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A Straightforward Approach for Engineering Efficacy and Selectivity at GPCRs

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Dopamine D_1-like (D_1 and D_5) and D_2-like (D_2, D_3, and D_4) receptors regulate a number of physiological functions including cognition and emotion. The dopaminergic system is also implicated in neurological diseases (e.g., Parkinson's disease, schizophrenia, neuropsychiatric disorders) and in cocaine craving. Indeed most of the drugs used for the treatment of resistant or nonresistant schizophrenic patients successfully modulate the dopaminergic system, and D_2-like receptor ligands may be proposed to reduce the effects (craving and relapse) of drugs of abuse associated with environmental stimuli.[1]

In the past decade the D_3 receptor (D_3R) has attracted a lot of interest mainly for its specific distribution in the mesolimbic area of the brain, leading to claim the D_3R as a druggable molecular target for neuropsychiatric disorders and drug addiction. To date, the role of D_3R is still unclear, since clean D_3R agonists and antagonists have no significant behavioral effects and have a controversial effect in drug addiction.[2] On the other hand, with D_3R functions mainly related to limbic rather than nigrostriatal dopaminergic system, D_3R antagonism may contribute to improve cognitive deficits of resistant schizophrenic patients poorly treated with currently available therapeutics and may be useful for craving and relapse. Because of the neurochemical complexity of CNS disorders and emotional impairment induced by drug addiction, the design of new drugs should include a fine balance of potency and efficacy toward GPCRs. However, highly selective ligands for GPCRs may be useful pharmacologic tools to explore functions of different receptors and their subtypes and their role in pathological conditions.[3]

In the past 5 years the availability of X-ray structures of adrenergic β_2 and β_1 and adenosine A_{2A} receptors[4] and more recently of the D_3R[5] has opened a new scenario for the rational design of selective and multireceptor affinity "profiled" molecules.

In a study reported by Newman et al.,[6] a straightforward and successful approach for the identification of the molecular determinants of efficacy, potency, and selectivity at D_3R is described. Their strategy, which may be extended to other GPCRs, is based on a comprehensive study including molecular deconstruction, computational simulations, and binding and efficacy studies, using the flexible arylpiperazine system of known D_3R ligands as the template (Figure 1).

The analysis exploits the information derived from the crystallographic structure of the D_3R in complex with an inverse agonist (inactive form), and a D_2R homology model was built and used to investigate the selectivity of the molecules. Also, a homology model of the active form of the D_3R was generated, based on the X-ray structure of the β_2 receptor in complex with

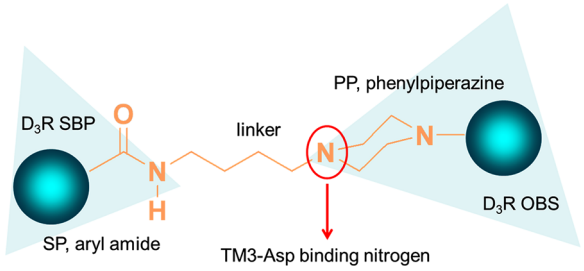

Figure 1. Schematic representation of the flexible dopamine receptor arylpiperazinecarboxamides binding the OBS and SBP of D_3R.

an agonist (active form). A molecular deconstruction of the original full-length molecules **4** and **5** (Figure 2) coupled to docking studies (by using an induced fit docking (IFD) procedure) and to functional assays allowed the identification of the primary pharmacophore (PP), binding the orthosteric binding site (OBS, enclosed by TM3, and TM5−7) and the secondary pharmacophore (SP) interacting with the secondary binding pocket (SBP, located at the interface of TM1,2,3,7 and

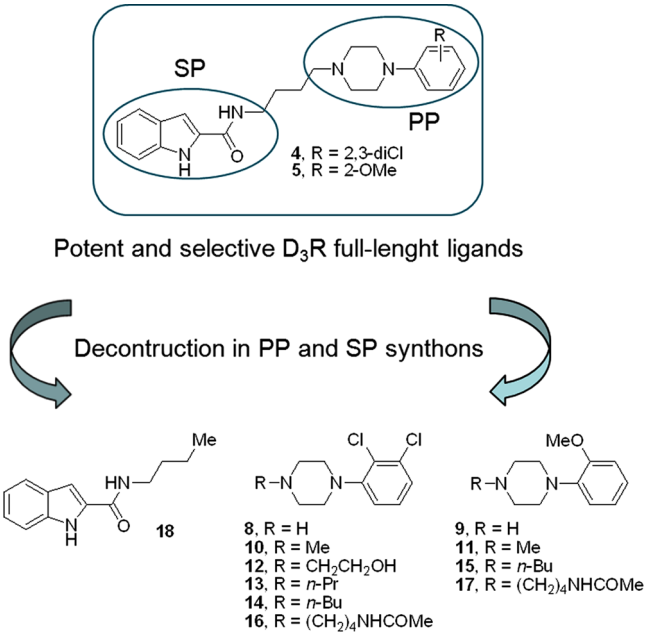

Figure 2. Schematic representation of the full-length ligand deconstruction.

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the first and second extracellular loops (EL1 and EL2), Figure 1).

Binding studies, performed by using [^{125}I]IABN, outlined the potency and selectivity ratios of compounds **2**, **4**, and **5** (**2**, K_{iD2} = 433 nM, K_{iD3} = 1.12 nM, D_2/D_3 = 394; **4**, K_{iD2} = 103 nM, K_{iD3} = 1.4 nM, D_2/D_3 = 73; **5**, K_{iD2} = 37.4 nM, K_{iD3} = 0.32 nM, D_2/D_3 = 117). The molecular synthons (**8**−**18**, Figure 2) generated by rational deconstruction were subjected to the same binding studies.

Consistent with the D_3R predicted binding modes, the arylpiperazine moieties (**8**−**17**) maintained a D_3R affinity in the submicromolar/nanomolar range, losing D_3R selectivity (D_2R/D_3R ranged from 0.30 to 3.25) with respect to the full length compounds **4** and **5**. Docking studies highlighted different binding modes of PP into OBS. On the other hand, **18** showed a loss of affinity at D_2R and D_3R. Taken together, the binding data indicate that the PP, interacting with OBS, highly conserved among D_2-like receptors, is critical for ligand potency and that the SP, binding to the SBP, structurally less conserved among the different receptor subtypes, is of pivotal importance for determining receptor subtype selectivity.

An interesting interpretation of functional assays is given. BRET-based assay of compounds **2**, **4**, and **5** uncovered a weak partial agonist profile at both D_3R and D_2R with E_{max} ranging from ∼8% to 20%. These latter data, coupled with the results obtained using synthons **8** and **9** (**8**, E_{maxD3R} = 57.27, E_{maxD2R} = 40.43; **9**, E_{maxD3R} = 6.27, E_{maxD2R} = 14.02), proved the hypothesis that the contribution to the efficacy is dependent upon the accommodation and substituent orientation of the PP inside the OBS rather than the SP into the SBP. This hypothesis was confirmed by the lack of efficacy of **18** in the same tests. A convincing validation of this hypothesis was achieved through an accurate structure−based analysis. Electrostatic potential surface calculations performed on **8** and **9** highlighted a significant difference in charge distribution in the phenyl ring depending on the presence of the chlorine atoms or of the methoxy group. Only **8** with the chlorine atom at position 3 might establish H-bonding with the serine stretch (Ser43) at TM5 (active form), while the OMe of analogue **9** only forms hydrophobic interactions, bringing back TM5 to the inactive form. This may explain the differences in efficacy between **8** and **9** binding OBS. Thus, dockings performed in the generated homology model of the active form of the D_3R offered a clear-cut structure-based explanation of the observed efficacies.

The study by Newman et al.[6] represents an elegant example of a structure-based rationalization of both D_3R/D_2R selectivity and efficacy. In general this approach might be successfully extended to other GPCRs, making possible today a more rational development of receptor specific ligands.[7] The possibility to manage potency, selectivity, and efficacy on a single GPCR and on a selected panel of GPCRs might pave the way to the development of drugs characterized by a fine-tuning of their pharmacodynamic properties. In particular, this work will help discovery and development of therapeutics for brain diseases, which benefit from an optimally balanced multi-receptor affinity and efficacy profile.

However, the outcome of this approach applied to molecules such as bishomo(hetero)arylpiperazines that share the pharmacodynamic profile with the arylpiperazinecarboxamides studied by Newman et al.[6] could be of interest. Probably, for these arylpiperazines a dual binding mode at D_3R and other GPCRs could not be ruled out yet.

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REFERENCES

(1) Ferraro, L.; Frankowska, M.; Marcellino, D.; Zaniewska, M.; Beggiato, S.; Filip, M.; Tomasini, M. C.; Antonelli, T.; Tanganelli, S.; Fuxe, K. A novel mechanism of cocaine to enhance dopamine d(2)-like receptor mediated neurochemical and behavioral effects. An in vivo and in vitro study. *Neuropsychopharmacology* **2012**, *37*, 1856−1866.

(2) (a) Cervo, L.; Carnovali, F.; Stark, J. A.; Mennini, T. Cocaine-seeking behavior in response to drug-associated stimuli in rats: involvement of D3 and D2 dopamine receptors. *Neuropsychopharmacology* **2003**, *28*, 1150−1159. (b) Volkow, N. D.; Skolnick, P. New medications for substance use disorders: challenges and opportunities. *Neuropsychopharmacology* **2012**, *37*, 290−292.

(3) Joyce, J. N.; Millan, M. J. Dopamine D3 receptor antagonists as therapeutic agents. *Drug Discovery Today* **2005**, *10*, 917−925.

(4) Topiol, S.; Sabio, M. X-ray structure breakthroughs in the GPCR transmembrane region. *Biochem. Pharmacol.* **2009**, *78*, 11−20.

(5) Chien, E. Y.; Liu, W.; Zhao, Q.; Katritch, V.; Han, G. W.; Hanson, M. A.; Shi, L.; Newman, A. H.; Javitch, J. A.; Cherezov, V.; Stevens, R. C. Structure of the human dopamine D3 receptor in complex with a D2/D3 selective antagonist. *Science* **2010**, *330*, 1091−1095.

(6) Newman, A. H.; Beuming, T.; Banala, A. K.; Donthamsetti, P.; Pongetti, K.; LaBounty, A.; Levy, B.; Cao, J.; Michino, M.; Luedtke, R. R.; Javitch, J. A.; Shi, L. Molecular determinants of selectivity and efficacy at the dopamine D3 receptor. *J. Med. Chem.* **2012**, DOI: 10.1021/jm300482h.

(7) Boeckler, F.; Gmeiner, P. Dopamine D3 receptor ligands: recent advances in the control of subtype selectivity and intrinsic activity. *Biochim. Biophys. Acta* **2007**, *1768*, 871−887.