Identification of novel dopamine D_2 receptor ligands – a combined in silico / in vitro approach

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Objective The dopamine receptor D_2 (D_2R) has been shown to be involved in CNS diseases. While different D_2R-targeting drugs have been approved by the FDA, they all suffer from major drawbacks due to promiscuous receptor activity leading to adverse effects. In dire need of novel D_2R ligands for drug development, combined in silico / in vitro approaches have been shown to be efficient strategies discovering potential drug candidates.

Conclusion With the combined approach using in silico pharmacophore modelling (both ligand (LB)- and structure-based (SB) approaches), in silico virtual screening (VS) and in vitro methods we were able to identify six novel D_2R ligands with low micro- to nanomolar activities. The developed workflow and successfully identified ligands could aid in developing novel therapeutics for D_2R-associated pathologies.

Methods

SB and LB pharmacophore models based on D_2R structure and ligands → In silico VS of large compound libraries (≥ 200 000) → In vitro validation of pharmacophore models – HTRF-based approach (selection of 90 virtual hits) → Identification of six novel D_2R ligands

Results

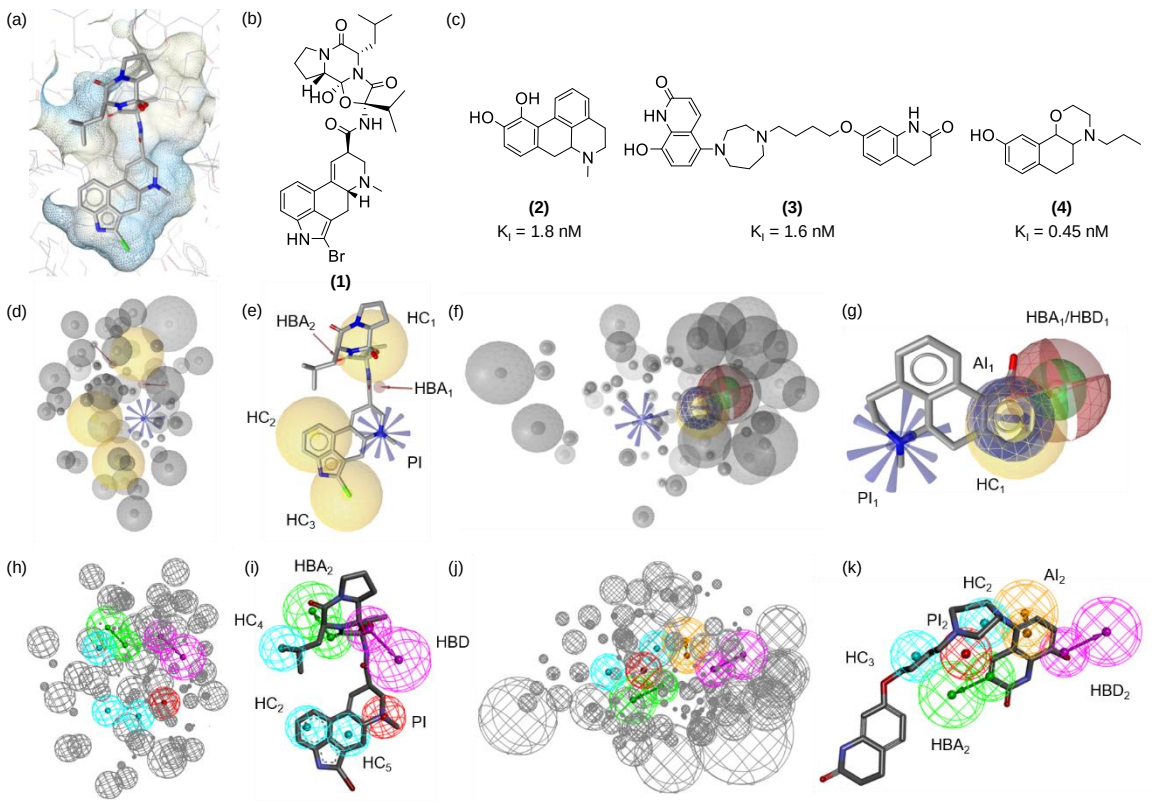
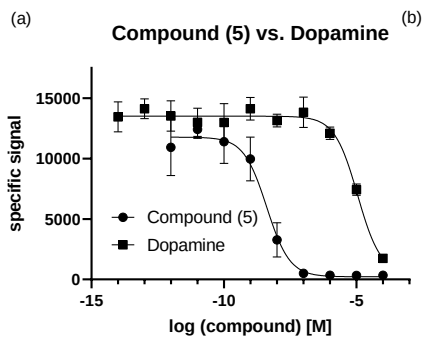


Figure 1. SB and LB pharmacophore models generated in LigandScout (LS) and DiscoveryStudio (DS). (a) Bromocriptine (ligand) in the binding pocket of the D_2R. (b) 2D structure of bromocriptine. (c) 2D structures of active D_2R ligands used for LB approaches. (d, f) SB an LB models from LS highlighting model features and exclusion volumes (XVOLs). (e, g) SB and LB models from LS superimposed with **(1)** and **(2)**. (h, j) SB an LB models from DS highlighting model features and XVOLs. (l, k) SB and LB models from DS superimposed with **(1)** and **(3)**. Equal pharmacophore feature indices in (e, g, i, k) indicate similar coordinates of the features in the different models. Hydrophobic contacts (HC, yellow and cyan). Hydrogen-bond acceptor (HBA, red arrows, green spheres) and donor (HBD, red and purple spheres) features. Positively ionizable (PI, blue and red (DS)) interactions. XVOLs (grey).



(b)

Compound (ID)	fold-difference	K_i [µM]
(5)	2700	0.004108
(6)	2.6	4.318
(7)	1.1	10.04
(8)	9.0	1.240
(9)	4.2	2.630
(10)	34.7	0.3203
Dopamine	-	11.12

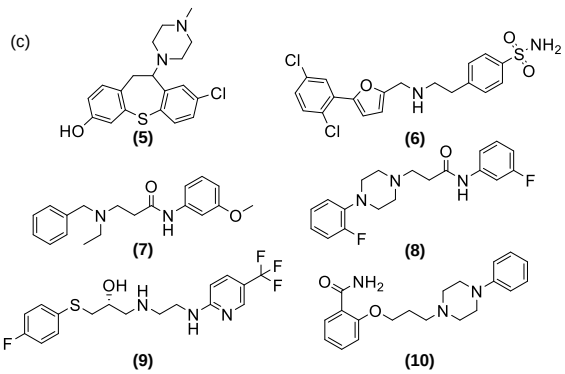


Figure 2. In vitro overview of the identified D_2R ligands. (a) Exemplary comparison of K_i values of dopamine (endogenous ligand) and **(5)** (highest activity of identified ligands). (b) Overview of the K_i of novel D_2R ligands determined in vitro. Fold-differences were calculated based on dopamine activity. K_i values were calculated with n = 6. (c) 2D structures of the identified, novel D_2R ligands.

Acknowledgements

L. Z. is funded by the FFG project 872425.