Tickling the 5-HT$_3$ receptor; potential therapeutic opportunities for patients with diarrhea-predominant irritable bowel syndrome from the selective 5-HT$_3$ receptor partial agonist, CSTI-300

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HYPOTHESIS

The 5-HT$_3$ receptor partial agonist CSTI-300 will provide symptomatic relief to patients with diarrhoea predominant irritable bowel syndrome (IBS-d) but have a better side effect profile compared to the 5-HT$_3$ receptor antagonist, alosetron.

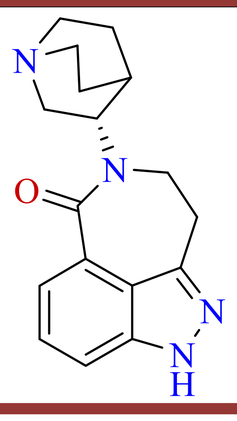
INTRODUCTION

- In diarrhoea predominant irritable bowel syndrome (IBS-d), elevated levels of 5-HT are evident in the gut.
- Current treatment with 5-HT$_3$ receptor antagonists (e.g. alosetron) deliver efficacy to IBS-d patients but sometimes have intolerable side effects including constipation and ischemic colitis due to high levels of 5-HT$_3$ receptor inhibition.
- Therefore, a 5-HT$_3$ receptor partial agonist might be a better alternative by reducing 5-HT$_3$ receptor activation in the presence of elevated levels of endogenous 5-HT, yet by not completely inhibiting receptor function should display a better side-effect profile.

METHODS

- HEK293 cells stably expressing 5-HT$_3$A receptor[1].
- Fluorescent $[Ca^{2+}]_i$ as a read out for 5-HT$_3$ receptor activity[1].
- Radioligand binding to the 5-HT$_3$A receptor[1].
- Conscious rat IBS-d model, *in vivo*[2].

CSTI-300



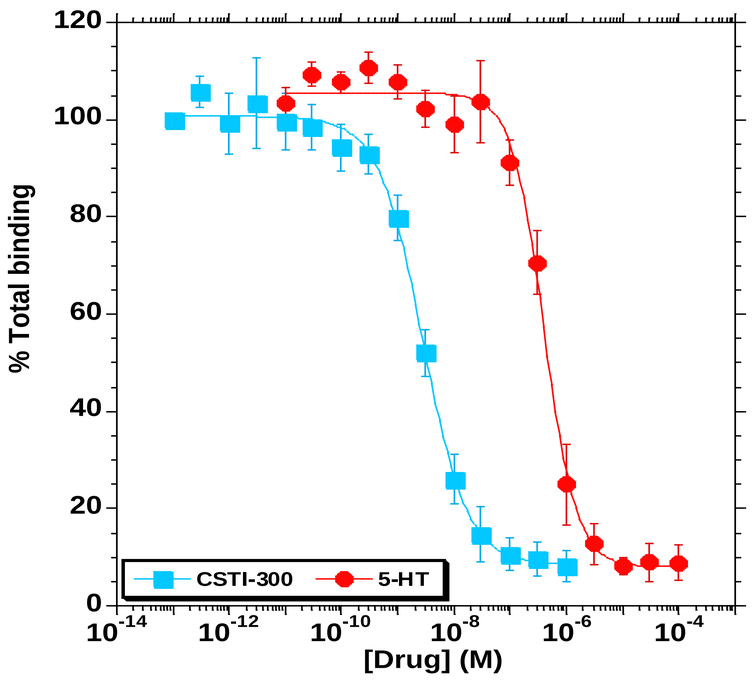


Figure 1: CSTI-300 is a high-affinity ligand for the human 5-HT$_3$ receptor.
Ability of CSTI-300 (K_i=2.26±0.48 nM, Hill number=1.19±0.16) or 5-HT (K_i=327±62 nM, Hill number=1.49±0.19) to compete for [^{3}H]-granisetron binding to human 5-HT$_3$A receptors stably expressed in HEK293 cells. Data represents mean±SEM, n=5.

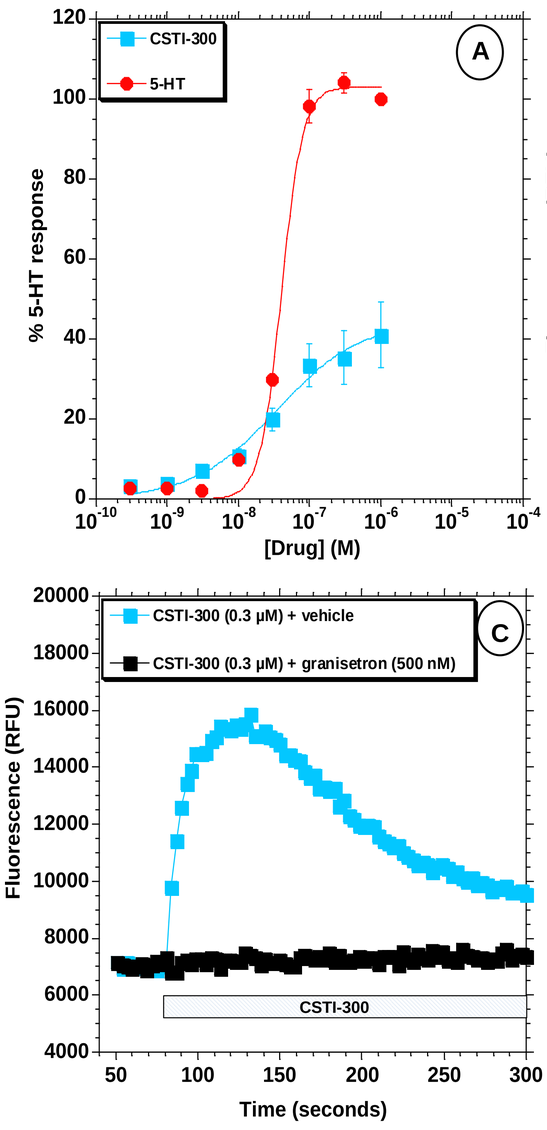


Figure 2: CSTI-300 is a potent partial agonist at the human 5-HT$_3$ receptor.
CSTI-300 (E_{max}=41±7% [relative to 5-HT], EC_{50}=25±5 nM) or 5-HT (EC_{50}=400±17) displays intrinsic activity at the human 5-HT$_3$A receptor, measured by an increase in $[Ca^{2+}]_i$ in HEK293 cells stably expressing the 5-HT$_3$A receptor. A: concentration-response curves (mean ± SEM, n=5) B: Representative traces showing changes in fluorescence over time. C, D; Representative traces (n=5) showing the ability of granisetron (selective 5-HT$_3$ receptor antagonist, 30 minutes pre-treatment) to block CSTI-300 or 5-HT induced activation of the human 5-HT$_3$A receptor.

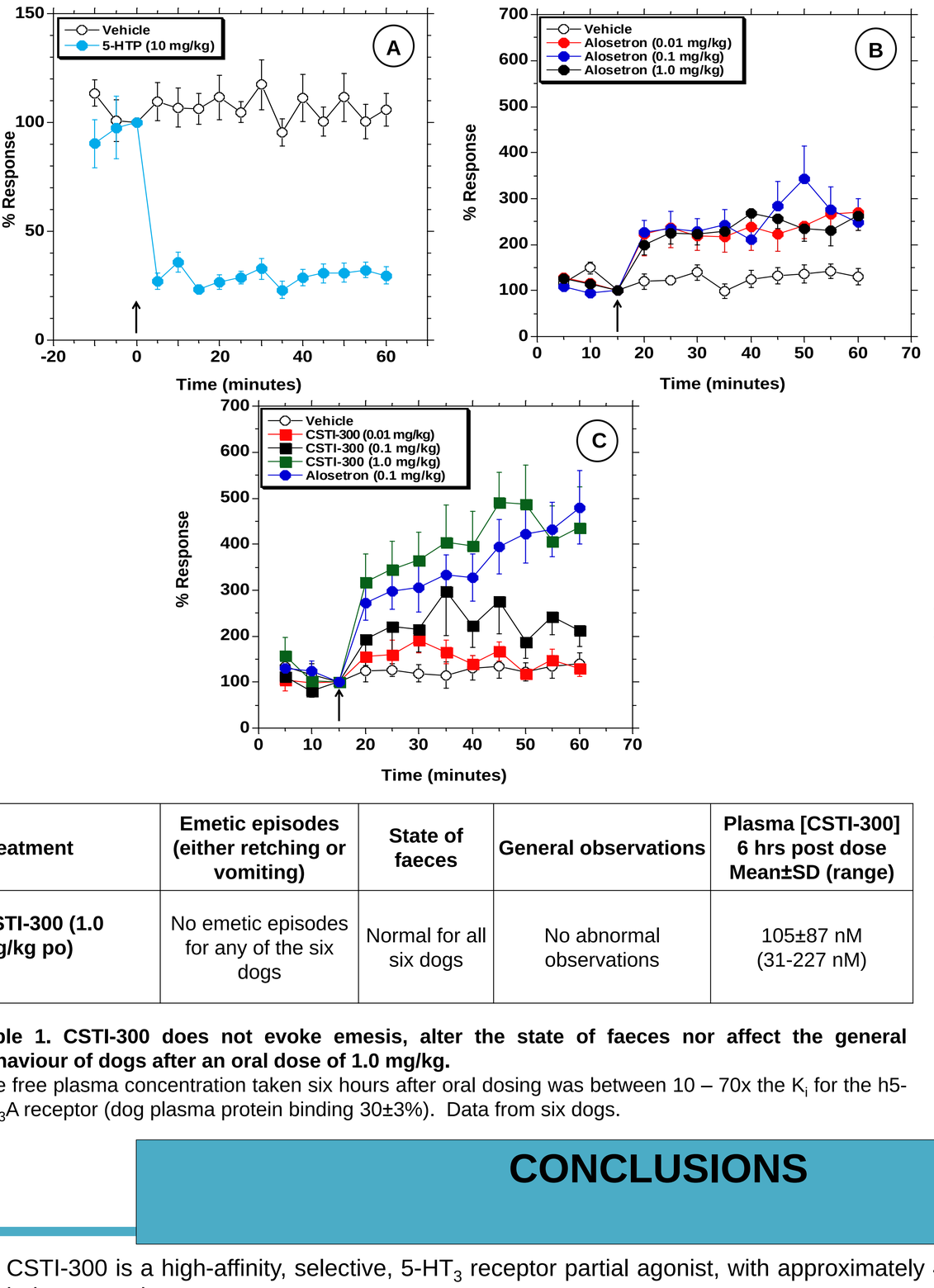


Figure 3: CSTI-300 displays comparable efficacy to the 5-HT$_3$ receptor antagonist alosetron in a conscious rat model of IBS-d, *in vivo*.
Inflation of a balloon in the colon of conscious rats allows colon sensitivity to be quantified by the balloon pressure required to evoke abdominal contractions. A: 5-HTP (10 mg/kg, s.c. arrow indicates administration), the precursor of 5-HT, increases the sensitivity of rats to colon distension. The colon sensitivity was reversed by the selective 5-HT$_3$ receptor antagonist alosetron (B, arrow indicates administration of alosetron) and the selective 5-HT$_3$ receptor partial agonist CSTI-300 (C, arrow indicates administration of CSTI-300 or alosetron). Data plotted as mean ± SEM, n=10. The impact of alosetron or CSTI-300 was statistically significant (p<0.01 to <0.0001).

Treatment	Emetic episodes (either retching or vomiting)	State of faeces	General observations	Plasma [CSTI-300] 6 hrs post dose Mean±SD (range)
CSTI-300 (1.0 mg/kg po)	No emetic episodes for any of the six dogs	Normal for all six dogs	No abnormal observations	105±87 nM (31-227 nM)

Table 1. CSTI-300 does not evoke emesis, alter the state of faeces nor affect the general behaviour of dogs after an oral dose of 1.0 mg/kg.
The free plasma concentration taken six hours after oral dosing was between 10 – 70x the K_i for the h5-HT$_3$A receptor (dog plasma protein binding 30±3%). Data from six dogs.

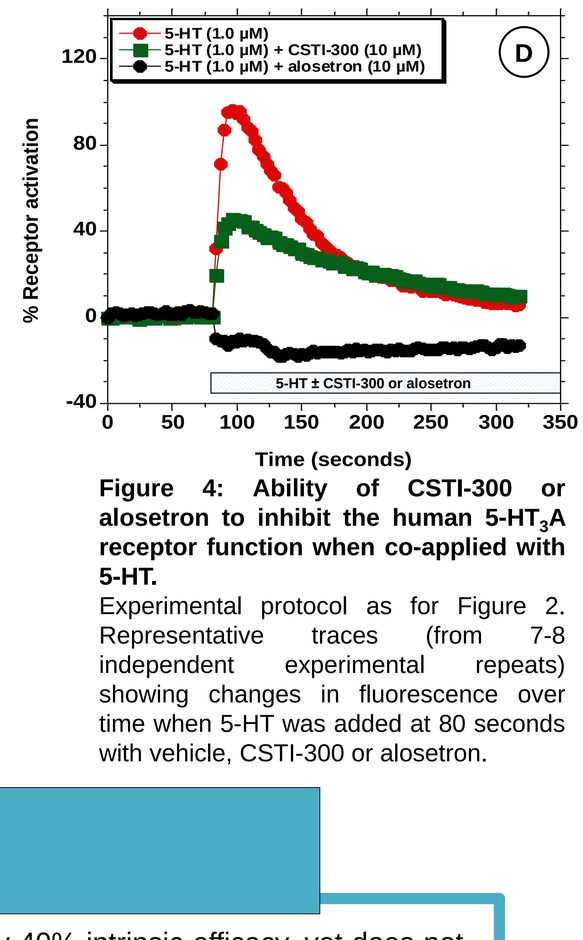


Figure 4: Ability of CSTI-300 or alosetron to inhibit the human 5-HT$_3$A receptor function when co-applied with 5-HT.
Experimental protocol as for Figure 2. Representative traces (from 7-8 independent experimental repeats) showing changes in fluorescence over time when 5-HT was added at 80 seconds with vehicle, CSTI-300 or alosetron.

CONCLUSIONS

- CSTI-300 is a high-affinity, selective, 5-HT$_3$ receptor partial agonist, with approximately 40% intrinsic efficacy, yet does not induce emesis.
- CSTI-300 (and alosetron) display comparable efficacy in a rat model of IBS-d, *in vivo*.
- CSTI-300 displays attractive therapeutic potential to treat IBS-d, with a predicted reduced side effect profile compared to 5-HT$_3$ receptor antagonists like alosetron.

REFERENCES

1. **Powell A.D. et al. *Br J Pharmacol* 114 (2016). 173, 3467–3479.**
2. **Banner S.E. and Sanger G.J. (1995). *Br J Pharmacol* 114, 558-562**