-sampler. The instrument was calibrated for energy and temperature using certified indium. Typically 0.5-1.5 mg of each sample, in a pin-holed aluminum pan, was heated at 10 °c.min-1 from 25 °C to 300 °C. A nitrogen purge at 50 ml/min-1 was maintained over the sample. Instrument control and data analysis were performed using the stare v 9.10 software package.

X-Ray Powder Diffraction (XRPD)

Method 1

X-Ray Powder Diffraction patterns were collected on a Siemens D5000 diffractometer using Cu Kα radiation (40kV, 40mA), θ-θ goniometer, divergence of V20 and receiving slits, a graphite secondary monochromator and a scintillation counter. The instrument is performance checked using a certified Corundum standard (NIST 1976). The software used for data collection was Diffrac Plus XRD Commander v2.3.1 and the data were analysed and presented using Diffrac *Plus* EVA v 11,0.0.2 or v 13.0.0.2.

Samples were run under ambient conditions as flat plate specimens using powder as received. Approximately 30 mg of the sample was gently packed into a cavity cut into polished, zerobackground (510) silicon wafer. The sample was rotated in its own plane during analysis. The details of the data collection are:

- Angular range: 2 to 42 °2θ
- Step size: 0.05 °2θ or 0.1 °2θ
- Collection time: 4 $s.step^{-1}$

Method 2

X-Ray Powder Diffraction patterns were collected on a Bruker AXS C2 GADDS diffractometer using Cu Kα radiation (40 kV, 40 mA), automated XYZ stage, laser video microscope for auto-sample positioning and a HiStar 2-dimensional area detector. X-ray optics consists of a single Göbel multilayer mirror coupled with a pinhole collimator of 0.3 mm.

The beam divergence, i.e. the effective size of the X-ray beam on the sample, was approximately 4 mm. A θ-θ continuous scan mode was employed with a sample – detector distance of 20 cm which gives an effective 2θ range of 3.2 ° – 29.7 °. Typically the sample would be exposed to the X-ray beam for 120 seconds. The software used for data collection was GADDS for WNT 4.1.16 and the data were analysed and presented using Diffrac *Plus* EVA v 9.0.0.2 or v 13.0.0.2.

Samples run under ambient conditions were prepared as flat plate specimens using powder as received without grinding. Approximately 1-2 mg of the sample was lightly pressed on a glass slide to obtain a flat surface. Samples run under non-ambient conditions were mounted on a silicon wafer with heatconducting compound. The sample was then heated to the appropriate temperature at ca. 10 °C.min^{-1} and subsequently held isothermally for ca 1 minute before data collection was initiated.

Single Crystal X-Ray Diffraction (SCXD)

Data were collected on a Bruker AXS 1K SMART CCD diffractometer equipped with an Oxford Cryosystems Cryostream cooling device. Structures were solved using either the SHELXS or SHELXD programs and refined with the SHELXL program as part of the Bruker AXS SHELXTL suite. Unless otherwise stated, hydrogen atoms attached to carbon were placed geometrically and allowed to refine with a riding isotropic displacement parameter. Hydrogen atoms attached to a heteroatom were located in a difference Fourier synthesis and were allowed to refine freely with an isotropic displacement parameter.

Example 1. Synthesis of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide

To a suspension of (2S,3R)-3-amino-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]octane (20 mg, 0.092 mmol, prepared as described in PCT WO 09/018505, herein incorporated by reference with regard to such synthesis), o-(benzotriazol-1-yl)-N,N,N,1-tetramethyluronium hexafluorophosphate (HBTU, 41.7 mg, 0.110 mmol) and 3,5-difluorobenzoic acid (17.4 mg, 0.110 mmol) in N,N-dimethylformamide (DMF, 2 ml) was added triethylamine (28 mg, 0.28 mmol) at room temperature. The reaction mixture was stirred overnight at room temperature, diluted with ethyl acetate (200 ml) and washed with 20% aqueous potassium carbonate. The residue was purified by silica gel chromatography with the eluent methanol:triethylamine = 300:1. The solvent was removed to give (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide (30 mg, 76%), purity by HPLC: 100% (214 nm), 98.2% (254 nm); ^{1}H NMR (400 MHz, $CDCl_3$) d 8.48 (d, j = 2.0 Hz, 1H), 8.36 (dd, j = 1.7 Hz, j = 4.9 Hz, 1H), 7.56-7.60 (m, 1H), 7.14-7.20 (m, 1H), 7.05-7.14 (m, 2H), 6.87-6.96 (m, 1H), 6.23 (d, j = 7.8 Hz, 1H), 3.88-3.96 (m, 1H), 3.02-3.13 (m, 1H), 2.82-3.00 (m, 4H), 2.65-2.82 (m, 2H), 1.97-2.05 (m, 1H), 1.58-1.84 (m, 3H), 1.43-1.55 (m, 1H); ESI-MS 358.1$(MH)^+$.

Example 2. Synthesis of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-

azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide fumarate

To a solution of 3,5-difluorobenzoic acid (9.36 g, 59.2 mmol), chloroform (200 mL) and triethylamine (16.34 g, 161.5 mmol) at 25 °C was added HBTU (22.5 g, 59.2 mmol). The mixture was heated to 40-42 °C for 45 min resulting in the formation of a white suspension. The suspension was cooled to 10 °C and a solution of (2S,3R)-3-amino-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]octane (11.7 g, 53.8 mmoles) in chloroform (50 mL) was added over a 15-20 min period and stirred for 1.5 h. The reaction mixture was heated to 40-42 °C and additional 3,5-difluorobenzoic acid (2.0 g, 13 mmol) and HBTU (4 g, 11 mmol) were added, followed by stirring at 40-42 °C for 2 h and then at ambient temperature for 16 h. The reaction mixture was quickly quenched with water (200 mL), and under stirring, the pH of the aqueous layer was adjusted to pH = 10-11 with 10 wt % aqueous sodium hydroxide. The layers were separated and the organic layer was washed twice with water (100 mL). The solvent was removed in vacuo to afford 22.9 g of a viscous orange solid. The strength of the product in the crude oil was determined at 66.0 wt % by quantitative HPLC against a reference standard; this corresponds to a yield of 15.1 g (78%). The oil was dissolved in methyl ethyl ketone (50 mL) which was subsequently distilled off in vacuo; this process was repeated a total of three times. A 500 mL three-necked, round-bottomed flask, equipped with an overhead stirrer, temperature probe, dropping funnel and condenser, was charged with fumaric acid (4.9 g, 42 mmol) and methyl ethyl ketone (150 mL). The suspension was heated to 78 °C which led to complete dissolution of the acid. A solution of the (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide in methyl ethyl ketone (50 mL) was added slowly, keeping the internal temperature above 75 °C. After completion of the addition, the suspension was stirred for 30-45 min at 78 °C and the heat source was turned off. The suspension was stirred overnight, filtered and the cake was dried at 50 °C in vacuo for 16 h to afford (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide fumarate as a light yellow, crystalline solid (99.6 % pure by HPLC), mp 208-210 °C. Yield: 65% for two steps. ^{1}H NMR (D_2O, 400 MHz): δ 8.30 (d, J = 5.6 Hz, 1H); 8.06 (d, J = 7.5 Hz, 1H); 7.48 (dd, J = 8.7 Hz, J = 5.6 Hz, 1 H); 6.97-7.06 (m, 1H); 6.75-6.85 (m, 2 H); 6.49 (s, 2H); 4.15 (d, J = 7.5 Hz, 1H); 3.69-3.81 (m, 1H); 3.40-3.59 (m, 2H); 3.17-3.40 (m, 4 H); 2.03-2.18 (m, 2H); 1.91-2.03 (m, 2 H), 1.78-1.91 (m, 1H).

Example 3. Synthesis of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide mono-hydrochloride

Procedure A: To a solution of 250 mg (0.7 mmol) of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide in 10 mL of isopropyl acetate was added aqueous hydrochloric acid (65 µL of a 37% (w/w), 0.78 mmol). The solution was heated to 50 °C and cooled to 0 °C over a 4 h period. The mono-hydrochloride sample was a mixture of gum and white powder at 0 °C. The sample was then heated to 20 °C and cooled again to 0 °C (cooling ramp 5 °C /min). The resulting solids were collected and dried under vacuum at 25 °C for 24 h. mp (DSC) = 274.8 °C.

Procedure B: Concentrated hydrochloric acid (0.54 mL of 37% (w/w), 6.6 mmol) was added drop-wise, with ice bath cooling, to tetrahydrofuran (THF , ~4 mL) and diluted to 5 mL volume with THF. This solution was added drop-wise (over a 5 min period) to a warm (45-50 °C) solution of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide (2.43 g of 96.8% purity, 6.58 mmol) in acetone (20 mL). Solids began to precipitate. The mixture was heated near boiling for 15 min, cooled to ambient temperature and allowed to sit 16 h. The solids were collected by suction filtration under nitrogen, washed with acetone and dried in a vacuum oven (85 °C, 3 h). This left 2.26 g of material that was 93% pure by LCMS. The entire sample was digested in hot (near boiling) 2-propanol (25 mL) for 10 min. The mixture was cooled to ambient temperature and allowed to stand for 3 h. The solids were collected by suction filtration under nitrogen and dried in a vacuum oven (85 °C, 2.5 h). The resulting white crystals were >99% pure by HPLC, weighed 2.03 g (78.4% yield) and melted at 273-276 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.45 (broad s, 1H), 8.66 (d, 1H), 8.54 (s, 1H), 8.28 (d, 1H), 7.76 (d, 1H), 7.41 (m, 1H), 7.24 (m, 3H), 4.14 (m, 1H), 4.07 (m, 1H), 3.46 (m, 2H), 3.10-3.35 (m, 4H), 2.06 (m, 3H), 1.90 (m, 1H), 1.69 (m, 1H). Elemental analysis: Calculated for $C_{20}H_{21}ON_3F_2 \cdot HCl$ (C, 60.99%; H, 5.63%, N, 10.67%); Found (C, 60.94%, 60.91%; H, 5.64%, 5.66%; N, 10.63%, 10.67%).

Example 4. Synthesis of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide mono-phosphate

To a solution of 250 mg (0.7 mmol) of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide in 5 mL of isopropyl alcohol was added 780 µL (0.78 mmol, 1.1 eq) of a 1M phosphoric acid in THF solution. The solution was heated to 50 °C and cooled to 0 °C over a 4 hour period. A white immobile slurry formed at 0 °C, which remained after warming the sample to room

temperature. Evaporation of the solvent yielded crystalline material that was collected and dried under vacuum at 25 °C for 24 h. mp (DSC) = 219.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (s, 1H), 8.34 (d, 1H), 8.28 (d, 1H), 7.68 (d, 1H), 7.43 (m, 1H), 7.24 (m, 3H), 5.04 (br s), 3.84 (m, 1H), 2.70-3.35 (m, 7H), 1.60-1.90 (m, 4H), 1.40 (m, 1H).

Example 5. Synthesis of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide mono-4-hydroxybenzoate

To a solution of 250 mg (0.70 mmol) of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide in 5 mL of isopropyl acetate was added 780 μL (0.78 mmol, 1.1 eq) of a 1M 4-hydroxybenzoic acid in THF solution. The solution was heated to 50 °C and cooled to 0 °C over a 4 hour period. The mono-4-hydroxybenzoate was obtained as a gum. The crystallization was obtained after seeding with the hemi-4-hydroxybenzoate and 48 hours of maturation between 50 °C and room temperature (4 hours cycle) of an evaporated mixture of gum and solvent (only a quarter of the starting volume was remaining). The solid was then isolated by evaporation of the solvent under nitrogen. The resulting solids were collected and dried under vacuum at 25 °C for 24 h. mp (DSC) = 144.0 °C.

Example 6. Synthesis of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide hemi-4-hydroxybenzoate

To a solution of 71.5 mg (0.20 mmol) of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide in 3.5 mL of isopropyl acetate was added 100 μL (0.1 mmol, 0.5 eq) of a 1M 4-hydroxylbenzoic acid in THF solution. The isopropyl acetate was evaporated to yield (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide mono-4-hydroxybenzoate as a solid which was collected and dried under vacuum at 25 °C for 24 h. mp(DSC) = 106.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.23 (br s), 8.43 (s, 1H), 8.29 (s, 1H), 8.28 (s, 1H), 7.78 (d, 1H), 7.61 (m, 1H), 7.41 (m, 3H), 7.22 (m, 1H), 6.80 (d, 1H), 3.66 (m, 1H), 2.70-3.20 (m, 7H), 1.50-1.90 (m, 4H), 1.20 (m, 1H).

Example 7. Synthesis of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide monohydrate

To a solution of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide (993 mg, 2.80 mmol) water (5 mL) was added chloroform (15 mL). The pH of the aqueous layer was adjusted to pH = 10-11 with 10 weight % sodium hydroxide. The biphasic mixture was shaken vigorously, and the layers

were allowed to separate. The chloroform layer was isolated, and the aqueous layer was extracted once more with chloroform (9 mL). The combined chloroform layers were washed once with water (7 mL), filtered over a bed of anhydrous magnesium sulfate and concentrated in vacuo to afford a colorless, clear oil with a tendency to foam. The material was treated with methyl tert-butyl ether (MTBE, 10-15 mL) followed by solvent distillation in vacuo; this process was repeated once more. The material was dissolved in MTBE (10-15 mL) and heptane was added until a white cloudiness appeared. At this point, a slow distillation of volatiles at 50-55 °C at ambient pressure was started and additional solid material separated out. The distillation was halted and the material was collected by filtration and washed with a small amount of heptane. The material was dried in vacuo at 55 °C under a vacuum/nitrogen bleed for 60 h and at 70-85 °C for 40 h to afford 400 mg of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide mono-hydrate as a white brittle solid: $\alpha_D^{26.6°C} = 40°$; elemental analysis, calc: C (63.99); H (6.18); N (11.19), H_2O (4.8 weight %), found: C (64.23); H (6.27); N (11.18); H_2O (4.48). ^1H NMR ($CDCl_3$, 400 MHz): δ 8.46 (d, J = 2 Hz, 1H); 8.35 (dd, J = 4.8 Hz, J = 2 Hz, 1 H); 7.56-7.61 (m, 1H); 7.08-7.19 (m, 3H); 6.87-6.95 (m, 1H), 6.32 (d, J = 8.1 Hz, 1H); 3.89-3.95 (m, 1H); 3.00-3.12 (m, 1H), 2.84-2.99 (m, 4H); 2.68-2.84 (m, 2H); 1.98-2.04 (m, 1H); 1.83 (s, 2H); 1.58-1.79 (m, 3H); 1.44-1.54 (m, 1H). Decomposes at 240 °C.

Example 8. Synthesis of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide hemi-galactarate

To a stirred solution of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide (357 mg, 1.00 mmol) in absolute ethanol (10 mL) at 70-72 °C was added galataric acid (neat) (105 mg, 0.50 mmol) in small portions. Heating was continued for an additional 15 min after completion of acid addition. The solution was slowly cooled to ambient temperature. After standing for 2 h, the solids were collected by vacuum filtration, washed with ethanol, and dried under a nitrogen cone for 30 min. The resulting material was dried for 3 h at 75 °C in a vacuum oven to remove residual ethanol. The results of XRPD analysis are shown in Figure 11.

Example 9. Salt screening of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide

Stock solutions of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide free base were prepared as follows:

• 20 mg/ml in IPA, 25mg/ml in i-ProAc - Counter-ions 1-12

• 25 mg/ml in i-ProAc - Counter-ions 13-20

Each vial was charged with 2 ml of free base stock solution at ambient (40-50 mg of free base/vial). To each vial was then added appropriate volumes of stock acid solution, (1M in THF, unless otherwise stated) at either 1.1 or 2.2 equivalents at ambient. Insoluble acids were added as solids accordingly. All samples were the warmed to 50 °C prior to cooling to 0 °C over 10 hours. All amorphous solids, including gums and oils, were placed on maturation (ambient-50 °C in 4 hour cycles over 2 days) followed by XRPD re-analysis. To those samples which remained amorphous post maturation 2 ml of methyl ethyl ketone was added and the samples further matured for 3 days. Clear solutions were sequentially evaporated to approximately to half and then to quarter volume at 50 °C. Remaining solutions were further cooled to 5 °C prior to the complete removal of the solvent under vacuum. All resulting solids were analyzed by XRPD and any crystalline samples with unique XRPD patterns were analyzed further by ^{1}HNMR/Ion chromatography, solid state stability at 40 °C/75%RH for 1 week and aqueous solubility (target 10 mg/ml at 25 °C, unbuffered)

Acidic counter-ions selected for the salt selection study

Nr.	Acid	Class	pKa			LogP	MW
			1	2	3		
1	Hydrochloric acid 37 wt% (12M)	1	-6.10			-	36.46
2	Sulphuric acid	1	-3.00	1.92		-1.03	98.08
3	Methane sulfonic acid	2	-1.20			-1.89	96.10
4	Maleic acid	1	1.92	6.23		-0.01	116.07
5	Phosphoric acid	1	1.96	7.12	12.32	-2.15	98.00
6	L-Tartaric acid	1	3.02	4.36		-1.43	150.09
7	Fumaric acid *used as powder*	1	3.03	4.38		-0.01	116.07
8	Citric acid	1	3.13	4.76	6.40	-1.72	192.12
9	L-Malic acid	1	3.46	5.10		-1.26	134.09
10	1-Hydroxy-2-Naphthoic acid *used as powder*	2	2.70	13.50		3.29	188.17
11	4-Hydroxy benzoic acid	2					
12	Succinic acid 1M in MeOH	1	4.21	5.64		-0.59	118.09
13	Benzene sulfonic acid	2	0.70			0.47	158.18
14	p-Toluene sulfonic acid 1M in EtOH	2	-1.34			0.93	190.22
15	Hippuric acid *used as powder*	1	3.55			0.31	179.17

16	D-Gluconic acid 50% in water	1	3.76			-3.18	196.16
17	Acetic acid	1	4.76			-0.29	60.05
18	Benzoic acid 1M in IPA	2	4.19				
19	Propionic acid	2	4.87			0.25	74.07
20	L-Aspartic acid *used as powder*	1	1.88	3.65		-0.67	133.11

Table -- Salt screen summary

Counter-ion	Solvent	Target Stoichiometry	Observation on addition of acid at RT	Observation at 0°C	XRPD analysis after filtration	XRPD after 48 hours of maturation	XRPD after evaporation and maturation in MEK	Observation on 2nd maturation
Hydrochloride	2-propanol	mono salt	Clear solution	White powder	Crystalline♣	Crystalline♣	n/a	n/a
		bis salt	Clear solution	Clear solution	n/a	Clear solution	Crystalline♣	n/a
	Isopropyl acetate	mono salt	Precipitate	White powder	Crystalline♣	Crystalline♣	n/a	n/a
		bis salt	Precipitate	White powder	gum♠	Crystalline♣	n/a	n/a
Sulphate (Sulfate)	2-propanol	mono salt	Precipitate	White powder	gum♠	Crystalline♣	n/a	n/a
		bis salt	Precipitate	gum	gum♠	gum	gum	gum
	Isopropyl acetate	mono salt	Precipitate	White powder	Amorphous♠	Amorphous♠	Amorphous♠	White powder
		bis salt	Precipitate	White powder	gum♠	Clear solution	gum♠	gum♠
Mesylate	2-propanol	mono salt	Clear solution	Clear solution	n/a	n/a	n/a	n/a
		bis salt	Clear solution	Clear solution	n/a	Amorphous♠	Amorphous♠	White powder
	Isopropyl acetate	mono salt	Precipitate	gum	n/a	Crystalline♣	n/a	n/a
		bis salt	Precipitate	gum	n/a	Crystalline♣	n/a	n/a
Maleate	2-propanol	mono salt	Clear solution	Clear solution	n/a	Clear solution	n/a	gum
		bis salt	Clear solution	Clear solution	n/a	Clear solution	n/a	gum
	Isopropyl acetate	mono salt	gum	gum	n/a	gum♠	n/a	gum
		bis salt	gum	gum	n/a	gum♠	n/a	gum
Phosphate	2-propanol	mono salt	Precipitate	White powder	n/a Not enough material	Crystalline♣	n/a	n/a
		bis salt	Precipitate	White powder	gum♠	White powder	Amorphous♠	White powder
	Isopropyl acetate	mono salt	Precipitate	gum	n/a	Crystalline♣	n/a	n/a
		bis salt	Precipitate	gum	n/a	Crystalline♣	n/a	n/a

Counter-ion	Solvent	Target Stoichiometry	Observation on addition of acid at RT	Observation at 0°C	XRPD analysis after filtration	XRPD after 48 hours of maturation	XRPD after evaporation and maturation in MEK	Observation on 2nd maturation
Fumarate	2-propanol	mono salt	Clear solution	Clear solution	n/a	Clear solution	Crystalline♣	n/a
		bis salt	Stopped experiment ♥					
	Isopropyl acetate	mono salt	Clear solution	Precipitate	Mainly amorphous♠	Crystalline♣	n/a	n/a
		bis salt	Clear solution	Precipitate	Amorphous♠	Crystalline♣	n/a	n/a
Citrate	2-propanol	mono salt	Precipitate	White powder	gum♠	n/a	gum♠	gum♠
		bis salt	Precipitate	White powder	gum♠	n/a	gum♠	gum♠
	Isopropyl acetate	mono salt	Precipitate	White powder	Amorphous♠	Amorphous♠	Amorphous♠	White powder
		bis salt	Precipitate	White powder	Amorphous♠	Amorphous♠	Amorphous♠	White powder
Malate	2-propanol	mono salt	Clear solution	Clear solution	n/a	Clear solution	gum♠	gum♠
		bis salt	Clear solution	Clear solution	n/a	Clear solution	gum♠	gum♠
	Isopropyl acetate	mono salt	Precipitate	White powder	Amorphous♠	Amorphous	Amorphous♠	White powder
		bis salt	Precipitate	White powder	gum♠	Low Crystallinity♣	n/a	n/a
Xinafoate	2-propanol	mono salt	Clear solution	Clear solution	n/a	Clear solution	gum♠	gum♠
		bis salt	Clear solution	Clear solution	n/a	Clear solution	gum♠	gum♠
	Isopropyl acetate	mono salt	Clear solution	Clear solution	n/a	Clear solution	gum♠	gum♠
		bis salt	Clear solution	Clear solution	n/a	Clear solution	gum♠	gum♠
4-Hydroxybenzoate	2-propanol	mono salt	Clear solution	Clear solution	n/a	Clear solution	gum♠	gum♠
		bis salt	Clear solution	Clear solution	n/a	Clear solution	gum♠	gum♠
	Isopropyl acetate	mono salt	Clear solution	Clear solution	n/a	Crystalline♣	n/a	n/a
		bis salt	Clear solution	Clear solution	n/a	Clear solution	gum♠	gum♠
Succinate	2-propanol	mono salt	Clear solution	Clear solution	n/a	Clear solution	gum♠	gum♠
		bis salt	Clear solution	Clear solution	n/a	Clear solution	gum♠	gum♠
	Isopropyl acetate	mono salt	Clear solution	Clear solution	n/a	Clear solution	gum♠	gum♠
		bis salt	Clear solution	Clear solution	n/a	Clear solution	gum♠	gum♠

Counter-ion	Solvent	Target Stoichiometry	Observation on addition of acid at RT	Observation at 0°C	XRPD analysis after filtration	XRPD after 48 hours of maturation	XRPD after evaporation and maturation in MEK	Observation on 2nd maturation	XRPD post evaporation and storage at 5°C
Benzylate	Isopropyl Acetate	mono salt	Precipitate	White powder	Amorphous♠	Low Crystallinity♠	n/a	n/a	n/a
Tosylate	Isopropyl Acetate	mono salt	Clear solution	Clear solution	n/a	Clear solution	Crystalline♣	n/a	n/a
Hippurate	Isopropyl Acetate	mono salt	Clear solution	Clear solution	n/a	Clear solution	gum♠	gum♠	n/a
Gluconate	Isopropyl Acetate	mono salt	Clear solution	Clear solution	n/a	Clear solution	gum♠	gum♠	n/a
Acetate	Isopropyl Acetate	mono salt	Clear solution	Clear solution	n/a	Clear solution	gum♠	gum♠	n/a
Benzoate	Isopropyl Acetate	mono salt	Clear solution	Clear solution	n/a	Clear solution	gum♠	gum♠	Crystalline♣
Propionate	Isopropyl Acetate	mono salt	Clear solution	Clear solution	n/a	Clear solution	gum♠	gum♠	n/a
Aspartate	Isopropyl Acetate	mono salt	Non-dissolved	Non-dissolved	Crystalline same as the acid♥	Crystalline same as the acid♥	Crystalline same as the acid ♥	n/a	n/a

Key: ♣ Crystalline
♠ Amorphous
♥ No further analysis performed
n/a Not applicable

Table -- Characterization after primary salt screening

Salt	Target Stoichiometry	XRPD after filtration	Stoichiometry (Acid:Base)		XRPD 40C/75%RH 1 Week (See Figure 12)	Aqueous Solubility
			1H NMR	Ion Chromatography		
Hydrochloride	mono salts	Pattern1	Confirmation of the salt formation	1.0:1	Pattern1♣	> 10 mg/mL
	bis salts	Pattern2 Pattern3	Confirmation of the salt formation	n/a	deliquescent♠	n/a
Phosphate	mono salts	Pattern1	Confirmation of the salt formation	1.0:1	Pattern1♣	> 10 mg/mL
	mono and bis salts	Pattern2	Confirmation of the salt formation	n/a	Pattern1♣ + Pattern2♣	> 10 mg/mL
Fumarate	mono salt	Pattern1	Confirmation of the formation of a mono salt 1.0:1	n/a	Pattern1♣	> 10 mg/mL
	bis salt	Pattern2	Confirmation of the formation of a bis salt 1.9:1	n/a	Pattern2♣	> 10 mg/mL
4-Hydroxybenzoate	mono salt	Pattern1	Confirmation of the formation of a hemi salt 0.5:1	n/a	Pattern1♣	> 10 mg/mL
Benzoate	mono salt	Pattern1	Confirmation of mono salt formation	n/a	Pattern2♣	0.8 mg/mL
Sulphate	mono salt	Pattern1	Confirmation of the salt formation	n/a	deliquescent♠	n/a
Mesylate	mono and bis salts	Pattern1	Confirmation of the formation of a mono salt	n/a	deliquescent♠	n/a
Malate	bis salts	Pattern1 Low crystallinity	Confirmation of the salt formation 1.4:1	n/a	deliquescent♠	n/a
Tosylate	mono salts	Pattern1	Confirmation of the salt formation 1:1	n/a	deliquescent♠	n/a

Key: ♣ Crystalline
♠ Amorphous
♥ No further analysis performed
n/a Not applicable

Example 10. Crystal Structure of the Hydrochloride Salt

Crystals of hydrochloride salt were obtained by maturation between room temperature and 50°C of a methanol solution of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide mono-hydrochloride. The single crystal structure data are indicated in the table below. The sample was checked to be representative of the bulk

Single Crystal Structure of the mono hydrochloride salt

Molecular formula	$C_{20}H_{22}ClF_2N_3O$				
Molecular weight	393.86				
Crystal system	Monoclinic				
Space group	$P2_1$	*a*	10.049(1)Å	α	90°
		b	8.872(1)Å	β	94.088(3)°
		c	10.491(1)Å	γ	90°
V	933.07(15)Å^3				
Z	2				
D_c	1.402g.cm^{-1}				
μ	0.239mm^{-1}				
Source, λ	Mo-K(alpha), 0.71073Å				
F(000)	412				
T	120(2)K				
Crystal	colourless prism, 0.3 x 0.15 x 0.11mm				
Data truncated to	0.80 Å				
θ_{max}	26.37°				
Completeness	99.4%				
Reflections	7986				
Unique reflections	3750				
R_{int}	0.0135				
Flack parameter	-0.04(3)				
R_{all}	0.0236				
R_1	0.0231				

The structure solution was obtained by direct methods, full-matrix least-squares refinement on F^2 with weighting $w^{-1} = \sigma^2(F_o^2) + (0.0435P)^2 + (0.1500P)$, where $P = (F_o^2+2F_c^2)/3$, anisotropic displacement parameters, empirical absorption corrections were applied, absolute structure parameter = -0.04(3). Final $wR^2 = \{\Sigma[w(F_o^2-F_c^2)^2]/\Sigma[w(F_o^2)^2]^{1/2}\} =$ 0.0636 for all data, conventional $R_1 = 0.0231$ on F values of 3684 reflections with $F_o > 4\sigma(F_o)$, S = 1.004 for all data and 252 parameters. Final Δ/σ(max) 0.001, Δ/σ(mean), 0.000. Final difference map between +0.195 and -0.136 e Å^{-3}.

The value of the absolute structure parameter enabled the determination of the configuration of the chiral centers. This configuration is indicated in Figure 10.

Example 11. Biological Assays

Radioligand Binding at CNS nAChRs α4β2 NNR Subtype

Preparation of membranes from rat cortex: Rats (female, Sprague-Dawley), weighing 150-250 g, were maintained on a 12 h light/dark cycle and were allowed free access to water and food supplied by PMI Nutrition International, Inc. Animals were anesthetized with 70% CO_2, and then decapitated. Brains were removed and placed on an ice-cold platform. The cerebral cortex was removed and placed in 20 volumes (weight:volume) of ice-cold preparative buffer (137 mM NaCl, 10.7 mM KCl, 5.8 mM KH_2PO_4, 8 mM Na_2HPO_4, 20 mM HEPES (free acid), 5 mM iodoacetamide, 1.6 mM EDTA, pH 7.4); PMSF, dissolved in methanol to a final concentration of 100 μM, was added and the suspension was homogenized by Polytron. The homogenate was centrifuged at 18,000 x g for 20 min at 4 °C and the resulting pellet was re-suspended in 20 volumes of ice-cold water. After 60 min incubation on ice, a new pellet was collected by centrifugation at 18,000 x g for 20 min at 4 °C. The final pellet was re-suspended in 10 volumes of buffer and stored at -20 °C.

Preparation of membranes from SH-EP1/human α4β2 clonal cells: Cell pellets from 40 150 mm culture dishes were pooled, and homogenized by Polytron (Kinematica GmbH, Switzerland) in 20 milliliters of ice-cold preparative buffer. The homogenate was centrifuged at 48,000 g for 20 minutes at 4 °C. The resulting pellet was re-suspended in 20 mL of ice-cold preparative buffer and stored at -20 °C.

On the day of the assay, the frozen membranes were thawed and spun at 48,000 x g for 20 min. The supernatant was decanted and discarded. The pellet was resuspended in Dulbecco's phosphate buffered saline (PBS, Life Technologies) pH 7.4 and homogenized with the Polytron for 6 seconds. Protein concentrations were determined using a Pierce BCA Protein Assay Kit, with bovine serum albumin as the standard (Pierce Chemical Company, Rockford, IL).

Membrane preparations (approximately 50 μg for human and 200-300 μg protein for rat α4β2) were incubated in PBS (50 μL and 100 μL respectively) in the presence of competitor compound (0.01 nM to 100 μM) and 5 nM [^{3}H]nicotine for 2-3 hours on ice. Incubation was terminated by rapid filtration on a multi-manifold tissue harvester (Brandel, Gaithersburg, MD) using GF/B filters presoaked in 0.33% polyethyleneimine (w/v) to reduce non-specific binding. Tissue was rinsed 3 times in PBS, pH 7.4. Scintillation fluid was added to filters containing the washed tissue and allowed to equilibrate. Filters were then counted to determine radioactivity bound to the membranes by liquid scintillation counting (2200CA Tri-Carb LSC, Packard Instruments, 50% efficiency or Wallac Trilux 1450 MicroBeta, 40% efficiency, Perkin Elmer).

Data were expressed as disintegrations per minute (DPMs). Within each assay, each point had 2-3 replicates. The replicates for each point were averaged and plotted against the log of the drug concentration. IC_{50}, which is the concentration of the compound that produces 50% inhibition of binding, was determined by least squares non-linear regression. Ki values were calculated using the Cheng-Prussof equation (1973):

$$Ki = IC_{50}/ (1 + N/Kd)$$

where N is the concentration of [^{3}H]nicotine and Kd is the affinity of nicotine (3 nM, determined in a separate experiment).

α7 NNR Subtype

Rats (female, Sprague-Dawley), weighing 150-250 g, were maintained on a 12 h light/dark cycle and were allowed free access to water and food supplied by PMI Nutrition International, Inc. Animals were anesthetized with 70% CO_2, and then decapitated. Brains were removed and placed on an ice-cold platform. The hippocampus was removed and placed in 10 volumes (weight:volume) of ice-cold preparative buffer (137 mM NaCl, 10.7 mM KCl, 5.8 mM KH_2PO_4, 8 mM Na_2HPO_4, 20 mM HEPES (free acid), 5 mM iodoacetamide, 1.6 mM EDTA, pH 7.4); PMSF, dissolved in methanol to a final concentration of 100 μM, was added and the tissue suspension was homogenized by Polytron. The homogenate was centrifuged at 18,000 x g for 20 min at 4 °C and the resulting pellet was re-suspended in 10 volumes of ice-cold water. After 60 min incubation on ice, a new pellet was collected by centrifugation at 18,000 x g for 20 min at 4 °C. The final pellet was re-suspended in 10 volumes of buffer and stored at -20 °C. On the day of the assay, tissue was thawed, centrifuged at 18,000 x g for 20 min, and then re-suspended in ice-cold PBS (Dulbecco's Phosphate Buffered Saline, 138 mM NaCl, 2.67 mM KCl, 1.47 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 0.9 mM $CaCl_2$, 0.5 mM $MgCl_2$, Invitrogen/Gibco, pH 7.4) to a final concentration of approximately 2 mg protein/mL. Protein was determined by the method of Lowry et al., *J. Biol. Chem.* **193**: 265 (1951), using bovine serum albumin as the standard.

The binding of [^{3}H]MLA was measured using a modification of the methods of Davies et al., *Neuropharmacol.* **38**: 679 (1999). [^{3}H]MLA (Specific Activity = 25-35 Ci/mmol) was obtained from Tocris. The binding of [^{3}H]MLA was determined using a 2 h incubation at 21 °C. Incubations were conducted in 48-well micro-titre plates and contained about 200 μg of protein per well in a final incubation volume of 300 μL. The incubation buffer was PBS and the final concentration of [^{3}H]MLA was 5 nM. The binding reaction was terminated by filtration of the protein containing bound ligand onto glass fiber filters (GF/B, Brandel) using a Brandel Tissue Harvester at room temperature. Filters were soaked in de-ionized water containing 0.33 % polyethyleneimine to reduce non-specific binding. Each filter was washed

with PBS (3 x 1 mL) at room temperature. Non-specific binding was determined by inclusion of 50 μM non-radioactive MLA in selected wells.

The inhibition of [^{3}H]MLA binding by test compounds was determined by including seven different concentrations of the test compound in selected wells. Each concentration was replicated in triplicate. IC_{50} values were estimated as the concentration of compound that inhibited 50 percent of specific [^{3}H]MLA binding. Inhibition constants (Ki values), reported in nM, were calculated from the IC_{50} values using the method of Cheng et al., Biochem. Pharmacol. 22: 3099-3108 (1973).

Selectivity vs. Peripheral nAChRs

Interaction at the Human Muscle nAChR Subtype

Activation of muscle-type nAChRs was established on the human clonal line TE671/RD, which is derived from an embryonal rhabdomyosarcoma (Stratton et al., Carcinogen 10: 899 (1989)). These cells express receptors that have pharmacological (Lukas, *J. Pharmacol. Exp. Ther.* **251**: 175 (1989)), electrophysiological (Oswald et al., *Neurosci. Lett.* **96**: 207 (1989)), and molecular biological profiles (Luther et al., *J. Neurosci.* **9**: 1082 (1989)) similar to the muscle-type nAChR.

TE671/RD cells were maintained in proliferative growth phase according to routine protocols (Bencherif et al., *Mol. Cell. Neurosci.* **2**: 52 (1991) and Bencherif et al., *J. Pharmacol. Exp. Ther.* **257**: 946 (1991)). Cells were cultured in Dulbecco's modified Eagle's medium (Gibco/BRL) with 10% horse serum (Gibco/BRL), 5% fetal bovine serum (HyClone, Logan UT), 1mM sodium pyruvate, 4 mM L-Glutamine, and 50,000 units penicillin-streptomycin (Irvine Scientific). When cells were 80% confluent, they were plated to 12 well polystyrene plates (Costar). Experiments were conducted when the cells reached 100% confluency.

Nicotinic acetylcholine receptor (nAChR) function was assayed using $^{86}Rb^+$ efflux according to the method described by Lukas et al., *Anal. Biochem.* **175**: 212 (1988). On the day of the experiment, growth media was gently removed from the well and growth media containing ^{86}Rubidium chloride (10^6 μCi/mL) was added to each well. Cells were incubated at 37 °C for a minimum of 3 h. After the loading period, excess $^{86}Rb^+$ was removed and the cells were washed twice with label-free Dulbecco's phosphate buffered saline (138 mM NaCl, 2.67 mM KCl, 1.47 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 0.9 mM $CaCl_2$, 0.5 mM $MgCl_2$, Invitrogen/Gibco, pH. 7.4), taking care not to disturb the cells. Next, cells were exposed to either 100 μM of test compound, 100 μM of L-nicotine (Acros Organics) or buffer alone for 4 min. Following the exposure period, the supernatant containing the released $^{86}Rb^+$ was removed and transferred to scintillation vials. Scintillation fluid was added and released radioactivity was measured by liquid scintillation counting.

Within each assay, each point had 2 replicates, which were averaged. The amount of $^{86}Rb^+$ release was compared to both a positive control (100 μM L-nicotine) and a negative control (buffer alone) to determine the percent release relative to that of L-nicotine.

When appropriate, dose-response curves of test compound were determined. The maximal activation for individual compounds (Emax) was determined as a percentage of the maximal activation induced by L-nicotine. The compound concentration resulting in half maximal activation (EC_{50}) of specific ion flux was also determined.

Interaction at the Rat Ganglionic nAChR Subtype

Activation of rat ganglion nAChRs was established on the pheochromocytoma clonal line PC12, which is a continuous clonal cell line of neural crest origin, derived from a tumor of the rat adrenal medulla. These cells express ganglion-like nAChR s (see Whiting et al., *Nature* **327**: 515 (1987); Lukas, *J. Pharmacol. Exp. Ther.* **251**: 175 (1989); Whiting et al., *Mol. Brain Res.* **10**: 61 (1990)).

Rat PC12 cells were maintained in proliferative growth phase according to routine protocols (Bencherif et al., *Mol. Cell. Neurosci.* **2**: 52 (1991) and Bencherif et al., *J. Pharmacol. Exp. Ther.* **257**: 946 (1991)). Cells were cultured in Dulbecco's modified Eagle's medium (Gibco/BRL) with 10% horse serum (Gibco/BRL), 5% fetal bovine serum (HyClone, Logan UT), 1mM sodium pyruvate, 4 mM L-Glutamine, and 50,000 units penicillin-streptomycin (Irvine Scientific). When cells were 80% confluent, they were plated to 12 well Nunc plates (Nunclon) and coated with 0.03% poly-L-lysine (Sigma, dissolved in 100mM boric acid). Experiments were conducted when the cells reached 80% confluency.

Nicotinic acetylcholine receptor (nAChR) function was assayed using $^{86}Rb^+$ efflux according to a method described by Lukas et al., *Anal. Biochem.* **175**: 212 (1988). On the day of the experiment, growth media was gently removed from the well and growth media containing ^{86}Rubidium chloride (10^6 μCi/mL) was added to each well. Cells were incubated at 37 °C for a minimum of 3 h. After the loading period, excess $^{86}Rb^+$ was removed and the cells were washed twice with label-free Dulbecco's phosphate buffered saline (138 mM NaCl, 2.67 mM KCl, 1.47 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 0.9 mM $CaCl_2$, 0.5 mM $MgCl_2$, Invitrogen/Gibco, pH. 7.4), taking care not to disturb the cells. Next, cells were exposed to either 100 μM of test compound, 100 μM of nicotine or buffer alone for 4 min. Following the exposure period, the supernatant containing the released $^{86}Rb^+$ was removed and transferred to scintillation vials. Scintillation fluid was added and released radioactivity was measured by liquid scintillation counting

Within each assay, each point had 2 replicates, which were averaged. The amount of $^{86}Rb^+$ release was compared to both a positive control (100 μM nicotine) and a negative control (buffer alone) to determine the percent release relative to that of L-nicotine.

When appropriate, dose-response curves of test compound were determined. The maximal activation for individual compounds (Emax) was determined as a percentage of the maximal activation induced by L-nicotine. The compound concentration resulting in half maximal activation (EC_{50}) of specific ion flux was also determined.

Novel Object Recognition

Memory was assessed by using the three-trial novel object recognition test. On the first day (exploratory trial), rats were allowed to explore an open arena (44.5 x 44.5 x 30.5 cm) for 6 min. On the second day (acquisition trial), rats were allowed to explore the same arena in the presence of two identical objects (both object A) for 3 minutes. On the third day (retention or recall trial), performance was evaluated by allowing the same animal to re-explore the arena for 3 minutes in the presence of two different objects: the familiar object A and a novel object B. An inter-trial interval of 24 hours was imposed between the three NOR trials. Recognition memory was assessed by comparing the time spent exploring a novel (object B) versus a familiar (object A) object during the recall trial. Recognition index was assessed for each animal and expressed as a ratio ((time B / time A + time B) x 100).

Summary of Biological Data

***In vitro* pharmacology**

A summary of the *in vitro* primary pharmacology data for (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide, or a pharmaceutically acceptable salt thereof, is presented in Table 1 and discussed in detail below.

Primary pharmacology and selectivity: (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide inhibited the binding of [^{3}H]methyllycaconitine (MLA) to rat native α7 receptors in rat hippocampal membranes with a K_i of 100 nM.

(2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide inhibited the binding of [^{3}H]-nicotine to human recombinant $\alpha4\beta2$ nicotinic receptors with a K_i of 1470 nM and [^{3}H]epibatidine to rat native $\alpha4\beta2$ receptors with a K_i of 4120 nM. (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide also displayed reduced affinity for human native ganglion-type nicotinic receptors (likely $\alpha3\beta4$), inhibiting the binding of [^{3}H]epibatidine to receptors in SH-SY5Y membranes with a K_i of 48 μM, and reduced affinity for human native muscle-type nicotinic receptors (likely $\alpha1\beta1\gamma\delta$), inhibiting the binding of [^{3}H]epibatidine to receptors in TE-671 membranes with a K_i of 136 μM. (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide inhibited the binding of [^{3}H]epibatidine to the human recombinant $\alpha4\beta4$ nicotinic receptors in SH-EP1 membranes with a K_i of 19 μM.

Table 1. Summary of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide *in vitro* pharmacology

Target affinity and activation	
Rat hippocampus (α7), K_i	0.1 μM
Rat cortex binding K_i	4.12 μM
Human recombinant (SH-EP 1) $\alpha4\beta2$ binding K_i	1.47 μM
Human ganglionic (SH-SY5Y), K_i	48 μM
Human (TE671/RD) muscle, K_i	136 μM
Human recombinant (SH-EP1) $\alpha4\beta4$, K_i	19 μM

***In vivo* pharmacology**

A summary of the *in vivo* pharmacology data for (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide, or a pharmaceutically acceptable salt thereof, is presented in Table 2 and discussed in detail below.

Table 2
Summary of NOR results for (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide, or a pharmaceutically acceptable salt thereof

Novel Object Recognition Model (NOR)	Result
Minimum Effective Dose (MED)	MED= 0.084 μmol/kg
Duration of Effect	Duration 6 h (@ 0.1mg/kg)
	Duration 18 h (@ 0.3mg/kg)

(2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide improved long-term visual episodic/declarative memory as assessed by novel object recognition (NOR) task following oral dosing in normal rats. The results of these studies are presented in Figure 1. The recognition index of the vehicle-treated group 24 h after the acquisition trial was 54±1% demonstrating the inability of this group to recognize the familiar object after this delay. By contrast, animals treated with (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide exhibited recognition indexes of 70±4% at the 0.84 μmol/kg dose level and 74±3% and the 0.28 μmol/kg dose level.

In a follow-up NOR study (Figure 2), the minimum effect dose (MED) level for (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide was determined to be 0.084 μmol/kg suggesting that the rats are able to recognize the familiar object at all doses levels tested. In the "recall only" session; a subset of animals were orally dosed with water on day 1 (i.e., exploratory session) and day 2 (i.e., acquisition session) and then orally dosed with 0.28 μmol/kg (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide on day 3 (i.e., recall session). Even following a single oral administration, (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-

difluorobenzamide demonstrated pro-cognitive effects at this dose level. (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide exhibited recognition indexes significantly above controls, indicating recognition of the familiar object following acute dosing. The dashed line at 65% denotes our criteria for biological cognitive enhancing activity. *P < 0.05.

(2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide was evaluated for its duration of effect in the NOR task in normal rats. The results of these studies are presented in Figure 3. The recognition index of the vehicle-treated group at 0.5 h and 24 h following dosing on the recall trial was 51±1% and 53±4%, respectively, demonstrating the inability of this group to recognize the familiar object after this delay. By contrast, animals treated with (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide (0.28 μmol/kg: oral) exhibited recognition indexes of 68±4% at 0.5h, 71±2% at 2h and 62±2% at 6 h suggesting that rats are able to recognize the familiar object for up to 6 h after dosing.

Furthermore, animals treated with (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide (0.84 μmol/kg: oral) exhibited recognition indexes of 59±2% at 0.5h, 63±2% at 2h, 68±3% at 6h, and 68±3% at 18h suggesting that rats are able to recognize the familiar object for up to 18h after dosing at this dose level (Figure 4). The dashed line at 65% denotes our criteria for biological cognitive enhancing activity (*P < 0.05).

Electrophysiology

Compound A, (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide, and Compound B, (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-4-fluorobenzamide are both partial agonist at the α7 NNR. However dramatic differences exist between the two compounds in their ability to induce so-called hump currents. Hump currents are defined as the tail current observed during co-application with endogenous ACh following agonist removal. As demonstrated herein, Compound A provides an improved profile and a greater potential to modulate α7 function in conditions, such as psychotic disorders, where this neurotransmission is compromised.

The dose-response of Compounds A and B with α7 nicotinic ACh receptors was analyzed. Both Compound B and Compound A are partial agonists at α7 nicotinic receptors (EC_{50} = 664 nM, 1.6 μM and E_{MAX} = 46.6%, 54.4%). As shown in Figure 5, both EC_{50} and E_{MAX} are comparable between these ligands.

Co-application of compounds with ACh, however, revealed substantial differences between these two ligands, as illustrated in Figure 6. Compound B inhibited current produced by ACh, presumably due to competitive inhibition, whereas Compound A

enhanced ACh-induced current. One hypothesis for this enhancement is Compound A's ability for orthosteric modulation.

Additionally, substantial differences were found when ACh was co-applied with nanomolar concentrations of Compound B or Compound A, as shown in Figures 7 and 8.

Figure 7A represents an experimental design of loading Dynaflow chip to measure interaction of the ligand (Compound A, 200 nM) with acetylcholine (100 μM) regarding activation of nicotinic α7 receptor. The channels were preared as follows: Control solution (channel #2), application ligand itself (channel #3), application of acetylcholine itself (channel #1), and application of mixture of acetylcholine and ligand (channel #4).

Figure 7B shows four representative current curves obtained with different application sequences:

Curve 1, Figure 7B: The bar above the curve indicates time of ACh application. The curve represents current induced by a one second application of 70 μM ACh. The curve illustrates the result from moving the cell from channel #2 to channel #1 for a 1 second application of ACh and back to channel 2 (washout). The application of ACh produced robust activation of current with fast recovery after washout.

Curve 4, Figure 7B: Curve 4 represents a repetition of Curve 1 at the end of measurements after application of the ligand and ACh/ligand mixture (recovery).

Curve 2, Figure 7B: The down/up arrows indicates time of application. Curve 2 represents a 5 second application of 200 nM of Compound A. The curve illustrates the result of moving the cell from channel #2 to channel #3 for 5 seconds. Compound A in concentration of 200 nM alone do not produce robust macro currents.

Curve 3, Figure 7B: Curve 3 represents the interaction of the application of ACh and Compound A. Curve 3 illustrates the results from moving the cell from channel #2 to #3 (2 sec), to #4 (1 sec) and back to #3 (2 sec) and back to #2 (washout). A profound "hump" current is created due to application of Compound A following application of ACh. This current was not a result of ACh, as seen when comparing Curves 1 and 4, or Compound A, as seen in Curve 2, activation of α7 receptors alone. Rather Curve 3 illustrates an example of interaction of application of both ACh and Compound A.

Figure 7C represents an average (n=4) of absolute values of hump currents obtained with different concentrations of Compound A (100 - 500 nM range). We observed a concentration dependent increase of current (EC_{50} = 120 nM) with E_{MAX} at approximately 500 nM.

Similarly, Figures 8A, 8B, and 8C represent the results obtained for Compound B. Upon comparing Figures 7A-C and with Figures 8A-C, substantial differences may be noted

when ACh was co-applied with Compound A as compared to Compound B. Compound A enhances ACh-induced current.

Formalin Test

One of the most clinically predictive screening models of acute pain is the formalin test in mice (LeBars et al., 2001). In this paradigm, originally described by Dubuisson and Dennis (1977), a diluted solution of formalin is injected into the plantar surface of a subject's (rat or mouse) rear paw and nociceptive behavior is measured; for instance, licking and biting of the injected paw. Two phases of the response are observed. First an early phase, starting immediately after injection and lasting 5-10 minutes, followed by a late phase that can last from 15-60 minutes after injection. Nociceptive response is attributed to direct chemical stimulation in the early phase and inflammation / persistent pain in the later phase (Dubuisson and Dennis, 1977). The response in the late phase also depends on changes in processing of information in the spinal cord due to the afferent barrage during the early phase (Coderre et al., 1990). An advantage of the test is that two different types of stimuli are employed in the same assay to study the possibility of varying analgesic effects of a drug in the two phases of the test (Tjølsen and Hole, 1997).

Subjects (adult male CD-1 mice (Charles River, Raleigh, NC) weighing approximately 20-25 grams) were removed from their home cage and weighed, then placed in a clear Plexiglas™ observation box for an acclimation period of 20-30 minutes. Mice were then removed from the observation chambers and (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide (as the hydrochloride salt in 0.9% saline) (1, 3 or 10 mg/kg s.c. (calculated with respect to the free base)), morphine (5mg/kg; s.c.) or 0.9% saline vehicle was administered subcutaneously in a volume of 1 mL/kg. Mice were then returned to the chamber for the predetermined pretreatment time of 30 minutes for (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide and morphine.

After the test compound pretreatment time, the animals were injected with formalin solution (2.5% derived as a 1:4 dilution of 10% phosphate buffered formalin solution (Sigma):distilled water). The subject's assigned paw was grasped gently and formalin solution was injected into the paw intra-dermally in the middle on the dorsal side. Once injected, the subject was immediately returned to its observation chamber and a timer was started to mark the beginning of Phase I. Each subject was videotaped for the entire 40-minute session. When scoring the tapes, each subject was observed for 1 min at 5-min intervals over a 40-minute session. The time spent licking during that 1 min interval was recorded, and the presence or absence of paw favoring was noted.

For data analyses, phase I of the test was defined as 0 to 5 minutes after formalin injection, and phase II was defined as 20 to 40 minutes after formalin injection. The time spent licking during the 1 minute intervals during those time frames was recorded and graphed as mean ± S.E.M. For comparisons across treatment groups, 1-way analyses of variance (ANOVAs) were performed for each phase of the session with treatment as the dependent variable. Post-hoc analyses were performed when appropriate to determine specific group differences.

The results demonstrate that although there was no statistically significant dose of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide in reducing time spent licking in phase I, nevertheless, 10 mg/kg (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide was significant in reducing the time spent licking the paw in phase II of the formalin test ($P<0.05$). The positive control morphine (5 mg/kg; s.c.) was efficacious in both phases of the test. These data indicate that (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide has analgesic potential with respect to chemically-induced inflammatory / persistent pain.

Subsequent analysis of the original videotapes wherein each animal was scored across the entire time period for phase I (0-5 min after formalin) and phase II (20-40 min) revealed a similar trend for the data, but failed to achieve statistical significance for the effect of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide on the reduction of time spent licking the affected paw in either phase I or phase II.

Reference is made to: Coderre TJ, Vaccarino AL, Melzack R (1990), Central nervous system plasticity in the tonic pain response to subcutaneous formalin injection, *Brain Res.* 535:155-158; Dubuisson D and Dennis SG (1977), The formalin test: A quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats, *Pain* 4: 161-174; Malmberg AB and Bannon AW (2002), Unit 8.9: Models of nociception: hot-plate, tail-flick, and formalin tests in rodents, *Current Protocols in Neuroscience*; and Tjølsen A and Hole K (1997), Animal models of analgesia, In: *Handbook of Experimental Pharmacology Volume 130: The Pharmacology of Pain* (Eds. A. Dickenson and J.-M. Besson), Springer Verlag, New York pp. 1-20.

Complete Freund's Adjuvant (CFA)-Induced Thermal Hyperalgesia

Injection of complete Freund's adjuvant (CFA) in rats is commonly used to evaluate compounds with potential for use as drugs in treatment of mono-arthritis (osteo-arthritis) and other inflammatory conditions. Signs of hyperalgesia develop within 24h (Schaible and Grubb, 1993).

(2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide was evaluated for possible analgesia effect in the CFA – induced thermal hyperalgesia test in rats using methods similar to those described by Walker and colleagues (2003). Briefly, adult male Sprague-Dawley rats (BioLasco, Taiwan) weighing 180±20 g on receipt were randomly assigned to treatment groups of n=8 per group. Animals each received a sub plantar injection (0.1 mL) of CFA (DIFCO, 264010; 0.1% solution) to the right hind paw at 24h prior to experimental testing. Thermal hyperalgesia was tested using a Paw / Tail stimulator analgesia meter (IITC Model-336G, IITC, USA) with a thermally regulated glass floor set at 30 °C. A subject was placed within a plastic box atop an elevated glass floor and a light beam located under the glass floor was directed at the plantar surface of the right hind paw. The time required for the animal to withdraw the paw from the thermal stimulus was automatically recorded. The intensity of the light was adjusted to evoke an average group baseline latency from 12-14 seconds (pre-CFA) and a cut-off latency of 20 seconds was imposed. The latency for paw withdrawal was obtained for each rat and defined as the heat pain threshold.

Twenty-four hours after CFA injection, subjects were pre-selected (for clear presence of thermal hyperalgesia) for experimentation only if the latency to withdrawal was less than 75% of the baseline. Test substance, morphine and vehicle were administered by subcutaneous (s.c.) injection at time 0. The post-treatment level of thermal hyperalgesia was then measured at 60 minutes post-treatment. One-way ANOVA followed by Dunnett's test was applied for comparison between test substance treated groups and vehicle control group. Activity was considered significant at the $P<0.05$ level.

Overall, subcutaneous (s.c.) administration of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide at 0.1, 1 or 10 mg/kg was not associated with any significant analgesic effect at 1 hour post-dose on CFA-induced thermal hyperalgesia in rats compared with the vehicle (0.9% saline) control group. In contrast, the concurrently run reference standard morphine (3 mg/kg s.c.) produced significant analgesic activity at 1 hour after dosing. See Figure 13. Reference is made to: Schaible H-G and Grubb BD (1993), Afferent and spinal mechanisms of joint pain, *Pain* 55: 5-54; and Walker KM, Urban L, Medhurst SJ, Patel S, Panesar M, Fox AJ and Mcintyre P (2003), The VR1 antagonist capsazepine reverses mechanical hyperalgesia in models of inflammatory and neuropathic pain, *JPET* 304: 56-62.

Streptozotocin (STZ) – Induced Diabetic Neuropathy (As evidenced by allodynia)

Peripheral neuropathy, a major complication of diabetes, often results in spontaneous pain or the perception of pain from contact with a normally non-noxious stimulus. Such neuropathic pain is experienced by 20-24% of diabetic patients, or approximately 30 million

people worldwide (Schmader, 2002). The streptozotocin (STZ) – induced diabetes model in rats provides a means to evaluate the efficacy of test compounds that offer therapeutic potential for peripheral neuropathy and to understand their putative mechanism of action. In this model, a single injection of STZ, an antibiotic that mimics clinical diabetes by causing irreversible damage to the pancreatic β and α-cells leads to chronic hyperglycemia, nerve dysfunction and pain sensitivity. The current study utilizes the STZ rat model of diabetic neuropathy to investigate the effects of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide on mechanical allodynia, an assessment of pain.

Diabetes was induced by a 0.5 ml injection of streptozotocin (60 mg/kg) dissolved in citrate buffer (pH=6) into the tail vein of each rat. The development of diabetes was confirmed by measuring the blood glucose levels (BGL) of all animals on study day 3 (BGL> 300 mg/dL). BGL was measured again on study day 14 and only the animals that showed tactile allodynia were tested again for their BGL on study day 21. BGL was measured on study day 16 for animals that did not show tactile allodynia on study day 14. These animals were tested again for their BGL at study day 23.

(2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide (0.1, 1 or 10 mg/kg p.o.) was administered as a solution of the hydrochloride salt in water once daily starting on study day 14 or study day 16 and continuing through study day 21 or 23, respectively. The control article gabapentin (in 0.9% saline, 150 mg/kg i.p.) was only administered on allodynia test days. Test item, vehicle or control article administration was based on evaluation of allodynia on study day 14. If allodynia was not present on study day 14, the animal was evaluated again at study day 16. Pain response was measured either on study days 14 and 21 or 16 and 23, 30 minutes after Test Item administration.

For the allodynia assessments, Von Frey filaments were used according to the methods of Chaplan and colleagues (1994). Briefly, the rats were placed in an enclosure and positioned on a metal mesh surface, but allowed to move freely. The rats' cabins were covered with red cellophane to diminish environmental distributions. The test began after cessation of exploratory behavior.

Rodents exhibit a paw withdrawal reflex when its paw is unexpectedly touched. When the tip of a Von Frey fiber of given length and diameter was pressed against the skin at right angles, the force of application increases as long as the researcher continued to advance the probe until the fiber bent. After the fiber bent, the probe was advanced, causing the fiber to bend more, but without additional force being applied. The animal would indicate sensation by pulling back its paw. In the absence of a paw withdrawal response to the initially selected filament, a stronger stimulus was presented; in the event of paw withdrawal,

the next weaker stimulus was chosen. In this fashion, the resulting pattern of positive and negative responses was used to determine the paw withdrawal threshold.

The set of Von Frey monofilaments provide an approximate logarithmic scale of actual force and a linear scale of perceived intensity. Below is a table showing the force (g) and its corresponding size of monofilaments.

Size	1.65	2.36	2.44	2.83	3.22	3.61	3.84	4.08	4.17	4.31	4.56	4.74	4.93	5.07	5.18	5.46	5.88	6.10	6.45	6.65
Force (g)	0.008	0.02	0.04	0.07	0.16	0.40	0.60	1.00	1.40	2.00	4.00	6.00	8.00	10	15	26	60	100	180	300

All normally distributed data are presented as means±SEM, as well as the animals' individual values followed by a student T-test (Software: Microsoft® Excel). A p value < 0.05 is considered to represent a significant difference. Due to the non-normal distribution of the allodynia data, descriptions of those data are provided as both mean (±SEM) and median values in order to represent their imprecise nature and skewed distribution.

The Von Frey data are presented as the minimum force (g) needed to withdraw each hind leg. A decrease in pain threshold was recorded 14/16 days post STZ injection. This decrease was expressed as an increase in the animal's sensitivity to the Von Frey filaments. The average and group median withdrawal force of the vehicle treated animals at baseline before STZ injection was 57.57 ± 2.43 (group median=60g). On study days 14/16, the median paw withdrawal force was significantly lower (20.5 - 22.14 ± 2.36 g; <0.01 vs. baseline; median = 20.5g) indicating tactile allodynia prior to (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide treatment. At study termination (study days 21/23), tactile allodynia was still observed post treatment (20.46 ± 3.31 g; p<0.01 vs. baseline; median=8g).

Overall, Treatment with 1 mg/kg (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide inhibited allodynia 30 minutes after its administration on study days 14/16 as compared to pretreatment (p<0.01) or to the Vehicle control (p<0.05). Treatment with (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide at a dose of 10 mg/kg inhibited allodynia 30 minutes after its administration on study days 14/16 as compared to pretreatment (p=0.012). Treatment with (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide at a dose of 1 mg/kg inhibited allodynia 30 minutes after their administration on study days 21/23 as compared to pretreatment (p=0.012). Treatment with (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide at a dose of 10 mg/kg (Group 11M) inhibited allodynia 30 minutes after its administration on study days 21/23 as compared to the Vehicle control (p<0.05). Treatment with the positive control, gabapentin,

reversed the tactile allodynia significantly in all treatment days as compared to pretreatment (study days 14/16 and 21/23; $p<0.01$) or as compared to the Vehicle control (study days 21/23; $p<0.01$). At study termination (study day 21/23), insulin levels in the serum were analyzed. No significant differences in insulin levels were observed. (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide at all doses was administered every day starting on study day 14 or 16 through study day 21 or 23, respectively. The pain test was performed prior to Test Article injection (pre-TI injection) and 30 minutes after Test Article administration (post-TI injection). The positive control, gabapentin, was administered 2 hours before pain testing on study days 14 or 16 and 21 or 23. Treatment with the positive control, gabapentin, reversed the tactile allodynia significantly in all treatment days as compared to pretreatment and to the vehicle: 22.77±3.77 g (median=15 g) vs. 45.62±4.24 g (median=60 g) in pre and post treatment, respectively, on study days 14/16, $p<0.01$; 28.23±4.91 g (median=20.5 g) vs. 50.88±4.12 g (median=60 g) in pre and post treatment, respectively, on study days 21/23, $p<0.01$; 45.62±4.24 g (median=60 g) vs. 26.61±4.41 g (median=15 g) in the vehicle group on study days 14/16, $p<0.01$; 50.88±4.12 g (median=60 g) vs. 20.46±3.31 g (median=10 g) in the vehicle group on study days 21/23, $p<0.01$.

Immediately after the Von Frey testing on the termination days, blood was collected. At the end of the study, the animals were euthanized with ketamine /xylazine solution (IP). Approximately 0.5-0.7 ml of blood was collected via cardiac puncture in tubes containing the anti-coagulant (K3 EDTA). The blood samples were kept chilled on ice and centrifuged within 30 minutes of collection. To obtain plasma, blood was centrifuged for 10 minutes at 3000 rpm. Plasma was transferred into labeled tubes and stored upright and frozen at approximately -20 °C until shipment. Each sample was labeled with the compound number and animal number.

All animals gained weight during the study. There were no significant differences in body weight gain between the groups.

The mean blood glucose levels increased in all animals. Baseline was 108.86±1.03 mg/dl and increased to 390.99±6.47 mg/dl on study day 3. No statistical differences were found between groups. High glucose levels were also measured on study days 14/16 and 21/23 based on the results for allodynia at study day 14. At the end of the study (study day 21 and 23) the mean blood glucose level was 403.86±8.45 mg/dl.

At study termination, insulin levels in serum were analyzed. The insulin level in the Vehicle control at study termination was 0.79±0.41 µg/l. No significant differences in insulin levels between treatments were observed.

The results of Von Frey assessment indicate that (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide is effective in reducing diabetic neuropathy pain at doses of 1 mg/kg and 10mg/kg compared to the Vehicle treated group. See Figure 14.

Reference is made to: Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL (1994), Quantitative assessment of tactile allodynia in the rat paw, *J. Neurosci. Methods* 53: 55-63; Schumader KE (2002), Epidemiology and impact on quality of life of postherpetic neuralgia and painful diabetic neuropathy, *Clinical Journal of Pain* 18: 350-354; and Sommer C (2003). Painful neuropathies, *Curr. Opin. Neurol.* 16: 623-628.

Murine Model of Type 2 Diabetes Mellitus

The *db/db* mouse, a well established model of type 2 diabetes mellitus, is a leptin-deficient mutant that expresses an obese phenotype and also commonly expresses metabolic symptoms including hyperglycemia, hyperlipidemia and hyperinsulinemia (Halaas et al., 1995 and Lee et al., 1996). This experimental animal model of diabetes was employed in a study designed to determine the effects of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide on body mass and several additional metabolic parameters.

In this study, (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide was repeatedly administered orally (via gavage) at 1.0 mg/kg once daily starting from the age of approximately 3 weeks and continuing throughout the 7-week study. Nondiabetic heterozygote littermate mice (*db/+*; designated "Db") were used as controls. Body weight and food intake were determined twice weekly. The α7 antagonist methyllycaconitine (MLA) was also given concurrently via gavage at 3 mg/kg daily to selected cohorts of *db/db* (designated "db") or *db/+* mice. At the end of the 7-week dosing regimen, total growth rates (overall body weight gain) and average daily food intake were calculated. In addition, glucose levels were assessed in mice fasted overnight. Furthermore, blood sample analytes from mice fasted overnight were collected for measurements of tumor necrosis factor-a (TNF-α), triglycerides and glycosylated hemoglobin (HbA1c). All data are expressed as mean ± SEM. For each parameter investigated, differences among all groups were compared by one-way ANOVA with post-hoc Neuman-Keuls multiple comparison test.

Overall, daily administration over the course of 7 weeks of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide to obese *db/db* mice resulted in a significant decrease in all parameters measured compared with control obese *db/db* mice treated with vehicle. With respect to total body weight gain, average daily food consumption, glycosylated HbA1c levels and plasma concentration of TNF-α, co-

administration of MLA attenuated the effect. Although attenuation of plasma glucose and triglycerides was not significantly attenuated by co-administration of MLA with (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide, there was a trend toward that reversal.

As illustrated in Figure 15, At the end of seven weeks of treatment, between ages 3 and 10 weeks, total body weight gain in the vehicle control-treated obese group ("db") was significantly greater than that of lean vehicle control animals ("Db"). By comparison, weight gain was significantly lower in the (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide-treated obese ("db-Test Article") mice. Notably, animals that were co-administered MLA with (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide failed to show the reduced weight gain exhibited by the obese rats administered (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide alone.

As shown in Figure 16, The daily food intake in vehicle control obese group ("db") was significantly greater than that of lean vehicle controls ("Db"). Average food consumption was significantly lower in the TC-(2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide -treated obese mice ("db-Test Article") than in the obese controls. The food consumption of the lean mice was unaffected by (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide ("Db-Test Article"). Animals that were co-administered MLA with (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide failed to show the reduced daily average food consumption exhibited by the obese rats administered (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide alone.

As shown in Figure 17, (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide significantly inhibited fasting plasma glucose levels in obese mice ("db-Test Article"). However, this effect was not reversed by co-administration with MLA.

As shown in Figure 18, (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide significantly inhibited glycosylated HbA1c levels in obese mice ("db-Test Article"). The reduction in glycosylated HbA1c by (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide was attenuated by co-administration of MLA.

As shown in Figure 19, (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide significantly reduced the pro-inflammatory cytokine TNF alpha in obese mice ("db-Test Article"). These effects were inhibited by co-administration of the alpha7 antagonist MLA.

As shown in Figure 20, (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide resulted in significantly lower triglyceride levels in obese mice ("db-Test Article") compared with vehicle-treated controls ("db"). The reduction in triglycerides by (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide was not attenuated by co-administration of MLA.

Pulmonary, Airway Hyperresponsiveness, Penh Measurement

Using the method of Hamelmann et al, (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide was evaluated for possible inhibition of airway hyper-responsiveness in mice. Breifly, ovalbumin (OVA)-sensitized animals, 12 animals per group, were challenged by nasal inhalation with aerosolized 5% OVA for 25 min on days 21, 23, and 25. The mice were treated with vehicle or (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide subcutaneously (s.c.) twice daily from day 21 to day 26 or once daily intratracheally (i.t.), preceding ovalbumin aerosol challenge by 30 min on days 21, 23 and 25 as well as methacholine challenge or bronchoalveolar lavage fluids (BALF) harvest on day 26. Dexamethasone, the reference standard, was administered at 3 mg/kg orally (p.o.) once daily 60 min before OVA challenge on day 21, 23 and 25 and 60 min before methacholine provocation or BALF harvest on day 26. Noninvasive measurements of airway responsiveness were performed by using whole body plethysmography, in which increases in enhanced pause (Penh) serve as an index of airway obstruction. Responses to inhaled methacholine were measured and calculated as percentage of respective baseline values. Unpaired Student's *t*-test was used for comparison between the vehicle control and the sham group; one-way ANOVA and Dunnett's post-hoc analyses were applied for comparison between the vehicle control and treated groups. Statistical significance is considered at $P<0.05$.

Figure 21 illustrates the effect of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide on % changes in Penh response to methacholine challenge in ovalbumin-sensitized mice. The Penh response to methacholine (10 and 30 mg/mL) was significantly augmented in OVA-sensitized animals compared to sham control. Dexamethasone at 3 mg/kg PO caused a significant inhibition of the methacholine (10 and 30 mg/mL)-induced increase in Penh values, both in absolute and % values compared to vehicle-treated OVA animals, indicating efficacy against airway hyperresponsiveness. (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide at 0.1, 1, and 10 mg/kg bid s.c. caused significant inhibition of the methacholine-induced increase in Penh values; 10 mg/kg IT was also associated with significant inhibition.

The effect of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide on white blood cell counts/differential cell counts and % white blood cell count/differential cell counts in ovalbumin sensitized mice are illustrated in Figures Y and Z, respectively. A significant increase in total WBC, neutrophils, lymphocytes, monocytes and eosinophils was noted in BALF in OVA-sensitized animals vs. sham control, which was inhibited significantly by dexamethasone. (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide at 0.1 and 1 mg/kg SC, but not at 10 mg/kg SC, significantly reduced total WBC and eosinophils in BALF; (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide at 10 mg/kg SC reduced monocytes; lymphocytes were reduced at 0.1 and 1 mg/kg SC as well as at 10 mg/kg IT.

These results demonstrate that multiple administrations of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide at 0.1, 1 and 10 mg/kg bid s.c. and at 10 mg/kg i.t. vs dexamethasone affords significant protection against airway hyper-responsiveness in OVA sensitized mouse model (as evidenced by reduced Penh response to methacholine challenge using whole body plethysmography in mice) and is associated with significant reduction in eosinophils and white blood cells in BALF (which, however, lacks a consistent dose-response relationship).

Figure 21 illustrates the effect of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide on % changes in Penh response to methacholine challenge in ovalbumin-sensitized mice. (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide and vehicle were administered subcutaneously bid or given intratracheally qd for 6 consecutive days from day 21 to day 25 at 30 min before OVA challenge and the last dosing was administrated at 30 min before MCh provocation on day 26. The Penh values were determined. One-way ANOVA followed by Dunnett's test was applied for comparison between the OVA immunized vehicle and other treatment groups. *P<0.05 vs. OVA-vehicle control.

Figure 22 illustrates the effect of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide on white blood cell counts and differential cell counts in ovalbumin sensitized mice. (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide and vehicle were administered subcutaneously bid or were given intratracheally qd for 6 consecutive days from day 21 to day 25 at 30 minutes before OVA challenge and the last dosing was administrated at 30 minutes before bronchoalveolar lavage fluid harvest on day 26. The total white blood cell count and differential cell counts were determined. One-way ANOVA followed by Dunnett's test was applied for comparison between the OVA immunized vehicle and other treatment groups. *P<0.05 vs. OVA-vehicle control.

Figure 23 illustrates the effect of (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide on % white blood cell count and differential cell counts in ovalbumin sensitized mice. (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide and vehicle were administered subcutaneously bid or were given intratracheally qd for 6 consecutive days from day 21 to day 25 at 30 minutes before OVA challenge and the last dosing was administrated at 30 minutes before bronchoalveolar lavage fluid harvest on day 26. The total white blood cell count and differential cell counts were determined. One-way ANOVA followed by Dunnett's test was applied for comparison between the OVA immunized vehicle and other treatment groups. *P<0.05 vs. OVA-vehicle control.

Reference is made to: Hamelmann E, Schwarze J, Takeda K, Oshiba A, Larsen GL, Irvin CG, and Gelfand EW, Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography, *Am J Respir Crit Care Med*, 156:766–775,1997.

Test compounds for the experiments described herein were employed in free or salt form. Unless otherwise specified, the compound provided for in vivo testing was (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide hydrochloride, with dosage amounts given assuming the free base form.

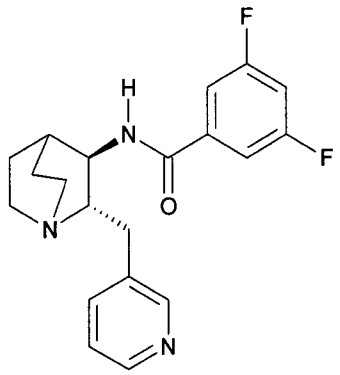
The specific pharmacological responses observed may vary according to and depending on the particular active compound selected or whether there are present pharmaceutical carriers, as well as the type of formulation and mode of administration employed, and such expected variations or differences in the results are contemplated in accordance with practice of the present invention.

Although specific embodiments of the present invention are herein illustrated and described in detail, the invention is not limited thereto. The above detailed descriptions are provided as exemplary of the present invention and should not be construed as constituting any limitation of the invention. Modifications will be obvious to those skilled in the art, and all modifications that do not depart from the spirit of the invention are intended to be included with the scope of the appended claims.

Claims

That which is claimed is:

1. A compound (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide (Formula I) or a pharmaceutically acceptable salt thereof.

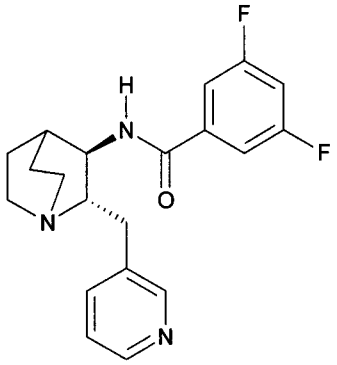


Formula I

2. The compound of claim 1, which is substantially free of one or more of (2R,3S)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide, (2R,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide, and (2S,3S)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide.
3. The compound of claim 1 or 2, as an acid addition salt, wherein the acid is selected from hydrochloric acid, methanesulfonic acid, maleic acid, phosphoric acid, 1-hydroxy-2-naphthoic acid, malonic acid, L-tartaric acid, fumaric acid, citric acid, L-malic acid, R-mandelic acid, S-mandelic acid, succinic acid, 4-acetamidobenzoic acid, adipic acid, galactaric acid, di-p-toluoyl-D-tartaric acid, oxalic acid, D-glucuronic acid, 4-hydroxybenzoic acid, 4-methoxybenzoic acid, (1S)-(+)-10-camphorsulfonic acid, (1R,3S)-(+)-camphoric acid, and p-toluenesulfonic acid, or a hydrate or solvate thereof.
4. The compound of claim 3, wherein the molar ratio of acid to (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide is 1:2 or 1:1.
5. A compound selected from:
(2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide mono-hydrochloride or a hydrate or solvate thereof;
(2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide mono-phosphate or a hydrate or solvate thereof;
(2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide mono-4-hydroxybenzoate or a hydrate or solvate thereof; and
(2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide

hemi-4-hydroxybenzoate or a hydrate or solvate thereof.

6. A compound, (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide or a pharmaceutically acceptable salt thereof containing less than 25% (2R,3R)-, (2S,3S)-, or (2R,3S)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide, either individually or in combination, by weight.
7. The compound of claim 6, containing less than 15% (2R,3R)-, (2S,3S)-, or (2R,3S)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide, either individually or in combination, by weight.
8. The compound of claim 6, containing less than 5% (2R,3R)-, (2S,3S)-, or (2R,3S)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide, either individually or in combination, by weight.
9. The compound of claim 6, containing less than 2% (2R,3R)-, (2S,3S)-, or (2R,3S)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide, either individually or in combination, by weight.
10. The compound of claim 6, containing less than 1% (2R,3R)-, (2S,3S)-, or (2R,3S)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide, either individually or in combination, by weight.
11. A compound (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide (Formula I) or a pharmaceutically acceptable salt thereof



Formula I

which is substantially crystalline.

12. A polymorphic form of a compound (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide hydrochloride characterized by an x-ray diffraction pattern comprising one or more peaks within ± 0.5 degrees 2θ of the following peaks:

2θ
8.4
8.8
11.9
13.2
15.2
16.0
17.6
18.4
18.9
19.9
20.1
21.3
23.1
25.4
26.2

13. A polymorphic form of a compound (2S,3R)-N-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)-3,5-difluorobenzamide hydrochloride characterized by an x-ray powder diffraction pattern that substantially corresponds to Figure 9.

14. Use of a compound of any one of claims 1-13, in the manufacture of a medicament for the treatment or prevention of an α7-mediated disease or dysfunction.

15. A method for treating or preventing an α7-mediated disease or dysfunction, comprising administering a therapeutically effective amount of a compound of any one of claims 1-13.

16. A compound as claimed in any one of claims 1-13, for use in treating or preventing an α7-mediated disease or dysfunction.

17. Use of a compound of any one of claims 1-13, in the manufacture of a medicament for the treatment or prevention of a disease or dysfunction selected from the group consisting of:

i) pain, including one or more of acute, neurologic, inflammatory, neuropathic, chronic pain, severe chronic pain, post-operative pain, pain associated with cancer, angina, renal or biliary colic, menstruation, migraine, gout, arthritis, rheumatoid disease, teno-synovitis, vasculitis, trigeminal or herpetic neuralgia, diabetic neuropathy pain, causalgia, low back pain, deafferentation syndromes, and brachial plexus avulsion;

ii) metabolic syndrome, weight gain, type I diabetes mellitus, type II diabetes mellitus, or diabetic neuropathy;

iii) inflammation, including one or more of psoriasis, asthma, atherosclerosis, idiopathic pulmonary fibrosis, chronic and acute inflammation, psoriasis, endotoxemia, gout, acute pseudogout, acute gouty arthritis, arthritis, rheumatoid arthritis, osteoarthritis, allograft rejection, chronic transplant rejection, asthma, atherosclerosis, mononuclear-phagocyte dependent lung injury, atopic dermatitis, chronic obstructive pulmonary disease, adult respiratory distress syndrome, acute chest syndrome in sickle cell disease, inflammatory bowel disease, Crohn's disease, ulcerative colitis, acute cholangitis, aphteous stomatitis, pouchitis, glomerulonephritis, lupus nephritis, thrombosis, and graft vs. host reaction; and

iv) cognition, including one or more of age-associated memory impairment, mild cognitive impairment, pre-senile dementia, early onset Alzheimer's disease, senile dementia, dementia of the Alzheimer's type, mild to moderate dementia of the Alzheimer's type, Lewy body dementia, vascular dementia, Alzheimer's disease, stroke, AIDS dementia complex, attention deficit disorder, attention deficit hyperactivity disorder, dyslexia, schizophrenia, schizophreniform disorder, schizoaffective disorder, cognitive deficits in schizophrenia, and cognitive dysfunction in schizophrenia.

18. A method for treating or preventing a disease or dysfunction, comprising administering a therapeutically effective amount of a compound of any one of claims 1-13 wherein the disease or dysfunction selected from the group consisting of:

i) pain, including one or more of acute, neurologic, inflammatory, neuropathic, chronic pain, severe chronic pain, post-operative pain, pain associated with cancer, angina, renal or biliary colic, menstruation, migraine, gout, arthritis, rheumatoid disease, teno-synovitis, vasculitis, trigeminal or herpetic neuralgia, diabetic neuropathy pain, causalgia, low back pain, deafferentation syndromes, and brachial plexus avulsion;

ii) metabolic syndrome, weight gain, type I diabetes mellitus, type II diabetes mellitus, or diabetic neuropathy;

iii) inflammation, including one or more of psoriasis, asthma, atherosclerosis, idiopathic pulmonary fibrosis, chronic and acute inflammation, psoriasis, endotoxemia, gout, acute pseudogout, acute gouty arthritis, arthritis, rheumatoid arthritis, osteoarthritis, allograft rejection, chronic transplant rejection, asthma, atherosclerosis, mononuclear-phagocyte dependent lung

injury, atopic dermatitis, chronic obstructive pulmonary disease, adult respiratory distress syndrome, acute chest syndrome in sickle cell disease, inflammatory bowel disease, Crohn's disease, ulcerative colitis, acute cholangitis, aphteous stomatitis, pouchitis, glomerulonephritis, lupus nephritis, thrombosis, and graft vs. host reaction; and

iv) cognition, including one or more of age-associated memory impairment, mild cognitive impairment, pre-senile dementia, early onset Alzheimer's disease, senile dementia, dementia of the Alzheimer's type, mild to moderate dementia of the Alzheimer's type, Lewy body dementia, vascular dementia, Alzheimer's disease, stroke, AIDS dementia complex, attention deficit disorder, attention deficit hyperactivity disorder, dyslexia, schizophrenia, schizophreniform disorder, schizoaffective disorder, cognitive deficits in schizophrenia, and cognitive dysfunction in schizophrenia.

19. A compound as claimed in any one of claims 1-13, for use in treating or preventing a disease or dysfunction wherein the disease or dysfunction selected from the group consisting of:

i) pain, including one or more of acute, neurologic, inflammatory, neuropathic, chronic pain, severe chronic pain, post-operative pain, pain associated with cancer, angina, renal or biliary colic, menstruation, migraine, gout, arthritis, rheumatoid disease, teno-synovitis, vasculitis, trigeminal or herpetic neuralgia, diabetic neuropathy pain, causalgia, low back pain, deafferentation syndromes, and brachial plexus avulsion;

ii) metabolic syndrome, weight gain, type I diabetes mellitus, type II diabetes mellitus, or diabetic neuropathy;

iii) inflammation, including one or more of psoriasis, asthma, atherosclerosis, idiopathic pulmonary fibrosis, chronic and acute inflammation, psoriasis, endotoxemia, gout, acute pseudogout, acute gouty arthritis, arthritis, rheumatoid arthritis, osteoarthritis, allograft rejection, chronic transplant rejection, asthma, atherosclerosis, mononuclear-phagocyte dependent lung injury, atopic dermatitis, chronic obstructive pulmonary disease, adult respiratory distress syndrome, acute chest syndrome in sickle cell disease, inflammatory bowel disease, Crohn's disease, ulcerative colitis, acute cholangitis, aphteous stomatitis, pouchitis, glomerulonephritis, lupus nephritis, thrombosis, and graft vs. host reaction; and

iv) cognition, including one or more of age-associated memory impairment, mild cognitive impairment, pre-senile dementia, early onset Alzheimer's disease,

senile dementia, dementia of the Alzheimer's type, mild to moderate dementia of the Alzheimer's type, Lewy body dementia, vascular dementia, Alzheimer's disease, stroke, AIDS dementia complex, attention deficit disorder, attention deficit hyperactivity disorder, dyslexia, schizophrenia, schizophreniform disorder, schizoaffective disorder, cognitive deficits in schizophrenia, and cognitive dysfunction in schizophrenia.

20. A pharmaceutical composition comprising a compound of any one of claims 1-13 and one or more pharmaceutically acceptable carrier.

21. A method of enhancing acetylcholine-induced current comprising administering an effective amount of a compound of any one of claims 1-13.