

In-vivo Pharmacology Assays - Efficacy

1. Addiction Assay

1.1 Schedule Induced Ethanol Polydipsia

In order to evaluate antiaddictive property of NCE under evaluation, four groups of animals (Control, Positive Control Group, Two dose levels of test compound; n=10) will be subjected to schedule induced ethanol polydipsia and alcohol consumption will be estimated.

2. Anxiety Assays

2.1 Elevated Plus maze

In order to evaluate anxiogenic or anxiolytic property of NCE under evaluation, four groups of animals (Control, Positive Control Group, Two dose levels of test compound; n=10) will be subjected to elevated plus maze assay and time spent in the open arms, % time in open arm, time in closed arm, number of open arm entries and % open arm visit will be analyzed.

2.2 Hole Board

In order to evaluate anxiolytic property of NCE under evaluation, four groups of animals (Control, Positive Control Group, Two dose levels of test compound; n=10) will be subjected to hole board assay and latency of head dips, number of head dips and cumulative time of head dips will be analyzed.

2.3 Vogel conflict

In order to evaluate anxiogenic or anxiolytic property of NCE under evaluation, six groups of animals (Control, Positive Control Group I, Positive Control Group II, Three dose levels of test compound; n=10) will be subjected to conflict test assay and number of shocks received will be analyzed.

3. Cognition Assays

3.1 Water maze – Spatial memory

In order to evaluate cognition-enhancing property of NCE under evaluation, five groups of animals (Control, Positive Control Group, Three dose levels of test compound; n=12) will be subjected to test and latency to reach the platform will be the dependant measure during acquisition trials. Swim speed and path length will be measured in acquisition trials. Latency to reach the target, time spent in the target quadrant and latency to the quadrant, which previously contained the platform, will be measured in probe trial will be analyzed.

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3.2 Water maze – Working memory

In order to evaluate cognition-enhancing property of NCE under evaluation, five groups of animals (Control, Positive Control Group, Three dose levels of test compound; n=12) will be subjected to test and latency to reach the platform, swim speed and path length will be analyzed.

3.3 NORT – Scopolamine induced

In order to evaluate cognition-enhancing property of NCE under evaluation, three groups of animals (Control, Positive Control Group, One dose levels of test compound; n=12) will be subjected to test and time spent with the novel, familiar object and discriminative index will be analyzed.

3.4 NORT – Time induced

In order to evaluate cognition-enhancing property of NCE under evaluation, four groups of animals (Control, Positive Control Group, Two dose levels of test compound; n=12) will be subjected to test and time spent with the novel, familiar object and discriminative index will be analyzed.

3.5 Radial arm maze

In order to evaluate cognition-enhancing property of NCE under evaluation, three groups of animals (Control, Positive Control Group, One dose levels of test compound; n=12) will be subjected to test and choice accuracy and the total error will be analyzed.

3.6 T-maze

In order to evaluate cognition-enhancing property of NCE under evaluation, three groups of animals (Control, Positive Control Group, One dose levels of test compound; n=12) will be subjected to test and choice accuracy will be analyzed.

4. Depression Assays

4.1 Apomorphine induced hypothermia

In order to evaluate antidepressant property of NCE under evaluation, four groups of animals (Control, Chemical challenger, Positive Control Group, one dose levels of test compound; n=10) will be subjected to test and change in body temperature will be analyzed.

4.2 Dominant submissive assay

In order to evaluate antidepressant property of NCE under evaluation, three groups of animals (Control, Two dose levels of test compound; n=10) will be subjected to test and dominance level and feeding scores will be analyzed.

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4.3 DRL-72s assay

In order to evaluate antidepressant property of NCE under evaluation, four groups of animals (Control, Positive Control Group, two dose levels of test compound; n=12) will be subjected to test and response efficiency, IRT, response and reinforces will be analyzed.

4.4 Forced swim test

In order to evaluate antidepressant property of NCE under evaluation, three groups of animals (Control, Positive Control Group, one dose levels of test compound; n=10) will be subjected to test and immobility time will be analyzed.

4.5 Reserpine induced hypothermia

In order to evaluate antidepressant property of NCE under evaluation, four groups of animals (Control, Positive Control Group, two dose levels of test compound; n=10) will be subjected to test and change in body temperature will be analyzed.

5. Schizophrenia Assays

5.1 Prepulse Inhibition

In order to evaluate antipsychotic property of NCE under evaluation, five groups of animals (Control, Chemical challenge Group, Positive Control Group, Two dose levels of test compound; n=12) will be subjected to Prepulse inhibition test and % prepulse inhibition and acoustic startle response will be analyzed.

5.2 MK-801 antagonism

In order to evaluate antipsychotic property of NCE under evaluation, four groups of animals (Control, Positive Control Group, Two dose levels of test compound; n=10) will be subjected to open field test and distance traveled will be analyzed.

6. Pain Models

6.1 Formalin induced Nociception.

In order to evaluate analgesic property of NCE under evaluation, four groups of animals (Control, Positive Control Group, three dose levels of test compound; n=10) will be subjected to formalin induced pain and hind paw licking and biting will be analyzed.

6.2 Spinal Nerve (L5) Ligation Model of Neuropathic Pain.

In order to evaluate analgesic property of NCE under evaluation, four groups of animals subject to spinal nerve ligation (Control, Positive Control Group, Two dose levels of test compound; n=10) will be subjected to tactile allodynia, measured by assessing rat hind paw withdrawal thresholds in response to mechanical stimulation using a dynamic plantar Aesthesiometer.

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6.3 Inflammatory Mechanical hyperalgesia

In order to evaluate analgesic property of NCE under evaluation, four groups of animals (Control, Positive Control Group, Two dose levels of test compound; n=10) will be subjected to mechanical hyperalgesia, measured by assessing rat hind paw withdrawal thresholds in response to mechanical stimulation using Randall–Selitto (after administration of Freund's complete adjuvant).

6.4 Diabetes - Induced Neuropathic Pain

In order to evaluate analgesic property of NCE under evaluation, four groups of animals (Control, Positive Control Group, Two dose levels of test compound; n=10) will be subjected to mechanical hyperalgesia, measured by assessing rat hind paw withdrawal thresholds in response to mechanical stimulation using Randall–Selitto (after administration of streptozotocin).

6.5 Chemotherapy- Induced Neuropathic Pain

In order to evaluate analgesic property of NCE under evaluation, four groups of animals (Control, Positive Control Group, Two dose levels of test compound; n=10) will be subjected to mechanical hyperalgesia, measured by assessing rat hind paw withdrawal thresholds in response to mechanical stimulation using Randall–Selitto (after administration of Vincristine).

6.6 Capsaicin-Induced Allodynia

In order to evaluate analgesic property of NCE under evaluation, four groups of animals (Control, Positive Control Group, Two dose levels of test compound; n=10) will be subjected to tactile allodynia, measured by assessing rat hind paw withdrawal thresholds in response to mechanical stimulation using a dynamic plantar Aesthesiometer (after administration of capsaicin).

7. Brain Microdialysis Assays (Rats and Guinea pigs)

7.1 Modulation of Acetylcholine Levels using Brain Microdialysis Assay

In order to evaluate modulation of basal acetylcholine levels in specific brain region after administration of NCE under evaluation, four groups of animals (Control, Positive Control Group, Two dose levels of test compound; n=8), subjected to stereotaxic surgery to implant a semi-permeable membrane probe into specific region of brain (hippocampus, cortex, striatum, etc), perfused with aCSF and collected dialysates (five basals followed by four hours post administration) will be quantitated for acetylcholine concentrations using LC-MS/MS method. % change compared to basal levels will be compared between groups.

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7.2 Modulation of Glutamate Levels using Brain Microdialysis Assay

In order to evaluate modulation of basal glutamate levels in specific brain region after administration of NCE under evaluation, four groups of animals (Control, Positive Control Group, Two dose levels of test compound; n=8), subjected to stereotaxic surgery to implant a semi-permeable membrane probe into specific region of brain (hippocampus, cortex, striatum, etc), perfused with aCSF and collected dialysates (five basals followed by four hours post administration) will be quantitated for glutamate concentrations using HPLC-Flu method. % change compared to basal levels will be compared between groups.

7.3 Modulation of GABA Levels using Brain Microdialysis Assay

In order to evaluate modulation of basal GABA levels in specific brain region after administration of NCE under evaluation, four groups of animals (Control, Positive Control Group, Two dose levels of test compound; n=8), subjected to stereotaxic surgery to implant a semi-permeable membrane probe into specific region of brain (hippocampus, cortex, striatum, etc), perfused with aCSF and collected dialysates (five basals followed by four hours post administration) will be quantitated for GABA concentrations using HPLC-Flu method. % change compared to basal levels will be compared between groups.

7.4 Modulation of Histamine Levels using Brain Microdialysis Assay

In order to evaluate modulation of basal histamine levels in specific brain region after administration of NCE under evaluation, four groups of animals (Control, Positive Control Group, Two dose levels of test compound; n=8), subjected to stereotaxic surgery to implant a semi-permeable membrane probe into specific region of brain (hippocampus, cortex, striatum, etc), perfused with aCSF and collected dialysates (five basals followed by four hours post administration) will be quantitated for histamine concentrations using HPLC-Flu method. % change compared to basal levels will be compared between groups.

7.5 Modulation of Serotonin and its Metabolite Levels using Brain Microdialysis Assay

In order to evaluate modulation of basal serotonin and 5-HIAA levels in specific brain region after administration of NCE under evaluation, four groups of animals (Control, Positive Control Group, Two dose levels of test compound; n=8), subjected to stereotaxic surgery to implant a semi-permeable membrane probe into specific region of brain (hippocampus, cortex, striatum, etc), perfused with aCSF and collected dialysates (four basals followed by four hours post administration) will be quantitated for serotonin and 5-HIAA concentrations using HPLC- ECD method. % change compared to basal levels will be compared between groups.

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7.6 Monitoring Extracellular Concentrations of Test Compound Levels using Brain Microdialysis Assay

In order to monitor the extracellular test compound concentrations levels in specific brain region after administration of NCE under evaluation, animals (n=6 per group) will be subjected to stereotaxic surgery to implant a semi-permeable membrane probe into specific region of brain (hippocampus, cortex, striatum, etc), perfused with aCSF and collected dialysates (one pre dose sample followed by post administration samples) will be quantitated for test compound concentrations using LC-MS/MS method. Pharmacokinetic parameters for extracellular test compound concentrations will be calculated.

7.7 Monitoring Extracellular Concentrations of Test Compound and Modulation of Acetylcholine Levels using Brain Microdialysis Assay

In order to monitor the extracellular test compound concentrations and resulting modulation of basal acetylcholine levels in specific brain region after administration of NCE under evaluation, four groups of animals (Control, Positive Control Group, Two dose levels of test compound; n=8), subjected to stereotaxic surgery to implant a semi-permeable membrane probe into specific region of brain (hippocampus, cortex, striatum, etc), perfused with aCSF and collected dialysates (five basals followed by four hours post administration) will be simultaneously quantitated for test compound concentrations and acetylcholine concentrations using LC-MS/MS method. % change compared to basal levels will be compared between groups. Pharmacokinetic parameters for extracellular test compound concentrations will be calculated.

8. In-vivo Receptor Occupancy Study

8.1 In-vivo Receptor Occupancy Study – D₃

One test item, three doses, oral or sc or ip administration followed by specific D₃ *i.v.* tracer administration. Sacrifice of rats at predetermined time points to scoop specific and nonspecific brain regions and quantifying tracer concentration using LC-MS/MS method to evaluate the receptor occupancy.

8.2 In-vivo Receptor Occupancy Study – D₂

One test item, three doses, oral or sc or ip administration followed by specific D₂ *i.v.* tracer administration. Sacrifice of rats at predetermined time points to scoop specific and nonspecific brain regions and quantifying tracer concentration using LC-MS/MS method to evaluate the receptor occupancy.

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8.3 *In-vivo* Receptor Occupancy Study – 5-HT_{2a}

One test item, three doses, oral or sc or ip administration followed by specific 5-HT_{2a} *i.v.* tracer administration. Sacrifice of rats at predetermined time points to scoop specific and nonspecific brain regions and quantifying tracer concentration using LC-MS/MS method to evaluate the receptor occupancy.

8.4 *In-vivo* Receptor Occupancy Study – SRI

One test item, three doses, oral or sc or ip administration followed by specific SRI *i.v.* tracer administration. Sacrifice of rats at predetermined time points to scoop specific and nonspecific brain regions and quantifying tracer concentration using LC-MS/MS method to evaluate the receptor occupancy.

8.5 *In-vivo* Receptor Occupancy Study – 5-HT_{2a}/SRI dual method

One test item, three doses, oral or sc or ip administration followed by specific combination of 5-HT_{2a}/SRI *i.v.* tracer administration. Sacrifice of rats at predetermined time points to scoop specific and nonspecific brain regions and quantifying tracer concentration using LC-MS/MS method to evaluate the receptor occupancy.

8.6 *In-vivo* Receptor Occupancy Study – 5-HT_{1A}

One test item, three doses, oral or sc or ip administration followed by specific 5-HT_{1A} *i.v.* tracer administration. Sacrifice of rats at predetermined time points to scoop specific and nonspecific brain regions and quantifying tracer concentration using LC-MS/MS method to evaluate the receptor occupancy.

8.7 *In-vivo* Receptor Occupancy Study – D₂/5-HT_{2A} dual method

One test item, three doses, oral or sc or ip administration followed by specific combination of D₂/5-HT_{2a} *i.v.* tracers administration. Sacrifice of rats at predetermined time points to scoop specific and nonspecific brain regions and quantifying tracer concentration using LC-MS/MS method to evaluate the receptor occupancy.

8.8 *In-vivo* Receptor Occupancy Study – D₂/5-HT_{2A}/5-HT_{1A} Triple Tracer method

One test item, three doses, oral or sc or ip administration followed by specific combination of D₂/5-HT_{2a}/5HT_{1A} *i.v.* tracer administration. Sacrifice of rats at predetermined time points to scoop specific and nonspecific brain regions and quantifying tracer concentration using LC-MS/MS method to evaluate the receptor occupancy.

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8.9 *In-vivo* Receptor Occupancy Study – $\alpha_4\beta_2$

One test item, three doses, oral or sc or ip administration followed by specific $\alpha_4\beta_2$ *i.v.* tracer administration. Sacrifice of rats at predetermined time points to scoop specific and nonspecific brain regions and quantifying tracer concentration using LC-MS/MS method to evaluate the receptor occupancy.

8.10 *In-vivo* Receptor Occupancy Study – H_3

One test item, three doses, oral or sc or ip administration followed by specific H_3 *i.v.* tracer administration. Sacrifice of rats at predetermined time points to scoop specific and nonspecific brain regions and quantifying tracer concentration using LC-MS/MS method to evaluate the receptor occupancy.

9. *Ex-vivo* Assay

9.1 5-HT_{2B} Rat fundus assay

In order to evaluate functional activity of NCE under evaluation at 5-HT_{2B} receptors, four tissues will be used for each group (Control, Positive Control Group, Two dose levels of test compound; n=10), contractile response will be recorded.

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