

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=6), subject 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=8), subject to elevated plus maze 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 Geller conflict

In order to evaluate anxiogenic or anxiolytic property of NCE under evaluation, three groups of animals (Control, Positive Control Group, one dose level of test compound; n=8), subject to conflict test and conflict responses will be analyzed.

2.3 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=8), subject to hole board and latency of head dips, number of head dips and cumulative time of head dips will be analyzed.

2.4 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=8), subject to conflict test and number of shocks received will be analyzed.

Suven Life Sciences Limited

Serene chambers, Road No - 5, Avenue-7 Banjara Hills, Hyderabad 500 034, INDIA

Tel: 91-40-23556039/38 Fax: 91-40-23541152

Contact : nvsrk@suven.com, knvishu@suven.com

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=10), subject 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.

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=10), subject 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=10), subject 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), subject 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=8), subject 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=10), subject to test and choice accuracy will be analyzed.

Suven Life Sciences Limited

Serene chambers, Road No - 5, Avenue-7 Banjara Hills, Hyderabad 500 034, INDIA

Tel: 91-40-23556039/38 Fax: 91-40-23541152

Contact : nvsrk@suven.com, knvishu@suven.com

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=8), subject 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=8), subject to test and dominance level and feeding scores will be analyzed.

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=8), subject 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=8), subject 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=8), subject 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=10), subject 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=8), subject to open field test and distance traveled will be analyzed.

Suven Life Sciences Limited

Serene chambers, Road No - 5, Avenue-7 Banjara Hills, Hyderabad 500 034, INDIA

Tel: 91-40-23556039/38 Fax: 91-40-23541152

Contact : nvsrk@suven.com, knvishu@suven.com

6. General Behavioral Assays

6.1 Social Interaction Test

In order to evaluate anxiogenic or anxiolytic property of NCE under evaluation, three groups of animals (Control, Positive Control Group, One dose levels of test compound; n=8), subject to social interaction and interaction time will be analyzed.

7. Brain Microdialysis Assays

7.1 Modulation of Acetylcholine Levels using Brain Microdialysis Assay

In order to evaluate modulation of basal acetylcholine levels in specific brain region due to 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 three hours post administration) will be quantitated for acetylcholine concentrations using LC-MS/MS method. % change compared to basal levels will be compared between groups.

7.2 Modulation of Glutamate Levels using Brain Microdialysis Assay

In order to evaluate modulation of basal glutamate levels in specific brain region due to 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 three hours post administration) will be quantitated for glutamate concentrations using HPLC-Flu method. % change compared to basal levels will be compared between groups.

8. In-vivo Receptor Occupancy Study

8.1. In-vivo Receptor Occupancy Study – D3

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

8.2 In-vivo Receptor Occupancy Study – D2

One test item, three doses, oral or sc administration followed by specific D2 iv tracer administration. Sacrifice of rats at predetermined time points to scoop specific and

Suven Life Sciences Limited

Serene chambers, Road No - 5, Avenue-7 Banjara Hills, Hyderabad 500 034, INDIA

Tel: 91-40-23556039/38 Fax: 91-40-23541152

Contact : nvsrk@suven.com, knvishu@suven.com

nonspecific brain regions and quantifying tracer concentration to evaluate the receptor occupancy on concentrations using LC-MS/MS method.

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

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

8.4 In-vivo Receptor Occupancy Study – SRI

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

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

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

Suven Life Sciences Limited

Serene chambers, Road No - 5, Avenue-7 Banjara Hills, Hyderabad 500 034, INDIA

Tel: 91-40-23556039/38 Fax: 91-40-23541152

Contact : nvsrk@suven.com, knvishu@suven.com