In Vivo Pharmacology Platform

Customized in vivo pharmacology services to accelerate drug discovery process
WuXi AppTec's *in vivo* Pharmacology

WuXi AppTec is building a world leading *in vivo* pharmacology service platform. This platform provides global drug hunters different animal disease models for the pharmacology evaluation of lead compound and preclinical candidates. Our animal experiments are supervised by experienced oversea experts and executed in AAALAC certified animal facilities. Our key research areas are mainly focused on diseases of central nervous system (especially on chronic pain), metabolic diseases like obesity, diabetes and diabetic complications (hepatitis and cardiovascular diseases). Considering the specificity of oncology drug discovery, services of this area are executed by another team in WuXi. Our vision is to design and execute pharmacology assays to provide qualified data to our clients. Keep providing high standard services that exceed our customers’ expectation, we have received lots of awards and high satisfaction feedbacks from all over the world.

| In vivo PK/PD platform (PK/PD screening model or translational science model) | • IV infusion in living animals with behavioral test  
• Behavioral test, Biomarker, RO in the same animal  
• Microdialysis, cpd exposure in the same animal |
| --- | --- |
| • PD/stroke  
• Psychiatry  
• Chronic pain | CNS and pain efficacy model platform |
| Metabolic disease *in vivo* platform (Efficacy or PK/PD screening model) | • Diabetes (including Diabetic complications)  
• Obesity  
• Dyslipidemia (including NAFLD and NASH) |
**General in vivo pharmacology team introduction**

40 core staff. PhD: 5; MS 14 (10 over 3ys in WuXi)

Supporting team: animal care, operation, cell culture etc.

**Gang Lu, M.D., Ph.D.**
Dir., Pharmacology
14 years of experience in neuroscience research including 6 years of *in vivo* pharmacology in pharmaceutical CRO (WuXi Apptec)

**Zhijing Hu, MS.c**
Assistant Dir., Pharmacology
9 years of experience in neuroscience research including 6 years of *in vivo* pharmacology in pharmaceutical CRO (WuXi Apptec)

**Zhou Zhou, Ph.D.**
Principle scientist, Pharmacology
11 years of experience in diabetes and metabolism research. PhD in pharmacology from Shanghai Institute of Material Medica.

**Juntao Kan, Ph.D.**
Senior scientist in metabolic, hepatic and cardiovascular models.
6 years of experience in pharmacological research including 1 year of drug discovery in pharmaceutical CRO (WuXi Apptec).

**Major clients and award**

![AstraZeneca](image)
![Novartis](image)
![Lundbeck](image)
![Progress in Mind](image)

![Pfizer](image)
![Lilly](image)
![GlaxoSmithKline](image)
### CNS and pain in vivo screening and efficacy models

#### AD/PD/stroke
- **Disease models**
  - 6-OHDA PD model
  - Ischemia model (MCAO)
- **Measurements**
  - Novel object recognition
  - Rota rod
  - AIM score

#### Psychiatry
- **Disease models**
  - Acute PCP/amphetamine
  - SubPCP/NeoPCP model
  - Learned helplessness
- **Measurements**
  - CAR
  - Locomotor activity
  - Prepulse inhibition
  - Attentional set shifting
  - Vogel
  - Forced swimming

#### Chronic pain
- **Disease models**
  - OA pain (MIA)
  - Fibromyalgia syndrome (ICS)
  - Neuropathic pain (Chung, CCI)
  - Inflammation pain (Formalin)
  - Postsurgical pain (Brennan)
- **Measurements**
  - Weight bearing
  - Von Frey hair
  - Cold allodynia (acetone)
  - Heat hyperalgesia

### Metabolic disease models and tests

#### Diabetes
- oGTT (mouse/rat), ipGTT (mouse/rat), ivGTT (rat);
- Glucosuria experiment;
- Chronic efficacy studies in db/db, Zucker and other models
- Chronic insulin sensitizing experiment
- DIO/STZ mice
- Thrombus

#### Obesity
- Acute food intake;
- Chronic food intake;
- BW studies;
- Growing or established DIO mice;
- Biochemical biomarkers;
- Pathology;

#### Dislipidemia
- Acute lipid tolerance;
- Chronic studies to monitor; TG, cholesterol
- NAFLD (DIO mice);
- NASH (STZ+DIO mice)
**Case study**

**In vivo pharmacology achievements**

- Provided drug discovery expertise for program leadership
- *In vivo* target engagement assay identified and fully validated in 14 weeks
- PKPD strategy identified & assay validated in 2 weeks
- Rodent disease model identified & assay validated in 4 weeks
- Biomarker strategy identified and evaluated

**Objective:**

To identify a potent, selective, adequately stable, brain penetrable, enzyme inhibitor — lead compound.

**Period:** 12 months

**Achievement:** Lead generation milestone achieved

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**Collaboration with a Major Pharma Partner**

**Objective:**

To identify a potent, selective, adequately stable, brain penetrable, receptor modulator.

**Period:** 15 months

**Achievement:** Lead generation milestone achieved

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**In vivo pharmacology achievements**

- Provided drug discovery expertise for program leadership
- *In vivo* target engagement assay identified and fully validated in 2 weeks
- PKPD strategy identified
- Rodent disease model supporting disease indication

**Objective:**

To identify a potent, selective, adequately stable, limited brain penetrable, ion channel blocker.

**Period:** 24 months

**Achievement:** Lead generation milestone achieved
Introduction of CNS/Pain drug discovery services

• We are building a translational science based CNS/Pain in vivo pharmacology platform, which can provide qualified data to increase success rate of testing drug in Phase 2 clinic trials.

• Currently, we have already set up a series of animal models and assays, including receptor occupancy, PK/PD, behavior test, biomarkers and pathology.

Animal model and measurements

PK/PD
- Receptor Occupancy
- PK/PD analysis in moving animals

Motor function
- Spontaneous locomotor activity test (sLMA)
- Rotarod test

Schizophrenia
- Drug evoked locomotor activity test
- Conditioned avoidance response (CAR)
- Prepulse inhibition
- Attentional set-shifting test in sub-PCP rats

Anxiety and Depression
- Depression: Forced swimming test; Learned helplessness
- Anxiety: Vogel conflict test; Elevated plus maze test

Parkinson’s Disease
- Unilateral 6-OHDA lesion model
- L-DOPA induced dyskinesia in PD model

Pain model and measurements
- Acute pain: Tail flick test
- Inflammation pain: Complete Freund’s adjuvant (CFA) model; Formulain Model
- Neuropathic Pain: Spinal nerve ligation; Chronic constriction injury model
- Fibromyalgia-like Pain: Intermittent cold stress (ICS) model
- Osteoarthritis: Monosodium iodoacetate induced osteoarthritis (MIA) model

Animal model and measurements

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**PK/PD**

**Target engagement — Receptor occupancy (RO)**
- Measurement of free ligand binding to its intended receptor *in vivo* at the site of action
- Supporting discovery program by providing confidence on compound MOA
- Providing bases for the dose-regimen of preclinical and clinical studies

**Species:** Mouse or rat

**Endpoint:** %Receptor occupancy *in vivo* with PK correlation
- Radio-labeled ligand detection by scintillation method
- Nonradioactive tracer detection by LC-MS/MS

**Throughput:** Fully DRC of compound within the same day

**Groups in a standard assay:** Naïve (Vehicle), Testing compounds with 6-8 doses; n=3-5/group

**Turnaround time:** ~3 days/each study; 2 assays/week

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**Dose-dependent dopamine D2 receptor occupancy by Haloperidol *in vivo***

![Graph showing dopamine D2 receptor occupancy](image)

<table>
<thead>
<tr>
<th>Assay method</th>
<th>ED50 (dose) mg/kg</th>
<th>ED50 (plasma) ng/ml (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC-MS/MS (PO)</td>
<td>0.4</td>
<td>1.3 (3nM)</td>
</tr>
<tr>
<td>Isotope method (s.c)</td>
<td>0.015</td>
<td>0.7 (1.5nM)</td>
</tr>
</tbody>
</table>

- D2 receptor occupancy of haloperidol as determined by the two methods
- Data were presented as % occupancy vs. plasma exposure in individual animals
- ED50 presented as mean of the group (n=3-5)

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**Body composition analysis moving animals with behavioral test**

- Assess PK and PD end point in the same animal
- Sample below was formalin test in free moving animals with i.v. infusion

**Species:** Mouse or rat

**Endpoint:** Motion counts

**Throughput:** Testing 8 animals at the same time

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**Data Sample**
Neuroscience

Motor Function — Spontaneous locomotor activity test (sLMA)

- A high-throughput, robust and sensitive behavioral assay to assess spontaneous motor activity

Species: Mouse or rat
Endpoint: Distance traveled (video tracking)
Throughput: Testing 18 mice or 16 rats at the same time

Groups in a standard assay: Naïve (Vehicle), Testing compounds with 3 doses; n=10/group
Turnaround time: ~2 days/each study; 3 assays/week

Data Sample

Effect of Cpd A (p.o.) on sLMA test

Data were presented as Mean±SEM, n=10/group. *** p < 0.001 vs. Veh group One way ANOVA followed by Dunnett’s post hoc

Effect of Cpd B (p.o.) on sLMA test

Data were presented as Mean±SEM, n=10/group. one way ANOVA followed by Dunnett’s post hoc

Motor Function —Rotarod test

- A high-throughput, robust and sensitive behavioral assay for testing motor coordination and exercise capacity.

Species: Mouse
Endpoint: Fall-off latency (90s Cutoff)
Throughput: Testing 8 animals at the same time

Groups in a standard assay: Naïve (Vehicle), Positive control, Testing compounds with 3 doses; n=12/group
Turnaround time: ~3 days/each study; 2 assays/week

Data Sample

Effect of Diazepam (p.o.) on rotarod test

Data were presented as Mean±SEM, n=10/group. * p < 0.05, *** p < 0.001 vs. Vehicle group, One way ANOVA by Dunnett’s post hoc
**Acute Pain — Tail flick test (TF)**

- A high-throughput, robust and sensitive nociceptive assay to measure sensitivity to heat

**Species:** Rat or Mouse  
**Endpoint:** Tail flick latency (TFL)  
**Throughput:** 90 animals per day

**Groups in a standard assay:** Naïve (Vehicle), Positive control, Testing compounds with 3 doses; n=10~12/group  
**Turnaround time:** ~3 days/each study; 2 assays/week

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**Inflammation Pain — Complete Freund’s adjuvant (CFA) model**

- A widely used model to assess CFA induced inflammation pain in paw

**Species:** Rat  
**Endpoint:** Thermal hyperalgesia, Cold hyperalgesia, paw thickness (digital caliper), mechanical hyperalgesia (Randle Salido device), mechanical allodynia  
**Throughput:** Compound test in one day

**Groups in a standard assay:** Naïve (Vehicle), Positive control, Testing compounds with 3 doses; n=8/group

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Data were presented as Mean±SEM, n=7 per group, *p<0.05, ***p<0.001 vs saline, One way ANOVA, Dunnett’s test.

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Data were presented as Mean±SEM, n=10/group. **p < 0.01, ***p < 0.001 vs Vehicle group, One way ANOVA by Dunnett’s post hoc test.
**Acute Pain — Formalin Model**

- Formalin is injected into the dorsal surface of the right hind paw to induce acute pain.
- A high-throughput, robust and sensitive acute pain behavioral assay

**Species:** Mouse or rat  
**Endpoint:** Motion counts  
**Throughput:** 8 sets of instrument to be able to test 8 animals at the same time

**Groups in a standard assay:** Naïve (Vehicle), Positive control, Testing compounds with 3 doses; n=10~12/group

---

Data Sample

**Data were presented as Mean±SEM, n=8/group. ** p< 0.01, *** p < 0.001 vs. vehicle group, One way ANOVA by Dunnett’s post hoc**

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**Neuropathic Pain — Chung Model (Spinal nerve ligation, SNL)**

- A commonly used model to measure surgical induced neuropathic pain
- Gabapentin & Doluxetine showed robust anti-allodynia and anti-hyperalgesia effects in the model

**Species:** rat  
**Endpoint:** mechanical allodynia, thermal hyperalgesia, cold allodynia  
**Throughput:** Testing 60 animals at the same time point

**Groups in a standard assay:** Naïve/sham, Vehicle control, Positive control, Testing compounds with 3 doses; n=8~10/group  
**Turnaround time:** ~2 weeks/each study

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**Data were presented as Mean±SEM, n=10-12/group. * p < 0.05, ** p < 0.01 vs. PBS group, One way ANOVA by Dunnett’s post hoc**
**Neuropathic pain — Chronic constriction injury model (CCI)**

- A commonly used model to measure surgical induced neuropathic pain
- Pregabalin showed robust effects in the model

**Species:** rat  
**Endpoint:** mechanical allodynia, thermal hyperalgesia, cold allodynia  
**Throughput:** Testing 60 animals at the same time point

**Groups in a standard assay:** Naïve/sham, Vehicle control, Positive control, Testing compounds with 3 doses; n=8~10/group  
**Turnaround time:** ~2 weeks/each study

![Data Sample](image)

Data were presented as Mean±SEM, n=9-10/group. ** p< 0.01, *** p < 0.001 vs. vehicle group, One way ANOVA by Dunnett’s post hoc

**Fibromyalgia-like Pain — Intermittent cold stress (ICS) model**

- A specific model to assess stress induced fibromyalgia pain  
- Mechanical allodynia lasted>12 days, thermal hyperalgesia lasted>15 days

**Species:** Mouse  
**Endpoint:** mechanical allodynia, thermal hyperalgesia  
**Throughput:** Compound test in one day

**Groups in a standard assay:** Naïve, Vehicle, Positive control, Testing compounds, n=10/group

![Data Sample](image)

Data were presented as Mean±SEM, n=10/group. *** p < 0.001 vs. Vehicle group, One way ANOVA by Dunnett’s post hoc
**Osteoarthritis — Monosodium iodoacetate induced osteoarthritis (MIA) model**

- A model to measure MIA induced osteoarthritis (OA) to measure joint inflammation and pain

**Species:** rat  
**Endpoint:** mechanical allostynia, grip force, weight bearing using Tekscan® system and pathological evaluation

**Groups in a standard assay:** Naïve (Vehicle), Positive control, Testing compounds with 3 doses; n=8/group

**Data Sample**

The Tekscan® system showed weight bearing asymmetry in MIA-injected rats.

① : left front paw  
② : right front paw  
③ : left hind paw  
④ : right hind paw (MIA injected)

Histopathological analysis for HE staining of MIA rats

Data were presented as Mean±SEM, n=8/group. * p< 0.05, *** p < 0.001 vs. Vehicle group , One way ANOVA by Dunnett’s post hoc

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**Motor Function — Drug evoked locomotor activity test**

- A high-throughput, robust and sensitive behavior al assay to assess evoked motor activity

**Species:** Mouse or rat  
**Endpoint:** Distance traveled  
**Throughput:** Testing 18 mice or 16 rats at the same time  
**Turnaround time:** ~3 days/each study; 2 assays/week

**Groups in a standard assay:** Naïve (Vehicle), Positive control, Testing compounds with 3 doses; n=10/group

**Data Sample**

Data were presented as Mean±SEM, n=10/group. *** p < 0.001 vs. Saline+Amphetamine group, Two-Way Repeated Measures ANOVA followed by Dunnett’s post hoc
Schizophrenia — Conditioned avoidance response (CAR) test

A high-throughput, robust and sensitive behavioral assay to access antipsychotics

**Species:** Rat  
**Endpoint:** Avoidance (%), Escape Failure (%)  
**Throughput:** Testing 8 animals at the same time

**Groups in a standard assay:** Naïve (Vehicle), Positive control, Testing compounds with 3 doses; n=10/group  
**Turnaround time:** ~3 days/each assay; 2 assays/week

**Data Sample**

Effect of Risperidone (s.c.) on avoidance in CAR test (Fish-344 Rat)

Data were presented as Mean ± SEM, n=8 per group, ***p<0.001 vs. Vehicle group, Two-way RM ANOVA followed by Dunnett’s post hoc

Schizophrenia — Prepulse inhibition (PPI)

- Measure inhibition of startle response to a strong stimulus following a weak preceding stimulus  
- Assess sensory motor activity often impaired in schizophrenia patients  
- Anti-psychotics (such as Haloperidol or clozapine) reduced amphetamine or PCP-induced PPI deficits

**Species:** Rat  
**Endpoint:** Prepulse inhibition of startle  
**Throughput:** Testing 8 animals at the same time

**Groups in a standard assay:** Naïve (Vehicle), Positive control, Testing compounds with 3 doses; n=12/group  
**Turnaround time:** ~3 days/each study; 2 assays/week

**Data Sample**

Effect of Clozapine (s.c.) on PCP-induced deficits in Prepulse Inhibition (5, 10 & 15 dB above background, collapsed data)

Data were presented as Mean ± SEM, n=12 per group, ***p<0.001 vs. saline/PCP group, Two-way RM ANOVA followed by Dunnett’s post hoc

Effect of Haloperidol (s.c.) on amphetamine-induced deficits in Prepulse Inhibition (5, 10 & 15 dB above background, collapsed data)

Data were presented as Mean ± SEM, n=12 per group, *p<0.05, **p<0.01, ***p<0.001 vs. saline/Amphet group, Two-way RM ANOVA followed by Dunnett’s post hoc
In Vivo Pharmacology

**Schizophrenia — Attentional set-shifting test in sub-PCP rats**

- A unique preclinical model to measure executive function deficit induced by PCP

**Species:** Rat

**Endpoint:** Trials to criteria of finishing tasks

**Throughput:** Testing 6 animals in one day

**Groups in a standard assay:** Naive (Vehicle), Model, Positive control, Testing compounds with 3 doses; n=12/group

**Turnaround time:** 1.5 month/study

**Data Sample**

![Effect of Modafinil (64 mg/kg, p.o.) on sub-PCP induced attentional set-shifting deficits in Long Evans rats](image)

Data were presented as Mean ± SEM, n=12 per group, ***p<0.001 vs. Vehicle group, **p<0.01 vs. PCP group, Two-way RM ANOVA followed by Dunnett’s post hoc test.

**Anxiety and Depression — Forced swimming test (FST)**

- A high-throughput, robust and sensitive behavior assay to measure forced swimming evoked stress

**Species:** Mouse

**Endpoint:** Immobility (s)

**Throughput:** Testing 4 animals at the same time

**Groups in a standard assay:** Naive (Vehicle), Positive control, Testing compounds with 3 doses; n=10~12/group

**Turnaround time:** ~3 days/each assay; 2 assays/week

**Data Sample**

![Effect of Imipramine (p.o.) on immobility in forces swim test](image)

Data were presented as Mean ± SEM, n=12 per group, **p<0.01, ****p<0.0001 v.s. saline, one way ANOVA followed by Dunnett’s test.
**Anxiety and Depression — Learned helplessness (LH)**

- A high-throughput assay to measure depression-like behaviors
- Antidepressants (such as imipramine) to decrease the escape failure in the assay

**Species:** Rat

**Endpoint:** Escape failure

**Throughput:** Testing 8 animals at the same time (8 boxes)

**Groups in a standard assay:** Vehicle (sham), IS (helplessness), Positive control, Testing compounds with 3 doses; n=10~12/group

**Turnaround time:** ~7 days/each study

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Data Sample

**Effect of Imipramine (p.o.) on escape failure in learned helplessness test**

Data were presented as Mean ± SEM, n=10-12 per group, ***p<0.001 vs NS-Saline; #p<0.05 vs IS-Saline, one way ANOVA followed by Dunnett’s test

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**Anxiety and Depression — Vogel conflict test**

- A high-throughput, robust and sensitive assay to measure anxiety-like behavior
- Anxiolytics (such as diazepam) increases the licks in the assay to reduce animal’s anxiety

**Species:** Rat or mouse

**Endpoint:** Licks/shocks

**Throughput:** 16 animals at the same time (16 cages)

**Groups in a standard assay:** Vehicle (no-shock), IS (shock), Positive control, Testing compounds with 3 doses; n=10~12/group

**Turnaround time:** ~7 days/each study

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Data Sample

**Effect of Diazepam (DZP, i.p.) on shocks in Vogel test**

Data were presented as Mean ± SEM, n=12 per group, ****p<0.0001 v.s. vehicle (no shock); **p<0.01 v.s. vehicle (shock), one way ANOVA followed by Dunnett’s test
Anxiety and Depression — Elevated plus maze test (EPM)

- A standard behavior assay of fear and anxiety by testing the proportion of time/entries spent in the open arms
- Anxiolytic (such as diazepam) increases the proportion of time/entries

Species: Rat or mouse

Endpoint: %OE (open arms entries), %TO (time in open arms), %DO (distance in open arms), Total travelled distance

Throughput: 50 animals per day

Groups in a standard assay: Naïve (Vehicle), Positive control, Testing compounds with 3 doses; n=10~12/group

Turnaround time: ~3 days/each study; 2 assays/week

Data Sample

Effect of Diazepam (DZP, i.p.) on behaviors of plus maze test

Data were presented as Mean ± SEM, n=12 per group, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 v.s. vehicle, one way ANOVA followed by Dunnett's test

Parkinson’s Disease (PD) — Unilateral 6-OHDA lesion model (EPM)

- The model is induced by unilateral 6-OHDA injection into specific brain areas via stereotaxic surgery
- It is a complete DA lesion model
- >80% successful rate

Species: Rat

Endpoint: Apomorphine-induced rotation, ICH of tyrosine hydroxylase (TH) neuron

Throughput: 30 rats/day for surgery

Turnaround time: 1 month for developing model

Data Sample

TH neuron loss in lesioned substantia nigra

Apomorphine induced contralateral rotations
**Parkinson’s Disease (PD) — L-DOPA induced dyskinesia in PD model (PD-LID)**

- This model is used for testing the compound effects on chronic L-DOPA induced dyskinesia in PD model

**Species:** Rat  
**Endpoint:** abnormal involuntary movement (AIM) score  
**Throughput:** Evaluate 40 PD-LID animals in one day

**Groups in a standard assay:** Naïve (Vehicle), Model group, Testing compounds with 3 doses; n=8/group  
**Turnaround time:** 2 month/each study

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### Data Sample

**Effect of Buspirone (2 mg/kg, i.p.) on AIMs in rat PD-LID model**

Data were presented as Mean ± SEM, n=12 per group, ***p<0.001 v.s. vehicle, t test

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### Remark

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Introduction of metabolic and hematology disease drug discovery services

- Animal model driven metabolic and hematology disease pharmacology platform provides IND enabling preclinical pharmacology data.
- Validated animal disease model including Obesity, Diabetes and Thrombosis.

Animal disease models and measurements

**Obesity and Hyperlipemia**
- High Fat Diet Induced Obesity (DIO) model
- Food intake
- High Fructose Diet (HFD) model
- Lipid Tolerance Test

**Diabetes**
- Glucose Tolerance Test
- Urine Glucose Assay
- db/db mouse model
- ZDF rat model

**Thrombosis**
- Platelet aggregation
- Tail bleeding test
- Arterio-venous shunt model
- Laboratory hematology testing
- Inflammation agents
Metabolic

**Obesity — High Fat Diet Induced Obesity (DIO) model**

- Mice fed with HFD (Research Diet D12492) for 12–14 weeks to assess anti-obesity agents
- 300 DIO mice /month ready to use

**Species:** Mouse

**Endpoint:** Body weight, Food intake, Total cholesterol (TC), Triglyceride (TG), ALT, AST, Tg in, liver/muscle Insulin, GLP-1, Leptin, HE/Oil red O stain, Fat pad, etc.

**Throughput:** 8 mice/group; 5–8 groups

**Groups in a standard assay:** Vehicle, Positive control, Testing compounds with 3 doses

**Turnaround time:** ~1.5 month/each study

**Data Sample**

Data were presented as Mean ± SEM, n=8 per group, *p<0.05, **p<0.01 vs. vehicle, one way ANOVA followed by Dunnett’s test

**Obesity — Food intake**

- Acute food intake measurement

**Species:** Mouse or rat

**Endpoint:** Body weight, food intake

**Throughput:** 8 animals/group; 5–8 groups

**Groups in a standard assay:** Vehicle, Positive control, Testing compounds with 3 doses

**Turnaround time:** 4 days/each study (baseline record included)

**Data Sample**

Data were presented as Mean ± SEM, n=8 per group, **p<0.01, ***p<0.001 vs. vehicle, one way ANOVA followed by Dunnett’s test

**Obesity - Body composition analysis**

- Fat mass, lean mass, body free water, body total water measurement

**Species:** Mouse

**Device:** Echo MRI

**Throughput:** 8 animals/group; 8~10 groups /day

**Data Sample**

Minor variation between tests with same samples

Significant difference of fat/lean mass in lean and DIO mice

Data were presented as Mean ± SD, n=10

Data were presented as Mean ± SEM; ****p<0.0001, t test
**Dyslipidemia — High Fructose Diet (HFD) model**

- A model induced by feeding with High Fructose Diet (HFD) to assess dyslipidemia

**Species:** Rat  
**Endpoint:** Body weight, Food intake, Total cholesterol (TC), Triglyceride (TG), HDL-C, LDL-C, etc.  
**Throughput:** 8 rats/group; 5~8 groups

**Groups in a standard assay:** Vehicle, Positive control, Testing compounds with 3 doses  
**Turnaround time:** ~1month/each study

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**Data Sample**

![Effect of EPA (p.o) on reducing Plasma TG Levels (mmol/L)](image)

Data were presented as Mean ± SEM, n=8 per group, *p<0.05, **p<0.01, ***p<0.001 vs. vehicle, one way ANOVA followed by Dunnett's test

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**Dyslipidemia — Lipid Tolerance Test**

- A test to assess lipid tolerance. Corn oil challenge is used to increase the blood TG levels

**Species:** Mouse or rat  
**Endpoint:** Blood Triglyceride (TG)  
**Throughput:** 8 animals/group; 5~8 groups

**Groups in a standard assay:** Vehicle, Positive control, Testing compounds with 3 doses  
**Turnaround time:** 1day/each study

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**Data Sample**

![Effect of Cpd A (p.o) on reducing Plasma TG Concentration Time course (mg/dl)](image)

Data were presented as Mean ± SEM, n=8 per group, *P<0.05, **P<0.01, ***P<0.001 vs. vehicle, two-way ANOVA followed by Newman-keuls test
### Hyperglycemia — Glucose Tolerance Test (Acute)

- An assay to assess glucose tolerance including oral glucose (OGTT), intraperitoneal glucose (IPGTT) or intravenous glucose (IVGTT) to fasted animals

**Species:** Mouse or rat  
**Endpoint:** Blood glucose and Insulin  
**Throughput:** 8 animals/group; 5~8 groups

**Groups in a standard assay:** Vehicle, Positive control, Testing compounds with 3 doses  
**Turnaround time:** ~5 days/each study

#### Data Sample

**Effect of Cpd A (s.c) on Blood Glucose lowering time course in IPGTT**

Data were presented as Mean±SEM, n=5 per group, ***p<0.001 vs vehicle, one way ANOVA followed by Dunnett’s test

### Diabetes — Urine Glucose Assay

- An assay to measure urine glucose

**Species:** Rat  
**Endpoint:** Urine volume; Urine glucose; Blood glucose

**Groups in a standard assay:** Naive (Vehicle), Positive control, Testing compounds with 3 doses; n=4~6/group  
**Turnaround time:** 2 days/each study

#### Data Sample

**Effect of Canagliflozin (p.o.) on urine volume in 24 hrs post treatment**

Data were presented as Mean±SEM, n=5 per group, ***p < 0.001 vs vehicle group, one way ANOVA followed by Dunnett multiple comparison test

**Effect of Canagliflozin (p.o.) on the urine glucose exclusion in 24 hrs post treatment**

Data were presented as Mean±SEM, n=5 per group, ***p < 0.001 vs vehicle group, one way ANOVA followed by Dunnett multiple comparison test
**Diabetes — db/db mouse model**

- A commonly used model to assess the anti-diabetic effect in db/db mice

**Species:** Mouse  
**Endpoint:** Body weight, Food intake, water intake, blood glucose, insulin, GTT, HBA1C/fructosamine, pancreases histology stain.  
**Throughput:** 10~12 animal/group; 5~8 groups

**Groups in a standard assay:** Vehicle, Positive control, Testing compounds with 3 doses  
**Turnaround time:** ~1.5 month/each study

![Data Sample](image1)

Data were presented as Mean ± SEM, n=12 per group, *p<0.05,**P<0.01, ***p<0.001 vs. vehicle, one way ANOVA followed by Dunnett’s test.  
9 weeks old db/db mice were treated with Cpd B for three weeks.

**Diabetes — ZDF rat model**

- A commonly used model to assess the anti-diabetic effect in ZDF rat

**Species:** Rat  
**Model:** Zuker fa/fa male rats  
**Endpoint:** Body weight, Food intake, water intake, blood glucose, insulin, GTT, HBA1C/fructosamine, pancreases histology stain.  
**Throughput:** 8 animal/group; 5~8 groups

**Groups in a standard assay:** Vehicle, Positive control, Testing compounds with 3 doses  
**Turnaround time:** ~1.5 month/each study

![Data Sample](image2)

Data were presented as Mean ± SEM, n=8 per group, *p<0.05 vs. vehicle, one way ANOVA followed by Dunnett’s test.  
12 weeks old ZDF rats were treated with Rosiglitazone for three weeks; age of model animal is critical in efficacy evaluation.
Hematology

**Thrombosis — Platelet aggregation**

- A classical and sensitive assay for evaluating novel anti-platelet drug to measure platelet aggregation and biomarkers (TXB2, PGI2, PGE etc)
- Aggregation inducer: ADP, arachidonic acid (AA), and collagen
- In vitro, ex vivo and in vivo PK/PD study

**Species:** Rat or Rabbit  
**Endpoint:** Platelet aggregation (%), platelet aggregation inhibition (%) and biomarkers

**Throughput:** For in-vitro study: 40 samples/day  
PK/PD study: 12 rats/day.  
**Turnaround time:** 1 week for one in vitro study; 2 week for one in vivo study

**Data Sample**

The correlation between rabbit platelet aggregation inhibition and TXB2 (the biomarker) production inhibition by ASA (Aspirin in vitro)

Data were expressed as Mean ± SEM (n=5), Sigmoidal dose-response (variable slope)

**Thrombosis — Tail bleeding test**

- A simple and quick in vivo assay for evaluating antithrombotic effects of compounds

**Species:** Rat  
**Endpoint:** Bleeding time  
**Throughput:** 36 rats/day

**Groups in a standard assay:** Vehicle, Positive control, Testing compounds with 3 doses  
**Turnaround time:** 1 weeks for one study

**Data Sample**

Effect of Rivaroxaban on rat tail-transection bleeding time after oral administration

Data were presented as Mean ± SEM, n=7 per group, *p<0.05, **p<0.01 vs. vehicle, one way ANOVA followed by Dunnett's test
**Thrombosis — Arterio-venous shunt model**

- A classical efficacy model to test agents to prevent thrombus formation

**Species:** Rat  
**Endpoint:** The weight of thrombus; Inhibition of thrombus formation (%)  
**Throughput:** 24 rats/day  

**Groups in a standard assay:** Vehicle, Positive control, Testing compounds with 3 doses  
**Turnaround time:** 1 week for one study  

**Data Sample**

![Graph showing effect of Rivaroxaban (p.o.) on thrombus formation in SD rats](image)

Data were presented as Mean ± SEM, n=7 per group, **P<0.01 vs. vehicle, one way ANOVA followed by Dunnett's test.

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**Laboratory hematology testing — ADVIA 2120i Hematology System**

- The ADVIA 2120i hematology systems provide accurate, first-pass results for laboratory hematology test

**Species:** Rat, mouse, Rabbit  
**Turnaround time:** 1 day  

**Parameters**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CBC results</th>
<th>Differential results (absolute and %)</th>
<th>Platelet results</th>
<th>Reticulocyte results (absolute and %)</th>
<th>CSF assay results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WBC, RBC, HB⁺, HCT, MCV, MCH, MCHC, CHCM, RDM, HDM, CH, CHDW, PLT</td>
<td>NEUT, LYMPH, MONO, EOS, BASO, LUC(Large Unstained Cells)</td>
<td>PLT, MPV, PDW, PCT</td>
<td>RETIC, MCVr, CHCMr, RDWr⁺⁺, HDWr⁺⁺, Chr, CHDWr⁺⁺</td>
<td>Optional</td>
</tr>
</tbody>
</table>
**Inflammation**

**Inflammation — Inflammation agents (IL-1b, IL-6 and TNF-α)**

- A high-throughput, robust and sensitive assay to detect inflammatory biomarkers
- Anti-inflammatory drugs significantly inhibit LPS induced cytokines production

**Species:** Mouse, Rat, and Human samples  
**Endpoint:** Cytokines (IL-1b, IL-6, and TNF-α) level in plasma and tissue  
**Throughput:** 40 samples in one plate

**Groups in a standard assay:** Saline + Vehicle, LPS + Vehicle, LPS + Testing compounds with 3 doses; n=8/group  
**Turnaround time:** ~3 days/each study; 2 assays/week

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**Data Sample**

Effect of compound A (s.c.) on mouse brain cytokines induced by LPS

Data were expressed as Mean ± SEM, ####p<0.0001 vs. Saline + veh group, *p<0.05, **p<0.01, ****p<0.0001 vs. LPS + veh group, One way ANOVA, Fisher’s LSD test

Effect of compound B (p.o.) on rat plasma cytokine induced by LPS

Data were expressed as Mean ± SEM, ####p<0.0001 vs. Saline + veh group, ****p<0.0001 vs. LPS + veh group, One way ANOVA, Fisher’s LSD test
Non-Alcoholic SteatoHepatitis

**STZ & high fat diet induced NASH model**

- Neonatal male mice exposed to low-dose streptozotocin (STZ).
- From week 4 to feed high fat diet (HFD; 60% kcal) for up to 16 weeks.

**Species:** Mouse

**Endpoint**

- Pathological analysis: liver oil-red, HE, sirius-red stain & NASH/fibrosis scoring.
- Biomarker analysis: liver inflammation, fibrosis and cancer related protein/gene expression

**Data Sample**

**Liver HE stain**

![Liver HE stain comparison](image)

Normal, Vehicle, Telmisartan

- Macrovesicular and microvesicular steatosis
- Ballooning degeneration, Inflammation foci

**Liver oil-red stain**

![Liver oil-red stain comparison](image)

Normal, Vehicle, Telmisartan

**Sirius red stain**

![Sirius red stain comparison](image)

Normal, Vehicle, Telmisartan

**Total NAS score**

![Total NAS score graph](image)

**Steatosis score**

![Steatosis score graph](image)

**Fibrosis Score**

![Fibrosis Score graph](image)

**Data were expressed as Mean ± SEM, N=10; #p<0.0001, ##p<0.01 compare to normal group; *p<0.05 compared to vehicle group; One-way ANOVA**