# Comprehensive Assessment of Inhibitory Effects of CNS-Acting Drugs on Major Neurotransmitter Transporters

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## ABSTRACT

**Purpose:** To assess the inhibitory effects of 30 CNS-acting drugs on major physiologically important neurotransmitter reuptake transporters; and to correlate the transporter inhibition data to therapeutic and adverse effects of the drugs.

**Method:** Cell-based assays for 15 major neurotransmitter transporters, such as SERT, DAT, NET, Glyt, EAATs, GATs, xCT, and "promiscuous" reuptake transporters, such as OCT3 and PMAT, have been developed and characterized. Thirty structurally diverse CNS-acting drugs, from nine therapeutic classes, were tested at high concentration (50 μM) to screen for inhibitors of these transporters. Hits from the screening were further assessed for $IC_{50}$. The inhibition data was correlated to the known clinical effects of the drugs.

**Result:** We compared our screening results with literature data for SERT, DAT and NET (very little information is available on other transporters). All previously known inhibitors of the three monoamine transporters were confirmed in our screens. It is noteworthy that our cell-based assay data suggested that some SSRIs were not as selective as previously reported based on binding assays. For example, the inhibition potency difference between SERT and DAT of Sertraline was less than five-fold to 13 fold in our study (in contrast to >100 fold as previously reported), which is in accordance with clinical results showing Sertraline exhibiting DAergic properties in addition to its primary effect on blocking 5-HT reuptake. A number of novel drug-transporter interactions were discovered from our studies. For example, Trazodone, a serotonin antagonist and reuptake inhibitor (SARI) that is often prescribed at high dose as an antidepressant, was found to inhibit all three classic monoamine transporters SERT, DAT and NET, plus OCT3, a recently discovered "uptake 2" transporter for 5-HT, at clinically relevant concentrations. In contrast, none of other SSRI or TCA antidepressants inhibited OCT3. Interestingly, another SARI drug, Nefazodone, was also demonstrated to inhibit OCT3 by another group. Together, inhibiting OCT3 could be a new MOA of Trazodone's distinct therapeutic properties compared to other antidepressants.

**Conclusion:** We have developed an assay platform for systematic assessment of drugs' inhibitory effects on key neurotransmitter transporters; the comprehensive transporter interaction profiles can be used to better understand drugs' therapeutic and adverse effects on the CNS.

## BACKGROUND

Although there are still a lot of ongoing efforts to develop anti-depressants targeting SERT, NET and DAT, it is now well established that monoamine neurotransmitters re-uptake occur through two distinct mechanisms called uptake 1 and 2. Uptake1 is actually mediated by the $Na^+Cl^-$ dependent transporters SERT, NET and DAT. The low affinity uptake2 system which is $Na^+$ independent, displays broad substrate specificity towards monoamine neurotransmitters and comprises members of the organic cation transporter family (OCTs) and the Plasma Membrane Monoamine Transporter (PMAT).

Residing in the remote extra-synaptic loci, OCTs and PMAT are capable of clearing biogenic amines from extracellular space and may serve to buffer the effects of currently marketed anti-depressants, such as SSRI, TCA and others. Several teams have already observed the impact of extra-synaptic transporters on the clinical outcome of currently marketed drugs. For instance, anti-depression action for Trimipramine, Venlafaxine, and Imipramine have been re-analyzed by taking into account the role of extrasynaptic transporters in an attempt to explain surprising clinical outcomes.

Furthermore, the role of extra-synaptic transporters has been associated with the pathophysiology and the treatment of several CNS disorders like bipolar disorder, all forms of anxiety and schizophrenia, ADHD, epilepsy, drug abuse and others. These new developments have major implications on designing therapies for CNS diseases, on the understanding of current therapies, and more importantly, on the predictability of potential neurotoxicity of both CNS and peripheral drug.

## OBJECTIVES

1) To establish and validate uptake assays for 15 human CNS transporters including monoamine transporters, EAATs, GlyTs, GATs, asc-1, xCT and promiscuous PMAT and OCT3.

2) To screen 30 structurally diverse marketed drugs against major CNS transporters. Hits (defined as >50% inhibition) were subject to further assessment for $IC_{50}$.

3) To attempt to correlate the inhibition data with previously reported unexpected clinical effects of the drugs.

## MATERIALS AND METHODS

MDCK-II cells were maintained in DMEM and seeded in Millipore Millicell 96-well insert plate (PCF-0.4 μm). Cells were transfected using a novel *in situ* transfection technology, Opti-Expression™, which allows consistent and effective transfection of polarized cell monolayers. Cells were transfected with plasmids encoding either one of the 15 human transporters including SERT, DAT, NET, GlyT1b, GlyT2, GAT1, GAT2, GAT3, EAAT1, EAAT2, EAAT3, xCT, asc-1, and PMAT. OCT3 uptake of 5-HT was also characterized. A plasmid encoding GFP was used as a mock control.

Thirty compounds were selected from nine therapeutic classes including TCA, SSRI, SNRI, MAOI, anticonvulsant, nootropics, VMAT inhibitors, GluT modulators and $Na^+$ channel blockers. Compounds were tested at 50 μM in the screening studies.

Uptake assays started 48 hours after transfection. Cells were pre-incubated with assay buffer for the appropriate amount of time. Transport was initiated by adding radio-labeled probe substrate or substrate / testing compound mixture. Following the incubation, cells were washed with PBS and solubilized with 50% acetonitrile to measure the intracellular accumulation of substrate. The amount of substrate was quantified by radiometric counting. All data are expressed as mean±SD

## RESULTS

### 1. Establishment of MDCK cell based uptake assay involving 15 human CNS transporters

By using radio-labeled substrates, uptake assays for 15 CNS transporters were successfully established in MDCK-II cells. The fold of activity (signal to background ratio) ranged from 3 to 40, depending on the transporter transfected, substrate used and the expression level of endogenous canine homolog. The uptake was substantially blunted when the standard inhibitors (other than the screening compounds) were co-applied with the substrates (Table 1).

| | SERT | DAT | NET | GlyT1b | GlyT2 | GAT1 | GAT2 | GAT3 | EAAT1 | EAAT2 | EAAT3 | xCT | asc-1 | PMAT | OCT3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Probe Substrate | 5-HT | Dopamine | | Glycine | | GABA | | | Glutamic Acid | | | | Glycine | MPP+ | MPP+ |
| Standard Inhibitor | | | | | | R-Nipecotic Acid | | | Cysteic Acid | | Way213613 | Sulfosalazine | | | Lanzoprozole |

### 2. Screening of 30 CNS drugs against major CNS transporters

None of the 30 CNS drugs were found to dramatically inhibit EAAT1, EAAT2, GAT1, GAT2 GAT3, GlyT1b or GlyT2. Their effects on classical monoamine transporters and OCT3 are shown in Fig 1.

As to monoamine transporters, TCA and SSRI were observed to significantly suppress SERT, DAT and NET. However, the selectivity of SSRI toward SERT in our inhibitory functional studies was not as great as previously reported in binding assays, regardless at the concentration tested (50 μM) in screening assays (Fig 2), or in terms of $IC_{50}$ values. For example, Sertraline, an SSRI, demonstrated only 13 fold preference toward SERT over DAT (Fig 3).

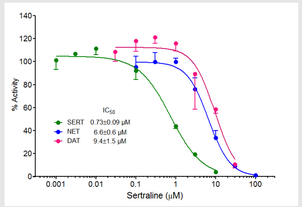


Fig 3. $IC_{50}$ values (mean±SEM) of Sertraline, an SSRI, on SERT, DAT and NET (substrates were used at the concentration of 20 nM)

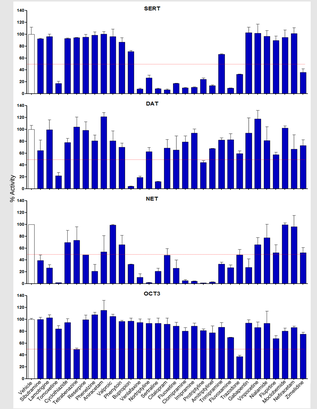


Fig 1. Inhibitory profiles of 30 CNS acting drugs on serotonin transporters including the classical SERT, DAT, NET and the "promiscuous" OCT3.

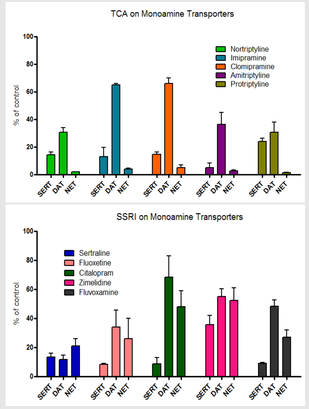


Fig 2. Selectivity of the inhibitory effects of TCA and SSRI tested at 50 μM on SER, DAT and NET.

## RESULTS (cont'd)

### 3. Trazodone'e effects on OCT3

In addition to inhibiting the transport of 5-HT and DA mediated by SERT and DAT/NET respectively, Trazodone at 50 μM significantly suppressed the uptake of MPP+ into cells through OCT3 (Fig 4).

In addition to its prototypical substrate MPP+, OCT3 also transported 5-HT into the cells with a $K_m$ of 430±105 μM and a $V_{max}$ of 193±15 pmoles/cm²/min (mean±SEM) (Fig 5).

Trazodone concentration-dependently inhibited both 5-HT and MPP+ uptake mediated by OCT3 (Fig 6).

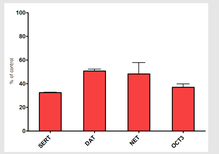


Fig 4. Besides SERT, DAT and NET, Trazodone (50 μM) inhibited OCT3 mediated uptake of MPP+.

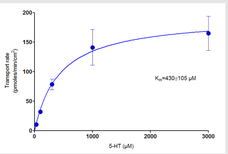


Fig 5. OCT3 mediated transport of 5-HT in MDCK-II cells.

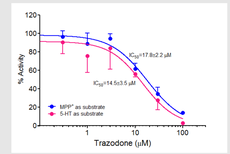


Fig 6. Trazodone concentration-dependently inhibited both 5-HT and MPP+ uptake mediated by OCT3.

## CONCLUSIONS

The MDCK-cell based assays for 15 CNS transporters were successfully established and validated (or are ready for validation).

In the screening study involving 30 CNS acting drugs against monoamine transporters, the inhibitory preference of SSRI toward SERT versus DAT/NET was less than the reported data from binding studies. Sertraline, an SSRI, was found to also inhibit DAT and NET. The suppression of DAT is consistent with its clinical DAergic effects.

Trazodone is unique by significantly inhibiting the OCT3 mediated uptake of 5-HT and MPP+. OCT3 is believed to play a role in the overall regulation of neurotransmission in the CNS, the inhibition of OCT3 may at least in part contribute to the pharmacological effects of Trazodone.

## ACKNOWLEDGEMENTS

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