RESEARCH HIGHLIGHT

Piecing together aminergic polypharmacology

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Neuropsychiatric medications are often designed to have affinity for multiple ligand-binding sites across different receptor types to increase therapeutic efficacy. In a recent paper in *Cell,* Chen and colleagues implement a flexible scaffold-based cheminformatics approach to design bi-target therapeutics for dementia-related psychosis.

Drug development began as a molecule-to-target approach; one chemical was developed to hit one disease target[1] and researchers typically explored potential therapeutics by trial and error, with serendipity being a key element of success. What began as a linear process has now evolved into a more complex, yet efficient system. Currently, rational drug design is the most refined method available, consisting of two branches: ligand-based (LB) and structure-based (SB). LB design relies on pre-existing knowledge of chemical structures that bind to a target of interest and the corresponding structure-activity relationships inform compound optimization. SB design requires known 3D target structures that function as a model against which biochemically compatible ligands are designed.

It has since been established that binding to more than one biological target (e.g. polypharmacology) tends to be more efficacious, particularly for neuropsychiatric disorders which are typically polygenic in origin.[1] Current neuropsychiatric medications often target G-protein coupled receptors (GPCRs), a wide family of transmembrane receptors that modulate a plethora of intracellular cascades.[2] For example, treatment for the positive symptoms of schizophrenia (hallucinations) heavily relies on atypical antipsychotics that antagonize serotonin subtype 2A receptor (5-HT_{2A}R) and dopamine 2 receptor.[3] These medications have been prescribed to patients with dementia-related psychosis (DRP) to attenuate their symptoms (delusions, aggression and agitation), unfortunately resulting in severe adverse side effects such as increased mortality rates and increased cognitive decline.[4] Interestingly, preclinical evidence has shown that serotonin subtype 1A receptor (5-HT_{1A}R) agonism can combat cognitive decline and psychosis.[5] This inspired Chen and colleagues to identify features from compounds effective in blocking psychosis and from 5-HT_{1A}R agonists with pro-cognitive effects. They then aimed to repurpose these features for potential DRP therapeutics using a novel SB design approach.[6] Considering the structural overlap between the binding pockets of 5-HT_{2A}Rs and 5-HT_{1A}Rs, DRP presents itself as a promising case study to employ a flexible scaffold cheminformatics approach (FSCA). FSCA takes note of receptor-binding pocket features from both agonists and antagonists and applies cheminformatic approaches to identify suitable scaffolds that meet criteria to generate flexible, dual-targeting scaffolds amongst available databases.

To first understand ligand properties with affinity for multiple serotonin receptors, the authors analyzed crystal structures of lysergic acid diethylamide (LSD)-bound 5-HT_{2A}R and 5-HT_{2B}R. They found a flat conformation of LSD in a shallow binding pocket (SBP) in a "stretching up" position. Conversely, the antipsychotic lumateperone, a 5-HT_{2A}R antagonist, was found to target the deep binding pocket (DBP) in a "bent" conformation, further stabilized by an interaction between a 4-fluorophenyl group and selected amino acids that are canonically targeted by receptor antagonists. Additionally, both LSD and lumateperone have flexible tetracyclic scaffolds that can adopt distinct (*cis*/*trans*) conformations exhibiting selective pharmacology at different serotonin receptors.

This flexibility was a key filter property for a high-throughput screening campaign against 2.2 million compounds from the ChEMBL database and ~250,000 commercially available building blocks from the Enamine library. Selection criteria included: (1) tetracyclic or tricyclic ring scaffolds; (2) the first ring must be an aromatic or an indole ring; (3) the second ring must be non-aromatic ring containing a sp^3 hybridized nitrogen atom; (4) the third ring must have a protonated nitrogen atom suitable for fragment linking and salt bridge formation with a conserved $D^{3.32}$ amino acid in all aminergic receptors. This screen resulted in eight tricyclic rings and two tetracyclic rings that selected for further virtual synthesis screening using fragment linking. A final 9 and 7 compounds for 5-HT_{2A}R and 5-HT_{1A}R, respectively, were advanced to docking studies. From these 16 compounds, one tricyclic (IHCH-7162) and one tetracyclic (IHCH-7179) scaffold were selected for functional assessment. Indeed, these compounds were conformationally selective agonists of 5-HT_{1A}R. Tetracyclic IHCH-7179 was antagonistic of 5-HT_{2A}R in both G-protein and β-arrestin2 signaling pathways. Moreover, the two enantiomers for IHCH-7179 both exhibited 5-HT_{1A}R agonism and 5-HT_{2A}R antagonism. Based on these results, the authors performed follow-up in vitro and in vivo experiments using IHCH-7179.

Cryo-EM structures validated a flat conformation of (***R***)-IHCH-7179 in the SBP and the tetracyclic scaffold in the orthosteric binding pocket of the active 5-HT_{1A}R–G_i-protein complex. The crystal structure of the inactive 5-HT_{2A}R-bound complex reported IHCH-7179 in the DBP, adopting a bent pose (Fig. 1), quite like lumateperone–5-HT_{2A}R structures. Mutagenesis studies and functional validation across both structures confirmed the importance of interacting residues for proper bitonic activity.

Using an animal model for Alzheimer's disease (*APP/PS1* mice), Chen and colleagues put IHCH-7179 to the test employing a variety of behavioral assays that model the symptoms seen in DRP such as hallucinogenic effects, psychosis-like behaviors,

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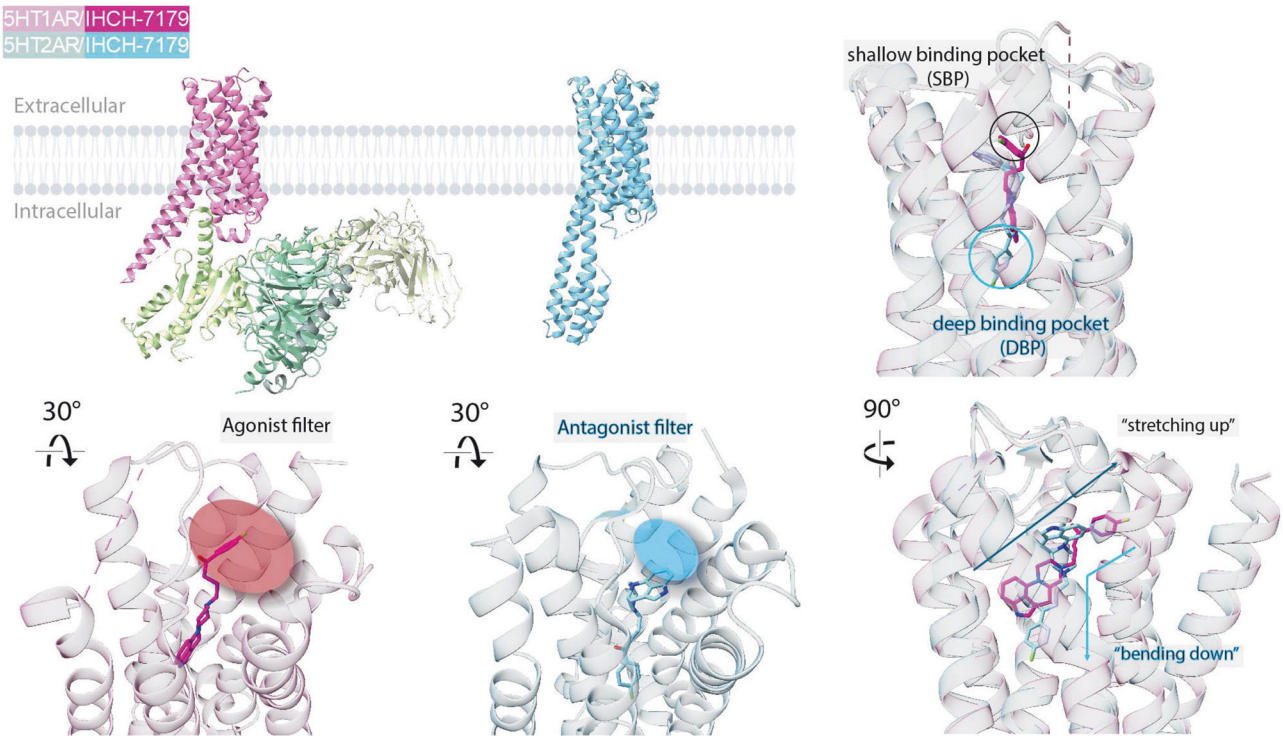

Fig. 1 Elements of the "conformational shaper". Top left, structures of active 5-HT_{1A}R-bound IHCH-7179 and inactive 5-HT_{2A}R-bound IHCH-7179 at the cell membrane. Top right, overlapped close-up view of the SBP and DBP with respect to compound conformation. Bottom left and middle, overlapped view of the agonist and antagonist filter positioning at the 5-HT_{1A}R and 5-HT_{2A}R binding pocket entrances, respectively. Bottom right, overlapped flat view of compound trajectories resembling a "stretching up" action for IHCH-7179 agonism and a "bending down" position for IHCH-7179 antagonism.

hyperactivity, impairments in spatial learning and long-term memory. IHCH-7179 displayed a dose-dependent decrease in head twitch response, a behavioral model commonly used to measure hallucinogenic activity in mice. IHCH-7179 restored sensorimotor gating function in LSD-administered mice as well. Additionally, IHCH-7179 at 4 mg/kg rescued hyperactivity in a pharmacologically induced mouse model of schizophrenia. To assess the pro-cognitive effects of IHCH-7179, spatial learning, and long-term memory were measured using the Morris water maze (MWM) test and the novel object recognition (NOR) test, respectively. To mimic cognitive decline, mice were exposed to sub-chronic MK-801 administration (0.2 mg/kg per dose, twice a day, 10 days). On day 11, mice were administered IHCH-7179 (0.1–1.0 mg/kg, intraperitoneal injection) for the next 6 days and evaluated on MWM performance. For the NOR paradigm, animals were exposed to sub-chronic MK-801 administration (0.3 mg/kg twice a day, 7 days), followed by one dose of IHCH-7179 prior to NOR testing one week later. The cognitive improvements seen across the MWM and NOR tests were abolished when animals were treated with 5-HT_{1A}R antagonist WAY-100635 (10 mg/kg), suggesting that the pro-cognitive benefits of IHCH-7179 is due to 5-HT_{1A}R agonism alone.

Lastly, Chen and colleagues outlined a set of general principles to aid future bifunctional ligand design: (1) compound conformation is influenced by the opening of the receptor-binding pocket; (2) interactions between receptor amino acid residues and compound functional groups determine overall pharmacology. For example, biochemical interactions between ligand and receptor determine whether the compound will bind in a "stretching up" or "bending down" position. Chen et al. showed that the orientation of the chemical, flat and "stretching up" or bent and "bending down", directly corresponds to whether the compound will function as an agonist or antagonist of 5-HT_{1A}R and 5-HT_{2A}R, respectively.

Together, these factors create what Chen and colleagues refer to as the "conformational shaper" of the receptor: the attributes that directly influence chemical binding and functional properties thereof (Fig. 1). The authors highlight the importance in identifying the components of a receptor's "conformational shaper" for future pursuits in rationally designed polypharmacology. Future drug design efforts should incorporate FSCA, particularly when known targets have published structural data and are relatively compatible, as with 5-HT_{2A}Rs and 5-HT_{1A}Rs. Within this context, Chen and colleagues developed a bona fide approach to designing flexible scaffolds for polypharmacology that will advance the entire field of drug design.

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ADDITIONAL INFORMATION

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