Title: USE OF N-DESMETHYLCLOzapINE TO TREAT PSYCHOSIS

Abstract: Disclosed herein are methods to treat neuropsychiatric diseases including psychosis. Treatment is carried out by administering a therapeutically effective amount of N-desmethyliclozapine to a patient suffering from a neuropsychiatric disease.
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.
USE OF N-DESMETHYLCLOZAPINE TO TREAT PSYCHOSIS

Related Application

[0001] This application claims the benefit of U.S. Provisional Application No. 60/817,010, filed June 27, 2006 and entitled "USE OF N-DESMETHYLCLOZAPINE TO TREAT HUMAN NEUROPSYCHIATRIC DISEASE," which is incorporated herein by reference in its entirety.

Field of the Invention

[0002] The present invention relates to administering N-desmethylclozapine for the treatment of pain, glaucoma, dementia, affective disease, and psychosis.

Background of the Invention

[0003] The physiological actions of the hormone/neurotransmitter acetylcholine are mediated, in part, by muscarinic acetylcholine receptors. Muscarinic receptors comprise a family of five (M1-M5) transmembrane proteins that mediate slow, modulatory signalling in cells and tissues expressing these genes. Muscarinic receptors are the targets of a number of therapeutically useful agents (1, 2). Peripherally, muscarinic receptors mediate the actions of acetylcholine in the parasympathetic nervous system. Peripherally acting muscarinic receptor agonists are therapeutically useful in lowering intra-ocular pressure in patients with glaucoma (3). Compounds that potentiate the central actions of acetylcholine as well as centrally acting muscarinic receptor agonists have both demonstrated clinical utility in the treatment of a number of neuropsychiatric diseases (1, 2, 4-7).

[0004] The actions of acetylcholine are terminated by degradation of the molecule by acetylcholinesterase enzymes. Inhibition of these enzymes within the central nervous system leads to increased concentrations of acetylcholine at muscarinic receptors. A number of acetylcholinesterase inhibitors have been developed and are in routine clinical use as cognitive enhancing agents in dementia (4).
[0005] A number of centrally acting muscarinic agonist have been the subject of clinical testing. One of these, Xanomeline, has been shown to possess efficacy in controlling psychosis and related behavioral disturbances observed in Alzheimer’s Disease patients (5). Further, it has recently been demonstrated that xanomeline is efficacious in treating schizophrenia (6). Interestingly, it displayed efficacy against both positive and negative symptoms, and did not induce adverse motoric effects in initial clinical studies in schizophrenics. These data suggest that compounds with muscarinic receptor agonist properties are likely to be efficacious in treating the behavioral disturbances common to neurodegenerative disease such as Alzheimer’s Disease and as antipsychotics to treat human psychoses, but only if they are tolerated in these patient populations. Additionally, muscarinic receptor agonists have shown activity in pre-clinical models of neuropathic pain states (7).

Summary of the Invention

[0006] One embodiment disclosed herein includes a method of treating psychosis of any origin, comprising orally administering to a subject about 100 mg of N-desmethylclozapine twice daily.

[0007] Another embodiment disclosed herein includes a method of treating psychosis of any origin, comprising orally administering to a subject about 200 mg of N-desmethylclozapine twice daily.

[0008] In one embodiment, the N-desmethylclozapine is administered via capsule or tablets, each comprising about 100 mg of N-desmethylclozapine.

[0009] Another embodiment disclosed herein includes a method of treating psychosis of any origin, including orally administering to a subject about 100 mg of N-desmethylclozapine per day and subsequently orally administering to a subject about 200 mg of N-desmethylclozapine per day. In one embodiment, administering the about 100 mg of N-desmethylclozapine comprises administering about 50 mg of N-desmethylclozapine twice daily to the subject. In one embodiment, administering the about 100 mg of N-desmethylclozapine comprises administering a tablet or capsule comprising about 50 mg of N-desmethylclozapine twice daily to the subject. In one embodiment, administering the
about 200 mg of N-desmethylclozapine comprises administering about 100 mg of N-desmethylclozapine twice daily to the subject. In one embodiment, administering the about 200 mg of N-desmethylclozapine comprises administering a tablet or capsule comprising about 100 mg of N-desmethylclozapine twice daily to the subject.

[0010] Another embodiment disclosed herein includes a method of treating psychosis of any origin, including orally administering to a subject about 100 mg of N-desmethylclozapine per day, subsequently orally administering to a subject about 200 mg of N-desmethylclozapine per day, subsequently orally administering to a subject about 300 mg of N-desmethylclozapine per day, and subsequently orally administering to a subject about 400 mg of N-desmethylclozapine per day. In one embodiment, administering the about 100 mg of N-desmethylclozapine comprises administering about 50 mg of N-desmethylclozapine twice daily to the subject. In one embodiment, administering the about 100 mg of N-desmethylclozapine comprises administering a tablet or capsule comprising about 50 mg of N-desmethylclozapine twice daily to the subject. In one embodiment, administering the about 200 mg of N-desmethylclozapine comprises administering about 100 mg of N-desmethylclozapine twice daily to the subject. In one embodiment, administering the about 200 mg of N-desmethylclozapine comprises administering a tablet or capsule comprising about 100 mg of N-desmethylclozapine twice daily to the subject. In one embodiment, administering the about 300 mg of N-desmethylclozapine comprises administering about 150 mg of N-desmethylclozapine twice daily to the subject. In one embodiment, administering the about 300 mg of N-desmethylclozapine comprises administering a first tablet or capsule comprising about 100 mg of N-desmethylclozapine and second tablet or capsule comprising about 50 mg of N-desmethylclozapine twice daily to the subject. In one embodiment, administering the about 400 mg of N-desmethylclozapine comprises administering about 200 mg of N-desmethylclozapine twice daily to the subject. In one embodiment, administering the about 400 mg of N-desmethylclozapine comprises administering a first tablet or capsule comprising about 100 mg of N-desmethylclozapine and second tablet or capsule comprising about 100 mg of N-desmethylclozapine twice daily to the subject.
Another embodiment disclosed herein includes a kit comprising a tablet or capsule comprising about 100 mg of N-desmethyclozapine and instructions for administering the tablet or capsule. In some embodiments, the instructions indicate to administer the tablet or capsule twice daily. In some embodiments, the instructions indicate to administer two of the tablets or capsules twice daily. In some embodiments, the instructions indicate to administer the tablet or capsule twice daily for a first period of time and then subsequently administer two of the tablets or capsules twice daily. In some embodiments, the instructions indicate to administer the tablet or capsule once daily for a first period of time and the subsequently administer the tablet or capsule twice daily.

Brief Description of the Drawings

[0012] Figure 1 is a graph showing the results of agonist activity of N-desmethyclozapine at M1 muscarinic acetylcholine receptors in R-SAT Assays.

[0013] Figure 2 is a graph showing the results of agonist activity of N-desmethyclozapine at M1 muscarinic acetylcholine receptors in Phosphatidylinositol Assay.

[0014] Figure 3 shows photographs of MAP kinase activation in rat hippocampus following parenteral administration of N-desmethyclozapine.

[0015] Figure 4A shows a graph of the muscarinic M1 receptor agonist activity of a library of 462 compounds as determined by R-SAT assays. M1 receptor efficacy data shown are derived from the 1-micromolar concentration of compound, and are reported as percentage efficacy relative to the maximal response observed for a saturating 40-micromolar concentration of carbachol (100%). Figures 4B-D shows a graph of PI hydrolysis data utilizing Chinese Hamster Ovary cells stably transfected with the human M1 receptor gene. Figure 4B depicts agonist responses reported as the percentage response observed for carbachol. Drugs depicted are carbachol (squares), clozapine (triangles), and N-desmethyclozapine (circles), with observed potencies (pEC_{50}) of: carbachol (5.7), N-desmethyclozapine (6.7), and clozapine (no response). Figure 4C depicts competitive antagonist responses obtained in the presence of a 3-micromolar concentration of carbachol, and are reported as the percentage response observed for atropine (100%). Drugs depicted are atropine (squares), clozapine (triangles), and N-desmethyclozapine (circles), with
observed potencies (pKi) of: atropine (8.5), N-desmethylclozapine (no response), and clozapine (7.1). Figure 4D depicts competitive antagonist responses obtained in the presence of a 0.15-micromolar concentration of N-desmethylclozapine, and are reported as the percentage response observed for atropine (100%). Drugs depicted are atropine (squares), and clozapine (triangles), with observed potencies (pKi) of: atropine (8.4), and clozapine (7.6).

[0016] Figure 5 shows M1 muscarinic receptor agonist activity of N-desmethylclozapine in mouse hippocampus. Phospho-MAPK immunoreactivity in the cell bodies and proximal dendrites of CA1 pyramidal cells (highlighted by arrows) is shown following the administration of vehicle (A), clozapine at 30 mg/kg (B), N-desmethylclozapine at 10 (C), 30 (D), 100 (E), or N-desmethylclozapine (30mg/kg) and scopolamine (0.3 mg/kg, i.p.)(F).

[0017] Figure 6 shows the quantification of M1 muscarinic receptor agonist activity of N-desmethylclozapine in mouse hippocampus. Quantification of phospho-MAPK immunoreactivity was performed via computer calculated optical density measurements of the CA1 region of the hippocampus from four mice, where (*) indicates a significant difference to vehicle treatment using a one factor ANOVA post-hoc Dunnett's test (F (5,23) =10.88; P<0.0001).

[0018] Figure 7 shows the results of an R-SAT assay with a combination of 150 nM NDMC and varying concentrations of clozapine.

[0019] Figure 8 shows the results of a PI hydrolysis assay with a combination of 150 nM NDMC and varying concentrations of clozapine.

**Detailed Description of the Preferred Embodiment**

**Definitions**

[0020] N-desmethylclozapine, 8- chloro -11- (1-piperazinyl) -5H- dibenzo [b,e] [1,4] diazepine, also known as NDMC, is defined as the compound having the molecular structure depicted in Formula (I):
[0021] An "agonist" is defined as a compound that increases the basal activity of a receptor (i.e. signal transduction mediated by the receptor).

[0022] An "antagonist" is defined as a compound that competes with an agonist or inverse agonist for binding to a receptor, thereby blocking the action of an agonist or inverse agonist on the receptor. However, an antagonist (also known as a "neutral" antagonist) has no effect on constitutive receptor activity.

[0023] A partial agonist is defined as an agonist that displays limited, or less than complete, activity such that it fails to activate a receptor in vitro, functioning as an antagonist in vivo.

[0024] The term "subject" refers to an animal, preferably a mammal, and most preferably a human, who is the object of treatment, observation or experiment.

[0025] The term "therapeutically effective amount" is used to indicate an amount of an active compound, or pharmaceutical agent, that elicits the biological or medicinal response indicated. This response may occur in a tissue, system, animal or human that is being sought by a researcher, veterinarian, medical doctor or other clinician, and includes alleviation of the symptoms of the disease being treated.

[0026] In certain embodiments, the method disclosed herein includes administering a therapeutically effective amount of NDMC to a subject for the purpose of treating psychosis of any origin.

[0027] In certain embodiments, the above method for treating psychosis comprises identifying a subject suffering from one or more symptoms of psychosis; and
contacting the subject with a therapeutically effective amount of N-desmethylclozapine; whereby the one or more symptoms of psychosis are ameliorated.

[0028] In some embodiments, the symptom is cognitive impairment associated with psychosis. In other embodiments, the subject suffering from psychosis exhibits more than one symptom of psychosis. In certain embodiments, one of the symptoms is cognitive impairment while another symptoms is one or more of hallucinations, delusions, disordered thought, behavioral disturbance, aggression, suicidality, mania, anhedonia, or flattening of affect.

[0029] In a further embodiment, the method includes administering a therapeutically effective amount of NDMC to a subject for the purpose of treating depression or mania.

[0030] In a still further embodiment, the method includes administering a therapeutically effective amount of NDMC to a subject for the purpose of treating the psychiatric and other behavioral disturbances characteristic of dementia or cognitive impairment of any origin.

[0031] In a still further embodiment, the method includes administering a therapeutically effective amount of NDMC to a subject for the purpose of treating neuropathic pain.

[0032] Administration of clozapine to human subjects results in the formation of two major metabolites N-desmethylclozapine (NDMC) and clozapine-N-oxide (11). However, clozapine-N-oxide is a polar metabolite that is rapidly excreted and likely does not contribute to the biological activity of the parent compound. A correlation exists between the dose of clozapine administered to a subject, and the serum levels of total clozapine moieties, yet the levels of NDMC can vary widely between individual subjects (12). Generally, NDMC constitutes 40-75% of the total serum clozapine concentrations during steady state kinetics in humans (13). Conflicting data exists as to the ability of NDMC to penetrate the blood brain barrier and impart centrally mediated activity (14, 15). These observations demonstrate that NDMC has been routinely administered to human subjects, and is well tolerated. Few data exist as to the molecular properties of NDMC. NDMC has been shown
to possess antagonist activity at 5HT$_{2C}$ and D2 receptors (16), but no data on its interaction with muscarinic receptors has been reported.

[0033] Surprisingly, and unlike the closely related compound clozapine, it has been found that the compound N-desmethyloclozapine (NDMC) possesses heretofore unappreciated functional activity as a muscarinic receptor agonist. Ex vivo experiments have demonstrated that NDMC crosses the blood brain barrier and acts as an agonist at central muscarinic receptors in rats. These observations have practical applications that support the use of NDMC as an antipsychotic, antimania agent, antidementia agent, and as a therapeuptic agent to treat glaucoma or neuropathic pain. Thus, in one aspect, disclosed herein is a method of agonizing the activity of a muscarinic receptor comprising contacting the receptor with an effective amount of NDMC. In another aspect, disclosed herein is a method of treating a subject suffering from a muscarinic receptor related disorder comprising indentifying a subject in need thereof and administering to the subject a therapeutically effective amount of NDMC.

[0034] By “muscarinic related disorder,” it is meant a disorder whose symptoms are ameliorated by agonizing a muscarinic receptor.

[0035] In another aspect, disclosed herein is a method of ameliorating one or more symptoms associated with schizophrenia or psychosis of any origin in a subject, comprising administering to the subject a therapeutically effective amount of NDMC. In some embodiments, the method comprises contacting a subject with a pharmacologically active dose of NDMC, for the purpose of controlling the positive (hallucinations and delusion) and negative (apathy, social withdrawal, anhedonia) symptoms of schizophrenia or related psychosis. In one embodiment, the NDMC administered to ameliorate one or more symptoms associated with schizophrenia or psychosis is essentially free of clozapine. By “essentially free of clozapine,” it is meant that no appreciable amount of clozapine may be detected in the blood stream of the subject at the same time that NDMC is detectable in the blood stream of the subject. In one embodiment, the amount of any clozapine administered with the NDMC is low enough such that the combined NDMC and clozapine administered result in a net agonism at muscarinic receptors. In one embodiment, some amount of
clozapine is administered but it is low enough such that the combined NDMC and clozapine administered result in a net agonism at muscarinic receptors. In one embodiment, the ratio of NDMC to clozapine is high enough to have a beneficial effect due to net agonism at muscarinic receptors. In various embodiments, the ratio of NDMC to clozapine is at least about 100:1, 50:1, 10:1, 9:1, 7:1, 5:1, or 3:1.

[0036] In another aspect, disclosed herein is a method of ameliorating one or more symptoms associated with affective disorders, including major depression, mania, bipolar disorder, and suicide, in a subject, comprising administering to the subject a therapeutically effective amount of NDMC. In some embodiments, the method comprises contacting a subject with a pharmacologically active dose of NDMC, for the purpose of controlling the symptoms observed during major depression or manic depression. In one embodiment, the NDMC administered to ameliorate one or more symptoms associated with affective disorders is essentially free of clozapine. In one embodiment, the amount of any clozapine administered with the NDMC is low enough such that the combined NDMC and clozapine administered result in a net agonism at muscarinic receptors. In one embodiment, some amount of clozapine is administered but it is low enough such that the combined NDMC and clozapine administered result in a net agonism at muscarinic receptors.

[0037] In another aspect, disclosed herein is a method of ameliorating one or more symptoms associated with Alzheimer's Disease and related neurodegenerative disorders in a subject, comprising administering to the subject a therapeutically effective amount of NDMC. In some embodiments, the method comprises contacting a subject with a pharmacologically active dose of NDMC, for the purpose of improving the cognitive deficits, and controlling the associated behavioral abnormalities, observed in degenerative dementias. In one embodiment, the NDMC administered to ameliorate one or more symptoms associated with dementia is essentially free of clozapine. In one embodiment, the amount of any clozapine administered with the NDMC is low enough such that the combined NDMC and clozapine administered result in a net agonism at muscarinic receptors. In one embodiment, some amount of clozapine is administered but it is low enough such that the combined NDMC and clozapine administered result in a net agonism at muscarinic receptors.
In another aspect, disclosed herein is a method of ameliorate one or more symptoms associated with neuropathic pain in a subject, comprising identifying a subject in need thereof and administering to the subject a therapeutically effective amount of NDMC. In some embodiments, the method comprises contacting a subject with a pharmacologically active dose of NDMC, for the purpose of controlling the dysthethetic, hyperalgesic, and other altered nociceptive symptoms observed in neuropathic pain states regardless of their etiology. In one embodiment, the NDMC administered to ameliorate one or more symptoms associated with neuropathic pain is essentially free of clozapine. In one embodiment, the amount of any clozapine administered with the NDMC is low enough such that the combined NDMC and clozapine administered result in a net agonism at muscarinic receptors. In one embodiment, some amount of clozapine is administered but it is low enough such that the combined NDMC and clozapine administered result in a net agonism at muscarinic receptors.

In another aspect, disclosed herein is a method of ameliorating one or more symptoms associated with glaucoma in a subject, comprising administering to the subject a therapeutically effective amount of NDMC. In some embodiments, the method comprises contacting a subject with a pharmacologically active dose of NDMC, for the purpose of controlling the raised intra-ocular pressure observed in glaucoma, regardless of its etiology. In one embodiment, the NDMC administered to ameliorate one or more symptoms associated with glaucoma is essentially free of clozapine. In one embodiment, the amount of any clozapine administered with the NDMC is low enough such that the combined NDMC and clozapine administered result in a net agonism at muscarinic receptors. In one embodiment, some amount of clozapine is administered but it is low enough such that the combined NDMC and clozapine administered result in a net agonism at muscarinic receptors.

Surprisingly, NDMC possesses potent agonist activity at the human muscarinic receptors. It is further disclosed herein that NDMC can cross the blood brain barrier, and function in vivo as a muscarinic receptor agonist measured via the activation of MAP kinase activity in rat hippocampus. The molecular activities of NDMC, as identified by the present methods, combined with the known clinical efficacy of compounds that possess a similar molecular pharmacological profile, indicate that NDMC can be used to alleviate or
treat disorders or conditions associated with human psychosis, affective disease, degenerative
dementia, glaucoma, and neuropathic pain.

[0041] In another aspect, disclosed herein is a method of activating an M1
muscarinic receptor comprising contacting the receptor with N-desmethyloclozapine.

[0042] In a further aspect, disclosed herein is a method of ameliorating at least
one symptom of a condition where it is beneficial to increase the level of activity of an M1
muscarinic receptor comprising administering N-desmethyloclozapine to a subject in need
thereof.

Preparation of N-desmethyloclozapine (NDMC)

[0043] NDMC is prepared as previously described (17). The dibenzo-diazepine-
lactam precursor (II) is converted to the thiolactam (III) using phosphorus pentasulfide,
followed by alkylation with e.g. dimethyl sulfate to give the imino thioether (IV). Aminolysis
of the thioether with an excess of piperazine gives the desired N-desmethyloclozapine (I).
Alternatively, the dibenzo-diazepine-lactam (II) may be converted into the imino-chloride (V)
by treatment with a halogenating agent such as phosphorus pentachloride and the product V
is converted to N-desmethyloclozapine (I) by reaction with piperazine.
[0044] NDMC may be formulated in pharmaceutical compositions comprising NDMC together with a pharmaceutically acceptable diluant or excipient. Such compositions may be formulated in an appropriate manner and in accordance with accepted practices such as those disclosed in *Remington's Pharmaceutical Sciences*, Gennaro, Ed., Mack Publishing Co., Easton PA, 1990. In some embodiments, a pharmaceutical composition comprising NDMC is provided that is essentially free of clozapine.

**Methods of Administration**

[0045] NDMC may be administered in a single daily dose, or the total daily dosage may be administered as a plurality of doses, (e.g., divided doses two, three or four times daily). Furthermore, NDMC may be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, or via topical use of ocular formulations, or using those forms of transdermal skin patches well known to persons skilled in the art.

[0046] In some embodiments, NDMC is administered orally in an amount totaling about 200 mg per day or about 400 mg per day. In some such embodiments, NDMC is Advantageously administered twice daily (e.g., about 100 mg twice daily or about 200 mg twice daily). While not being bound by any particular theory, twice daily administration is believed to attenuate fluctuations in blood levels and improve tolerability.

[0047] In some embodiments, NDMC is administered orally as a tablet or capsule comprising about 100 mg of N-desmethylclozapine. Thus, for example, about 100 mg capsules may be administered twice daily to achieve an about 200 mg per day dose. To achieve an about 400 mg per day dose, two about 100 mg capsules may be administered twice daily (totaling 4 capsules over the day).

[0048] In some embodiments, the dose administered to a subject is titrated to the final dose. For example, in some embodiments, a final about 200 mg per day dose is achieved by first administering about 100 mg per day (e.g., about 50 mg twice daily) for a certain period of time followed by administering about 200 mg per day (e.g., about 100 mg twice daily). In some embodiments, a final about 400 mg per day dose is achieved by first
administering about 100 mg per day (e.g., about 50 mg twice daily) for a certain period of
time, followed by administering about 200 mg per day (e.g., about 100 mg twice daily) for a
certain period of time, followed by administering about 300 mg per day (e.g., about 150 mg
twice daily) for a certain period of time, followed by administering the final about 400 mg per
day (e.g., about 200 mg twice daily). Prior to each dose escalation, a physician may evaluate
the patient to determine if continued dose escalation is warranted. In some cases, the
physician may decide to extend the amount of time that a lower dose is administered prior to
escalation. In some embodiments, the physician may decide to not increase the dosage any
further, thereby choosing as a final dose a dose less than the originally planned final dose. In
any of the embodiments described above, the desired administration doses may be achieved
by administering a single capsule or tablet. Alternatively, the doses may be achieved by
administering multiple capsules or tablets simultaneously or in sequence. In some
embodiments, all doses are achieved using tablets or capsules containing 50 mg or 100 mg of
NDMC or combinations thereof.

[0049] Some embodiments include kits comprising tablets or capsules comprising
NDMC along with instructions for administering NDMC according the procedures described
above. In some embodiment, the tablets or capsules in the kit comprise 100 mg of NDMC.
In some embodiments, the instructions indicate to take the tablets or capsules twice daily. In
some embodiments, the instructions indicate to take the two of the tablets or capsules twice
daily. In some embodiments, the instructions indicate to take one capsule or tablet per day
for a first period of time and subsequently to take two capsules or tablets per day. In some
embodiments, the instructions indicate to take one of the capsules or tablets twice daily for a
first period of time and subsequently to take two capsules or tablets twice daily.

[0050] In some embodiments, NDMC is administered in combination with one or
more additional therapeutic agents. The additional therapeutic agents can include, but are not
limited to, a neuropsychiatric agent. As used herein, a “neuropsychiatric agent” refers to a
compound, or a combination of compounds, that affects the neurons in the brain either
directly or indirectly, or affects the signal transmitted to the neurons in the brain. Neuropsychiatric agents, therefore, may affect a person’s psyche, such as the person’s mood,
perception, nociception, cognition, alertness, memory, etc. In certain embodiments, the neuropsychiatric agent may be selected from the group consisting of monoamine reuptake inhibitors, selective serotonin reuptake inhibitors, norepinephrine reuptake inhibitors, dual serotonin and norepinephrine reuptake inhibitors, dopamine agonists, antipsychotic agents, inverse serotonin agonists, serotonin antagonists, serotonin 2 inverse agonists, serotonin 2 antagonists, serotonin1A agonists, antiepileptic and peripherally acting muscarinic antagonists.

[0051] In some embodiments, the antipsychotic agent may be selected from the group consisting of a phenothiazine, phenylbutylpiperadine, debenzamine, benzisoxidil, and salt of lithium. The phenothiazine group of compounds may be selected from the group consisting of chlorpromazine (Thorazine®), mesoridazine (Serentil®), prochlorperazine (Compazine®), and thioridazine (Mellaril®). The phenylbutylpiperadine group of compounds may be selected from the group consisting of haloperidol (Haldol®), and pimoziade (Ora®). The debenzamine group of compounds may be selected from the group consisting of clozapine (Clozaril®), loxapine (Loxitane®), olanzapine (Zyprexa®) and quetiapine (Seroquel®). The benzisoxidil group of compounds may be selected from the group consisting of risperidone (Resperidal®) and ziprasidone (Geodon®). The salt of lithium may be lithium carbonate. In some embodiments, the antipsychotic agent may be selected from the group consisting of Aripiprazole (Abilify), Clozapine, Clozaril, Compazine, Etrafon, Geodon, Haldol, Inapsine, Loxitane, Mellaril, Moban, Navane, Olanzapine (Zyprexa), Ora, Permitil, Prolixin, Phenergan, Quetiapine (Seroquel), Reglan, Risperdal, Serentil, Seroquel, Stelazine, Taractan, Thorazine, Triavil, Trilafon, and Zyprexa, or pharmaceutically acceptable salts thereof.

[0052] In certain embodiments, the selective serotonin reuptake inhibitor is selected from the group consisting of fluoxetine, fluvoxamine, sertraline, paroxetine, citalopram, escitalopram, sibutramine, duloxetine, and venlafaxine, and pharmaceutically acceptable salts or prodrugs thereof.

[0053] In other embodiments, the norepinephrine reuptake inhibitor is selected from the group consisting of thionisoxetine and reboxetine.
[0054] In further embodiments, the dopamine agonist is selected from the group consisting of cabergoline, amantadine, lisuride, pergolide, ropinirole, pramipexole, and bromocriptine.

[0055] In another embodiment, the inverse serotonin 2A agonist is N-(1-methylpiperidin-4-yl)-N-(4-flourophenylmethyl)-N'-(4-(2-methylpropyloxy)phenylmethyl)carbamide, MDL 100,907, SR-43694B (eplivanserin), rtianserin, ketanserin, mianserin, cinanserin, mirtazepine, cyproheptadine and cinnarizine.

[0056] In another aspect, the present disclosure is directed to a method of treating neuropsychiatric disorder in a patient comprising identifying a patient in need thereof and administering to said patient a therapeutically effective amount of a pharmaceutical composition comprising a compound of Formula (I) and a neuropsychiatric agent. In yet another aspect, the present disclosure is directed to a method of treating neuropsychiatric disorder in a patient comprising identifying a patient in need thereof and administering to said patient a therapeutically effective amount of a compound of Formula (I) and a therapeutically effective amount of a neuropsychiatric agent.

[0057] In some embodiments, NDMC and additional therapeutic agent(s) are administered nearly simultaneously. These embodiments include those in which the compounds are in the same administrable composition, i.e., a single tablet, pill, or capsule, or a single solution for intravenous injection, or a single drinkable solution, or a single dragee formulation or patch, contains the compounds. The embodiments also include those in which each compound is in a separate administrable composition, but the patient is directed to take the separate compositions nearly simultaneously, i.e., one pill is taken right after the other or that one injection of one compound is made right after the injection of another compound, etc.

[0058] In other embodiments, one of NDMC and an additional therapeutic compound is administered first and then the other one of NDMC and the additional therapeutic compound is administered second. In these embodiments, the patient may be administered a composition comprising one of the compounds and then at some time, a few minutes or a few hours later, be administered another composition comprising the other one
of the compounds. Also included in these embodiments are those in which the patient is administered a composition comprising one of the compounds on a routine or continuous basis while receiving a composition comprising the other compound occasionally.

[0059] In some embodiments of combination administration, NDMC is administered in combination with another therapeutic agent, wherein at least a portion of the NDMC is administered by directly introducing NDMC to a subject. Thus, for example, clozapine may be administered in combination with NDMC wherein both clozapine and NDMC are directly administered to a subject. A portion of the NDMC administered to the patient will be due to metabolism of clozapine. However, another portion of NDMC will be due to direct administration of NDMC. In one embodiment, directly introducing NDMC to a subject may be accomplished by the subject orally ingesting NDMC. In one embodiment, directly introducing NDMC to a subject may be accomplished by intravenously injecting NDMC into the subject.

[0060] Defining the functional pharmacological activity of NDMC at a given receptor can be achieved by a variety of methodologies. A currently favored assay is the Receptor Selection and Amplification Technology (R-SAT) assay disclosed in US 5,707,798, the content of which is hereby incorporated by reference in its entirety.

[0061] Defining the functional pharmacological activity of NDMC at a given receptor can be achieved by a variety of methodologies. Another currently favored assay is the PI Hydrolysis assay (18).

[0062] Defining the ability of NDMC to penetrate the blood brain barrier and elicit a meaningful biological response can be achieved by a variety of methodologies. A currently favored assay is the hippocampal MAP kinase activation assay (19).

[0063] The present invention is further disclosed in the following examples, which are not in any way intended to limit the scope of the invention as claimed.

Examples

Example 1: Receptor Selection and Amplification Technology

[0064] The functional receptor assay, Receptor Selection and Amplification Technology (R-SAT), was used (essentially as disclosed in US 5,707,798, incorporated by
reference herein in its entirety) to investigate the functional pharmacological properties of known drugs, including many of their metabolites. These experiments have provided a molecular profile, or fingerprint, for each of these agents. Of all of the agents tested, only one, NDMC, displayed potent M1 acetylcholine receptor agonist activity. Figure 1 shows the concentration response relationship of clozapine (filled triangles) and N-desmethylclozapine (filled circles) to activate human M1 muscarinic receptors. Data was derived from R-SAT assays as previously described (20). Data is plotted as the percentage activation relative to the full muscarinic receptor agonist carbachol versus drug concentration. Veh denotes vehicle.

As shown in Figure 1, clozapine displays high potency (pEC\textsubscript{50} of 7.2) yet limited intrinsic efficacy (<25% relative efficacy) at human M1 receptors. Clozapine is thus defined as a weak partial agonist. Partial agonists lack sufficient intrinsic agonist activity to stimulate the receptor in a manner similar to full agonists. They thus behave as antagonists \textit{in vivo}. In contrast, NDMC also displays high potency (pEC\textsubscript{50} of 7.2) at human M1 receptors, yet it displays significantly greater intrinsic agonist activity at M1 receptors (65% relative efficacy to carbachol), behaving as a robust agonist in R-SAT assays. This increased efficacy suggests that NDMC will act as an agonist \textit{in vivo}, a functional profile distinct from that observed for clozapine.

To confirm the observation that NDMC displays increased agonist efficacy at M1 receptors, a PI hydrolysis assay was performed, the results of which are disclosed in Figure 2 and Table 1. The data in Figure 2 is derived from PI assays as described in (18). In Figure 2, the concentration response relationship of carbachol (filled squares), clozapine (filled triangles), and N-desmethylclozapine (filled circles) to activate human M1 muscarinic receptors is shown. Data are plotted as a radioactivity measured in counts per minute versus drug concentration.
Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>%Efficacy</th>
<th>M1</th>
<th>pEC50</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbachol</td>
<td>100%</td>
<td></td>
<td>6.04 ± 0.05</td>
<td>5</td>
</tr>
<tr>
<td>Clozapine</td>
<td>No Activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-desmethylclozapine</td>
<td>65 ± 10</td>
<td></td>
<td>7.01 ± 0.06</td>
<td>5</td>
</tr>
</tbody>
</table>

[0067] In Table 1, potency is reported as pEC50 values and efficacy is reported as that relative to the full agonist carbachol, both +/- standard deviation. “n” denotes number of experimental determinations. NDMC displays high potency as an M1 agonist in this system (pEC50 = 7.0), with full efficacy (>65% relative efficacy to carbachol). Thus, two distinct functional assays confirm that NDMC possesses previously unappreciated potent and fully efficacious agonist activity at human M1 muscarinic acetylcholine receptors. This significantly greater positive intrinsic activity of NDMC suggests that it behaves as an M1 receptor agonist in vivo.

[0068] Clozapine and NDMC were tested at the remaining muscarinic receptor subtypes. These data are disclosed in Table 2. The data in Table 2 are derived from R-SAT assays as previously described (20). Potency is reported as pEC50 values and efficacy is reported as that relative to the full agonist carbachol, both +/- standard deviation. N denotes number of experimental determinations.

Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>M1 Efficacy</th>
<th>pEC50</th>
<th>M2 Efficacy</th>
<th>pEC50</th>
<th>M3 Efficacy</th>
<th>pEC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clozapine</td>
<td>24±3</td>
<td>7.63±0.37</td>
<td>65±8</td>
<td>6.23±0.14</td>
<td>No response</td>
<td></td>
</tr>
<tr>
<td>N-desmethylclozapine</td>
<td>72±5</td>
<td>7.26±0.07</td>
<td>106±19</td>
<td>6.47±0.21</td>
<td>27±4</td>
<td>6.49±0.18</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>No response</td>
<td></td>
<td>No response</td>
<td></td>
<td>No response</td>
<td></td>
</tr>
<tr>
<td>N-desmethylolanzapine</td>
<td>No response</td>
<td></td>
<td>No response</td>
<td></td>
<td>No response</td>
<td></td>
</tr>
<tr>
<td>Xanomeline</td>
<td>121±6</td>
<td>7.20±0.08</td>
<td>106±9</td>
<td>6.30±0.23</td>
<td>66±6</td>
<td>6.63±0.21</td>
</tr>
<tr>
<td>Carbachol</td>
<td>101±2</td>
<td>6.11±0.03</td>
<td>101±5</td>
<td>6.23±0.09</td>
<td>102±3</td>
<td>6.53±0.04</td>
</tr>
<tr>
<td>Compound</td>
<td>M4 Efficacy</td>
<td>pEC_{40}</td>
<td>M5 Efficacy</td>
<td>pEC_{50}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------------</td>
<td>----------</td>
<td>-------------</td>
<td>----------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clozapine</td>
<td>57±5</td>
<td>7.35±0.10</td>
<td>No response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-desmethylclozapine</td>
<td>87±8</td>
<td>6.87±0.17</td>
<td>44±6</td>
<td>7.63±0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olanzapine</td>
<td>No response</td>
<td></td>
<td>No response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-desmethylolanzapine</td>
<td>No response</td>
<td></td>
<td>No response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xanomeline</td>
<td>116±9</td>
<td>7.46±0.14</td>
<td>86±12</td>
<td>6.59±0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbachol</td>
<td>96±3</td>
<td>6.53±0.05</td>
<td>105±3</td>
<td>6.76±0.12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[0069] NDMC displays increased intrinsic activity at all five muscarinic receptor subtypes when compared to clozapine. The profile of NDMC at human muscarinic receptors is most similar to that observed for the investigational agent Xanomeline, with one important distinction, a significantly lower efficacy at human m3 receptors.

[0070] To confirm aspects of this molecular profile in vivo, and to assess the ability of NDMC to access the central nervous system, NDMC was administered parenterally to rats, and the M1 receptor mediated activation of hippocampal MAP kinase (MAPK) activity was determined, and this is disclosed in Figure 3. NDMC treatment activates MAPK in CA1 pyramidal neurons. C57BL6 mice were treated s.c with vehicle, N-desmethylclozapine, clozapine, or NDMC and scopolamine (i.p.) at the doses described in Figure 3, and then subjected to labeling via immunohistochemistry. With NDMC treatment, cell bodies and proximal dendrites of CA1 pyramidal neurons showed increased phospho-MAPK immunoreactivity compared to either vehicle or clozapine treatment. Furthermore, scopolamine reduced NDMC induced MAPK activation in the CA1 region indicative of a muscarinic receptor mediated mechanism. Robust activation was observed, at a dose of 30 mg/kg. This confirms that NDMC penetrates the blood brain barrier, and function as a muscarinic receptor agonist in vivo.

Example 2: Nonclinical Pharmacology of NDMC

[0071] A comprehensive functional pharmacological screen of nearly all known antipsychotics, and many of their metabolites, at a majority of the known biogenic amine G-
protein-coupled receptors (GPCRs) identified NDMC as pharmacologically unique. NDMC is an antagonist of D₂ dopamine receptors and a potent inverse agonist of 5HT₂A receptors. However, unlike any other compound tested, NDMC is a potent and efficacious muscarinic receptor agonist. Specifically, NDMC is a potent partial agonist of M₁ (Kᵢ=50nM) and M₅ receptors (Kᵢ=25nM). NDMC also displays agonism of M₂, M₃, and M₄ receptors, however this interaction is 10-fold less potent than the interaction with other subtypes and indeed, under physiological conditions NDMC is able to competitively antagonize M₃ receptors. In comparison, clozapine is a potent competitive antagonist of M₁, M₃, and M₅ receptors, a weak agonist of M₂ receptors, and a potent partial agonist of M₄ receptors. Furthermore, olanzapine, an antipsychotic structurally related to NDMC and clozapine is an antagonist of all 5 muscarinic subtypes. Haloperidol, risperidone, and ziprasidone do not interact with any of these receptors at concentrations up to 1 μM. Thus, the agonist activity of NDMC at muscarinic receptors, particularly M₁ and M₅ receptors, is unique among antipsychotic drugs.

[0072] In addition to its activity at D₂, 5HT₂A, and muscarinic receptors, NDMC has affinity for α₁, α₂, D₁, H₁, δ₂, 5HT₁A, 5HT₁B, 5HT₃, 5HT₆, and 5HT₇ receptors, and Ca²⁺ channels in ligand binding assays. Functionally it is a potent competitive antagonist of 5HT₂C, H₁, and α₁A receptors and an inverse agonist of 5HT₆A and 5HT₇A receptors.

[0073] NDMC is orally active in two models thought to be predictive of antipsychotic activity. Like clozapine, NDMC attenuates both MK-801-induced and amphetamine-induced hyperactivity in mice at doses lower or similar to those that reduce spontaneous activity. Unlike clozapine and haloperidol, NDMC does not attenuate apomorphine-induced climbing in mice. This may reflect the reduced affinity of NDMC for D₂ receptors compared to these other antipsychotics. NDMC administration results in a dose-dependent activation of mitogen-activated protein kinase (MAPK) in the CA1 region of hippocampus and this activation can be blocked by the non-selective muscarinic antagonist scopolamine. Given that M₁ receptors are the predominant subtype of muscarinic receptor responsible for MAPK activation in the CA1 region of the hippocampus, this finding supports the in vivo agonism of M₁ receptors by NDMC. Clozapine administration does not result in MAPK activation. Additional evidence of pharmacological activity of NDMC.
comes from the observation that NDMC administration increases cFOS expression in the prefrontal cortex and nucleus accumbens, but not in the striatum. The lack of cFOS expression in the striatum suggests that NDMC is unlikely to produce extrapyramidal side effects.

Example 3: Nonclinical Pharmacokinetics and Metabolism of NDMC

[0074] The pharmacokinetics of NDMC and clozapine were investigated in rats and dogs. In both species, a single dose of NDMC was administered orally (10 mg/kg) or intravenously (1 mg/kg) and blood samples were taken at regular intervals post-dose. The data showed that the oral bioavailability of NDMC is 25% and 44% in rats and dogs, respectively. In comparison, the oral bioavailability of clozapine is 1.5% and 7% in rats and dogs, respectively. Thus these data indicate that NDMC has superior oral bioavailability relative to clozapine.

[0075] In animals that received clozapine, appreciable levels of NDMC were detected. In rats, NDMC levels at \( C_{\text{max}} \) were approximately 20-fold higher than the levels of clozapine at its \( C_{\text{max}} \). In dogs, peak NDMC levels were approximately 16% of the peak clozapine levels. These data confirm published studies that demonstrate the metabolism of clozapine to NDMC in several species including mice, rabbit, dog, pig, monkey, and human.

[0076] The brain-to-plasma ratio of NDMC was calculated in rats. The ratio was 1.0 at 240 minutes after oral administration of NDMC and 2.6 at 240 minutes after oral administration of clozapine. Together with data available in the literature, these results show that NDMC distributes into the CNS.

Example 4: In Vitro Pharmacology of NDMC

[0077] The affinity of NDMC for 50 receptors, ion channels, and transporters was evaluated at a single high dose (10 \( \mu M \)). This screen identified 16 sites at which NDMC caused 90% or greater inhibition of binding and these were \( \alpha_1, \alpha_2, D_1, D_{2S}, H_1, M_1, M_2, M_3, \delta_2, 5HT_{1A}, 5HT_{1B}, 5HT_{2A}, 5HT_3, 5HT_6, \) and 5HT\(_7\) receptors, and \( Ca^{2+}\) channels. The inhibition of ligand binding in these assays provides information regarding the binding of NDMC to these receptors, however does not indicate the nature of the interaction.
Example 5: Functional Screen of NDMC Against Multiple G-Protein-Coupled Receptors (GPCRs)

[0078] The pharmacological profile of NDMC was extensively studied in a wide range of functional GPCR assays using proprietary Receptor Selection and Amplification Technology (R-SAT; 2, 3). Table 3 reports the functional pharmacological activity of NDMC and leading typical and atypical antipsychotics at a subset of human monoaminergic receptor at which these drugs demonstrate the highest potencies.

Table 3 Antagonist and Inverse Agonist Activity of NDMC and Reference Antipsychotics in R-SAT Assays

<table>
<thead>
<tr>
<th>Compound</th>
<th>NDMC</th>
<th>Clozapine</th>
<th>Olanzapine</th>
<th>Haloperidol</th>
<th>Risperidone</th>
<th>Ziprasidone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptor</td>
<td>pKi</td>
<td>pKi</td>
<td>pKi</td>
<td>pKi</td>
<td>pKi</td>
<td>pKi</td>
</tr>
<tr>
<td>D&lt;sub&gt;2&lt;/sub&gt;</td>
<td>7.2 ± 0.1</td>
<td>7.7 ± 0.1</td>
<td>8.4 ± 0.2</td>
<td>10.0 ± 0.1</td>
<td>9.3 ± 0.1</td>
<td>8.3 ± 0.3</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;2A&lt;/sub&gt;</td>
<td>8.3 ± 0.2</td>
<td>8.3 ± 0.2</td>
<td>8.6 ± 0.1</td>
<td>7.3 ± 0.1</td>
<td>9.7 ± 0.1</td>
<td>8.6 ± 0.1</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;1A&lt;/sub&gt;</td>
<td>nr&lt;sup&gt;1&lt;/sup&gt;</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
<td>nr&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;2C&lt;/sub&gt;</td>
<td>7.8 ± 0.2</td>
<td>7.4 ± 0.2</td>
<td>7.4 ± 0.1</td>
<td>nr</td>
<td>7.2 ± 0.3</td>
<td>7.4 ± 0.2</td>
</tr>
<tr>
<td>H&lt;sub&gt;1&lt;/sub&gt;</td>
<td>8.2 ± 0.2</td>
<td>9.5 ± 0.2</td>
<td>8.4 ± 0.1</td>
<td>nr</td>
<td>7.0 ± 0.2</td>
<td>nr</td>
</tr>
<tr>
<td>M&lt;sub&gt;1&lt;/sub&gt;</td>
<td>nr*</td>
<td>7.8 ± 0.2</td>
<td>7.2 ± 0.2</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
</tr>
<tr>
<td>M&lt;sub&gt;2&lt;/sub&gt;</td>
<td>nr*</td>
<td>nr*</td>
<td>6.9 ± 0.1</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
</tr>
<tr>
<td>M&lt;sub&gt;3&lt;/sub&gt;</td>
<td>6.8 ± 0.7</td>
<td>8.2 ± 0.2</td>
<td>6.7 ± 0.5</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
</tr>
<tr>
<td>M&lt;sub&gt;4&lt;/sub&gt;</td>
<td>nr*</td>
<td>nr*</td>
<td>7.4 ± 0.3</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
</tr>
<tr>
<td>M&lt;sub&gt;5&lt;/sub&gt;</td>
<td>nr*</td>
<td>7.5 ± 0.3</td>
<td>7.2 ± 0.2</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
</tr>
<tr>
<td>D&lt;sub&gt;3&lt;/sub&gt;</td>
<td>nr</td>
<td>6.3 ± 0.1</td>
<td>7.6 ± 0.4</td>
<td>9.7 ± 0.1</td>
<td>7.9 ± 0.4</td>
<td>7.5 ± 0.3</td>
</tr>
<tr>
<td>α&lt;sub&gt;1A&lt;/sub&gt;</td>
<td>7.3 ± 0.1</td>
<td>8.1 ± 0.1</td>
<td>7.4 ± 0.2</td>
<td>7.4 ± 0.1</td>
<td>8.5 ± 0.1</td>
<td>7.4 ± 0.2</td>
</tr>
<tr>
<td>α&lt;sub&gt;2A&lt;/sub&gt;</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
<td>7.7 ± 0.1</td>
<td>nr</td>
</tr>
</tbody>
</table>

Inverse Agonist

<table>
<thead>
<tr>
<th></th>
<th>pEC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>pEC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>pEC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>pEC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>pEC&lt;sub&gt;50&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>5HT&lt;sub&gt;2A&lt;/sub&gt;</td>
<td>8.0 ± 0.3</td>
<td>8.0 ± 0.3</td>
<td>7.8 ± 0.1</td>
<td>6.8 ± 0.1</td>
<td>9.0 ± 0.3</td>
</tr>
<tr>
<td>5HT&lt;sub&gt;6A&lt;/sub&gt;</td>
<td>6.9 ± 0.1</td>
<td>7.0 ± 0.2</td>
<td>7.4 ± 0.2</td>
<td>nr</td>
<td>nr</td>
</tr>
<tr>
<td>5HT&lt;sub&gt;7A&lt;/sub&gt;</td>
<td>7.3 ± 0.1</td>
<td>7.4 ± 0.1</td>
<td>nr</td>
<td>nr</td>
<td>9.1 ± 0.2</td>
</tr>
</tbody>
</table>

<sup>1</sup>nr = no significant antagonist or inverse agonist activity up to 1μM.
The pharmacological activity of NDMC was similar to that of existing, clinically efficacious atypical antipsychotics. Like all atypical antipsychotics, NDMC showed high potency, competitive antagonist and inverse agonist activity at 5-HT$_{2A}$ receptors. It displayed lower potency as a dopamine D$_2$ receptor antagonist, than clozapine and therefore has a higher 5-HT$_{2A}$/D$_2$ receptor potency ratio. NDMC also displayed lower potency as an H$_1$ and α$_{1A}$ receptor antagonist than clozapine, suggesting that it may have less of a propensity to induce adverse clinical effects, including sedation and orthostatic hypotension, mediated by these receptor subtypes. Consistent with these data, published reports confirm the potent competitive antagonist activity of NDMC at D$_2$ and 5-HT$_{2C}$ receptors in vitro (Kouppamäki M, Syvälahti E and Hietala J (1993). Clozapine and N-desmethylclozapine are potent 5-HT$_{1C}$ receptor antagonists. Eur J Pharm, 245: 179-182), the lack of potent activity at histamine H$_3$ receptors (Alves-Rodrigues A, Leurs R, Willems E and Timmerman H (1996). Binding of clozapine metabolites and analogues to the histamine H$_3$ receptor in rat brain cortex. Arch Pharm Pharm Med Chem, 329: 413-416; Schlicker E and Marr I (1996). The moderate affinity of clozapine at H$_3$ receptors is not shared by its two major metabolites and by structurally related and unrelated atypical neuroleptics. Naunyn-Sch Arch Pharmacol, 353: 290-294), and only low potency interactions with GABA$_A$ receptors (Wong G, Kuoppamäki M, Hietala J, Lüddens H, Syvälahti E and Korpi ER (1996). Effects of clozapine metabolites and chronic clozapine treatment on rat brain GABA$_A$ receptors. Eur J Pharm, 314: 319-323).

Of the antipsychotics screened, only NDMC and clozapine possessed muscarinic receptor agonist properties (Table 2; Sur C, Mallorga PJ, Wittmann M, Jacobsen MA, Pascalella D, Williams JB, Brandish PE, Pettibone DJ, Scolnick EM and Conn PJ (2003). N-desmethylclozapine, an allosteric agonist at muscarinic 1 receptor, potentiates N-methyl-D-aspartate receptor activity. PNAS, 100: 13674-13679). NDMC was a potent, partial agonist of human M$_1$ and M$_5$ receptors and a less potent, full agonist of human M$_2$ and M$_4$ receptors (Table 2); it lacked antagonist activity at these receptors under similar conditions (Table 1). The physiological significance of M$_2$ and M$_5$ agonism in schizophrenia is unknown. However, agonism of M$_1$ and M$_4$ receptors is associated with antipsychotic activity
(Bymaster FP, Felder C, Ahmmed S and McKinzie D (2002). Muscarinic Receptors as a Target for Drugs Treating Schizophrenia. Curr Drug Targ CNS Neurol Dis, 1: 163-181; Felder CC, Bymaster FP, Ward J and DeLapp N (2000). Therapeutic Opportunities for Muscarinic Receptors in the Central Nervous System. J Med Chem, 43: 4333-4353). Furthermore, agonism of M1 receptors may confer cognition-enhancing activity on NDMC (Bymaster FP, Felder C, Ahmmed S and McKinzie D (2002). Muscarinic Receptors as a Target for Drugs Treating Schizophrenia. Curr Drug Targ CNS Neurol Dis, 1: 163-181). NDMC displays minimal, low potency agonist activity at M3 receptors and behaves as an antagonist at this site (Tables 3 and 4). Muscarinic M3 receptors are the predominant receptor subtype that mediate cholinergic effects of parasympathetic activation in humans, such that significant agonist activity would likely result in treatment-limiting parasympathetic side effects including sweating, ocular, and gastrointestinal dysfunction. The antagonist activity of NDMC at M3 suggests that severe parasympathetomimetic effects will not be observed in clinical testing. The pharmacological activity of NDMC at the muscarinic receptors has been observed by others (Sur et al. PNAS 2003).

Table 4  Muscarinic Receptor Agonist Activity of Dibenzodiazepine Antipsychotics

<table>
<thead>
<tr>
<th>Compound</th>
<th>M1 Efficacy</th>
<th>pEC50</th>
<th>M2 Efficacy</th>
<th>pEC50</th>
<th>M3 Efficacy</th>
<th>pEC50</th>
<th>M4 Efficacy</th>
<th>pEC50</th>
<th>M5 Efficacy</th>
<th>pEC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDMC</td>
<td>72 ± 2</td>
<td>7.3 ± 0.1</td>
<td>106 ± 19</td>
<td>6.5 ± 0.2</td>
<td>27 ± 4</td>
<td>6.5 ± 0.2</td>
<td>87 ± 8</td>
<td>6.9 ± 0.2</td>
<td>48 ± 6</td>
<td>7.6 ± 0.3</td>
</tr>
<tr>
<td>Clozapine</td>
<td>24 ± 3</td>
<td>7.3 ± 0.4</td>
<td>65 ± 8</td>
<td>6.5 ± 0.1</td>
<td>nr</td>
<td>nr</td>
<td>57 ± 5</td>
<td>7.4 ± 0.1</td>
<td>nr</td>
<td></td>
</tr>
<tr>
<td>Olanzapine</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbachol</td>
<td>101 ± 2</td>
<td>6.1 ± 0.1</td>
<td>101 ± 5</td>
<td>6.3 ± 0.1</td>
<td>102 ± 3</td>
<td>6.5 ± 0.1</td>
<td>96 ± 3</td>
<td>6.5 ± 0.1</td>
<td>105 ± 3</td>
<td>6.8 ± 0.1</td>
</tr>
</tbody>
</table>

1Efficacy is % carbachol activation of the receptor

2 Data are mean ± S.E.M.
3 nr=no significant agonist activity up to 10 μM

[0081] The pharmacological profile of NDMC at the muscarinic receptors is distinct from that of clozapine. Clozapine displayed potent agonist activity at M1 receptors, however the efficacy of this interaction was very low (Table 4) and under similar conditions
clozapine was a potent antagonist of M₁ receptor activation (Table 3). Also in contrast to NDMC, clozapine demonstrated potent M₃ and M₅ antagonism. At the M₂ and M₄ receptors, clozapine demonstrated partial agonism. These data predict that, whereas it is likely that NDMC will behave as an M₁ agonist in vivo, clozapine is likely to act as an M₁ antagonist.

**Example 6:** Effect of NDMC on Spontaneous Locomotion and Reversal of MK-801-Induced Hyperactivity in Non-Swiss Albino Mice

[0082] NDMC was administered subcutaneously (s.c.) or orally (p.o.) to male, adult Non-Swiss Albino (NSA) mice at 1, 10, or 30 mg/kg. Upon both s.c. and p.o. administration, NDMC significantly reduced spontaneous activity at 10 and 30 mg/kg. At 10 mg/kg s.c. the maximal reduction was achieved at 30 minutes post-administration and was maintained for the duration of the experiment, 120 minutes. This effect of NDMC was similar to that seen with clozapine, which reduced spontaneous locomotion at 3 and 10 mg/kg s.c. and p.o.

[0083] Clinically effective antipsychotic drugs can block the behavioral effects of non-competitive N-methyl-D-aspartate agonists, such as MK-801. NDMC was evaluated for its ability to attenuate MK-801-induced hyperactivity in male, adult, NSA mice and its activity in this assay was compared to that of clozapine. NDMC attenuated MK-801-induced hyperactivity with a minimal effective dose of 1 mg/kg s.c. and 10 mg/kg p.o., consistent with antipsychotic-like efficacy. These doses were lower than or similar to those that reduced spontaneous locomotion, suggesting that the antipsychotic-like effects can be differentiated from general locomotor behavioral disruption. Similarly, clozapine reduced MK-801-induced hyperactivity with a minimal effective dose of 1 mg/kg s.c. and 3 mg/kg p.o.

**Example 7:** Effect of NDMC on the Reversal of Amphetamine-induced Locomotor Behaviors in Non-Swiss Albino Mice

[0084] Similar to attenuation of hyperactivity induced by N-methyl-D-aspartate agonists, clinically effective antipsychotics also attenuate dopamine-mediated hyperactivity in rodents. Amphetamine-induced hyperactivity in mice is, therefore, a commonly used assay for in vivo antipsychotic-like activity. NDMC attenuated amphetamine-induced hyperactivity.
in male, adult NSA mice at 10 mg/kg after s.c. or p.o. administration. Clozapine also reduced amphetamine-induced hyperactivity with a minimal effective dose of 3 mg/kg p.o. These results are predictive of antipsychotic-like efficacy in humans.

Example 8: Effect of NDMC on Reversal of apomorphine-induced climbing in Non-Swiss Albino Mice

[0085] Another way to assess the blockade of dopamine-mediated behavior in rodents is the attenuation of apomorphine-induced climbing in mice. Direct D₂ receptor antagonists most effectively block climbing induced by the dopamine receptor agonist apomorphine. Haloperidol, a typical neuroleptic antipsychotic drug with high affinity for dopamine D₂ receptors, completely attenuated the apomorphine-induced climbing in male, adult, NSA mice at 0.1 mg/kg s.c. Clozapine also reduced apomorphine-induced climbing in a dose-dependent manner with the minimal effective dose at 10 mg/kg s.c. In contrast NDMC did not attenuate apomorphine-induced climbing at doses up to 100 mg/kg s.c. This may reflect the reduced affinity of NDMC for D₂ receptors as compared to clozapine and haloperidol.

Example 9: Effect of NDMC on MAPK Activation in Brain in C57BL/6 Mice

[0086] In an effort to confirm the muscarinic agonist properties of NDMC in vivo, the activation of mitogen-activated protein kinase (MAPK) in CA1 region of the hippocampus was examined. NDMC was administered s.c. at doses of 3, 10, 30, and 100 mg/kg to C57BL/6 mice. The animals were killed two hours later; whole brains were removed and subjected to immunodetection of MAPK activity in hippocampus. NDMC administration resulted in the stimulation of MAPK activity at all doses in a dose-dependent manner. In contrast, clozapine at 30 mg/kg did not result in MAPK activation in CA1 region of brain. The stimulation of MAPK activity induced by NDMC was blocked by the non-selective muscarinic receptor antagonist scopolamine (0.3 mg/kg, i.p.), confirming that NDMC acts as a muscarinic receptor agonist in vivo. It has been demonstrated in vitro that M₁ receptors are the predominant subtype of muscarinic receptor that is responsible for activation of MAPK in the forebrain (Hamilton SE and Nathanson NM (2001). The M₁

Example 10: Effects of Desmethylclozapine on Fos Protein Expression in the Forebrain: In vivo Biological Activity of the Clozapine Metabolite

[0087] The first in vivo demonstration of pharmacological activity of NDMC (desmethylclozapine) was a dose-dependent induction of the expression of the immediate early gene cFOS in rat brain (Young CD, Meltzer HY and Deutch AY (1997). Effects of desmethylclozapine on Fos protein expression in the forebrain: In vivo biological activity of the clozapine metabolite. *Neuropsychopharm.*, 19: 99-103). NDMC was administered to adult male Sprague-Dawley rats s.c. at doses of 7.5 and 30.0 mg/kg; the animals were sacrificed two hours later and homogenized tissue from various brain regions was subjected to immunodetection of cFOS by western blotting. NDMC resulted in the induction of cFOS expression in the pre-frontal cortex and nucleus accumbens, but not in striatum, and these effects were similar in magnitude and regional selectivity to those observed for clozapine. The lack of cFOS expression in the striatum of NDMC-treated animals may indicate a low propensity for NDMC to cause EPS.

Example 11: Pharmacokinetic Evaluation of Clozapine and N-Desmethylclozapine following Administration of a Single Intravenous Dose or Oral Dose to Conscious Sprague Dawley Rats

[0088] The pharmacokinetics of clozapine and N-desmethylclozapine (NDMC) was evaluated in rats after intravenous (i.v.) and oral (p.o.) dosing. C<sub>max</sub>, T<sub>max</sub> and bioavailability after p.o. dosing and the volume of distribution (V<sub>ss</sub>), terminal plasma half-
life (T½) and clearance (CLs) after i.v. dosing were determined. The brain-to-plasma ratio of NDMC after both intravenous and oral administration was also determined. A total of 18 male Sprague-Dawley rats were dosed with clozapine p.o. (N=6, 10 mg/kg), NDMC p.o. (N=6, 10 mg/kg), clozapine i.v. (N=6, 1 mg/kg), or NDMC i.v. (N=6, 1 mg/kg), and serum samples for bioanalytical analysis were obtained at regular intervals at between 0 and 240 minutes post dose. Animals were euthanised and brain and plasma samples obtained at 60 or 240 minutes post-dose, depending on study group. The levels of NDMC and clozapine were measured in each sample. Pharmacokinetic data for NDMC is presented in tables 5-8.

Table 5  Plasma Concentration (ng/mL) of NDMC in Rat after NDMC Administration

<table>
<thead>
<tr>
<th>Compound Measured (route)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>NDMC (p.o.)</td>
<td>305 ± 101</td>
</tr>
<tr>
<td>NDMC (p.o.)</td>
<td>277 ± 57</td>
</tr>
<tr>
<td>NDMC (l.v.)</td>
<td>540 ± 46</td>
</tr>
</tbody>
</table>

¹Mean ± SD; ²Dosages for oral administration were 10 mg/kg and 1mg/kg for intravenous administration; ³NS = no sample taken because study terminated at 60 minutes

Table 6  Plasma Concentration (ng/mL) of NDMC and Clozapine in Rat after Clozapine Administration

<table>
<thead>
<tr>
<th>Compound Measured (route)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Clozapine (p.o.)</td>
<td>3.8 ± 1.5</td>
</tr>
<tr>
<td>Clozapine (p.o.)</td>
<td>4.9 ± 1.7</td>
</tr>
<tr>
<td>Clozapine (l.v.)</td>
<td>112⁴</td>
</tr>
<tr>
<td>NDMC (p.o.)</td>
<td>77.1 ± 88.7</td>
</tr>
<tr>
<td>NDMC (p.o.)</td>
<td>241 ± 21.3</td>
</tr>
<tr>
<td>NDMC (l.v.)</td>
<td>3.5⁴</td>
</tr>
</tbody>
</table>

¹Mean ± SD; ²Dosages for oral administration were 10 mg/kg and 1mg/kg for intravenous administration; ³NS = no sample taken because study terminated at 60 minutes; ⁴N=2
### Table 7  Pharmacokinetic Parameters of NDMC in Rat after NDMC Administration

<table>
<thead>
<tr>
<th>Compound Measured (route)</th>
<th>Average AUC (min.ng (^{-1}).mL(^{-1}))</th>
<th>C(_{\text{max}}) (ng/mL)</th>
<th>T(_{\text{max}}) (min)</th>
<th>T(_{1/2}) (min)</th>
<th>BA(^2) (%)</th>
<th>V(_{ss}) (L/kg)</th>
<th>CL(_{s}) (mL.min(^{-1}).kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDMC (i.v.)</td>
<td>27331</td>
<td>756</td>
<td>0</td>
<td>39.3</td>
<td>-</td>
<td>1.47</td>
<td>36.2</td>
</tr>
<tr>
<td>NDMC (p.o.)</td>
<td>68227</td>
<td>582</td>
<td>60</td>
<td>ND(^3)</td>
<td>25.0 ± 7.5</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

\(^1\) Mean ± SD; \(^2\) BA = oral bioavailability; \(^3\) ND = not determined

### Table 8  Pharmacokinetic Parameters of NDMC and Clozapine in Rat after Clozapine Administration

<table>
<thead>
<tr>
<th>Compound Measured (route)</th>
<th>Average AUC (min.ng/mL)</th>
<th>C(_{\text{max}}) (ng/mL)</th>
<th>T(_{\text{max}}) (min)</th>
<th>T(_{1/2}) (min)</th>
<th>BA(^2) (%)</th>
<th>V(_{ss}) (L/kg)</th>
<th>CL(_{s}) (mL.min(^{-1}).kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDMC (i.v.)</td>
<td>489.7</td>
<td>3.99</td>
<td>60</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NDMC (p.o.)</td>
<td>16199</td>
<td>194</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Clozapine (i.v.)</td>
<td>8836</td>
<td>137</td>
<td>0</td>
<td>79.4</td>
<td>-</td>
<td>9.88</td>
<td>101</td>
</tr>
<tr>
<td>Clozapine (p.o.)</td>
<td>1347</td>
<td>10.8</td>
<td>60</td>
<td>ND(^3)</td>
<td>1.5 ± 0.6</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

\(^1\) Mean ± SD; \(^2\) BA = oral bioavailability; \(^3\) ND = not determined

[0089] These data demonstrate that NDMC was rapidly absorbed from the gastrointestinal tract following oral administration; a C\(_{\text{max}}\) of 582 ng/mL was achieved by 30 minutes. NDMC had low clearance from the circulation, a low volume of distribution, and was approximately 25% orally bioavailable. Clozapine reached much lower peak drug levels (10.8 ng/mL; 1/50\(^{th}\) that of NDMC), had higher clearance, and poorer bioavailability (1.5%) following oral administration. These data suggest that NDMC may have acceptable pharmacokinetic properties after oral administration in humans and may indeed have improved pharmacokinetic properties as compared to clozapine.

[0090] High plasma levels of NDMC were observed following oral administration of clozapine and peak plasma levels of NDMC were nearly 20-fold greater than those observed for clozapine (194 ng/mL versus 10.8 ng/mL). Similar observations have been made
by others (Weigmann H, Härter S, Fischer V, Dahmen N and Hiemke C (1999). Distribution of clozapine and desmethyleclozapine between blood and brain in rats. *Eur Neuropsychopharm*, 9: 253-256; Baldessarini RJ, Centorrino F, Flood JG, Volpicelli SA, Huston-Lyons D and Cohen BM (1993). Tissue concentrations of clozapine and its metabolite in the rat. *Neuropsychopharm*, 9: 117-124). Weigmann et al. (*Eur Neuropsychopharm* 1999) showed that following oral administration of 5 doses (20 mg/kg) of clozapine at 1.5-hour intervals to male Sprague-Dawley rats, plasma concentrations of NDMC exceeded those of clozapine by up to 2.2-fold. In another study, high levels of circulating NDMC were observed following intraperitoneal (i.p.) administration of varying (1-60 mg/kg) doses of clozapine to Sprague-Dawley rats (Baldessarini et al; *Neuropsychopharm* 1993). Thus, NDMC is a major chemical moiety formed after oral administration of clozapine in the rat. It is also been shown in vitro that NDMC is the primary clozapine metabolite formed by rat liver microsomes (Bun H, Disdier B, Aubert C and Catalin J (1999). Interspecies variability and drug interactions of clozapine metabolism by microsomes. *Fund Clin Pharm*, 13: 577-581).

[0091] The pharmacokinetic study described above included an initial assessment of the distribution of NDMC into brain. The ratio of brain-to-plasma levels of NDMC was 0.36 ± 0.16 at 60 minutes and 1.0 ± 0.4 at 240 minutes following oral administration of 10 mg/kg NDMC to Sprague-Dawley rats. Additionally, after oral administration of clozapine the brain-to-plasma ratio of NDMC was 0.26 ± 0.07 at 60 minutes and 2.6 ± 0.8 at 240 minutes. This latter result confirms previously published findings showing that oral administration of clozapine to male Sprague-Dawley rats resulted in NDMC levels in brain that were up to 3.9-fold higher than those observed in serum (Baldessarini et al.; *Neuropsychopharm* 1993) and intraperitoneal administration of 20, 30, and 60 mg/kg of clozapine to Sprague-Dawley rats resulted in the detection of NDMC in brain (Bun et al.; *Fund Clin Pharm* 1999). Together these in vivo data clearly document that NDMC distributes into the CNS after oral administration.
Example 12: Bioavailability Assessment of Clozapine and N-Desmethylclozapine in Male Beagle Dogs

The pharmacokinetics of clozapine and N-desmethylclozapine (NDMC) were evaluated in dogs after intravenous (i.v.) and oral (p.o.) dosing. $C_{\text{max}}$, $T_{\text{max}}$ and bioavailability after p.o. dosing and the volume of distribution (Vss), terminal plasma half-life ($T_{\text{1/2}}$) and clearance (CLs) after i.v. dosing were determined. A total of 6 beagle dogs were dosed with clozapine p.o. (N=3, 10 mg/kg), NDMC p.o. (N=3, 10 mg/kg), clozapine i.v. (N=3, 1 mg/kg), or NDMC i.v. (N=3, 1 mg/kg). Serum samples for bioanalytical analysis were obtained pre-dose and 10 min, 30 min, 1, 2, 3, 4, and 6 h post dose after p.o. administration and pre-dose, 2, 5, 10, 30 min, 1, 2, 3, and 4 h after i.v. administration. The levels of NDMC and clozapine were measured in each sample. Pharmacokinetic data for NDMC are presented in tables 9-12.

Table 9 Plasma Concentration (ng/mL$^1$) of NDMC in Dog after NDMC Administration$^2$

<table>
<thead>
<tr>
<th>Compound Measured (route)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-  10  30  60  120  180  240  360</td>
</tr>
<tr>
<td>NDMC (p.o.)</td>
<td>-  1.0  14 ± 12$^2$  67 ± 37  155 ± 95  249 ± 44  274 ± 44  261</td>
</tr>
<tr>
<td></td>
<td>2  5  10  30  60  120  180  240</td>
</tr>
<tr>
<td>NDMC (i.v.)</td>
<td>182.5 ± 90  73 ± 22  50 ± 10  35 ± 2  32 ± 6  28 ± 4  27 ± 7  27 ± 4</td>
</tr>
</tbody>
</table>

$^1$ Mean SD; $^2$ Dosages for oral administration were 10 mg/kg and 1mg/kg for intravenous administration.
Table 10  Plasma Concentration (ng/mL¹) of NDMC and Clozapine in Dog after Oral of Intravenous Clozapine Administration²

<table>
<thead>
<tr>
<th>Compound Measured (route)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
</tr>
<tr>
<td>NDMC (p.o.)</td>
<td>-</td>
</tr>
<tr>
<td>Clozapine (p.o.)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>NDMC (i.v.)</td>
<td>0.54 ± 0.12</td>
</tr>
<tr>
<td>Clozapine (i.v.)</td>
<td>166 ± 28</td>
</tr>
</tbody>
</table>

¹ Mean SD; ² Dosages for oral administration were 10 mg/kg and 1 mg/kg for intravenous administration.

Table 11  Pharmacokinetic Parameters¹ of NDMC in Dog after Oral or Intravenous NDMC and Clozapine Administration

<table>
<thead>
<tr>
<th>Compound Measured (route)</th>
<th>Average AUC (min-ng¹ .mL⁻¹)</th>
<th>Cmax (ng/mL)</th>
<th>Tmax (min)</th>
<th>T1/2 (min)</th>
<th>BA²</th>
<th>Vss (L/kg)</th>
<th>CLs (mL.min⁻¹ .kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDMC (i.v.)</td>
<td>134.8 ± 21.3</td>
<td>353.2 ± 242</td>
<td>-</td>
<td>13.2 ± 7.0</td>
<td>-</td>
<td>28202.1 ± 4919.8</td>
<td>1850 ± 1060.4</td>
</tr>
<tr>
<td>NDMC (p.o.)</td>
<td>597.6 ± 111.8</td>
<td>286.3 ± 25</td>
<td>3.3 ± 1.2</td>
<td>ND</td>
<td>44.3</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Clozapine (i.v.)</td>
<td>15.0 ± 3.9</td>
<td>5.3 ± 1.2</td>
<td>2.7 ± 0.58</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Clozapine (p.o.)</td>
<td>32.1 ± 24.0</td>
<td>19.2 ± 7.2</td>
<td>4.0 ± 0.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

¹ Mean ± SD; ² BA=oral bioavailability
Table 12 Pharmacokinetic Parameters\(^1\) of Clozapine in Dog after Clozapine Administration

<table>
<thead>
<tr>
<th>Compound Measured (route)</th>
<th>Average AUC (min ng(^1) .mL(^{-1}))</th>
<th>C(_\text{max}) (ng/mL)</th>
<th>T(_\text{max}) (min)</th>
<th>T(_{1/2}) (min)</th>
<th>BA(^2) (%)</th>
<th>Vss (L/kg)</th>
<th>CLs (mL/min .kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clozapine (i.v.)</td>
<td>266 ± 33</td>
<td>189 ± 18</td>
<td>-</td>
<td>3.3 ± 0.63</td>
<td>-</td>
<td>10335 ± 1636</td>
<td>2190 ± 295.9</td>
</tr>
<tr>
<td>Clozapine (p.o.)</td>
<td>186 ± 109.5</td>
<td>124.9 ± 58.3</td>
<td>3.0 ± 1.7</td>
<td>ND</td>
<td>7.0</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

\(^1\) Mean ± SD; \(^2\) BA=oral bioavailability

[0093] NDMC was absorbed from the gastrointestinal tract following oral administration with a C\(_\text{max}\) of 286.3 ng/mL achieved by 3.3 h. NDMC had low clearance from the circulation, a low volume of distribution, and was approximately 44% orally bioavailable. Clozapine had poorer oral bioavailability (7%). These data suggest that NDMC may have acceptable pharmacokinetic properties after oral administration in humans and may indeed have improved pharmacokinetic properties as compared to clozapine.

[0094] NDMC was readily detectable in plasma following both intravenous and oral administration of clozapine. The mean NDMC/clozapine AUC ratio was 0.056 after i.v. administration of clozapine and 0.161 (i.e., 16%) after oral administration. These data confirm recent studies that demonstrated the metabolism of clozapine to N-desmethyloclozapine in dog both in vitro (Bun et al. *Fund Clin Pharm* 1999) and in vivo (Mosier KE, Song J, McKay G, Hubbard JW and Fang J (2003). Determination of clozapine, and its metabolites, N-desmethyloclozapine and clozapine N-oxide in dog plasma using high-performance liquid chromatography. *J Chromat B, 783*: 377-382). Mosier and colleagues showed that following oral administration of clozapine to a dog the C\(_\text{max}\) of desmethyloclozapine was approximately 20% that of clozapine (i.e., the NDMC/clozapine ratio was approximately 0.2). An early study did not detect N-desmethyloclozapine in dog (Gauch R and Michaelis W (1970)). The metabolism of 8-chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo[b,e][1,4] diazepine (Clozapine) in mice, dogs, and human subjects.
Il Farmaco, 26: 667-681) after oral administration; however this may have been due to insensitive analytical techniques.

Example 13: The role of M1 muscarinic receptor agonism of N-desmethyleclozapine in the unique clinical effects of clozapine

Methods

Molecular profiling of clinically relevant drugs was performed at all known monoaminergic receptor subtypes except the Dopamine D4, Serotonin 5A, and Histamine H4 receptors using Receptor Selection and Amplification Technology (R-SAT) assays. Briefly, NIH/3T3 cells plated at 70-80% confluency were transfected with various receptor cDNA (10-100ng receptor and 20ng β-Gal reporter/well of a 96 well plate) using the Polyfect Reagent (Qiagen Inc.) as described in the manufacture's protocol. One day after transfection, ligands were added in Dulbecco’s modified Eagle’s medium supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml) and 2% Cyto-SF3. After four to six days, the media was aspirated off, the cells were lysed, O-Nitrophenyl-beta-D-Galactopyranoside (ONPG) was added and the resulting absorbance was measured spectrophotometrically. Concentration response curves were performed as eight-point concentration response experiments run in duplicate, where the maximal antipsychotic concentrations varied from 10-25 micromolar, and data were analyzed using Excel fit and Graph Pad Prism. Reported EC₅₀ values represent the concentration of a ligand that produces a half-maximal response from a receptor in the absence of other ligands, and IC₅₀ values represent the concentration of a ligand that inhibits half of the agonist-induced activity. Competitive antagonist IC₅₀ data were adjusted for agonist occupancy using the equation Ki = IC₅₀/[1+{agonist}/EC₅₀ agonist]. Data are reported as negative log values (pEC₅₀ and pKᵢ). Sources of the drugs utilized in this study are described in Weiner et al. (2001) and Wellendorph et al. (2002), with the exception of N-desmethyleclozapine, which was acquired from Sigma, Inc., and N-desmethylolanzapine, which was synthesized by ACADIA Pharmaceuticals. A list of the compounds screened can be found as supplemental information.
[0096] PI hydrolysis assays were performed on Chinese Hamster Ovary cells stably transfected with the human M1 muscarinic receptor cDNA as described in Spalding et al (2002), and the data are derived from six or eight-point concentration response experiments performed in duplicate.

[0097] MAP Kinase assays utilized C57BL6 mice treated subcutaneously with either vehicle, clozapine, or N-desmethylclozapine with or without scopolamine, sacrificed two hours later, and phospho-MAPK immunoreactivity was assayed as described in Berkeley et al (2001). Briefly, after treatments which were administered s.c. at 60 min., mice were perfused with 100 ml of 4% paraformaldehyde followed with 100 ml of 10% sucrose. Brains were removed and cryoprotected in 30% sucrose overnight at 4 °C. The next day, 50 µm slices were cut on a sliding microtome. Slices were rinsed, treated with 3% H₂O₂ for 10 minutes at room temperature and rinsed again. Slices were blocked in PBS containing 10 µg/ml avidin (Vector Laboratories Burlingame, CA), 0.1% triton-X and 4% normal goat serum (NGS) for 1 hour. Slices were rinsed and incubated in PBS containing 50 µg/ml biotin (Vector Laboratories Burlingame, CA), 2% NGS, and phospho-ERK1/2 antibody (Cell signal Technologies, Beverly, MA) at a concentration of 1:250 and allowed to incubate overnight at 4 °C. The next day, slices were rinsed and placed in PBS containing 2% NGS and biotinylated goat anti-rabbit (Vector Laboratories Burlingame, CA) at a concentration of 1:100 for 1 hour at 4 °C. Slices were rinsed and placed in horseradish peroxidase-conjugated avidin-biotin complex (Vector Laboratories Burlingame, CA) for 1 hour at 4 °C. Slices were rinsed and incubated in TSA Fluorescein tyramide for 10 min at room temperature. Slices were treated with 10 mM CuSO₄ for 30 minutes, mounted onto glass slides with Vectashield mounting media (Vector Laboratories Burlingame, CA). Slides were visualized via a fluorescence microscope and digital images were analyzed with Scion image analysis software (Scion Corp. Frederick, MD).

[0098] Stepwise multiple-regression analysis, including the dependent measure, dose, age, and gender was utilized to assess the contribution of NDMC to treatment response in schizophrenic subjects (Hasegawa et al 1993 and Lee et al 1999). The analysis was adjusted for baseline level of symptom severity, age, and dose, since dose was not fixed. The
plasma samples chosen for the analyses were obtained at 6 weeks and 6 months after initiation of therapy, were related to the clinical measures obtained at those times, and were drawn 12 hours after the last clozapine dose. Only subjects who had received at least 100 mg of clozapine per day were included in the analysis, and some data were unavailable for these subjects at some time points. Regarding co-treatment with anticholinergic agents, only two subjects in this sample were treated with benztropine. The results did not differ when data from these two subjects were omitted (data not shown). Lastly, ten of the patients in this study were treated with benzodiazepines at the time the levels of clozapine and NDMC were measured. Benzodiazepines have not been reported to affect the metabolism of clozapine.

[0099] Drugs screened, grouped according to clinical class, included:


[0103] Monoaminergic: 7-OH-DPAT, 8-OH-DPAT, Alpha Methyl Serotonin, Arecoline, Astemizole, Bethanacol, Carbachol, CGS 12066A, Cinanserin, Chlorpheniramine, Cimetidine, Clobenpropit, CPP, Dihydroergocristine, Dimaprit, Diphenhydramine, Doxylamine, Eltoprazine, Famotidine, Histamine, Imetit, Isomaltane, Ketanserin, Loperamide, L-Tryptophan, LY 53857, mCPP, Mesulergine, Metergoline, Methergine, Methiothepin, Methysergide, Mexamine, Mianserin, MK 212, Mepyramine, Pheniramine, Phenylbiguanide, Pimethixene, Piperazine, Pirenpirone, Prazosin, Promethazine, Pyrilamine,
Quiapazine, Ranitidine, Ritanserin, SB 204741, SB 206553, Serotonin, Spiroxatrine, Sumatriptan, Thioperamide, Trippellamine, Tripropidine, and WB 4101.


Results and Discussion

[0106] A library of 462 clinically relevant drugs were profiled for functional activity at 33 of the 36 known human monoaminergic G-protein coupled receptors using the mammalian cell-based functional assay Receptor Selection and Amplification Technology (R-SAT). Table 13 illustrates data on representative antipsychotic agents for receptors at which the most potent activities were observed. Potency data for five representative antipsychotics and the clozapine metabolite N-desmethylclozapine (NDMC) at 13 human monoamine receptor subtypes are shown. Potency data are reported as pKi values for the competitive antagonist studies, while inverse agonist data are reported as pEC_{50} values, both derived from three to eight separate determinations ± standard error. Asterixes (*) indicate the presence of agonist activity where the muscarinic receptor agonist potencies are reported in Table 14. Ziprasidone displays limited but detectable agonist efficacy at human 5-HT_{1A} receptors (<30% relative to 8-OH-DPAT), and a Ki > 1-micromolar when assayed as a competitive antagonist. Abbreviations used: NDMC-N-desmethylclozapine, 5-HT-serotonin, H- histamine, M-muscarinic, D-dopamine, and Alpha-alpha adrenergic, and nr-no response defined as no significant antagonist or inverse agonist activity at concentrations up to 1-micromolar.
Table 13  Pharmacological activities of antipsychotics at human monoamine receptors.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Histopside</th>
<th>Risperidone</th>
<th>Ziprasidone</th>
<th>Olanzapine</th>
<th>Clozapine</th>
<th>NDMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>D2</td>
<td>10.0±0.1</td>
<td>9.3±0.1</td>
<td>8.3±0.3</td>
<td>8.4±0.2</td>
<td>7.7±0.1</td>
<td>7.2±0.1</td>
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<tr>
<td>5-HT2A</td>
<td>7.3±0.1</td>
<td>9.7±0.1</td>
<td>8.6±0.1</td>
<td>8.6±0.1</td>
<td>8.3±0.2</td>
<td>8.3±0.2</td>
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<tr>
<td>5-HT1A</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
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<td>nr</td>
</tr>
<tr>
<td>5-HT2C</td>
<td>nr</td>
<td>7.2±0.3</td>
<td>7.4±0.2</td>
<td>7.4±0.1</td>
<td>7.4±0.2</td>
<td>7.8±0.2</td>
</tr>
<tr>
<td>H1</td>
<td>nr</td>
<td>7.0±0.2</td>
<td>nr</td>
<td>8.4±0.1</td>
<td>9.5±0.2</td>
<td>8.2±0.2</td>
</tr>
<tr>
<td>M1</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
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<td>7.8±0.2</td>
<td>nr</td>
</tr>
<tr>
<td>M2</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
<td>6.9±0.1</td>
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<td>nr*</td>
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<tr>
<td>M3</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
<td>6.7±0.5</td>
<td>8.2±0.2</td>
<td>6.8±0.7*</td>
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<tr>
<td>M4</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
<td>7.4±0.3</td>
<td>nr*</td>
<td>nr*</td>
</tr>
<tr>
<td>M5</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
<td>7.2±0.2</td>
<td>7.5±0.3</td>
<td>nr*</td>
</tr>
<tr>
<td>D3</td>
<td>9.7±0.1</td>
<td>7.9±0.4</td>
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<tr>
<td>Alpha1A</td>
<td>7.4±0.1</td>
<td>8.5±0.1</td>
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<td>7.4±0.2</td>
<td>8.1±0.1</td>
<td>7.3±0.1</td>
</tr>
<tr>
<td>Alpha2A</td>
<td>nr</td>
<td>7.7±0.1</td>
<td>nr</td>
<td>7.4±0.3</td>
<td>nr</td>
<td>nr</td>
</tr>
</tbody>
</table>

**Inverse Agonist**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>pEC50</th>
<th>pEC50</th>
<th>pEC50</th>
<th>pEC50</th>
<th>pEC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT2A</td>
<td>6.8±0.1</td>
<td>9.0±0.3</td>
<td>8.8±0.3</td>
<td>7.8±0.1</td>
<td>8.0±0.3</td>
</tr>
<tr>
<td>5-HT6A</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
<td>7.4±0.2</td>
</tr>
<tr>
<td>5-HT7A</td>
<td>nr</td>
<td>9.1±0.2</td>
<td>7.3±0.1</td>
<td>nr</td>
<td>7.4±0.1</td>
</tr>
</tbody>
</table>

[0107] Competitive antagonism of D$_2$ receptors, and inverse agonism of 5-HT$_{2A}$ receptors was nearly uniform throughout this class, with typical agents demonstrating low 5HT$_{2A}$/D$_2$ ratios, and atypical agents demonstrating high ratios (Meltzer et al 1989 and Weiner et al 2001). Inverse agonism of H$_1$ receptors was commonly observed, where clozapine and olanzapine displayed particularly high potency (Weiner et al 2001). Many compounds showed antagonist activity at alpha$_1$-adrenergic receptors, fewer agents exhibited potent 5-HT$_6$ activity, while many, particularly risperidone, displayed potent inverse agonist activity at 5-HT$_7$ receptors. Clozapine, olanzapine, and a number of typical agents (e.g. thioridazine, data not shown), were found to possess potent muscarinic receptor antagonist properties. Importantly, no single antagonist activity differentiated clozapine from all other agents.

[0108] In contrast to the widespread antagonist activity of these compounds, very few agents possessed agonist activity. Figure 4A reports the results of the functional agonist screen of this compound library at the human M1 muscarinic acetylcholine receptor. Only four compounds, the known muscarinic receptor agonists arecoline and carbachol, moperone
and N-desmethyclozapine (NDMC), the major metabolite of clozapine (Gauch and Michaelis 1971), were identified. Moperone displayed only a very low potency (EC50>1-micromolar) interaction. In contrast, NDMC displayed an EC50 of 100 nM with 80% efficacy (relative to carbachol) in this study. This result was further confirmed in a second functional assay, PI hydrolysis. As depicted in Figure 4B, clozapine displays limited agonist efficacy in this assay, precluding accurate potency determinations, whereas NDMC displayed high potency (93 +/- 22nM, n=3) and greater agonist efficacy (56 +/- 8%, n=3) relative to carbachol. In fact, when assayed against carbachol for competitive antagonist activity, clozapine behaved as an antagonist, while NDMC only partially reversed carbachol-induced PI hydrolysis (Figure 4C), consistent with the lack of an antagonistic response observed when NDMC was tested as a competitive antagonist at M1 receptors in R-SAT (Table 13). Finally, the agonist activity of NDMC was blocked by both atropine and clozapine (Figure 4D). These results confirm that NDMC is a potent, efficacious, M1 receptor agonist, distinguishing it from the M1 receptor antagonist properties of clozapine.

[0109] Having demonstrated the agonist activity of NDMC at human M1 receptors in multiple in vitro functional assays, we then profiled carbachol, clozapine, NDMC, olanzapine, the major olanzapine metabolite N-desmethylolanzapine, and the muscarinic agonist xanomeline (Shannon et al 1994), at all five human muscarinic receptor subtypes using R-SAT (Table 14).
Table 14  Muscarinic acetylcholine receptor agonist activity of antipsychotics.

Muscarinic receptor (M1-M5) agonist activity of clozapine, N-desmethylclozapine, olanzapine, N-desmethylolanzapine, xanomeline, and carbachol was determined using RSAT as previously described (Spalding et al 2002). Average efficacy (percentage relative to carbachol) and potency (pEC50) +/- standard error are reported for 3 or more replicate determinations. No response denotes the lack of agonist activity at concentrations up to 10-micromolar.

<table>
<thead>
<tr>
<th>Compound</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
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<tbody>
<tr>
<td></td>
<td>Efficacy</td>
<td>pEC50</td>
<td>Efficacy</td>
</tr>
<tr>
<td>Clozapine</td>
<td>24±3</td>
<td>7.63±0.37</td>
<td>65±8</td>
</tr>
<tr>
<td>N-desmethylclozapine</td>
<td>72±5</td>
<td>7.26±0.07</td>
<td>106±19</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>No response</td>
<td></td>
<td>No response</td>
</tr>
<tr>
<td>N-desmethylolanzapine</td>
<td>No response</td>
<td></td>
<td>No response</td>
</tr>
<tr>
<td>Xanomeline</td>
<td>121±6</td>
<td>7.20±0.08</td>
<td>106±9</td>
</tr>
<tr>
<td>Carbachol</td>
<td>101±2</td>
<td>6.11±0.03</td>
<td>101±5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compound</th>
<th>M4</th>
<th>M5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Efficacy</td>
<td>pEC50</td>
</tr>
<tr>
<td>Clozapine</td>
<td>57±5</td>
<td>7.35±0.10</td>
</tr>
<tr>
<td>N-desmethylclozapine</td>
<td>87±8</td>
<td>6.87±0.17</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>No response</td>
<td></td>
</tr>
<tr>
<td>N-desmethylolanzapine</td>
<td>No response</td>
<td></td>
</tr>
<tr>
<td>Xanomeline</td>
<td>116±9</td>
<td>7.46±0.14</td>
</tr>
<tr>
<td>Carbachol</td>
<td>96±3</td>
<td>6.53±0.05</td>
</tr>
</tbody>
</table>

[0110]  Clozapine was found to be a very weak partial agonist at M1 receptors, a more efficacious agonist at M2 and M4 receptors, and to lack agonist activity at M3 and M5 receptors. NDMC also displayed high potency interactions with all five human muscarinic receptors, but with increased agonist efficacy at M1, M4, and M5 receptors when compared to clozapine (Table 14). In contrast, olanzapine and N-desmethylolanzapine, both structurally related to clozapine and NDMC, lacked agonist activity at human muscarinic receptors. Interestingly, xanomeline displayed a muscarinic receptor profile that is similar to that observed for NDMC, with the notable exception of higher agonist efficacy at M3.
receptors. The agonist activities of clozapine, NDMC, and xanomeline at human muscarinic receptor subtypes are unique among all neuropsychiatric agents tested (Figure 4, and Tables 13 and 14).

[0111] The present inventors discovered that muscarinic receptor agonism, and M1 receptor agonism in particular, of NDMC can be achieved in vivo during pharmacotherapy with clozapine. Clozapine and NDMC were tested for their ability to increase the phosphorylation of mitogen-activated protein kinase (MAP kinase) in the CA1 region of mouse hippocampus, a response that has been shown to reflect M1 receptor activation (Berkeley et al 2001). As depicted in Figure 5, subcutaneous administration of vehicle (Figure 5A), clozapine (Figure 5B), or scopolamine alone (data not shown) fails to stimulate phosphorylation of hippocampal MAP kinase. In contrast, NDMC induced phosphorylation of MAP kinase in hippocampal neurons in a dose dependent manner (Figures 5C, 5D, and E), an effect that was blocked by pretreatment with scopolamine (Figure 5F). Quantification of this effect demonstrates statistically significant M1 receptor activation at NDMC doses of 30 mg/kg and greater (Figure 6). Clozapine fails to behave as an agonist under these experimental conditions, which likely reflects either insufficient metabolism to NDMC after acute administration in mouse, or direct antagonist effects at the M1 receptor as demonstrated in the in vitro studies. These data confirm that NDMC passes the blood brain barrier and activates hippocampal M1 receptors in vivo.

[0112] It has long been appreciated that antagonism of central muscarinic receptors can attenuate the EPS induced by antipsychotics (Miller and Hiley 1974). Initial investigations of the anti-muscarinic properties of antipsychotics defined the high potency of clozapine for these receptors in rodent brain, and elucidated the inverse correlation between muscarinic receptor antagonism and propensity to induce EPS (Snyder et al 1974). Following the elucidation of five muscarinic acetylcholine receptor subtypes (Bonner et al 1987), clozapine was described as a potent competitive antagonist (Bolden et al 1991). Functional studies in various cell lines subsequently documented that clozapine has significant agonist activity at M2 and M4 receptors, and low agonist efficacy at M1 receptors (Zorn et al 1994 and Olianias et al 1999), consistent with the results reported herein. In
humans, clozapine has two major metabolites, NDMC and clozapine-N-oxide (Gauch and Michaelis 1971). After steady state dosing, NDMC represents a large proportion of total detectable moieties, with concentrations ranging from 20-150% of that observed for clozapine, with mean values of 60-80% (Bondesson and Lindstrom 1988 and Perry et al 1991). That NDMC is an active metabolite is supported by the present data, as well as by prior reports documenting D₁, D₂, and 5-HT₂C receptor competitive antagonist activity (Kuoppamaki et al 1993), and a recent report of M₁ receptor agonist activity (Sur et al 2003). In contrast, the other major clozapine metabolite, clozapine-N-oxide, displays only very low potency (pKᵢ's < 6.0) functional activity at human monoaminergic receptors (data not shown). While varying degrees of brain penetration of NDMC have been reported in rodents (Baldessarini et al 1993 and Weigmann et al 1999), the present results, the observation that systemically administered NDMC activates cFOS expression in rodent brain (Young et al 1998), and the detection of NDMC in human cerebrospinal fluid following parenteral administration of clozapine (Nordin et al 1995), demonstrate that NDMC is brain penetrant and centrally active.

[0113] The present inventors have discovered that clozapine, acting through its predominant metabolite NDMC, functions as a direct acting muscarinic receptor agonist in vivo. During pharmacotherapy with clozapine, the agonist actions of NDMC is attenuated by the antagonistic actions of the parent compound. Thus, high NDMC levels, and particularly high NDMC/clozapine ratios, increases agonist efficacy at muscarinic receptors, as predicted by mass action and by agonist/antagonist mixing studies (Brauner-Osborne et al 1996). Clinical data support this notion. Not only does clozapine therapy usually lack the traditional anti-cholinergic side effects of dry mouth, blurred vision, and urinary retention common to classical muscarinic antagonists, it is unique in its ability to frequently produce sialorrhea (Baldessarini and Frankenburg 1991), an effect that can be blocked by the muscarinic antagonist pirenzepine (Fritze and Elliger 1995). Thus, the muscarinic receptor agonist activity of NDMC likely mediates this peripheral effect, while the muscarinic receptor subtype responsible is still unknown, receptor subtypes in addition to the M3 have been implicated (Bymaster et al 2003).
The muscarinic agonist properties of NDMC reported herein underlies some of the unique central effects of treatment with clozapine. Multiple lines of evidence support a pro-cognitive effect of potentiating central cholinergic neurotransmission, including the clinical effects of acetylcholinesterase inhibitors and direct acting muscarinic receptor agonists (Davis et al 1993). High dose clozapine therapy in treatment refractory schizophrenics may actually serve to raise brain levels of NDMC to achieve central muscarinic receptor agonist activity, particularly M1 receptor stimulation, rather than recruiting additional lower potency receptor interactions that clozapine and NDMC possess (Table 13). Thus, NDMC/clozapine ratios are a better predictor of therapeutic response to clozapine, particularly for cognition, than absolute clozapine levels.

The data on clozapine and NDMC plasma levels and clinical response that were prospectively gathered as part of two clinical trials which included 59 neuroleptic resistant patients (Hasegawa et al 1993), and 33 neuroleptic responsive patients (Lee et al 1999) with schizophrenia were re-analyzed. Patients were classified as treatment resistant or not by standard criteria (Kane et al 1988), and clinical ratings and neuropsychological test scores were obtained by trained raters who were blinded to plasma drug levels. The mean daily dosages of clozapine, as well as clozapine and NDMC serum levels, and NDMC/Clozapine ratios after 6 weeks and 6 months of treatment are reported in Table 15A.

**Table 15** Serum N-desmethylclozapine levels and clinical response in schizophrenia.

Statistical analysis of the correlation between clinical outcome and serum levels of clozapine and N-desmethylclozapine (NDMC) for a cohort of 92 clozapine treated schizophrenics are reported. Table 15A reports the clozapine dose, clozapine level, NDMC levels, and NDMC/clozapine ratios for all treatment resistant (TR) subjects, responders, non-responders, and all subjects at 6 weeks and 6 months. \( P^* \) reports statistically significant differences between responders and non-responders. Table 15B reports the major relationships of interest for the prediction of the contribution of NDMC to response to clozapine treatment, including quality of life, negative symptoms, and cognition, analyzed by multiple linear regression. \( R^{2*} \) refers to the model applied. Abbreviations used include: NS-not significant, BPRS-Brief Psychiatric Rating Scale, SANS-Scale for the Assessment of Negative Symptoms, SAPS- Scale for the Assessment of Positive Symptoms, WISC-Wisconsin Card Sorting Test.
### Table 15A

<table>
<thead>
<tr>
<th>Drug Measure</th>
<th>All TR Subjects (59)</th>
<th>Responders (26)</th>
<th>Non-Responders (25)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/day)</td>
<td>498+/=190</td>
<td>485+/=205</td>
<td>433+/=178</td>
<td>NS</td>
</tr>
<tr>
<td>NDMC Level (ng/ml)</td>
<td>260+/=203</td>
<td>308+/=243</td>
<td>171+/=123</td>
<td>0.01</td>
</tr>
<tr>
<td>Clozapine Level (ng/ml)</td>
<td>393+/=301</td>
<td>453+/=328</td>
<td>268+/=207</td>
<td>0.02</td>
</tr>
<tr>
<td>NDMC/Clozapine</td>
<td>0.75+/=0.36</td>
<td>0.70+/=0.22</td>
<td>0.81+/=0.48</td>
<td>NS</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drug Measure</th>
<th>All Subjects at 6 Weeks (86)</th>
<th>All Subjects at 6 Months (92)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/day)</td>
<td>369+/=169</td>
<td>417+/=197</td>
</tr>
<tr>
<td>NDMC Level (ng/ml)</td>
<td>194+/=136</td>
<td>235+/=190</td>
</tr>
<tr>
<td>Clozapine Level (ng/ml)</td>
<td>287+/=190</td>
<td>365+/=285</td>
</tr>
<tr>
<td>NDMC/Clozapine</td>
<td>0.83+/=1.08</td>
<td>0.71+/=0.30</td>
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</tbody>
</table>

### Table 15B

<table>
<thead>
<tr>
<th>Clinical Measure</th>
<th>Beta</th>
<th>F</th>
<th>P</th>
<th>r²**</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dependent Variable: 6 Weeks</td>
<td>-0.52</td>
<td>3.73</td>
<td>0.06</td>
<td>0.32</td>
<td>3.73</td>
</tr>
<tr>
<td>BPRS-Withdrawal/Retardation</td>
<td>-0.28</td>
<td>5.65</td>
<td>0.02</td>
<td>0.26</td>
<td>3.65</td>
</tr>
<tr>
<td>SANS Attentional Impairment</td>
<td>-1.00</td>
<td>3.87</td>
<td>0.05</td>
<td>0.60</td>
<td>3.55</td>
</tr>
<tr>
<td>SAPS Global Delusions</td>
<td>17.50</td>
<td>5.20</td>
<td>0.03</td>
<td>0.50</td>
<td>2.40</td>
</tr>
<tr>
<td>Quality of Life Scale: Total</td>
<td>2.91</td>
<td>7.10</td>
<td>0.01</td>
<td>0.43</td>
<td>2.40</td>
</tr>
<tr>
<td>Quality of Life Scale: Objects and Activities</td>
<td>13.80</td>
<td>14.84</td>
<td>0.01</td>
<td>0.54</td>
<td>2.39</td>
</tr>
<tr>
<td>Quality of Life Scale: Instrumental Role</td>
<td>2.27</td>
<td>4.10</td>
<td>0.05</td>
<td>0.75</td>
<td>4.33</td>
</tr>
<tr>
<td>WISC-R Maze</td>
<td>7.45</td>
<td>6.75</td>
<td>0.01</td>
<td>0.47</td>
<td>4.47</td>
</tr>
<tr>
<td>Dependent Variable: 6 Months</td>
<td>1.35</td>
<td>3.67</td>
<td>0.06</td>
<td>0.47</td>
<td>3.48</td>
</tr>
</tbody>
</table>

[0116] Both time points were analyzed because improvement in psychopathology and cognition with clozapine may take six months or longer (Hagger et al 1993). Thirteen of the 92 patients (14.1%) had NDMC/clozapine ratios >=1. Of these thirteen patients, the highest ratio was 1.77 and the median was 1.05. The Spearman rank order correlation between clozapine and NDMC levels was 0.82 and 0.89 at 6 weeks and 6 months, respectively (P=0.0001). The correlation between NDMC/clozapine ratios at 6 weeks and 6 months was 0.92 (P= 0.0001), indicating remarkable stability of NDMC/clozapine ratios.
within subjects. Importantly, dose and NDMC/clozapine ratios were not significantly correlated at either time point (rho<0.10) in neither the neuroleptic-resistant nor neuroleptic-responsive patients.

[0117] Stepwise multiple-regression were utilized to determine the best predictors of outcome from each of these measures, including baseline levels of the dependent measure, dose, age, and gender, since all have been shown to significantly predict response to clozapine (Table 15B).

[0118] In all the models tested, baseline levels of the dependent measure predicted the largest share of the variance in the model. The NDMC/clozapine ratio was the next most frequent predictor of response; the ratio significantly predicted response in 8/24 (33.3%) of the models, all in the expected direction: the higher the ratio, the better the outcome. This result contrasts with the lack of predictive power of clozapine levels alone, NDMC levels alone, or their sum. The exception was that higher NDMC levels alone predicted greater improvement in two subscales of the Quality of Life scale (Heinrichs et al, 1984) (data not shown). As shown in Table 15B, higher NDMC/clozapine ratio predicted improvement in multiple measures of cognition, as well as the Scale for the Assessment of Negative Symptoms-Attention subscale, which has been suggested to be more related to cognition than negative symptoms. The ratio also predicted improvement in Quality of Life-total score, including the Instrumental Role Function factor, which has been shown to be dependent upon cognitive status (Green 1996), and negative symptoms, which have been found to correlate with cognition. The ratio also predicted improvement in delusions, but not hallucinations, with clozapine treatment. Dose did not contribute to the prediction of any of the models in Table 15B. Dose is significantly correlated with plasma levels of clozapine and NDMC (P<0.01-0.001) but not, as noted above, with the NDMC/clozapine ratio. This provides further evidence that the absolute levels of clozapine and NDMC, while important in identifying responders and non-responders (Fabrazzo et al 2002) are not as important as their ratio when baseline levels of the dependent measure are included in the model. Although additional analyses in larger cohorts are necessary, this analysis, as well as recent reports (Frazier et al 2003 and Mauri et al 2003) all suggest that the NDMC/clozapine ratio is
a better predictor of clinical response to clozapine than clozapine levels alone, and support the hypothesis that NDMC is a critical mediator of clozapine action.

[0119] The muscarinic receptor agonist properties of NDMC also contribute to the efficacy of clozapine therapy against positive symptoms. Not only did high NDMC/clozapine ratios predict response to delusions as noted above, but additional support comes from the observation that there are several similarities between the central effects of muscarinic receptor agonists and dopamine D2 receptor antagonists (Pfeiffer and Jenney 1957 and Mirza et al 2003). For example, behavioral pharmacological experiments with mice harboring targeted deletions of each of the five muscarinic receptor subtypes have shown that the M1 receptors plays a central role in DA-mediated behaviors (Gerber et al 2001). In addition, xanomeline (which displays some selectivity for M1 and M4 receptors) inhibits amphetamine-induced locomotion (Shannon et al 2000). Clinically, xanomeline was found to diminish hallucinosis and aggression in Alzheimer’s Disease patients (Bodick et al 1997), and has been shown to display activity against both positive and negative symptoms in a recent, small, Phase 2 study in schizophrenia (Schekhar et al, unpublished data).

[0120] The central dopaminergic and muscarinic cholinergic systems are well known to be functionally interrelated (Miller and Hiley 1974). The muscarinic antagonist properties of clozapine are thought to contribute to its low propensity to cause EPS, yet the anti-EPS effects of clozapine are more robust than those obtained by the adjunctive use of anticholinergics agents like trihexyphenidyl, and some EPS producing antipsychotics, e.g. thioridazine, also possess potent muscarinic receptor antagonist properties. These observations suggest that although antagonism of central muscarinic receptors can confer anti-EPS effects, cholinergic modulation of the motoric effects of D2 receptor blockade are more complex than previously appreciated. Present data show that agonism, not antagonism, of certain muscarinic receptor subtypes expressed within critical basal ganglia structures (Weiner et al 1990), are a more efficacious mechanism to lessen these adverse motor effects. Further, the widespread use of adjunctive anticholinergics should be reevaluated in light of the present data on the pro-cognitive benefits conferred by the central muscarinic receptor agonist properties of NDMC.
[0121] In summary, functional characterization of therapeutically useful neuropsychiatric drugs has revealed the potent, efficacious, muscarinic receptor agonist activity of NDMC. This activity was found to be unique among neuropsychiatric agents as a class. It is demonstrated that NDMC can cross the blood brain barrier and function as an M1 receptor agonist in vivo. Consideration of the contribution of NDMC to improvement in cognition and quality of life in clozapine treated patients shows that NDMC mediates clinically relevant aspects of treatment response that differentiate clozapine from other agents used to treat schizophrenia. These findings show that muscarinic receptor agonism mediates the unique clinical properties of clozapine, and that M1 muscarinic receptor agonists (Spalding et al 2002), including NDMC itself, may be efficacious atypical antipsychotic agents.

Example 14: Net Agonism in N-desmethylclozapine/Clozapine Mixtures

[0122] The effect of mixtures of clozapine and N-desmethylclozapine was evaluated using an R-SAT assay as described above. 150 nM of N-desmethylclozapine was provided with varying concentrations of clozapine. Figure 7 depicts the results of the R-SAT assay as a function of clozapine concentration. As indicated by the dotted line in Figure 7, net agonistic activity was observed for clozapine concentrations of about 100 nM and below. Thus, ratios of NDMC to clozapine of about 1.5 and greater provide a net agonistic effect.

[0123] The results of the R-SAT assay were confirmed using a PI hydrolysis assay as described above. 150 nM of N-desmethylclozapine was again provided with varying concentrations of clozapine. Figure 8 depicts the results of the assay as a function of clozapine concentration. The dotted line in Figure 8 indicates the maximum concentration of clozapine for which a net agonistic effect is observed. Similar to the results of the R-SAT assay, net agonistic activity was observed for clozapine concentrations of about 100 nM and below, thus confirming that a ratio of NDMC to clozapine of about 1.5 and greater provide a net agonistic effect.

Example 15: Administration of Single Doses of NDMC to Schizophrenic Patients
[0124] A single-center, in-patient, randomized, double blind, placebo controlled, single dose study is conducted on two sequential group of patients. Different groups of 4-6 patients each are enrolled. Each patient receives single doses of placebo and two different doses of NDMC sequentially in random order with a washout between administrations. The doses range from 25 mg – 250 mg of NDMC. The NDMC and placebo is administered orally as a powder in a gelatin capsule. Male or female patients, 20 to 50 years of age, with a history of schizophrenia or schizoaffective disorder, who are otherwise in good health are selected for the study. The patients are not experiencing acute exacerbation of severe psychosis, defined as a Positive and Negative Syndrome Scale (PANSS) score greater than 75.

[0125] Patients are withdrawn from all centrally active medications during a lead-in period of 4-7 days prior to study start on Study Day -1. On Study Day -1, patients are randomized to a schedule of NDMC:placebo in a 2:1 manner. On Study Day 1, patients receive study drug or placebo, orally, in the morning, and serial blood samples are collected up to 24 h after dose administration. Patients are monitored for 8 hr post-dose by continuous lead II ECG monitoring. Clinical evaluation, clinical rating scales, and frequent assessments of safety including vital signs, ECGs, clinical labs, and adverse event recording are performed periodically throughout Study Day. No study drug is given on Study Days 2 and 3. On Study Day 4, subjects once again receive study drug or placebo, orally, in the morning, and serial blood samples are collected up to 24 h after dose administration. Patients are monitored for 8 h post-dose by continuous lead II ECG monitoring. Clinical evaluation, clinical rating scales, and frequent assessments of safety including vital signs, ECGs, clinical labs, and adverse event recording are performed periodically throughout the Study Day 4. No study drug or placebo is given on Study Days 5 and 6. On Study Day 8, patients receive study drug or placebo, orally, in the morning, and serial blood samples are collected up to 24 h after dose administration. Patients are monitored for 8 h post-dose by continuous lead II ECG monitoring. Clinical evaluation, clinical rating scales, and frequent assessments of safety including vital signs, ECGs, clinical labs, and adverse event recording are performed periodically throughout the Study Day 8. A final End of Study evaluation is performed 3-5
days following Study Day 8 and a clinical evaluation, administration of clinical rating scales, and a safety assessment are performed.

[0126] An interim analysis of the safety variables and pharmacokinetic data obtained from Group 1 is conducted after the End of Study evaluation and before the randomization of Group 2. Safety variables are reviewed by the PI in order to determine the doses to be administered in Group 2. If NDMC administration during Group 1 is deemed safe the second patient cohort is screened, randomized, and enrolled. If the doses of NDMC in Group 2 are greater than those administered in Group 1, then, during the lead-in period, a pre-conditioning dose of 25 mg of NDMC is given to each subject. This test dose is used to identify any patient who may be particularly sensitive to higher doses of NDMC, and is administered at least 3 days prior to Day -1. Study related procedures for Group 2 is identical to those of Group 1, with the exception of the NDMC dose.

Pharmacokinetic Analysis

[0127] Plasma samples are analyzed for concentrations of NDMC. Pharmacokinetic parameters are calculated including $C_{\text{max}}$ (maximum plasma concentration), $t_{\text{max}}$ (time to maximum plasma concentration), $\text{AUC}_{0-\infty}$ (area under the plasma concentration time curve from time zero to the last quantifiable timepoint, calculated by linear-log trapezoidal summation), $\text{AUC}_{0-\infty}$ (area under the plasma concentration time curve from time zero to infinity, calculated by linear-log trapezoidal summation and extrapolated to infinity by addition of the last quantifiable plasma concentration divided by the elimination rate constant $\lambda_Z$), $\lambda_Z$ (elimination rate constant, determined by linear regression of the terminal points of the log-linear plasma concentration-time curve), $t_{1/2}$ (terminal half-life, determined as $\ln(2)/\lambda_Z$), and $\text{CI}_{\text{po}}$ (apparent oral clearance, calculated by $\text{Dose} / \text{AUC(0-}\infty)$).

Tolerability

[0128] Tolerability of NDMS is determined by measuring extrapyramidal (EPS) motor effect using the Simpson and Angus Scale (SAS) and the Barnes Akathisia Scale (BAS). These scales are administered at baseline (Study Day -1), 3-5 hours after drug administration on Study Days 1, 4, and 8, and at the End of Study evaluation.
[0129] The results indicate that NDMC is well tolerated for single doses up to 200 mg and 250 mg.

**Antipsychotic efficacy**

[0130] Antipsychotic efficacy is measured using the PANSS and the Clinical Global Impression Scale-Schizophrenia (CGI-S) measures. These scales are administered at baseline (Study Day -1), on Study Days 1, 4, and 8, and at the End of Study evaluation.

[0131] Reductions in total PANSS score were evident even after single doses.

**Safety**

[0132] Safety is evaluated by measuring vital signs including 3-positional blood pressure and pulse rate (5 minute supine, 1 minute sitting, 3 minutes standing), respiratory rate, and oral temperature except during screening and post-study procedures.

[0133] 12-lead ECGs are recorded and standard electrocardiogram parameters including QRS, PR, QT, and QTc intervals are measured. In addition, continuous lead-II ECG monitoring is performed for the first 8 hours of Day 1, 4, and 8 following each NDMC or placebo dose administration.

[0134] A neurological screen is conducted by the clinically responsible physician at the clinic. The neurological screen consists of a qualitative assessment of muscle tone in the extremities, the presence of tremors, fasciculations, and nystagmus, and various tests of cerebellar coordination (finger nose test, dysdiadochokinesia, heel-shin test, and gait).

[0135] Clinical laboratories are measured after at least an 8-hour fast on Study Days 1, 4, 8, and the End of Study evaluation and include the following:

[0136] Erythrocytes: RBC count, hematocrit, hemoglobin, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), RDW, and reticulocyte count.

[0137] Leukocytes: WBC count and differential (basophils, eosinophils, lymphocytes, monocytes, and neutrophils) reported as absolute values.

[0138] Coagulation: platelet count, PT as INR, and aPTT.
Liver: alkaline phosphatase, ALT (serum glutamic-pyruvic transaminase [SGPT]), AST (serum glutamic-oxaloacetic transaminase [SGOT]), bilirubin (total, direct, indirect), gamma-glutamyl transferase (GGTP), creatine phosphokinase (CPK) and LDH.

Renal: blood urea nitrogen (BUN), creatinine, and uric acid.

Electrolytes: carbon dioxide, chloride, magnesium, potassium, and sodium.

General: albumin, calcium, glucose (fasting) phosphate, and protein (total).

Endocrine: prolactin.

Lipids: cholesterol (total), HDL cholesterol, LDL cholesterol, and triglycerides.

Macroscopic urinalysis: pH, specific gravity, glucose, ketones, leukocyte esterase, nitrites, occult blood, and protein.

Microscopic urinalysis: RBC/high powered field, WBC/high powered field, bacteria, castes, epithelial cells, mucous threads and crystals.

The results indicate that NDMC is safe at single doses up to 200 mg and 250 mg.

Example 16: Administration of Multiple Doses of NDMC to Schizophrenic Patients

A single-center, in-patient, randomized, double blind, placebo controlled, multiple dose study is conducted. Forty patients are enrolled and assigned to one of six cohorts. Each patient receives either placebo or NDMC daily for fourteen days (with a four day titration). The NDMC and placebo is administered orally as a powder in a gelatin capsule. Male or female patients, 20 to 50 years of age, with a history of schizophrenia or schizoaffective disorder, who are otherwise in good health are selected for the study. The patients are not experiencing acute exacerbation of severe psychosis, defined as a Positive and Negative Syndrome Scale (PANSS) score greater than 75.

Patients are withdrawn from all centrally active medications during the lead-in period of 4-7 days prior to study start on Study Day -1. During the lead-in portion of the study, patients not receiving placebo are administered a lower dose of NDMC for 4 days,
followed by a final dose for 10 days starting on Study Day 1. The dosages administered are indicated in Table 16.

Table 16. Lead-in and final doses administered in multiple dose study.

<table>
<thead>
<tr>
<th>Lead-in period dosage (4 days)</th>
<th>Final dosage (10 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 mg twice daily</td>
<td>50 mg twice daily</td>
</tr>
<tr>
<td>50 mg twice daily</td>
<td>100 mg twice daily</td>
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<tr>
<td>100 mg twice daily</td>
<td>150 mg twice daily</td>
</tr>
<tr>
<td>150 mg twice daily</td>
<td>200 mg twice daily</td>
</tr>
<tr>
<td>By titration</td>
<td>300 mg twice daily</td>
</tr>
<tr>
<td>By titration</td>
<td>400 mg twice daily</td>
</tr>
</tbody>
</table>

0150 On Study Day 1, patients receive study drug or placebo, orally, and serial blood samples are collected up to 24 hours after dose administration. Patients are monitored for 8 hr post-dose by continuous lead II ECG monitoring. Clinical evaluation, clinical rating scales, and frequent assessments of safety including vital signs, ECGs, clinical labs, and adverse event recording are performed periodically throughout Study Day 1.

0151 Patients receive study drug or placebo daily for the next nine days. Pre-dose serum sampling for pharmacokinetic analysis are obtained, and patients are monitored for 8 hr post-dose by continuous lead II ECG monitoring. Clinical evaluation, clinical rating scales, and frequent assessments of safety including vital signs, ECGs, clinical labs, and adverse event recording are performed periodically throughout Study Days 2-10.

0152 On Study Day 10, pre-dose serum sampling as well as serial blood samples collected up to 24 hours after study drug or placebo administration are obtained for pharmacokinetic analysis, and patients are monitored for 8 hr post-dose by continuous lead II ECG monitoring. Clinical evaluation, clinical rating scales, and frequent assessments of safety including vital signs, ECGs, clinical labs, and adverse event recording are performed periodically throughout Study Day 10.

0153 A final End of Study evaluation is conducted 5-7 days after the cessation of active dosing. A safety assessment is performed during this clinical evaluation, including vital signs, ECG, clinical labs, NDMC serum determination, and adverse event recording.
All patients are followed clinically, as in-patients, for as long as is indicated following the cessation of active dosing of NDMC.

[0154] An interim analysis of the safety variables and pharmacokinetic data from Group 1 is conducted after the End of Study evaluation, and before the randomization of Group 2. Safety variables are reviewed by the PI in order to determine the doses to be administered in Group 2. If NDMC administration during Group 1 is deemed safe, the second patient cohort is screened, randomized, and enrolled. Study related procedures for Group 2 are identical to those of Group 1, with the exception of NDMC dose and/or frequency of administration.

Pharmacokinetic Analysis

[0155] Plasma samples are analyzed for concentrations of NDMC. Pharmacokinetic parameters are calculated following the single dose administration on Day 1 including $C_{\text{max}}$ (maximum plasma concentration), $t_{\text{max}}$ (time to maximum plasma concentration), $AUC_{0-\infty}$ (area under the plasma concentration time curve from time zero to the last quantifiable timepoint, calculated by linear-log trapezoidal summation), $AUC_{0-\infty}$ (area under the plasma concentration time curve from time zero to infinity, calculated by linear-log trapezoidal summation and extrapolated to infinity by addition of the last quantifiable plasma concentration divided by the elimination rate constant $\lambda_z$), $\lambda_z$ (elimination rate constant, determined by linear regression of the terminal points of the log-linear plasma concentration-time curve), $t_{1/2}$ (terminal half-life, determined as $\ln(2)/\lambda_z$), and $CL_{po}$ (apparent oral clearance, calculated by $\text{Dose} / AUC(0-\infty)$).

[0156] Pharmacokinetic parameters are also calculated following the last dose including $C_{\text{max,ss}}$ (maximum steady-state plasma concentration), $C_{\text{min,ss}}$ (minimum steady-state plasma concentration), $C_{\text{avg,ss}}$ (average steady-state plasma concentration calculated as $AUC(0-\tau)_{\text{ss}}$ divided by the dosing interval $\tau$), $t_{\text{max,ss}}$ (time to maximum steady-state plasma concentration), $t_{\text{min,ss}}$ (time to minimum steady-state plasma concentration), $AUC_{0-\tau}$ (area under the plasma concentration time curve from time zero to the last quantifiable timepoint, calculated by linear-log trapezoidal summation), $AUC_{0-\tau ss}$ (area under the plasma concentration time curve from time zero to the end of the steady-state dosing interval,
calculated by linear-log trapezoidal summation), $\lambda_{Z,ss}$ (steady-state elimination rate constant, determined by linear regression of the terminal points of the log-linear plasma concentration-time curve), $t_{1/2,ss}$ (steady-state terminal half-life, determined as $\ln(2)/\lambda_{Z,ss}$), and $\text{CL}_{po,ss}$ (apparent oral clearance, calculated by $\text{Dose} / \text{AUC}(0-\infty)_{ss}$).

[0157] Pharmacokinetic analyses suggest that steady state is established after 6-7 days of dosing with an estimated half-life of 15-35 hours. The mean steady state trough levels at 200 mg twice daily are measured to be 362 ng/ml.

Tolerability

[0158] Tolerability of NDMS is determined by measuring extrapyramidal (EPS) motor effect using the Simpson and Angus Sacle (SAS) and the Barnes Akathisia Scale (BAS). These scales are administered at baseline (Study Day -1), 6 hours after drug administration on Study Days 1-10, and at the End of Study evaluation.

[0159] The results indicate that administration of 300 mg and less of NDMC twice daily is well tolerated.

Antipsychotic efficacy

[0160] Antipsychotic efficacy is measured using the PANSS and the Clinical Global Impression Scale-Schizophrenia (CGI-S) measures. These scales are administered at baseline (Study Day -1), on Study Days 1, 10, and at the End of Study evaluation.

[0161] An antipsychotic effect is apparent with subchronic dosing of 200 mg twice daily and higher.

Safety

[0162] Safety is evaluated by measuring vital signs including 3-positional blood pressure and pulse rate (5 minute supine, 1 minute sitting, 3 minutes standing), respiratory rate, and oral temperature except during screening and post-study procedures.

[0163] 12-lead ECGs are recorded and standard electrocardiogram parameters including QRS, PR, QT, and QTc intervals are measured. In addition, continuous lead-II ECG monitoring is performed for the first 8 hours of Days 1-10 following each NDMC or placebo dose administration.
[0164] A neurological screen is conducted by the clinically responsible physician at the clinic. The neurological screen consists of a qualitative assessment of muscle tone in the extremities, the presence of tremors, fasciculations, and nystagmus, and various tests of cerebellar coordination (finger nose test, dysdiadochokinesia, heel-shin test, and gait).

[0165] Clinical laboratories are measured after at least an 8-hour fast and include the following:

[0166] Erythrocytes: RBC count, hematocrit, hemoglobin, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), RDW, and reticulocyte count.

[0167] Leukocytes: WBC count and differential (basophils, eosinophils, lymphocytes, monocytes, and neutrophils) reported as absolute values.

[0168] Coagulation: platelet count, PT as INR, and aPTT.

[0169] Liver: alkaline phosphatase, ALT (serum glutamic-pyruvic transaminase [SGPT]), AST (serum glutamic-oxaloacetic transaminase [SGOT]), bilirubin (total, direct, indirect), gamma-glutamyl transferase (GGTP), creatine phosphokinase (CPK) and LDH.

[0170] Renal: blood urea nitrogen (BUN), creatinine, and uric acid.


[0174] Lipids: cholesterol (total), HDL cholesterol, LDL cholesterol, and triglycerides.

[0175] Macroscopic urinalysis: pH, specific gravity, glucose, ketones, leukocyte esterase, nitrites, occult blood, and protein.

[0176] Microscopic urinalysis: RBC/high powered field, WBC/high powered field, bacteria, casts, epithelial cells, mucous threads and crystals.

[0177] The results indicate that 300 mg and less of NDMC twice daily is safe with no dose-limiting adverse events.
Example 17: Administration of NDMC to Schizophrenic Patients

[0178] A randomized, 6-week, 3-arm, double-blind, placebo-controlled, multicenter study is conducted using subjects with schizophrenia who are experiencing an acute psychotic episode. The study includes a Screening Period of up to 8 days, a 6-week Treatment Period, and a 4-week Follow-Up Period. Subjects are enrolled who have a diagnosis of schizophrenia as defined by the Diagnostic and Statistical Manual of Mental Disorders (Text Revision) (DSM-IV-TR) Fourth Edition and as confirmed by administration of the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I). In order to be eligible to enter the study, a subject must have a Baseline PANSS total score of greater than or equal to 70 and a score of 4 (moderate) or higher on at least 2 of the following 4 positive symptom PANSS subscale items: delusions, conceptual disorganization, hallucinatory behaviors, and/or suspiciousness/persecution, and at least 1 of the 2 items must be hallucinations or delusions. Subjects also have a Baseline score of 4 (moderately ill) or greater on the CGI-S scale.

[0179] A total of approximately 246 subjects are randomized in a 1:1:1 ratio to receive either NDMC 100 mg bid (n = 82), NDMC 200 mg bid (n = 82), or placebo bid (n = 82).

[0180] The initial Screening Visit occurs no earlier than Day -8; therefore, the Screening Period may last as long as 8 days (Days -8 through -1). The Baseline Visit (Day 1) represents the first day of the 6-week Treatment Period; the final day of the Treatment Period is Day 43 (Week 6). A subsequent 4-week Follow-Up Period commences after completion of the Treatment Period (or Early Termination); the final Follow-Up Visit is scheduled to occur on Day 71 (Week 10). Therefore, each subject's participation in the study is anticipated to be a total of approximately 11 weeks. The end of the study is defined as the date of the last visit of the last subject in the study overall.

[0181] Subjects are admitted into the inpatient hospital setting at the initial Screening Visit and remain hospitalized for the entire Screening Period. Subsequently, all subjects remain in the inpatient hospital setting for at least the first 2 weeks (i.e., 15 days) of
the Treatment Period. If deemed clinically appropriate, subjects may be discharged from the inpatient hospital setting as early as Day 15 (after completion of scheduled Day 15 Visit assessments) and continue treatment with study drug on an outpatient basis for the remainder of the Treatment Period. Prior to discharge from the inpatient hospital setting, each subject must be deemed medically and psychiatrically stable and appropriate for the outpatient setting.

[0182] Screening Visit evaluations are performed do determine if any subject found to have a clinically significant laboratory abnormality at the Screening Visit should have the test repeated during the Screening Period. If an unacceptable abnormality is discovered, the subject is declared ineligible to enter the study. Subjects who are still receiving antipsychotic medications for psychosis at the initial Screening Visit are tapered off their existing antipsychotic medications during the Screening Period. Prior to beginning the Screening Period, subjects should not have been treated with any long-acting (depot) intramuscular (im) antipsychotic preparation within the time required for 1 cycle of treatment, plus 1 week (or 2 cycles plus 1 week for RISPERDAL CONSTA®). Subjects who at the initial Screening Visit are found not to be taking any antipsychotic medications due to non-compliance or otherwise will not require antipsychotic medication taper.

[0183] The Baseline Day (Day 1) will represent the first day of the Treatment Period (Days 1-43) and the first day of dosing with study drug. Baseline evaluations are conducted in the inpatient hospital setting on Day 1, prior to administration of the first dose of study drug on that day. Eligible subjects are randomized on Day 1.

[0184] For those subjects randomized to either of the 2 active drug treatment groups, the initial dose (double-blinded) of NDMC on Day 1 is 50 mg bid; subsequently, these subjects receive gradually increasing doses (double-blinded) of NDMC until the target dose (100 or 200 mg bid) of NDMC is reached. All effort is made for subjects to reach their designated target Maintenance dose of NDMC on or before the morning of Day 13.

[0185] The titration is flexible with a maximum duration of up to 13 days, allowing fixed dosing (maintenance) for at least 4 weeks. The titration doses of study medication are identified by Levels as shown in Table 17. All subjects start dosing at dose
Level 1 and continue for 2 days. After the initial 2 days of dosing at each dose Level, if well-tolerated, the dose is advanced to the next Level and the same procedure is followed through dosing Levels 1, 2, and 3. At the discretion of the Investigator and depending on the subject’s tolerance to that dose, each Level may be extended for up to an additional 2 days. Subjects requiring more than 2 days extension (i.e. more than 4 days total) at any dose Level due to AEs are withdrawn from the study. The maintenance dose may be reached as early as Day 7, but not later than Day 13. The maintenance dose is subsequently administered for the remainder of the Treatment Period.

Table 17. Titration schedule for 100 mg bid and 200 mg bid administration.

<table>
<thead>
<tr>
<th></th>
<th>100 mg bid</th>
<th>200 mg bid</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AM</td>
<td>PM</td>
<td>AM</td>
</tr>
<tr>
<td>Level 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extra Extra</td>
<td>Pbo 50 mg</td>
<td>Pbo 50 mg</td>
<td>Pbo 50 mg</td>
</tr>
<tr>
<td>Level 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extra Extra</td>
<td>Pbo 100 mg</td>
<td>Pbo 100 mg</td>
<td>Pbo 100 mg</td>
</tr>
<tr>
<td>Level 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extra Extra</td>
<td>Pbo 100 mg</td>
<td>Pbo 100 mg</td>
<td>Pbo 100 mg</td>
</tr>
<tr>
<td>Final Dose</td>
<td>Pbo 100 mg</td>
<td>Pbo 100 mg</td>
<td>100 mg 100 mg</td>
</tr>
</tbody>
</table>

AM = morning dose (2 capsules); PM = evening dose (2 capsules)
50 mg = NDMC 50 mg capsule; 100 mg = NDMC 100 mg capsule; Pbo = placebo capsule

[0186] After the target Maintenance dose is reached, subjects continue taking that fixed dose for the remainder of the Treatment Period; therefore, at Day 43, each active treatment subject will have received the target dose (NDMC 100 or 200 mg bid) for at least 4 weeks. During the titration and fixed-dose portions of the Treatment Period, subjects randomized to the placebo group receive capsules that are indistinguishable in appearance and number from those received by subjects in active treatment groups. All study drug is administered under double-blind conditions for all subjects throughout the entire 6-week Treatment Period (Days 1-43).
Throughout the Treatment Period, subjects take the supplied doses of study drug at approximately 8 AM and 8 PM each day; however, on study days that have scheduled Study Visit evaluations, the morning dose of study drug is delayed until after scheduled study assessments for that day are completed. The use of concomitant medication during the Treatment Period is minimal.

During the Treatment Period (Days 1-43), subjects undergo clinical evaluations at least weekly. Scheduled clinical evaluations during the Treatment Period occur at Baseline (Day 1) and at Study Visits on Days 8, 15, 22, 29, 36, and 43 (Weeks 1, 2, 3, 4, 5, and 6, respectively).

After cessation of administration of study drug (e.g., completion of Treatment Period or Early Termination), each subject completes 4 Follow-Up Visits to assess safety; Follow-Up Visits are scheduled weekly for 4 weeks after the last dose of study drug. Presuming a subject completes the Treatment Period, Follow-Up Visits are scheduled to occur on Days 50, 57, 64 and 71 (Weeks 7, 8, 9, and 10, respectively).

The primary efficacy parameter is the PANSS total score and the primary efficacy endpoint is the mean change from Baseline in the PANSS total score for each dose of NDMC versus placebo. The primary time point for assessing this primary efficacy parameter is the Endpoint, defined as the Day 43 (Week 6) Visit or the last PANSS total score collected during the Treatment Period (using the LOCF method).

Secondary efficacy parameters for this study are the change from Baseline in PANSS positive, negative, and general psychopathology subscales, CGI-S, and CDSS; additionally, treatment responders are identified using the PANSS total score (i.e., 20% decrease from Baseline) and the CGI-I (i.e., score of 1, 2, or 3). Change from Baseline and treatment responder scores are determined for the Day 43 (Week 6) Visit or the last assessment while on treatment using the LOCF method.

Exploratory parameters include the BACS (composite score) and FTND scales. Additionally, the PANSS-derived BPRS is explored. Post-Baseline cognition testing with the BACS for Early Termination subjects are conducted only if a subject has completed at least 4 weeks of treatment with study drug.
Literature Cited

[0193] Each of the following references is incorporated by reference herein in its entirety, including any drawings.

[0194] The following references are incorporated herein by reference in their entireties, including any drawings.


WHAT IS CLAIMED IS:

1. A method of treating psychosis of any origin, comprising orally administering to a subject about 100 mg of N-desmethylclozapine twice daily.

2. A method of treating psychosis of any origin, comprising orally administering to a subject about 200 mg of N-desmethylclozapine twice daily.

3. The method of claim 1 or 2, wherein the N-desmethylclozapine is administered via capsule or tablets, each comprising about 100 mg of N-desmethylclozapine.

4. A method of treating psychosis of any origin, comprising:
   orally administering to a subject about 100 mg of N-desmethylclozapine per day; and
   subsequently orally administering to a subject about 200 mg of N-desmethylclozapine per day.

5. The method of claim 4, wherein administering the about 100 mg of N-desmethylclozapine comprises administering about 50 mg of N-desmethylclozapine twice daily to the subject.

6. The method of claim 5, wherein administering the about 100 mg of N-desmethylclozapine comprises administering a tablet or capsule comprising about 50 mg of N-desmethylclozapine twice daily to the subject.

7. The method of claim 4, wherein administering the about 200 mg of N-desmethylclozapine comprises administering about 100 mg of N-desmethylclozapine twice daily to the subject.

8. The method of claim 7, wherein administering the about 200 mg of N-desmethylclozapine comprises administering a tablet or capsule comprising about 100 mg of N-desmethylclozapine twice daily to the subject.

9. A method of treating psychosis of any origin, comprising:
   orally administering to a subject about 100 mg of N-desmethylclozapine per day;
   subsequently orally administering to a subject about 200 mg of N-desmethylclozapine per day;
subsequently orally administering to a subject about 300 mg of N-desmethylclozapine per day; and
subsequently orally administering to a subject about 400 mg of N-desmethylclozapine per day.

10. The method of claim 9, where administering the about 100 mg of N-desmethylclozapine comprises administering about 50 mg of N-desmethylclozapine twice daily to the subject.

11. The method of claim 10, wherein administering the about 100 mg of N-desmethylclozapine comprises administering a tablet or capsule comprising about 50 mg of N-desmethylclozapine twice daily to the subject.

12. The method of claim 9, where administering the about 200 mg of N-desmethylclozapine comprises administering about 100 mg of N-desmethylclozapine twice daily to the subject.

13. The method of claim 12, wherein administering the about 200 mg of N-desmethylclozapine comprises administering a tablet or capsule comprising about 100 mg of N-desmethylclozapine twice daily to the subject.

14. The method of claim 9, where administering the about 300 mg of N-desmethylclozapine comprises administering about 150 mg of N-desmethylclozapine twice daily to the subject.

15. The method of claim 14, wherein administering the about 300 mg of N-desmethylclozapine comprises administering a first tablet or capsule comprising about 100 mg of N-desmethylclozapine and a second tablet or capsule comprising about 50 mg of N-desmethylclozapine twice daily to the subject.

16. The method of claim 9, where administering the about 400 mg of N-desmethylclozapine comprises administering about 200 mg of N-desmethylclozapine twice daily to the subject.

17. The method of claim 16, wherein administering the about 400 mg of N-desmethylclozapine comprises administering a first tablet or capsule comprising about 100
mg of N-desmethylclozapine and second tablet or capsule comprising about 100 mg of N-
desmethylclozapine twice daily to the subject.

18. The method of any one of the preceding claims, wherein the administration of
N-desmethylclozapine improves cognitive impairment.

19. The method of any one of the preceding claims, wherein the subject has
schizophrenia.

20. A kit, comprising:
   a tablet or capsule comprising about 100 mg of N-desmethylclozapine; and
   instructions to administer the tablet or capsule twice daily.

21. A kit, comprising:
   a tablet or capsule comprising about 100 mg of N-desmethylclozapine; and
   instructions to administer two of the tablets or capsules twice daily.

22. A kit, comprising:
   a tablet or capsule comprising about 100 mg of N-desmethylclozapine; and
   instructions to administer the tablet or capsule twice daily for a first period of
time and then subsequently administer two of the tablets or capsules twice daily.

23. A kit, comprising:
   a tablet or capsule comprising about 100 mg of N-desmethylclozapine; and
   instructions to administer the tablet or capsule once daily for a first period of
time and the subsequently administer the the tablet or capsule twice daily.
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. A61K31/5513 A61P25/18

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

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

A61K

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

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

EPO-Internal, WPI Data, EMBASE, BEILSTEIN Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
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</table>

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:

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

**E** earlier document but published on or after the international filing date

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

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

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

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

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

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

**A** document member of the same patent family

**Date of the actual completion of the international search**

13 November 2007

**Date of mailing of the international search report**

23/11/2007

**Name and mailing address of the ISA**

European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk

Tel: (+31-70) 340-2040, Telex 31 651 epo nl, Fax: (+31-70) 340-3016

Authorized officer

Collura, Alessandra
### INTERNATIONAL SEARCH REPORT

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

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

1. **Claims Nos.:**
   - because they relate to subject matter not required to be searched by this Authority, namely:
     - Although claims 1-19 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

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

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

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

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

1. **As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.**

2. **As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of additional fees.**

3. **As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:**

4. **No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:**

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**Remark on Protest**

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

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Form PCT/ISA/210 (continuation of first sheet (2)) (April 2005)
Disclosed herein are methods to treat neuropsychiatric diseases including psychosis. Treatment is carried out by administering a therapeutically effective amount of N-desmethylclozapine to a patient suffering from a neuropsychiatric disease.
<table>
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<th>Patent document cited in search report</th>
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<th>Patent family member(s)</th>
<th>Publication date</th>
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