Research proposal: Drug design of a third generation antipsychotic for schizophrenia treatment

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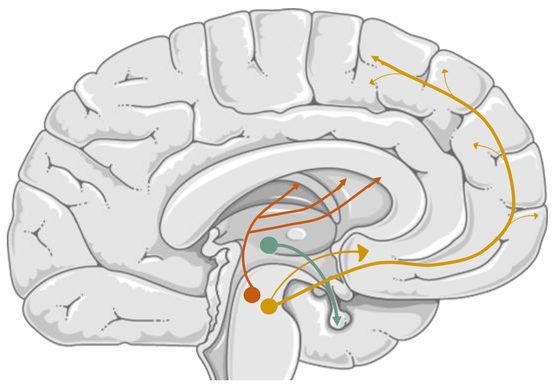
INTRODUCTION



Schizophrenia is a complex, debilitating, chronic psychiatric disorder that affects roughly 1% of the world's population; that is, 21 million people. Manifestations of the disease include positive symptoms (hallucinations, delusions) and negative symptoms: cognitive disturbances that include avolition, emotional dysregulation, apathy, lack of initiative and motivation, anhedonia and social withdrawal; and expressive deficits which include blunted affect and poverty of speech.

Although their clinical expression is less obvious than that of positive symptoms, negative symptoms can significantly impact patients' psychosocial functioning, quality of life and long-term outcome. Amongst others, dopaminergic dysregulation seems to be responsible for the pathophysiology of the disease. Antipsychotic medication mostly targets dopamine D2 receptors and is able to ameliorate positive symptoms, although they cause side effects that include hyperprolactinemia, extrapyramidal symptoms (EPS) and severe tardive dyskinesia (TD).

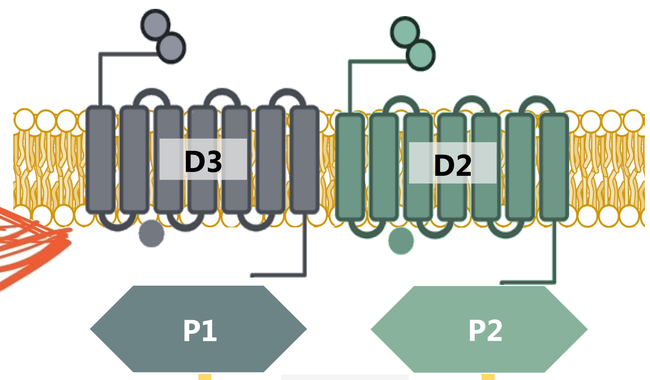
Despite its societal burden, no available treatment for schizophrenia is free of side effects or is able to successfully ameliorate positive and negative symptoms. Partial D2 agonists are believed to cause fewer EPS than total agonists. Recent research has found D3-preferring antagonists have an effect on negative symptoms. Also, D2-D3 heteromer formation has been suggested to have pathophysiological implications and proposed as a potential drug target.

TUBEROINFUNDIBULAR PATHWAY → Hyperprolactinemia

MESOLIMBIC PATHWAY → Positive symptoms

MESOCORTICAL PATHWAY → Negative symptoms

NIGROSTRIATAL PATHWAY → EPS + TD



METHODS

Google scholar · PDB PROTEIN DATA BANK · PubMed · UniProt · PubChem

Keywords: schizophrenia, bivalent ligand, GPCR, antipsychotics, negative symptoms, drug design, heterodimers, atypical antipsychotics, animal model, dopamine

HYPOTHESIS & RESEARCH OBJECTIVES

Hypothesis: Using previously-described dopamine receptor ligands, we can synthesize a bivalent ligand that targets the D_2-D_3 heterodimer and is able to ameliorate positive as well as negative symptoms of schizophrenia

MAIN GOAL: Design of a third generation antipsychotic for schizophrenia treatment

OBJECTIVES

1. Design a bivalent ligand that specifically binds to the D_2D_3R
2. Ensure that the ligand shows pharmacological activity *in vitro*
3. Assess its effect on positive and negative symptoms on an animal model of schizophrenia

BIVALENT LIGAND DESIGN

DETERMINING LINKER LENGHT

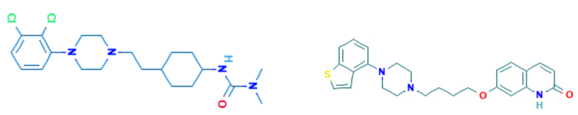
The ZDOCK protocol in Discovery studio 2.5 suite will be used to search the heterodimer interface of the minimized receptor crystal structures. We will use a computational tool designed specifically for this to calculate the preferred spacer length for the selected interface.

DESIGN

The selected pharmacophores, a D3-prefferrng partial agonist, and a D2 partial agonist, will be linked by PEG molecules, to avoid increases in hydrophobicity, using *Chemsketch* .

DOCKING

For all minimized_ligand-receptor pairs, the ligand will be docked and its stability will be assessed by 1 μs of unbiased molecular dynamics simulation.

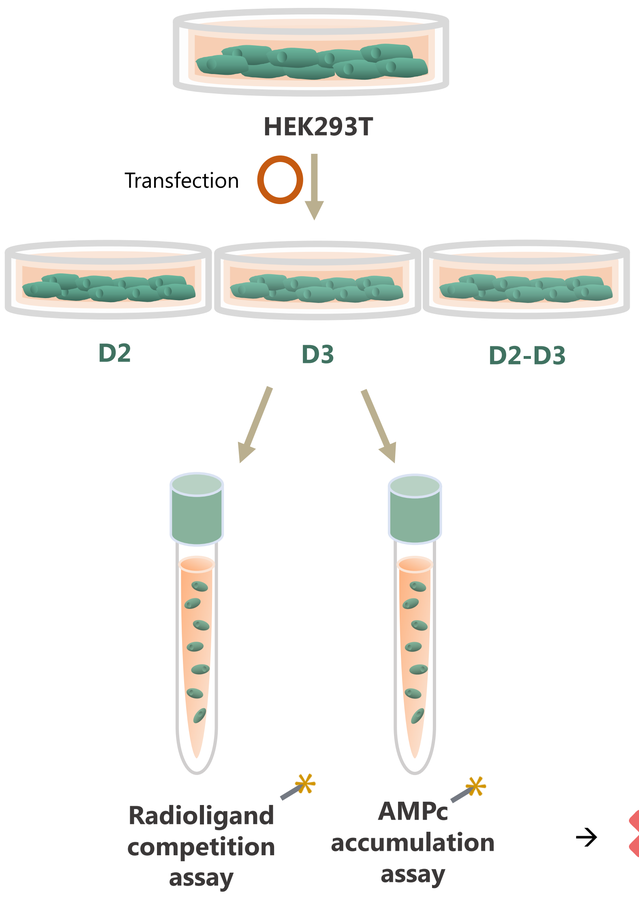


Cariprazine — Brexpiprazole

PEG

→ ✖ → Structure based pharmacophore → **Virtual Screening**

BIOLOGICAL EVALUATION



TRANSFECTION

HEK 293T cells will be cultured in standard conditions in DMEM + 10% FBS + antibiotics. Cells will be transfected with the plasmid DNA containing dopamine receptors and the chimeric AC-V/VI by the Polyethylenimine method.

RADIOLIGAND BINDING

Three days after transfection, HEK293T cells will be suspended in Tris·HCl pH 7.4 buffer with different concentrations of **[^{3}H]raclopride** with the added presence of **varying concentrations** of the **unlabeled bivalent ligand**. The nonspecific binding will be determined in the presence of haloperidol. Free and cell-bound ligand will be separated by rapid filtration. The radioactivity counts will be measured with a Tri-Carb 2800TR liquid scintillation analyzer.

cAMP ACCUMULATION

24h after transfection cells will be incubated for 2h with fresh growth medium containing **[^{3}H]adenine**, which will be replaced by medium containing **phosphodiesterase inhibitors.** After, they will be incubated with **forskolin** in the presence or absence of the bivalent receptor ligand. The amount of recovered **[^{3}H]cAMP** will be determined by a two-step column separation procedure,

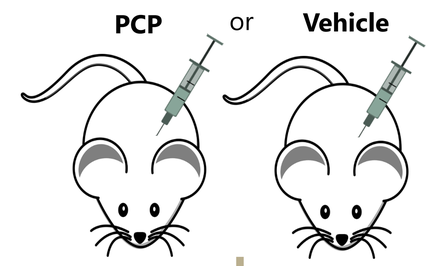
→ ✖ → **BiFC assay**: Are receptors dimerizing?

EFFECT ON SCHIZOPHRENIA SYMPTOMATOLOGY

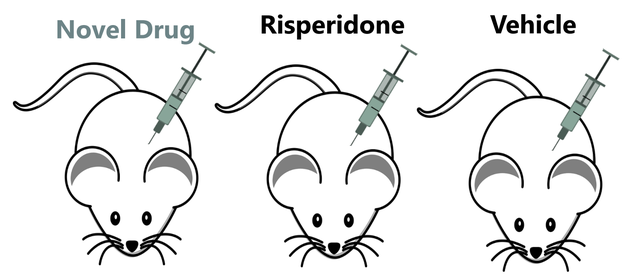
Novel Drug

Following single i.v. and p.o. administration of novel drug

***In vivo* pharmacokinetics**



PCP: NDMA antagonist that induces positive and negative-like symptoms



ANOVA ANALYSIS

MOTOR ACTIVITY

POSITIVE SYMPTOMS

Rats will be placed in square cages equipped with infrared photobeams along the bottom of the cage for an hour. Motor activity will be determined as the total number of beam interruptions during this period.

CATALEPSY

EPS

Animals will be placed with the forepaws on a high podium. Rats will be considered cataleptic if they do not correct their body posture within 30s.

NOVEL OBJECT RECOGNITION

NEGATIVE SYMPTOMS

Acquisition trial: animals placed in the test box and allowed to explore 2 identical objects. Retention trial: animals placed in the test box with 1 duplicate object from the acquisition and 1 novel object- Total object exploration time and discrimination index will be analysed.

SOCIAL INTERACTION

Two unfamiliar rats will be placed in a open-field box with black gridlines, with an unfamiliar object for a 10' period. We will score following, investigative sniffing behavior, avoidance, object exploration, and line crossings.

✖ → determine in vivo binding of [3H](+)-PHNO → demonstrate ***in vivo* D2/D3 binding affinity of novel drug**

DISSEMINATION PLAN

Scientific Journals

Social media

Conferences

DISSCUSSION AND CONCLUSIONS

Available treatment for schizophrenia presents serious side effects such as extrapyramidal symptoms and tardive dyskinesia and fails to ameliorate negative symptoms of the disease, which have an important impact on the patient's psychosocial and clinical long-term outcome.

1. Further research is necessary to find an effective treatment for all schizophrenia symptomatology.
2. Computational tools are useful for studying protein-protein interactions and drug design.
3. In vitro techniques allow us to study novel drug´s pharmacology.
4. Rats are a good model in which to study complex processes such as pharmacokinetics and behavioral changes.