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Development of a high-throughput neurotensin 1 receptor assay

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Introduction*:* The neurotensin 1 receptor (NTS1R) is a promising GPCR drug discovery target. There is potential to develop novel NTS1R pharmacotherapies for a range of indications that include pain, Alzheimer's disease, Parkinson's disease, schizophrenia, addiction, and cancer. Consequently, robust and high-throughput NTS1R assays are of high value to exploit this target.

Methods*:* We have developed a 384-well NTS1R calcium assay using FLIPRtetra. A CHO cell line stably expressing the human NTS1R was created, which provided a pure system in which to study the receptor.

Results*:* Responses to the endogenous agonist neurotensin were robust and reproducible, yielding an EC_{50} of 7.2 ± 1.4 nM (n=3), and assay Z' values at EC_{90} stimulation levels of consistently > 0.7. Characterisation of a diverse panel of agonists confirmed the amenability of the assay for pharmacological studies, revealing a range of potencies and intrinsic activities. Furthermore, in antagonist mode, we show that tool antagonist compounds display expected potencies. We will describe a number of ways in which this assay can be used to identify novel hit matter with activity at NTS1R and good drug-like properties. These include the Domainex "PharmaProfiler" library of 400 approved drugs, and our "LeadBuilder" virtual screening technology.

Conclusions*:* In summary, we have developed a reliable GPCR calcium assay, and successfully employed it for high-throughput screening. Interrogating these targets with the full suite of integrated drug discovery platforms at Domainex will offer exciting opportunities to develop novel GPCR medicines.