DOI: 10.1002/cmdc.200700067

GPCR Targeted Library Design: Novel Dopamine D_3 Receptor Ligands

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The design of G protein-coupled receptor (GPCR)-focused compound libraries sets a challenging scenario for the application of virtual screening tools for hit and lead structure finding. In this study, different computational techniques were strategically utilized to identify structurally novel ligands for the dopamine D_3 receptor.

Dopamine receptors belong to the subfamily of biogenic amine binding class A GPCR and are implicated in various neurological and neuropsychiatric disorders.[1,2] They are divided into dopamine D_1-like receptors, with its subtypes D_1 (D_{1a}) and D_5 (D_{1b}), and dopamine D_2-like receptors, including D_2, D_3, and D_4 receptor subtypes.[2] The dopamine D_3 receptor attracts intensive attention because of its restricted distribution in limbic brain areas associated with cognitive and emotional functions.[1] Consequently it has been proposed that the dopamine D_3 receptor is implicated in schizophrenia, Parkinson's disease, and drug abuse.[1,3] Currently, selective dopamine D_3 receptor antagonists and partial agonists are in clinical development as potential therapeutics for the aforementioned disorders.[3]

The unavailability of an experimentally determined three-dimensional (3D) structure of this GPCR limits the applicability of structure-based design techniques. Therefore, ligand-based clustering techniques were first applied to identify regions of interest in chemical space using sets of known dopamine D_3 receptor antagonists. Two hierarchical clustering methods, namely hierarchical *k*-means and NIPALSTREE,[4] and the self-organizing map (SOM)[5] approach were employed. Then, automated ligand docking was performed into a homology model of the transmembrane domain of the dopamine D_3 receptor. Two different binding modes of a new lead compound were observed. A pharmacophore model was constructed considering both predicted binding modes, and K_i values of the final candidate compounds were determined. The most affine ligands (**1**, **18**) yielded K_i values of 65 nM at dopamine D_3 receptors. Results clearly demonstrate the applicability of current chemoinformatic prediction techniques to early stages of GPCR drug discovery.

The starting point for our analysis was an intensive literature search for dopamine receptor antagonists and partial agonists with the aim to identify affine and selective dopamine D_3 receptor ligands. The dominant motif present in these molecules is summarized in Figure 1 showing as an example BP 897,[6] a selective dopamine D_3 partial agonist. The structural motif contains 1) an aryl residue, 2) an amide moiety, 3) a spacer region, and 4) an amine residue.[7] Mutagenesis studies of the dopamine D_2 receptor report the aspartic acid Asp 114 (corresponding to Asp 110 in D_3 receptors) as an essential interaction partner of the positively charged nitrogen in the amine moiety.[8] With the exception of the amide group and the charged nitrogen, the remaining parts of the molecules are characterized by aromatic or hydrophobic residues. A distance of 6–7 Å between the amide oxygen and the positively charged nitrogen seems to be responsible for selective binding to dopamine D_3 receptors.[7] A linear spacer region has been favored for receptor binding, although aromatic substitutions are tolerated.[7] The structure–activity relationship (SAR) of the amine rest is relatively steep, as large substitutions and differently substituted bioisosteric groups decrease K_i values.[9] In contrast, the aryl portion tolerates larger aromatic or hydrophobic substitutions.[7,9]

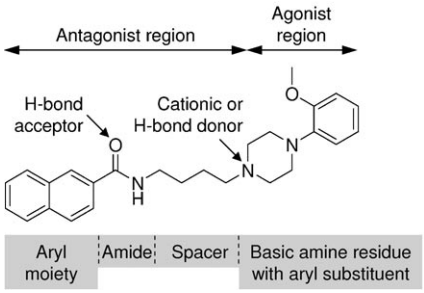

Figure 1. SAR of dopamine D_3 receptor antagonists. The example depicts BP 897, a clinical phase II partial agonist.[6,7]

Table 1. Data sets used for virtual screening.[a]

Data set	DS MOE	DS CATS3D
Number of compounds	230,130 (472 D_3 + 229,658 SPECS)	230,130 (472 D_3 + 229,658 SPECS)
Descriptor set	MOE2D	CATS3D
Original dimension of descriptor	146	420
UFS[b] R^2-based pruning	110	338
Entropy-based pruning	53	35
Threshold Θ	2.6	2.1

[a] See Supporting Information for details on descriptor pruning. [b] UFS: Unsupervised Forward Selection.

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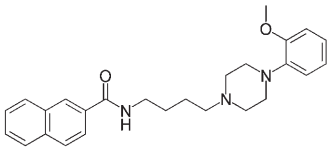
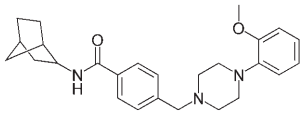
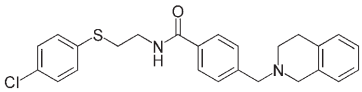
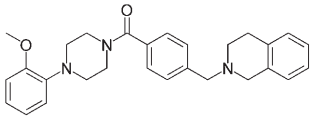
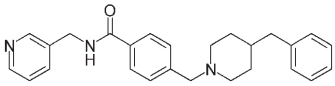
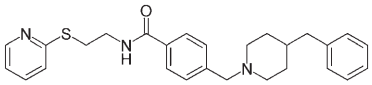
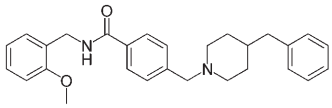
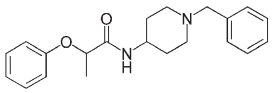
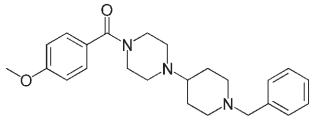
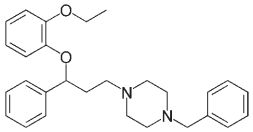
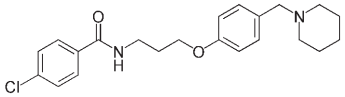
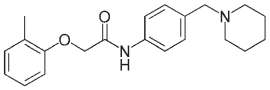
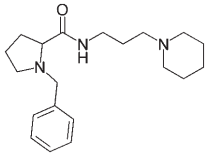
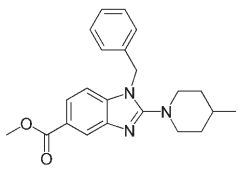
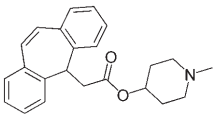
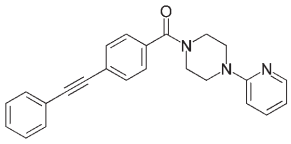
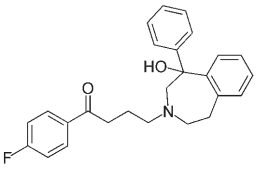
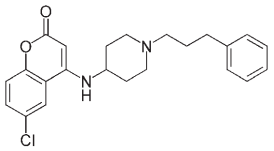
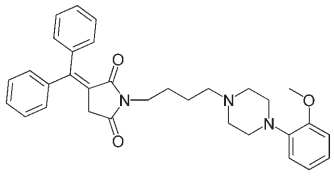
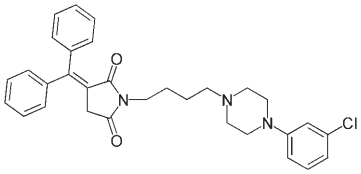
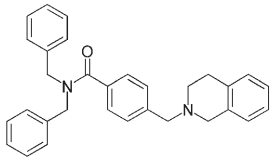
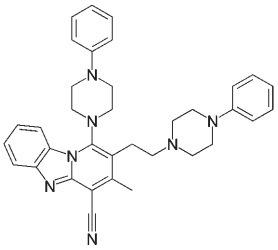
Supporting information for this article is available on the WWW under http://www.chemmedchem.org or from the author.

Table 2. Dopamine D_2 and D_3 receptor subtype affinities of compounds from the first and second virtual screening cycle.

Compd.	Chemical structure	K_i (D_2) [nM][a]	K_i (D_3) [nM][a]	Ratio K_i (D_2/D_3)[a]
BP 897		52 ± 12	0.91 ± 0.2	57
1		901, 730[b]	65 ± 7.3[c]	12.6
2		894, 666[b]	289, 244[b]	2.9
3		7750, 4900[b]	1367, 621[b]	6.4
4		11715 ± 6172[c]	3212, 1935[b]	4.6
5		3351, 4766[b]	214, 297[b]	15.9
6		2812, 2363[b]	573, 572[b]	4.5
7		1092, 779[b]	983, 938[b]	1.0
8		> 10000[b]	4526, 4280[b]	n.d.
9		801, 743[b]	264 ± 163[d]	2.9
10		> 10000[b]	2007, 2037[b]	n.d.
11		7013, 4431[b]	2299, 2268[b]	2.5
12		> 10000[b]	3466, 10320[b]	n.d.
13		> 50000[b]	> 5000[b]	n.d.

For our virtual screening experiments two data sets were compiled (Table 1). These contain 472 active compounds with known K_i values for D_2 and D_3 receptors, and the SPECS compound catalogue (version of June 2003; Specs, HT Delft, The Netherlands). All molecules were described by two-dimensional (2D) descriptors available from the software suite MOE (version 2005, Chemical Computing Group, Montreal, Canada), and by a 3D pharmacophore descriptor (CATS3D).[10] Both descriptor sets were clustered using the hierarchical techniques NIPALSTREE and hierarchical *k*-means.[4] We focused only on terminal clusters of the hierarchical dendrograms containing known dopamine D_3 receptor ligands, and analyzed the co-clustered SPECS molecules: From NIPALSTREE we selected 37 SPECS compounds, and from the hierarchical *k*-means analysis 144 SPECS molecules were selected. Then, a SOM[5] was trained containing 30×20 neurons (cluster centroids). In the resulting 2D map, a clustering of dopamine D_3 receptor ligands was visible (cf. Supporting Information). Molecules belonging to these activity islands (n = 1,551) were further analyzed by training a second SOM containing 15×10 neurons. From this more fine-grained map we picked 52 SPECS substances from neurons containing at least five already known dopamine receptor ligands. The resulting 37 + 144 + 52 = 233 candidate molecules were checked for duplicates (n = 26), and 17 of 207 compounds were manually extracted by considering drug-like properties,[11] presence of positively charged nitrogen essential for receptor binding,[12,13] and structural dissimilarity to the training set of well-known leads. Calculated log*P* values for these selected molecules are in the range of

Table 2. (Continued)

Compd.	Chemical structure	K_i (D_2) [nM][a]	K_i (D_3) [nM][a]	Ratio K_i (D_2/D_3)[a]
14		3212, 5549[b]	190 ± 58[c]	23.1
15		> 10000[b]	3016, 6542[b]	n.d.
16		216, 284[b]	2707, 2464[b]	0.1
17		495, 575[b]	1093, 621[b]	0.6
18		162 ± 26[d]	65 ± 9[c]	2.5
19		1359 ± 315[c]	498 ± 76[d]	2.7
20		1376 ± 285[c]	> 1000[b]	n.d.
21		4392 ± 348[c]	2636 ± 349[c]	1.7

[a] K_i values (mean value with standard deviation (SD)) were measured in CHO cells stably expressing hD_{2S} and hD_3 receptors in triplicates by using [^{3}H]spiperone. [b] Two independent experiments. [c] Three independent experiments. [d] Four independent experiments. n.d.: Not determined.

2.14–5.62 (cf. Supporting Information), which is characteristic of central nervous system penetrating drugs.[14] For experimental verification, binding affinities for these 17 selected ligands were determined in radioligand competition assays for dopamine D_2 and D_3 receptors. Results are listed in Table 2 for compounds **1**–**17**.

Our study was intended to provide potential new lead structures rather than classes of structural analogues. Therefore, we defined a hit as a compound yielding a $K_i < 1$ μM, and consequently found nine hits for dopamine D_3 and six for D_2 receptors. Five compounds exhibited a $K_i < 300$ nM at D_3 (**1**, **2**, **5**, **9**, **14**), and one compound (**16**) yielded a K_i value of 250 nM at the D_2 receptor.

Compound **1** was the overall most potent with $K_i = 65$ nM and a 13-fold preference for dopamine D_3 over D_2 receptors. Compound **14** displayed the overall best selectivity ratio (23-fold). By analyzing the structures we recognized a novel structural feature, a benzamide moiety. It acts as a spacer between the aryl moiety and the amine residue (Figure 1). Benzamides in this structural motif have been described as aryl moieties only; representatives are the atypical antipsychotics, sulpiride and raclopride, with high affinities for dopamine D_2 and D_3 receptors.[13] Synthesis of spacer benzamides is straightforward and enables the production of a large variety of derivatives by parallel synthesis.

Summarizing the first round of our study, both hierarchical clustering algorithms and the SOM were able to identify new dopamine D_3 receptor-preferring ligands.

In a previous study, a 3D homology model of the dopamine D_3 receptor had been shown to produce meaningful results.[15] We used this receptor model for automated docking of compounds **1**–**17** with the software GOLD (version 2.2, The Cambridge Crystallographic Data Centre, Cambridge, UK).[16] Only positive GOLD score values were obtained indicating that all compounds potentially fit into the putative binding pocket. As expected,[17] no correlation was observed between the docking

score and pK_i values (D_3) ($R^2 = 0.03$, cf. Supporting Information). Consequently, manual analysis and interpretation of the predicted binding modes was required.

We observed that the ligands could bind with their aryl moiety into two alternative binding pockets of the dopamine D_3 receptor model. This is exemplified in Figure 2a for BP 897, two binding modes of compound **1**, compound **14**, and a (phenylpiperazinyl) benzoxazinone, which was previously identified by us in a machine learning study.[15] Close proximity of the investigated ligands was observed to Asp110 (TM3), Phe345 (TM6), Phe346 (TM6), Ser192 (TM5), and Thr369 (TM7) (Figure 2b), which have been claimed to be important interaction partners of dopamine D_3 receptor ligands.[7,15,18]

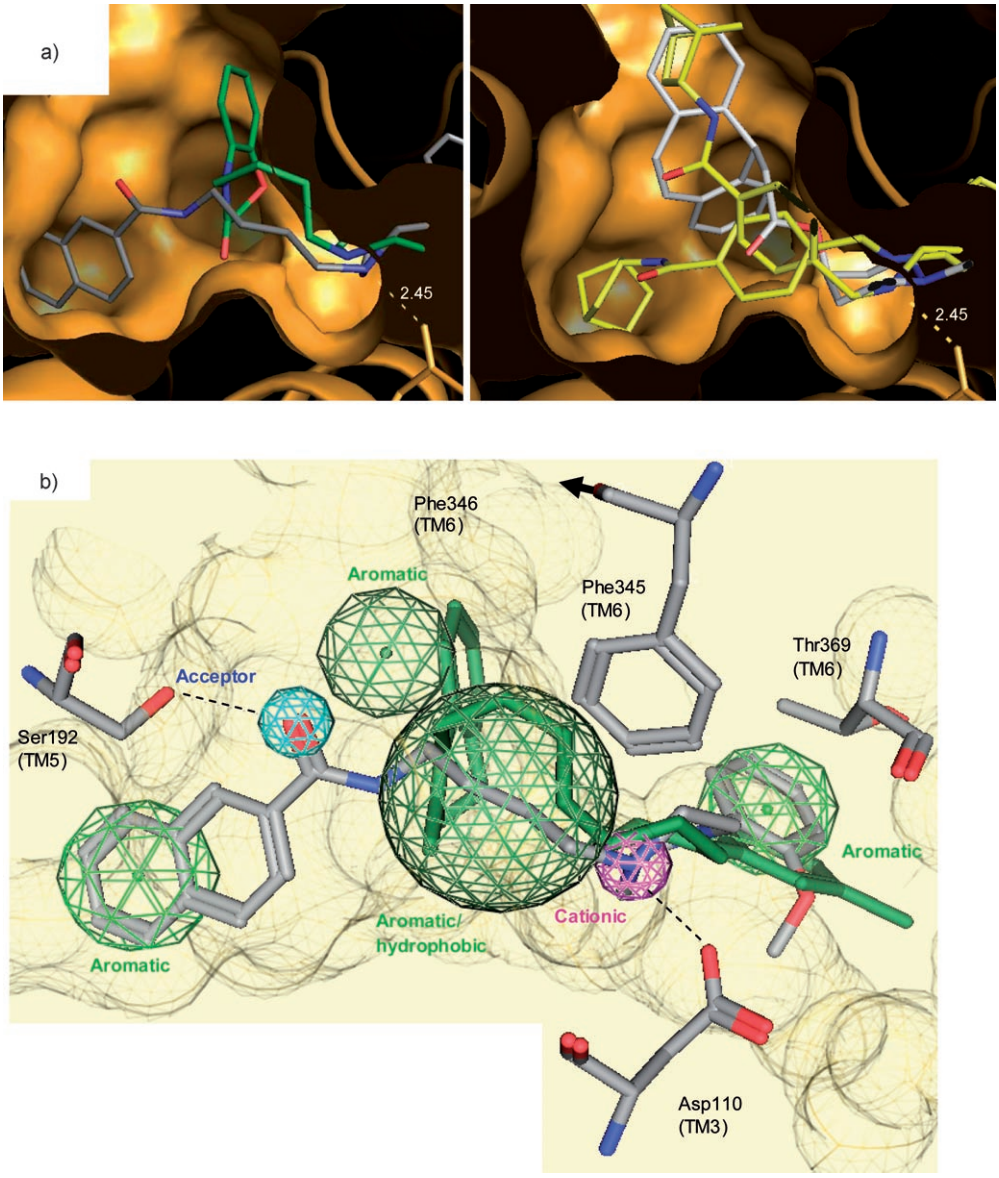


Figure 2. Docking and pharmacophore studies. a) BP 897 (gray), its morpholino analogue (green), two binding modes of compound **1** (yellow) and compound **14** (light gray) bind with the aryl moiety into alternative parts of the binding pocket of a dopamine D_3 homology model. b) Pharmacophore model used for virtual screening requiring both aryl moieties.[7,15,18]

The suggested docking modes provided the basis for building potential pharmacophore models. In a first experiment, a dopamine D_3 receptor antagonist pharmacophore model was created based on the motif shown in Figure 1. It contains an aromatic potential pharmacophore point (PPP) in the aryl moiety, a hydrogen-bond acceptor PPP at the position of the oxygen amide, a hydrophobic or aromatic PPP in the spacer region, an essential cationic and an aromatic PPP in the amine rest (we defined that three of the five PPPs had to match in a virtual hit as different binding modes of diverse leads could be implemented by this procedure). The model was validated employing two data sets: The first set contained 374 of the 472 dopamine D_3 receptor ligands (Table 1, the missing 98 compounds did not pass prior drug-likeness filtering implemented in the pharmacophore search routine of the software package MOE) and 1,473 randomly picked SPECS compounds. 305 (65%) dopamine D_3 receptor ligands were correctly predicted by the pharmacophore model, and only six (0.4%) additional SPECS compounds were retrieved. The 69 false-negative D_3 receptor ligands were mainly agonists, which had been described to require a different pharmacophore model.[19] The results encouraged us to continue our validation with compounds **1**–**17**. Setting the K_i (D_3) threshold to 3 μM, 15 compounds were correctly classified, and only two false-positives occurred. These results suggest that we constructed a useful antagonist model which could serve as a filter in a second round of virtual screening.

For identification of compounds binding into both predicted aryl pockets, the pharmacophore model was extended (Figure 2b). An additional aromatic PPP was introduced for the alternative aryl binding pocket. With this procedure four out of six PPPs were defined as essential (nonessential: the acceptor PPP and the PPP in the spacer region). Screening the entire SPECS catalogue, only 35 molecules were identified obeying the specified rules. To draw conclusions about the effect of the two aryl residues, three com-

pounds were selected, which differ from our ligand data set only in the aryl pocket. These molecules and an additional molecule were ordered and experimentally tested in radioligand binding assays. Results for these compounds **18**–**21** are given in Table 2.

Only structures **18** and **19**, both containing a benzhydrylidene substituted pyrrolidindione residue, were potent with a slight preference for the dopamine D_3 receptor. Most other data for receptor preference should be taken cautiously as numerous affinities were recorded for screening reasons in duplicate only. The more flexible dibenzylcarbamoylbenzyl substituted 1,2,3,4-tetrahydroisoquinoline **20**, and the bulky benzoimidazo-substituted phenylpiperazine **21** exhibited affinity at both receptor subtypes. The planar rigidized molecules **18** and **19** are favored, where compound **18** displayed low nanomolar binding at D_3 ($K_i = 65$ nM). To understand the lack of activity of compound **20** we aligned it to **18**. In the alignment, the branching point of the biphenyl structure is closer to the acceptor oxygen of **20** compared to that of **18**. This might provide an explanation for the observed differences. Noteworthy, **20** lacks an *ortho*-substituted phenylpiperazine, and the nitrogen is dibenzylic, which might result in a different protonation state compared to **18** at physiological pH. This provides an additional interpretation of the observed differences.

Different attempts to further optimize the models for dopamine D_3 versus D_2 receptor binding were unsatisfying. Apparently, differences between the two structurally undefined receptor subtypes are small, impeding successful application of advanced structure-based approaches. In addition, our ligand data did not permit successful machine learning (cf. Supporting Information).

In conclusion, an objective of our study was to examine the applicability of virtual screening methods in early stages of the drug discovery process for the generation of GPCR lead structures. By strategic combination of different techniques we succeeded in finding novel lead candidates for the dopamine D_3 receptor using hierarchical clustering methods and self-organizing maps. Docking studies suggested two potential binding modes of dopamine D_3 receptor antagonists and partial agonists. To enlighten the role of the two predicted binding modes, a pharmacophore model was constructed simultaneously requiring both predicted binding modes. Four such molecules were found, and the best compound **1** showed a K_i value of 65 nM at the D_3 receptor with a 13-fold preference over D_2, supporting our hypothesis of multiple aryl-accepting pockets in the antagonist binding region of the receptor. Compared to **18** ($K_i = 65$ nM), substance **1** presents a stronger structural character on novelty,[20] which renders it the preferred candidate for further optimization.

Experimental Section

Dopamine D_{2S} and D_3 receptor binding assay. Membrane preparations of CHO-cells stably expressing human D_{2short} and D_3 receptors were used for displacement studies.[21,22] In brief, [^{3}H]spiperone (0.2 nM) served as a radioligand and nonspecific binding was determined in the presence of BP 897 (10 μM). Stock solutions (10 mM) of test compounds were prepared with pure DMSO. They were diluted to give final concentration ranges either from 1 μM to 1 mM or from 10 nM to 10 μM, depending on the affinity of the test compound. The assay was incubated for 2 h at RT and terminated by rapid filtration through PerkinElmer GF/B glass fibre filters (PerkinElmer Life Sciences, Rodgau, Germany) coated with 0.3% polyethylenimine (Sigma–Aldrich, Taufkirchen, Germany) using an Inotech cell harvester (Inotech AG, Dottikon, Switzerland). Radioactivity was counted using a PerkinElmer MicroBeta®Trilux scintillation counter (PerkinElmer Life Sciences, Rodgau, Germany). For detailed screening the compounds have been tested at seven concentrations in triplicates carryed out in two to four independent experiments. Competition binding data were analyzed by GraphPad PrismTM (2000, version 3.02, San Diego, CA, USA), using nonlinear least squares fit. K_i values were calculated from the IC_{50} values according to Cheng-Prusoff equation.[23]

Acknowledgement

We thank Dr. P. Sokoloff (INSERM, Paris), and Dr. J. Shine (The Garvan Institute of Medical Research, Sydney) for providing cell lines expressing dopamine D_3 and D_2 receptors, respectively. Technical assistance of T. Kottke is greatly acknowledged. This work was supported by the Beilstein-Institut zur Förderung der Chemischen Wissenschaften, Frankfurt am Main.

Keywords: D_3 receptor · dopamine receptor · G-protein-coupled receptor · homology model · virtual screening

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Received: March 22, 2007
Revised: April 1, 2007
Published online on May 3, 2007

