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ML417: a potent and highly selective D3 dopamine receptor agonist

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Introduction: Dopamine receptors (DRs) are involved in the development and/or treatment of many neuropsychiatric disorders including schizophrenia and Parkinson's disease (PD). Many currently available dopaminergic drugs modulate both DRD2 and DRD3 due to high homology in their orthosteric binding sites, leading to potential unwanted side effects and also uncertainty as to the roles each DR subtype plays in normal and pathological processes. Thus, a highly selective DRD3 agonist may have great utility from multiple perspectives. As such, our lab has employed high throughput screening paradigms as an approach to discover highly selective modulators for the DRD3 for use as both research tool compounds and leads for therapeutic drug development.

Methods: The NIH Molecular Libraries Program 400,000+ small molecule library was initially screened in a 1536-well format using the DiscoveRx PathHunter® β-arrestin assay for compounds that activate the DRD3 without effects on the DRD2. Confirmation and counter-screens were performed to obtain an initial assessment of selectivity and mechanisms of action. We identified 62 potential agonists, and chose the most promising hit compound to perform a structure activity relationship (SAR) study to increase potency while maintaining selectivity. 375 analogs were synthesized and screened in β-Arrestin assays. The lead compound identified through this process, ML417 ((1H-indol-2-yl)(4-(2-(4-methoxyphenoxy)ethyl)piperazin-1-yl)methanone), was also characterized using bioluminescence resonance energy transfer (BRET)-based β-arrestin recruitment and G-protein activation assays (1) as well as p-ERK assays. Potential neuroprotective properties of this compound were assessed using a terminally differentiated SHSY5Y neuronal cell model.

Results: ML417 displays potent, DRD3-selective agonist activity in multiple functional assays.

Assay	**DRD3 EC_{50} (n≥4)**	**DRD2 EC_{50} (n≥4)**
DiscoveRx β-Arrestin	36 ± 5.6 nM	> 10,000 nM
β-Arrestin BRET	1.2 ± 0.5 nM	> 10,000 nM
Go BRET	0.21 ± 0.16 nM	> 10,000 nM
p-ERK	21 ± 6.6 nM	> 10,000 nM

In addition, radioligand inhibition binding and functional GPCR screens (>165 receptors) show ML417 has limited cross-reactivity with other GPCRs. ML417 also displays superior [compared to the reference compound pramipexole], dose-dependent protection against a decrease in neurite length induced by 10 μM of the neurotoxin, 6-hydroxydopamine in the SHSY5Y cell model.

Conclusions: A high throughput screening campaign and iterative medicinal chemistry efforts yielded ML417, a potent and highly selective DRD3 agonist. This compound will be useful as a research tool to identify biological processes governed by DRD3 activation, and may prove useful as a therapeutic drug lead.

References:

1. Sanchez-Soto M et al. (2016) *Mol.Pharmacol.* **89,** 457-466.