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SEARCHING FOR SAFER AND MORE EFFECTIVE MEDICATIONS IN THE MANAGEMENT OF SEIZURE DISORDERS: A 5-HT$_{2C}$ SUPERAGONIST

Randall Kaye,[1] Graeme Semple,[2] David Unett,[2] Ibragim Gaidarov,[2] Todd Anthony[2]

[1]Longboard Pharmaceuticals, La Jolla, CA, USA; [2]Eurofins Beacon Discovery, San Diego, CA, USA

LONGBOARD PHARMACEUTICALS

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BACKGROUND

- Superagonists are defined as ligands that produce a greater magnitude of response (ie, higher receptor output signaling) than that of the endogenous agonist[1-4]
  - Exogenously administered superagonists may allow for supraphysiological efficacy when used therapeutically
- LP352 is a potent and selective 5-hydroxytryptamine (5-HT)$_{2C}$ agonist designed to have increased selectivity for the 5-HT$_{2C}$ receptor (versus 5-HT$_{2A}$ and 5-HT$_{2B}$) compared with serotonergic agonists such as fenfluramine and lorcaserin
  - Increased selectivity may reduce the potential for adverse effects associated with 5-H$_{T2A}$ (eg, hallucinogenic activity)[5] and 5-HT$_{2B}$ agonism (eg, cardiovascular disease)[6,7]
- LP352 displays a binding affinity (Ki) of 44 nM at the human 5-HT$_{2C}$ receptor
- LP352 is currently in development for the treatment of seizures associated with developmental and epileptic encephalopathies

OBJECTIVES

- Explore the activity of LP352 at the 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ receptors in receptor binding and functional assays
- Compare relative activity of LP352 at the 5-HT$_{2C}$ receptor expressed recombinantly in HEK293 cells or endogenously in rat choroid plexus cells with that of the endogenous ligand (5-HT)

METHODS

Dynamic Mass Redistribution (DMR) Assays

- DMR assays were performed in HEK293 cells expressing the human 5-HT$_{2C}$ receptor and in rat choroid plexus epithelial cells (which express endogenous 5-HT$_{2C}$)
- Data were analyzed by measuring the change in the DMR response from baseline at a timepoint that produced a maximal response (typically 30-60 minutes following compound addition)
- DMR assays were performed using a Corning Epic® BT reader

Inositol Phosphate (IP) Accumulation Assays

- IP accumulation assays were performed in primary rat choroid plexus epithelial cells using [^{3}H]myo-inositol
- IP accumulation assays provide a more specific assessment of G protein activation by the test compounds

Radioligand Binding Assays

- Radioligand binding assays were performed using 5-HT$_2$ receptor–expressing HEK293 cell membranes and [125I]DOI as radioligand

RESULTS

Recombinant Human 5-HT Receptors

- Functional activity of LP352 was undetectable at human 5-HT$_{2A}$ or 5-HT$_{2B}$ receptors up to a test concentration of 10 µM (**Figure 1A-B**). In binding assays, modest displacement of [^{125}I]DOI at human 5-HT$_{2A}$ or 5-HT$_{2B}$ receptors was observed only at 10 µM (**Figure 2A-B**)
- At the human 5-HT$_{2C}$ receptor, LP352 demonstrated maximal activity exceeding that induced by the endogenous ligand 5-HT (**Figure 1C, Figure 2C**)
- Lorcaserin, 5-HT, and LP352 all generated positive dose responses in the DMR assay (**Figure 3**)
  - At increasing concentrations, the maximal cellular response of LP352 exceeded that of the partial agonist lorcaserin and the endogenous ligand 5-HT, consistent with classification as a superagonist at 5-HT$_{2C}$ receptors

Endogenous 5-HT Receptors

- LP352 demonstrated superagonist activity (activity greater than that of the endogenous ligand 5-HT) as measured by both IP accumulation and DMR assays (**Figure 4A-B**)
- Dose response of 5-HT in the presence and absence of 10 µM LP352 (**Figure 4C-D**)
  - Increasing concentrations of 5-HT reduced the activity of LP352, confirming that 5-HT has lower efficacy than LP352

Figure 1. Inositol Phosphate Accumulation Assay

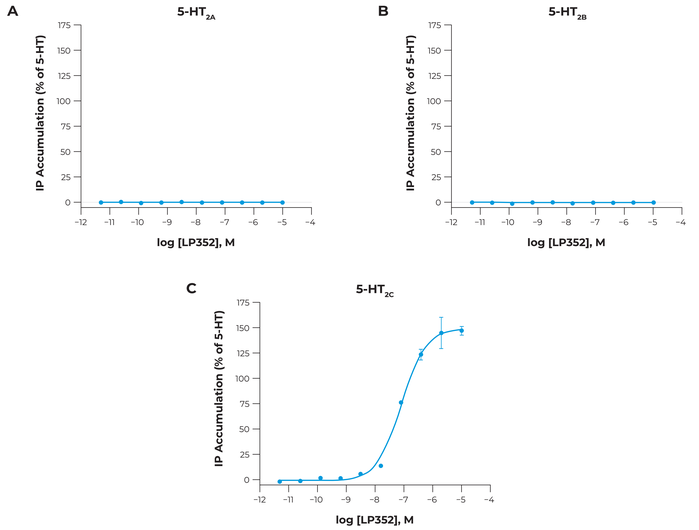


Figure 2. Radioligand Binding

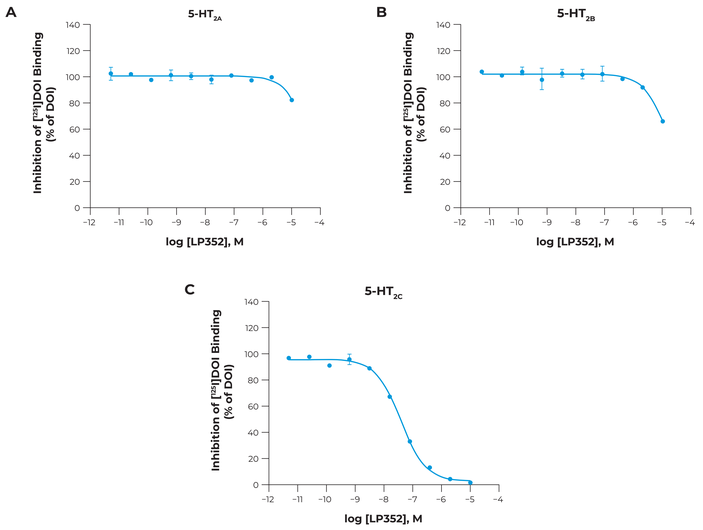


Figure 3. Dynamic Mass Redistribution Assay in Human 5-HT2C–Expressing HEK293 Cells

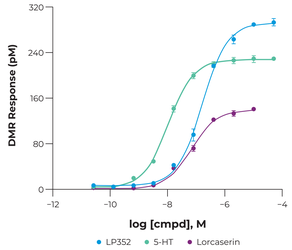
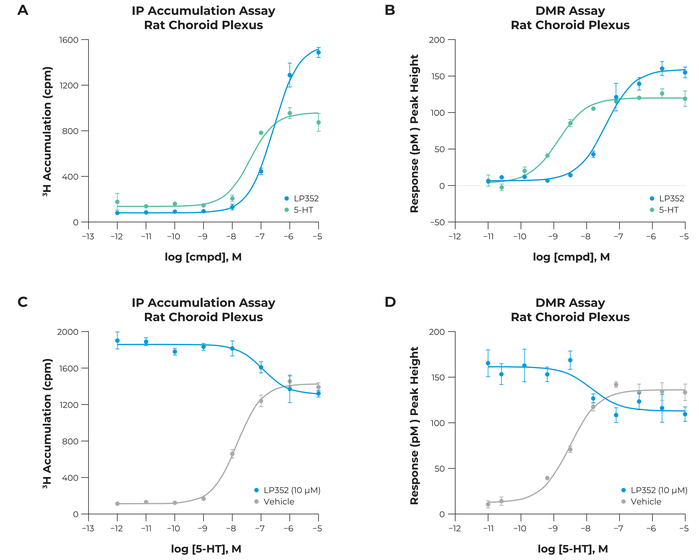


Figure 4. Rat Choroid Plexus Epithelial Cell Assays



CONCLUSIONS

- **LP352 is a potent 5-HT$_{2C}$ receptor agonist with high selectivity for the 5-HT$_{2C}$ receptor versus 5-HT$_{2A}$ and 5-HT$_{2B}$**
  - **LP352 shows no functional agonism at 5-HT$_{2A}$ or 5-HT$_{2B}$ at concentrations up to 10 µM**
  - **LP352 shows >200-fold selectivity at 5-HT$_{2C}$ versus 5-HT$_{2A}$ or 5-HT$_{2B}$ in radioligand binding assays**
- **LP352 is a 5-HT$_{2C}$–specific superagonist**
  - **Maximal LP352-induced cellular responses exceeded that of the endogenous agonist, 5-HT**
  - **LP352 superagonism may drive greater in vivo efficacy compared with 5-HT$_{2C}$ partial or full agonists**
- **Further clinical studies should be undertaken to determine if this highly targeted superagonism translates to safety and/or efficacy advantages in disorders likely to benefit from this unique pharmacology**

Abbreviations **5-HT**, 5-hydroxytryptamine; **cpm**, counts per minute; **cmpd**, compound; **DMR**, dynamic mass redistribution; **IP**, inositol phosphate.

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