# Characterization of the 5-HT$_6$ receptor antagonist, SB-742457, on cognitive test performance in the rat.

Guy A. Higgins[1,2], Leo Silenieks[1], Winnie Lau[1], Sandy Thevarkunnel[1], Elijus Undzys[1], Alan Henderson[3], Nicholas Moore[3] ([1] CanCog Technologies, Toronto ON, [2] Dept. of Pharmacology, University of Toronto, [3] Albany Molecular Research Inc., Albany, NY)

## Introduction

**Although considerable effort is currently directed toward identifying treatments that modify the neurodegeneration that contributes to Alzheimer's Disease (AD) aetiology, there is also a need to identify treatments that can relieve the symptoms of this disease. One approach that has gained significant recent interest is selective 5-HT$_6$ receptor antagonists. Indeed one 5-HT$_6$ antagonist, SB-742457, has demonstrated clinical efficacy in mild-moderate AD patients as measured using ADAS-cog and CIBIC+ scales, with an effect size equivalent to donepezil (see Upton et al, 2008). However results published from a recent Phase II study of 6 month duration conducted in a total of 371 patients randomized to treatment showed improvements in only a subset of efficacy endpoints compared to placebo (Maher-Edwards et al, 2010). In both studies, SB-742457 was reported to be well tolerated, even at exposures predicted to attain ~97% occupancy of central 5-HT$_6$ receptors.**

**In the present studies we have evaluated SB-742457 in a rodent model of cognition, the novel object recognition (NOR) task, and compared its profile to donepezil (Aricept®). Furthermore, we have conducted preliminary studies to followup on the finding that 5-HT$_6$ receptor antagonists may increase maximal electroshock seizure threshold in rats (Routledge et al, 2000; Stein et al, 2002). These studies were extended to the mouse as despite significant sequence homology between the rat, mouse and human 5-HT$_6$ receptor, the pharmacological profile of the mouse receptor appears distinct to that of the rat and human (Hirst et al, 2003). Also species differences may exist with respect to the regional expression of the 5-HT$_6$ receptor in mouse compared to rat and human (Hirst et al, 2003). Consequently, comparison of drug effect between these species offers a potential means to examine target based mechanism of action.**

## Methods

*Locomotor activity*: Male, CD-1 mice and SD rats were used as test subjects. Following a defined pretreatment time the animals are singly placed within test chambers where locomotor activity (defined as distance travelled) is measured by photocell interruptions. The spatial distribution of activity can also be measured.

*MES threshold test*: Male, CD-1 mice and SD rats were used as test subjects. Following a defined pretreatment time the animals were tested for MES seizure threshold using the 'up and down' method (Upton et al, 1997). In the mouse studies, sodium valproate was included as a positive control.

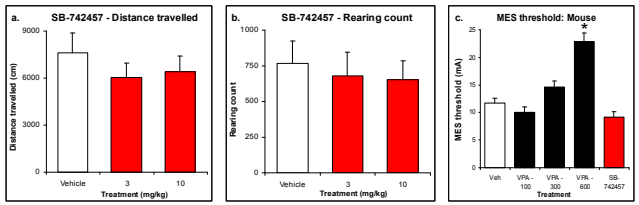
*Cholinergic side effects:* Male SD rats served as test subjects. Animals were checked for cholinomimetic signs (lacrimation, salivation, diarrhoea, tremor and hypothermia) at specific time points for up to 24 hours after drug treatment. General activity was measured at 1 hour after treatment (represented by distance travelled and rearing count).

*Novel Object recognition*: Male SD rats served as test subjects. Animals were habituated twice in the test chambers prior to test sessions. Each experiment was comprised of two trials, the sample trial (T1) and the choice trial (T2). Every exposure to the test chamber was preceded by a defined pretreatment. During T1 animals were singly placed within test chambers where two identical objects were presented. In the choice trial the animals were exposed to a duplicate of the object from T1 as well as a novel object. During both trials, animals were tracked digitally using video cameras and an automated tracking software (ANY-maze®). Comparing the amount of time spent interacting with either object during T2, it is possible to asses how well rats are able to discern between the familiar (a) and the novel objects (b). Due to the rat's spontaneous tendency to explore and interact with unfamiliar objects, this model is widely used as a measure of recognition memory, an important facet of human declarative memory (Winters et al, 2008), which is compromised in AD patients.

## Results

### A. Comparison between SB-742457 on locomotor activity & MES threshold in mouse vs. rat

**CD-1 mouse: SB-742457**



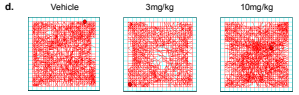
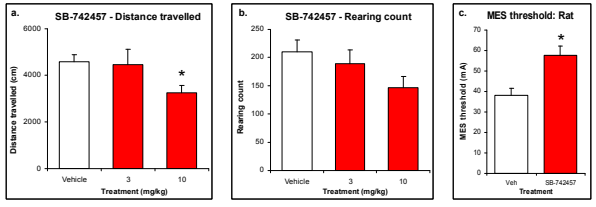




*Figure 1. Locomotor activity & MES threshold: Mouse*

(a,b) Dose response for SB-742457 (3-10mg/kg oral) on total distance travelled and rearing count in CD-1 mice recorded over a 60min activity test session. (c) Effect of SB-742457 (10mg/kg oral) on MES threshold in CD-1 mice. Sodium valproate (100-600mg/kg oral) was included in these experiments as a +ve control. (d) Representative path plots for individual mice treated with either vehicle, or SB-742457 at 3 or 10mg/kg from the activity study (a). In each study SB-742457 (3-10mg/kg) had no significant effect compared to vehicle controls.

**Sprague-Dawley rat: SB-742457**



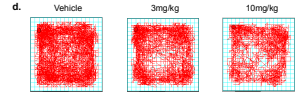




*Figure 2. Locomotor activity & MES threshold: Rat*

(a,b) Dose response for SB-742457 (3-10mg/kg oral) on total distance travelled and rearing count in Sprague-Dawley rats recorded over a 60min test session. (c) Effect of SB-742457 (10mg/kg oral) on MES threshold in SD rats. (d) Representative path plots for individual rats treated with either vehicle, or SB-742457 at 3 or 10mg/kg from the activity study (a). *P<0.05 vs. vehicle pretreatment. SB-742457 (10mg/kg) significantly reduced activity and increased MES threshold compared to vehicle controls.

**References:**
Hirst et al (2003) Mol. Pharmacology 64: 1295-1308.
Maher-Edwards et al (2010) Curr. Alzheimer Res. 7: 374-385.
Routledge et al (2000) Br. J. Pharmacology 130: 1606-1612.
Stean et al (2002) Pharmacol. Biochem. Behav. 71: 645-654.
Upton et al (1997) Br. J. Pharmacology 121: 1679-1686.
Upton et al (2008) Neurotherapeutics 5: 458-469.
Winters et al (2008) Neurosci. Biobehav. Reviews 32: 1055-1070

### B. Comparison of cholinergic related effects of donezepil (Aricept®) and SB-742457 in the SD rat

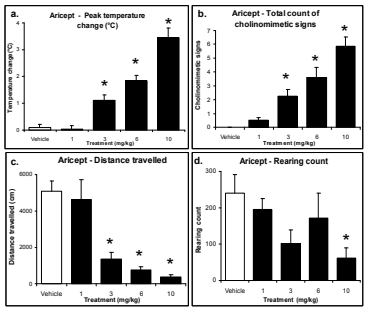



*Figure 3.* (a) Peak temperature changes recorded over a 6h period after the administration of donepezil (Aricept®). (b) Average count of cholinergic signs recorded over the same period. (c) Effect of donepezil on distance travelled and (d) rearing count. Donepezil (3-10mg/kg i.p.) produced hypolocomotion, hypothermia and increased incidence of cholinomimetic signs. *P<0.05 vs. vehicle control.

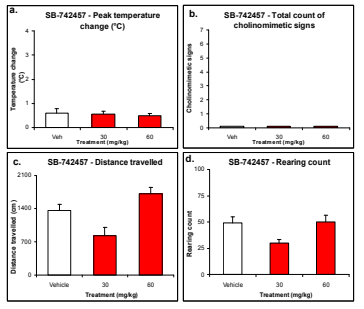
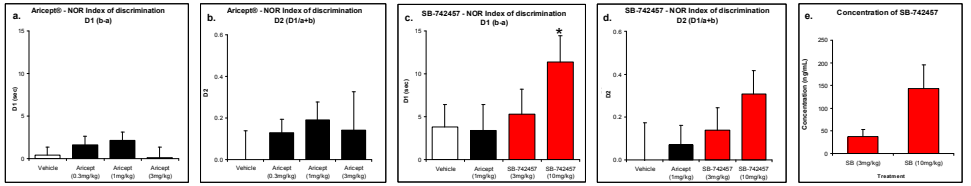



*Figure 4.* (a) Peak temperature changes over a 6h period after the administration of SB-742457. (b) Average count of cholinergic effects over the same period. (c) Effect of SB-742457 on distance travelled and (d) rearing count. SB-742457 failed to induce any obvious cholinomimetic signs. Interestingly, while activity was decreased at 30mg/kg, consistent with trend evident at 10mg/kg dose in the previous study, at the 60mg/kg dose no hypolocomotor effect was evident.

### C. Effect of donepezil (Aricept®) and SB-742457 on novel object recognition in the SD rat

**Donepezil (Aricept®)** **SB-742457**





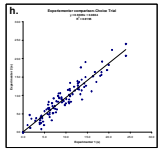




*Figure 5.* (a) Effect of donepezil (Aricept®) on the index of discrimination D1 (b-a). (b) Effect of donepezil on index of discrimination D2 ((b-a)/(a+b)). (c, d) Effect of SB-742457 on D1 and D2 respectively. (e) Plasma concentration of SB-742457 (3-10mg/kg) measured from blood plasma taken immediately post choice test phase. (f, g) Photographs to illustrate the objects used in this test and the arena used to conduct these studies. Time spent exploring each object is independently assessed by 2 trained observers, the graph (h) shows good inter-experimenter reliability.

## Summary and conclusions

1. **SB-742457 (3-10mg/kg oral) reduced motor activity and increased MES threshold in Sprague Dawley rats. The effect on MES threshold confirms previous studies by Routledge et al (2000); Stein et al (2002) using other structurally distinct 5-HT$_6$ antagonists.**
2. **Interestingly, SB-742457 (3-10 mg/kg oral) did not reliably reduce activity or increase MES threshold in CD mice. This may reflect species differences in affinity of SB-742457 for the rat vs. murine 5-HT$_6$ receptor, and/or regional species differences in expression pattern (Hirst et al, 2003).**
3. **In a 24h delay novel object recognition test, young vehicle pretreated rats failed to show a robust novel object preference.**
4. **SB-742457 (10mg/kg oral) pretreatment during the sample and choice test phases, resulted in a significant novel object preference. Mean plasma exposure immediately post test was measured at 143±15 ng/ml. In comparison, donepezil (0.3-1mg/kg i.p.) showed a modest, but inconsistent trend to produce an equivalent effect.**
5. **At doses from 3-10mg/kg, donepezil produced clear cholinomimetic signs which likely contributed to a decline in novel object test performance. In contrast, SB-742457 (30-60mg/kg oral) produced no cholinomimetic or other overt behavioral signs. These observations are consistent with a good tolerability reported in the clinic (Maher-Edwards et al, 2010).**
6. **The present studies support the hypothesis that 5-HT$_6$ receptor antagonists have pro-cognitive effects although current clinical reports seem mixed (Upton et al, 2008; Maher-Edwards et al, 2010).**