Discovery Research

In Vivo Receptor Occupancy Capabilities
## Contents

<table>
<thead>
<tr>
<th>S. No</th>
<th>Title</th>
<th>Page No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Overview</td>
<td>3-6</td>
</tr>
<tr>
<td>2</td>
<td>LC-MS/MS Based Receptor Occupancy</td>
<td>7-30</td>
</tr>
<tr>
<td>3</td>
<td>Receptor occupancy using [3H] tracers</td>
<td>31-32</td>
</tr>
<tr>
<td>4</td>
<td>Contacts</td>
<td>33</td>
</tr>
</tbody>
</table>


LC-MS/MS or LSA Based Receptor Occupancy
Overview: Methodology

Formulation

Test compound dosing
p.o./s.c./i.p./i.v.

Animal model: rat/ mouse/ guinea pig

Receptor occupancy and exposure correlation

Non-radiolabelled tracer (i.v.) dose at T_{max} of test compound

Cervical dislocation & trunk blood collection

Brain regional isolation

Sample homogenation, protein precipitation & centrifugation

LC-MS/MS quantification of tracer/ test compound (ng/g or ng/mL) - API 6500 Q Trap

Receptor occupancy calculation
(specific binding/ positive control method)

Radiolabelled ([^{3}H]) tracer (i.v.) dose at T_{max} of test compound

Cervical dislocation

Brain regional isolation

Sample homogenation & filtration or tissue digestion

Radioactivity determination by Liquid scintillation counter - Tricarb 3110TR (CPM or DPM)

Liquid scintillation analyzer (LSA) based

LC-MS/MS based
Currently practiced rat brain regions at Suven
### LC-MS/MS Based Receptor Occupancy

**Overview: Validated Targets**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Tracer</th>
</tr>
</thead>
<tbody>
<tr>
<td>#, * 5-HT$_{1A}$</td>
<td>WAY-100635■</td>
</tr>
<tr>
<td>5-HT$_{1B}$</td>
<td>AZ10419369</td>
</tr>
<tr>
<td>* 5-HT$_{2A}$</td>
<td>MDL-100907</td>
</tr>
<tr>
<td>5-HT$_{2C}$</td>
<td>SB242084</td>
</tr>
<tr>
<td>5-HT$_{4}$</td>
<td>SB207145</td>
</tr>
<tr>
<td>5-HT$_{6}$</td>
<td>Lu AE60157■</td>
</tr>
<tr>
<td>NET</td>
<td>S,S-MeNER</td>
</tr>
<tr>
<td>DAT</td>
<td>WIN 35428</td>
</tr>
<tr>
<td>#SERT</td>
<td>DASB</td>
</tr>
<tr>
<td>GABA$_A$</td>
<td>Flumazenil</td>
</tr>
<tr>
<td>CB$_1$</td>
<td>AM251</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Tracer</th>
</tr>
</thead>
<tbody>
<tr>
<td>A$_{2A}$</td>
<td>SCH442416</td>
</tr>
<tr>
<td>nAChR $\alpha_4\beta_2$</td>
<td>ZW-104</td>
</tr>
<tr>
<td>nAChR $\alpha_7$</td>
<td>Methyllycaconitine</td>
</tr>
<tr>
<td>Histamine H$_3$</td>
<td>GSK-189254</td>
</tr>
<tr>
<td>PDE10</td>
<td>AMG 7980</td>
</tr>
<tr>
<td>D$_1$</td>
<td>SCH39166</td>
</tr>
<tr>
<td>* D$_2$</td>
<td>Raclopride■</td>
</tr>
<tr>
<td>NK1</td>
<td>GR205171</td>
</tr>
<tr>
<td>mGluR5</td>
<td>mPEPy</td>
</tr>
<tr>
<td>Adrenergic $\alpha_1A$</td>
<td>Prazosin</td>
</tr>
</tbody>
</table>

All tracer are non-radiolabelled tracers; ■ Radiolabelled tracers; *# Dual or triple target receptor occupancy assay

5-HT: Serotonin; NERT: Nor epinephrine reuptake transporter; DAT: Dopamine reuptake transporter; SERT: Serotonin reuptake transporter; GABA: Gamma amino butyric acid; CB: Cannabinoid; A: Adenosine; nAChR: nicotinic acetylcholine receptor; PDE: Phosphodiesterase; D: Dopamine; NK: Neurokinin; mGluR: metabotropic glutamate receptor; MAO: monoamine oxidase
LC-MS/MS Based Receptor Occupancy
5-HT<sub>1A</sub> in Rats

Specific region: Frontal Cortex  Non specific region: Cerebellum  Tracer: WAY 100635

8-OHDPAT

Ki = 0.58 nM

SUVEN ED<sub>50</sub> = 0.95 mg/kg, s.c.
Reported ED<sub>50</sub> = 0.60 mg/kg, s.c.

Pindolol

Ki = 14.4 nM

SUVEN ED<sub>50</sub> = 1.27 mg/kg, i.v.
Reported ED<sub>50</sub> = 0.44 mg/kg, i.v.

Buspirone

Ki = 8.9 nM

SUVEN ED<sub>50</sub> = 3.74 mg/kg, i.v.
Reported ED<sub>50</sub> = 5.00 mg/kg, i.v.


The calculated % receptor occupancy ED<sub>50</sub> value from non-radiolabeled tracer is comparable to reported ED<sub>50</sub> from study measured using radiolabeled tracer
AZ10419369, shown high specific binding in hypothalamus and striatal regions and cerebellum as non specific region. This tracer can be employed in screening the ligands specific to 5-HT$_{1B}$ receptor using ratio method.

LC-MS/MS Based Receptor Occupancy
5-HT\textsubscript{2A} in Rats

Specific region: Frontal cortex
Non specific region: Cerebellum
Tracer: MDL 100907

**Ketanserin**

\[
\text{Ki} = 3 \text{ nM}
\]

\[
\begin{array}{cccccc}
\text{Ketanserin, mg/kg p.o.} & 0.1 & 0.3 & 1 & 3 & 10 & 30 \\
\% \text{ of Receptor Occupancy} & 20 & 40 & 60 & 80 & 100 & \\
\end{array}
\]

SUVEN ED\textsubscript{50} = 1.04 mg/kg, p.o.
Reported ED\textsubscript{50} = 0.32 mg/kg, p.o.

**Eplivanserin**

\[
\text{Ki} = 0.12 \text{ nM}
\]

\[
\begin{array}{cccccc}
\text{Eplivanserin, mg/kg p.o.} & 0.3 & 1 & 3 & 10 & 30 \\
\% \text{ of Receptor Occupancy} & 25 & 50 & 75 & 100 & \\
\end{array}
\]

SUVEN ED\textsubscript{50} = 1.79 mg/kg, p.o.
Reported ED\textsubscript{50} = 1.50 mg/kg, p.o.

---

The calculated % receptor occupancy ED\textsubscript{50} value from non-radiolabeled tracer is comparable to reported ED\textsubscript{50} from study measured using radiolabeled tracer
**LC-MS/MS Based Receptor Occupancy**

5-HT$_{2C}$ in Rats

Specific region: Choroid plexus

**Ritanserin**

SUVEN ED$_{50}$ = 0.08 mg/kg, s.c.

Ki = 0.33 nM

![Graph showing Ritanserin occupancy](image1)

**Mianserin**

SUVEN ED$_{50}$ = 0.04 mg/kg, s.c.

Ki = 0.67 nM

![Graph showing Mianserin occupancy](image2)

**Olanzapine**

SUVEN ED$_{50}$ = 0.68 mg/kg, s.c.

Ki = 11 nM

![Graph showing Olanzapine occupancy](image3)

Non specific region: Cerebellum

Tracer: SB 242084

Specific region: Choroid plexus

Non specific region: Cerebellum

Tracer: SB 242084
LC-MS/MS Based Receptor Occupancy
5-HT\textsubscript{4} in Rats

Specific region: Striatum
GR113808 (Antagonist)  
\[ Ki = 0.3 \text{ nM} \]

Non specific region: Cerebellum
Piboserod (Antagonist)  
\[ Ki = 0.12 \text{ nM} \]

Prucalopride (Agonist)  
\[ Ki = 25 \text{ nM} \]

PF04995274 (Partial agonist)  
\[ Ki = 0.3 \text{ nM} \]

Grimwood et al., Pfizer – Poster No: 11818 - Translational receptor occupancy for the 5-HT\textsubscript{4} partial agonist PF-04995274 in rats, non-human primates and healthy volunteers – ICAD-2011 - Paris
LC-MS/MS Based Receptor Occupancy
5-HT\textsubscript{6} in Rats

Specific region: Striatum

Obtained ED\textsubscript{50} = 0.29 mg/kg, p.o.
Reported ED\textsubscript{50} = 0.30 mg/kg, p.o.

Non specific region: Cerebellum

Obtained ED\textsubscript{50} = 5.16 mg/kg, p.o.
Reported ED\textsubscript{50} = 2.70 mg/kg, p.o.

Tracer: LuAE-60157


Idris et al., Sertindole improves sub-chronic PCP-induced reversal learning and episodic memory deficits in rodents. Psychopharmacology 2010; 23-36.


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LC-MS/MS Based Receptor Occupancy
NET in Rats

Specific region: Thalamus/Hypothalamus

Duloxetine

Ki = 10 nM

Atomoxetine

Ki = 5 nM

SUVEN ED$_{50}$ = 0.40 mg/kg, s.c.
Reported ED$_{50}$ = 1.00 mg/kg, s.c.

The calculated % receptor occupancy ED$_{50}$ value from non-radiolabeled tracer is comparable to reported ED$_{50}$ from study measured using radiolabeled tracer

**LC-MS/MS Based Receptor Occupancy**

**DAT in Rats**

Specific region: Striatum
Non specific region: Cerebellum
Tracer: WIN 35428

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**GR12909**

- **SUVEN ED\textsubscript{50} = 0.89 mg/kg, i.v.**
- **Reported ED\textsubscript{50} = 1.49 mg/kg, i.v.**

**Nomifensine**

- **SUVEN ED\textsubscript{50} = 0.65 mg/kg, i.v.**
- **Reported ED\textsubscript{50} = 2.2 mg/kg, i.v.**

LC-MS/MS Based Receptor Occupancy
SERT in Rats

Specific region: Frontal cortex
Tracer: DASB

<table>
<thead>
<tr>
<th>Drug</th>
<th>Ki (nM)</th>
<th>SUVEN ED$_{50}$ (mg/kg, i.p.)</th>
<th>Reported ED$_{50}$ (mg/kg, i.p.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paroxetine</td>
<td>0.16</td>
<td>1.27</td>
<td>1.00</td>
</tr>
<tr>
<td>Sertraline</td>
<td>0.7</td>
<td>1.97</td>
<td>2.60</td>
</tr>
<tr>
<td>Citalopram</td>
<td>0.8</td>
<td>2.48</td>
<td>4.70</td>
</tr>
</tbody>
</table>

The calculated % receptor occupancy ED$_{50}$ value from non-radiolabeled tracer is comparable to reported ED$_{50}$ from study measured using radiolabeled tracer.

The calculated % receptor occupancy ED$_{50}$ value from non-radiolabeled tracer is comparable to reported ED$_{50}$ from study measured using radiolabeled tracer.
**LC-MS/MS Based Receptor Occupancy**

**Cannabinoid-1 in Rats**

Specific region: Cerebellum/Brain stem/Frontal cortex

Tracer: AM-251

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### Rimonabant (SR141716A)

ED$_{50}$ at Suven = 1.09-1.53 mg/kg, p.o.

### AM281

ED$_{50}$ at Suven = 0.053-0.089 mg/kg, i.v.
LC-MS/MS Based Receptor Occupancy
Adenosine $A_{2A}$ in Rats

Specific region: Striatum
Non specific region: Cerebellum
Tracer: SCH442416

SUVEN $ED_{50} = 3.20$ mg/kg, i.v.

SUVEN $ED_{50} = 0.05$ mg/kg, i.p.

*Neurology.*, 2002 and 2003

The calculated % receptor occupancy $ED_{50}$ value from non-radiolabeled tracer is comparable to reported $ED_{50}$ from study measured using radiolabeled tracer
**LC-MS/MS Based Receptor Occupancy**

α4β2 nACh in Rats

**Specific region: Thalamus**

- **TC1734**
  - $K_i = 1.5 \text{ nM}$

**Non specific region: Cerebellum**

- **A366883**
  - $K_i = 1.77 \text{ nM}$

**Tracer: ZW-104**

- **Cytisine**
  - $K_i = 0.17 \text{ nM}$

The calculated % receptor occupancy $E_{D50}$ value from non-radiolabeled tracer is comparable to reported $E_{D50}$ from study measured using radiolabeled tracer.
MLA, shown high specific binding in hypothalamus and cerebellum as non specific region. This tracer can be employed in screening the ligands specific to α7 receptor using ratio method

LC-MS/MS Based Receptor Occupancy
Histamine H3 in Rats

Specific region: Frontal Cortex

Thioperamide

Ki = 4 nM

Ciproxifan

Ki = 0.5 nM

SUVEN ED$_{50}$ = 1.58 mg/kg, i.p.
Reported ED$_{50}$ = 2.70 mg/kg, i.p.

Non specific region: Cerebellum

Tracer: GSK-189254

GSK334429

Ki = 5.9 nM

SUVEN ED$_{50}$ = 0.14 mg/kg, p.o.
Reported ED$_{50}$ = 0.35 mg/kg p.o.

The calculated % receptor occupancy ED$_{50}$ value from non-radiolabeled tracer is comparable to reported ED$_{50}$ from study measured using radiolabeled tracer

JPET., 2008; Biochemical pharmacology., 2007
LC-MS/MS Based Receptor Occupancy
Phosphodiesterase 10 in Rats

Specific region: Striatum        Non specific region: Thalamus        Tracer: AMG 7980

SUVEN ED$_{50}$ = 0.57 mg/kg, p.o.
Reported ED$_{50}$ = 2.3 mg/kg, p.o.

SCH 39166, shown high specific binding in striatum & nucleus accumbens regions and cerebellum as non specific region. This tracer can be employed in screening the ligands specific to D1 receptor using ratio method.
**LC-MS/MS Based Receptor Occupancy**

**Dopamine D2 in Rats**

**Specific region:** Striatum  
**Non specific region:** Cerebellum  
**Tracer:** Raclopride

**Haloperidol**  
\[ \text{Ki} = 1.4 \text{ nM} \]

**Olanzapine**  
\[ \text{Ki} = 22.4 \text{ nM} \]

<table>
<thead>
<tr>
<th>Dose (mg/kg, p.o.)</th>
<th>% of Receptor Occupancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.03</td>
<td>25</td>
</tr>
<tr>
<td>0.10</td>
<td>25</td>
</tr>
<tr>
<td>0.30</td>
<td>75</td>
</tr>
<tr>
<td>1.00</td>
<td>75</td>
</tr>
<tr>
<td>3.00</td>
<td>75</td>
</tr>
<tr>
<td>10.00</td>
<td>75</td>
</tr>
</tbody>
</table>

SUVEN \( \text{ED}_{50} \) = 0.12 mg/kg, p.o.  
Reported \( \text{ED}_{50} \) = 0.20 mg/kg, p.o.


The calculated % receptor occupancy \( \text{ED}_{50} \) value from non-radiolabeled tracer is comparable to reported \( \text{ED}_{50} \) from study measured using radiolabeled tracer
LC-MS/MS Based Receptor Occupancy
Neurokinin 1 in Rats

Specific region: Striatum / Habenula
Non specific region: Cerebellum
Tracer: GR205171

ED_{50} = 0.30 mg/kg, p.o.

MK-869, mg/kg

% of Receptor Occupancy

0 25 50 75 100 125

0.3 1.0 3.0 10

MK-869, mg/kg p.o.

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LC-MS/MS Based Receptor Occupancy
Adrenergic alpha 1 in Rats

Specific region: Frontal cortex  Non specific region: Cerebellum  Tracer: Prazosin

ED<sub>50</sub> at Suven = 1.05 mg/kg, s.c.
LC-MS/MS Based Receptor Occupancy
mGlu5 in Rats

Specific region: Striatum
Non specific region: Cerebellum
Tracer: mPEPy or ABP688

ED$_{50}$ at Suven = 8.81 mg/kg, p.o.
**LC-MS/MS Based Receptor Occupancy**

**Sigma-1 in Rats**

Specific region: Frontal cortex/ midbrain/ pons

### Tracer: FTC-146

#### SA-4503

\( \text{ED}_{50} \text{ at Suven} \)

0.58 - 1.04 mg/kg, p.o

#### Fluspidine

\( \text{ED}_{50} \text{ at Suven} \)

0.09 - 0.11 mg/kg, p.o

#### Haloperidol

\( \text{ED}_{50} \text{ at Suven} \)

0.08 – 0.09 mg/kg, p.o

#### Donepezil

\( \text{ED}_{50} \text{ at Suven} \)

0.035 – 0.043 mg/kg, p.o
LC-MS/MS Based Receptor Occupancy
Monoamine oxidase (MAO)-A in Rats

Specific region: Striatum/ cingulate cortex/ thalamus
Tracer: Harmine

Clorgyline
ED$_{50}$ at Suven
0.041 – 0.081 mg/kg, i.v.

Tranylcypromine
ED$_{50}$ at Suven
0.525 – 0.620 mg/kg, p.o

Pargyline
ED$_{50}$ at Suven
5.0 – 5.6 mg/kg, p.o
LC-MS/MS Based Receptor Occupancy
Monoamine oxidase (MAO)-B in Rats

Specific region: Striatum/ cingulate cortex/ thalamus

Tracer: R (-) Deprenyl

Pargyline

ED$_{50}$ at Suven = 0.11 – 0.51 mg/kg, p.o

![Graph showing MAO-B occupancy for Pargyline with different doses and regions.]

Tranylcypromine

ED$_{50}$ at Suven = 0.25 – 0.48 mg/kg, p.o

![Graph showing MAO-B occupancy for Tranylcypromine with different doses and regions.]
We have added Tricarb 3110TR* to our fleet of high-end instruments.

This State-of-the-Art Technology Adds to Our Capability in Measurement of Pre-clinical Receptor Occupancy for Validated and Novel Targets

Suven has Capability to Measure RO by Labeled and Non-Labeled Tracers

*Tricarb 3110TR is a vial-based bench-top liquid scintillation analyzer (Make- PerkinElmer) and the most versatile and sensitive instrument available for detecting small amounts of alpha, beta and gamma radioactivity.
Receptor occupancy using [3H] tracers
D2 Receptor Occupancy Using [3H] Raclopride as a Tracer

Specific region: Striatum
Non specific region: Cerebellum
Tracer: $^3$H-Raclopride

RO ED$_{50}$ = 0.25 mg/kg, p.o.
Reported RO ED50 = 0.3 ± 1.1 mg/kg, p.o.
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