Characterisation of [^{3}H]-HTL45725, A Novel Radioligand for the Orphan Receptor GPR52

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Introduction

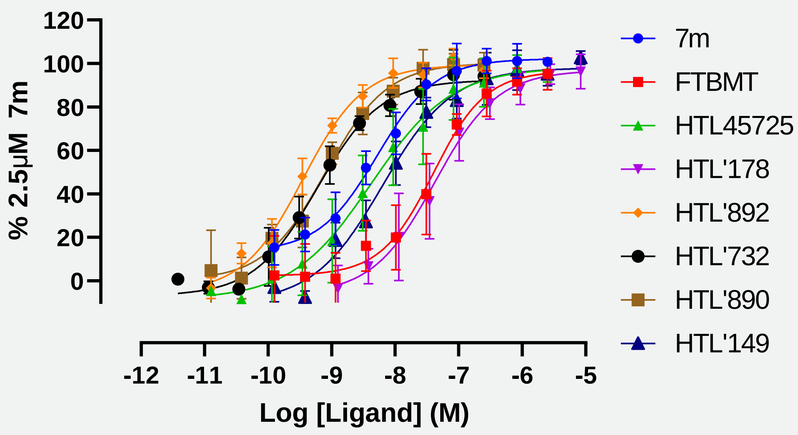
- GPR52 is a constitutively active, orphan G protein coupled receptor which is highly expressed within the brain. It has been shown to co-localise with the dopamine D2 receptor in the striatum and the dopamine D1 receptor in the cortex, leading to interest in GPR52 as a potential target for the treatment of schizoaffective and related disorders[1].
- There is no known endogenous ligand for GPR52 and only a handful of ligands have been reported in literature[2,3,4]. We therefore set out to generate a radioligand for GPR52 in order to characterise GPR52 ligands.

Methods

- *Cell culture and membrane generation:* HEK293T cells were infected with 5% v/v human or rat GPR52 BacMam for 24h prior to harvesting. Cell pellets were homogenised in harvesting buffer (20mM HEPES, 10mM EDTA, pH 7.4) and centrifuged (15min, 41,000*g*, 4°C). Pellets were then homogenised again and centrifuged (45min, 41,000*g*, 4°C). Membranes were resuspended in storage buffer (20mM HEPES, 0.1mM EDTA, pH7.4) and stored at -80°C until required.
- *GPR52 cAMP assays:* Frozen GPR52 expressing cells were seeded 4000 cells per well on proxiplates containing test compound and incubated for 30min at 37°C in stimulation buffer (Cisbio). cAMP was detected using HiRange cAMP kit (Cisbio) according to the manufacturer's instructions.
- *[^{3}H]-HTL45725 binding assays*: 25µg human or rat GPR52 membranes were incubated for 2h with [^{3}H]-HTL45725 and competing ligands in binding buffer (20mM HEPES + 10mM $MgCl_2$ + 0.1% BSA). Membranes were harvested by rapid filtration using TomTec harvester onto 0.1% PEI coated GF/B plates and washed 3x with cold PBS. For kinetic experiments, membranes were added at indicated time points. Non-specific binding was determined by 10µM HTL'892.

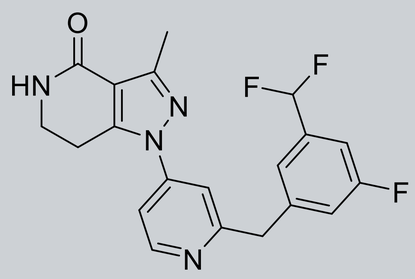
Results

- **Figure 1: Proprietary GPR52 agonists increase cAMP in GPR52-expressing HEK-f cells.** All GPR52 agonists tested were full agonists, including literature GPR52 compounds 7m[2] and FTBMT[3] with compound potencies summarised in the table. Data shown are pooled (mean ± SD) from at least 4 independent experiments.



Compound	pEC_{50}
7m	8.34 ± 0.25
FTBMT	7.57 ± 0.30
HTL45725	8.58 ± 0.30
HTL'178	7.54 ± 0.23
HTL'892	9.45 ± 0.09
HTL'732	9.63 ± 0.57
HTL'890	8.98 ± 0.22
HTL'149	8.14 ± 0.17

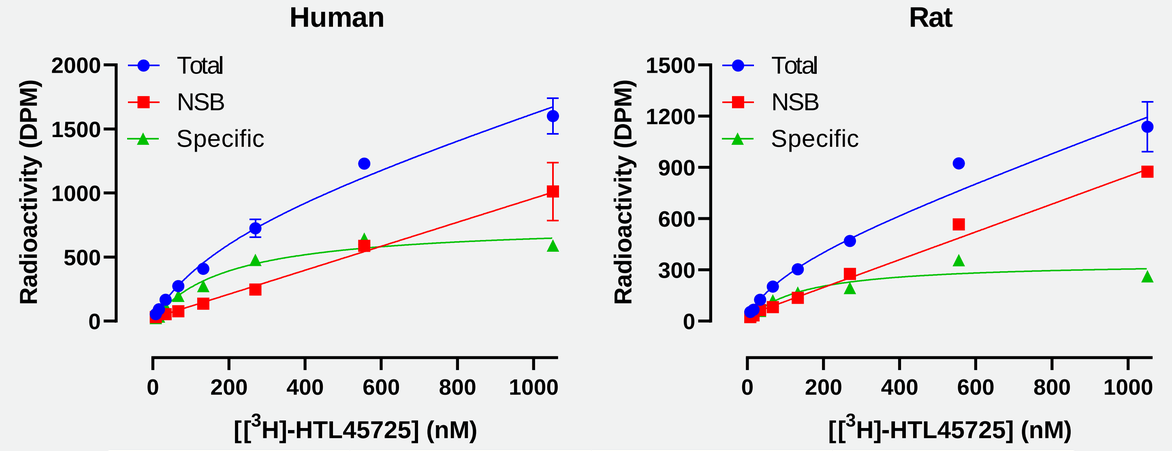
- **Figure 2: Structure and properties of HTL45725.** HTL45725 was selected for radiolabelling based on its high potency and favourable properties including good LLE and lower PPB than other test compounds. A CNS binding panel (Eurofins Drug Abuse Potential Panel), showed no significant activity (<50% binding at 10µM) to any of the CNS receptors tested, suggesting good selectivity for GPR52.



HTL45725
GPR52 pEC_{50} 8.6
MWt 386 clogP 2.7 PSA 59.8
rPPB 92.0% hPPB 93.6%
Patent WO2021/090030A1

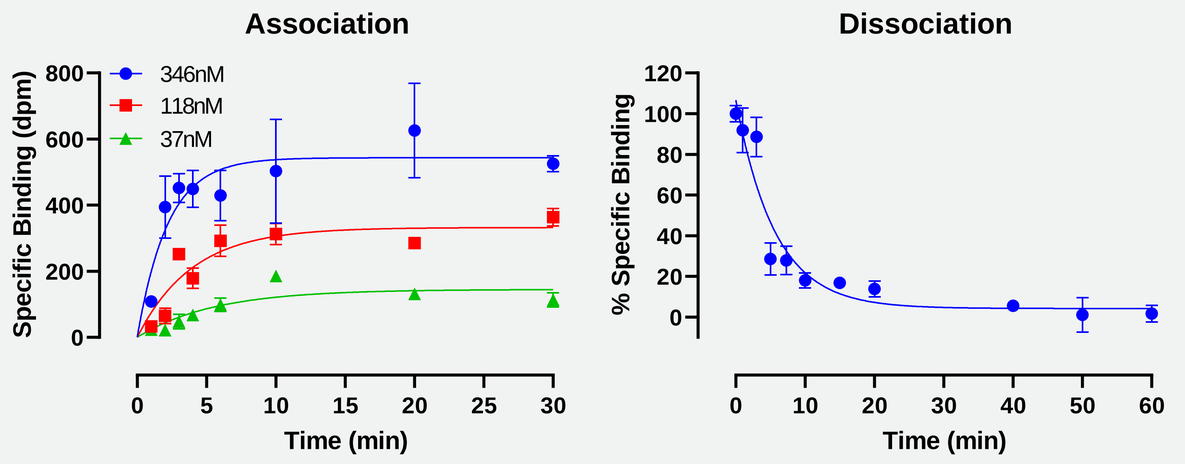
Results continued

- **Figure 3: [^{3}H]-HTL45725 shows saturable and specific binding to both human and rat GPR52 in recombinant HEK-GPR52 membranes.** pK_Ds were consistent between the human and rat receptor. No specific binding detected in untransfected HEK cell membranes. Data shown are representative example (mean ± SD) from 3-4 independent experiments.

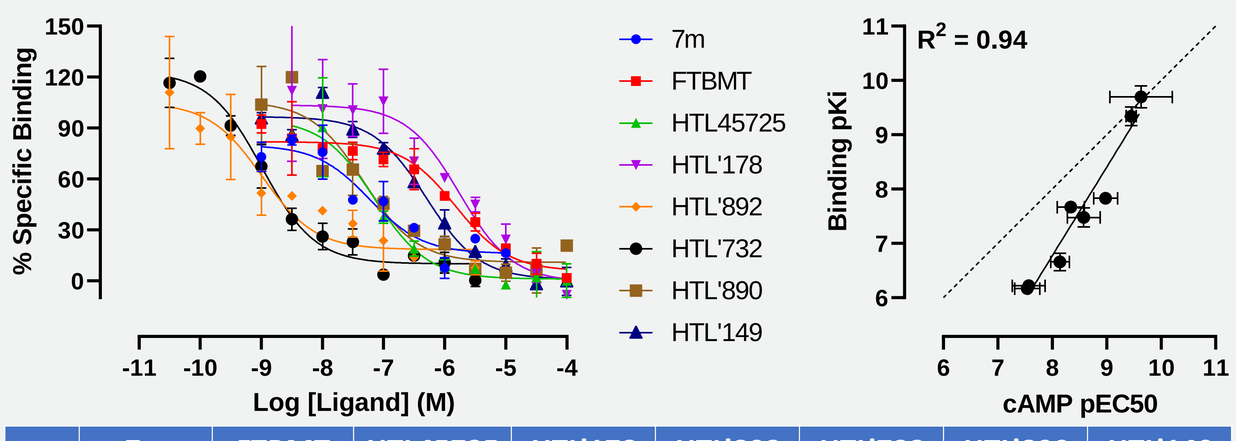


Species	pK_D	B_{max} (pmol/mg)	n
Human	7.0 ± 0.2	0.6 ± 0.2	4
Rat	7.1 ± 0.4	0.3 ± 0.1	3

- **Figure 4: [^{3}H]-HTL45725 binding kinetics were determined at human GPR52.** Kinetic parameters were calculated as following: k_{on} 8.52 ± 0.23 x10^5, k_{off} 0.15 ± 0.00 and kinetic pK_D 6.76 ± 0.01 (n=2). Data shown are representative example (mean ± SD) from 2 independent experiments.



- **Figure 5: Competition binding at human GPR52 derives compound affinities.** pK_is showed excellent correlation to pEC_{50}s from functional cAMP assays (R^2 = 0.94) and are summarised in the table below. Data shown are representative example (mean ± SD) from 2 independent experiments.



	7m	FTBMT	HTL45725	HTL'178	HTL'892	HTL'732	HTL'890	HTL'149
pK_i	7.7 ± 0.1	6.2 ± 0.1	7.5 ± 0.2	6.2 ± 0.1	9.3 ± 0.2	9.7 ± 0.2	7.8 ± 0.0	6.7 ± 0.2

Conclusions

- [^{3}H]-HTL45725 has been identified as a high affinity, selective radioligand for GPR52 and is the first radioligand to be described for this orphan receptor.
- [^{3}H]-HTL45725 allows the derivation of compound affinities for GPR52 and will hopefully aid further characterisation of this receptor in native tissue.

References

1. Komatsu *et al* (2014) PLoS ONE 9(2): e90134
2. Setoh *et al* (2014) J Med Chem 57:5226
3. Nishiyama et al (2017) J Pharmacol Exp Ther 363:253
4. Stott *et al* (2021) Heylion 7 (6): E07201

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