Multiway calibration in 3D QSAR
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Design, Syntheses and QSAR of a Series \textit{trans}-1,2,3,4,4a,5,6,10b-Octahydrobenzo[f]quinolines with Dopaminergic Affinity

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Summary

In order to investigate the influence of different substituents at the 4-N and 7-O positions of \textit{trans}-1,2,3,4,4a,5,6,10b-octahydrobenzo[f]quinolines (\textit{trans-OHB[f]Qs}) on the affinity for the dopamine D$_{2L}$, D$_3$ and D$_{4.2}$ receptors, a large number of compounds were generated theoretically, and characterized with physicochemical descriptors. Accordingly, a subset of compounds was selected by means of a factorial design in the descriptor space, subsequently synthesized and screened for dopamine D$_{2L}$, D$_3$ and D$_{4.2}$ receptor affinities.

In general, compounds with a hydroxy group at the seven position displayed significant high affinities for all three dopamine receptors while the compounds with a sulfon ester group were less potent. In addition, the sulfon ester group suppresses the affinity for the D$_4$ receptor. The nitrogen substituent may be as large as a phenylethyl group without detrimentally affecting the affinity for the dopamine receptors. Finally, a compound with a 7-OH group and an N-propargyl group lacks affinity for the dopamine D$_4$ receptor. The somewhat rigid N-propargyl group and the low pK$_a$ value (6.1) may be contributing factors to the low D$_4$ affinity.

In analogy with the 2-aminotetralins, where the affinity for the dopamine receptors resides in the (2S)-enantiomers, the potency of the \textit{trans-OHB[f]Qs} resides in the corresponding \textit{trans}-(4aS,10bS) enantiomer.

3.1 Introduction

The OHB[f]Qs\textsuperscript{1-7} (\textit{trans-17} and \textit{trans-1}) as rigid analogues of 2-aminotetralins\textsuperscript{8} (24) and 3-PPP\textsuperscript{5,6,9} (22) have, during the last two decades, achieved a lot of attention in the literature (see also Chapter 1). Attempts have been made to explain the structure-activity relationships\textsuperscript{2,10} between this class of compounds\textsuperscript{11,12} and other dopaminergic compounds like aporphines and ergolines.\textsuperscript{8} For the OHB[f]Q system it was found that the potency resided in the \textit{trans}-(4aS,10bS) enantiomer.\textsuperscript{8} As was pointed out,\textsuperscript{2,5} the \textit{trans} isomer is rigid, assuming a flat molecular conformation, whereas the \textit{cis} isomer is more flexible, with the piperidine-ring moiety protruding out of the plane, which may cause steric hindrance in the ligand-receptor interaction, and explain the higher affinity of the \textit{trans} isomer.
Compound *trans*-17 stimulated central presynaptic DA receptors at a low dose with no significant behavioral stimulation, reported by Wikström *et al.* However, when administrated at higher doses, *trans*-17 elicited typical and postsynaptic DA receptor stimulatory effects, *i.e.*, stereotypies, and increased locomotor activity.

Sonesson *et al.* found *trans*-1 to be inactive as an agonist even at high doses (50 μmol/kg). Instead, the striatal DOPAC levels in nonpretreated habituated rats were increased by 235 %, suggesting presynaptic DA receptor antagonistic properties. In the same assay, *trans*-1 decreased significantly the locomotor activity to 56 %.

As previously reported by Wikström *et al.*, when the N-n-propyl group was replaced by an N-n-butyl group or longer chains, these analogues of compounds 22 and *trans*-17 became more active in the biochemical and the behavior models used by the authors.

In the present chapter a large number of OHB[f]Qs were generated, using compound 1 as the template, and subsequently characterized with physicochemical parameters. A few of the most diverse compounds were then selected and synthesized. After testing the compounds for *in vitro* affinity at the dopamine D_{2L}, D_{3} and D_{4:2} receptor subtypes, these data plus the physicochemical descriptors will provide information to the structure-activity relationships.

### 3.2 Computational Chemistry

: **Tentative Compounds**

A large number of 4-N and 7-O substituted *trans*-OHB[f]Qs were designed by permuting all the combinations of the substituents listed in Table 3.1, at the R\(_1\) and R\(_2\) positions. On position R\(_1\) and R\(_2\), eight (ID R\(_1\) = a, b, c, d, e, f, g, h) and eleven (ID R\(_2\) = 1 to 11) different substituents were considered, respectively. Each compound can be identified by combining the IDs in Table 3.1. For example, compound h03 is a *trans*-OHB[f]Q with a triflate group on the 7 position (ID R\(_1\) is h) and an ethyl group on the 4-N position (ID R\(_2\) is 03).

: **Physicochemical descriptors**

Physicochemical descriptors were generated for all compounds listed in Table 3.1. The descriptors were obtained from different sources: Mopac AM1 single point calculations\(^{13,14}\), pKa and logP values were calculated using the Pallas 1.2 program\(^{15}\) and, finally, descriptors were obtained from the
The 19 descriptors in Table 3.2 were generated for all 88 compounds, and collected in the descriptor matrix $X$ ($88 \times 19$).

Table 3.1 The substituents permuted in order to generate the initial 88 compounds.

<table>
<thead>
<tr>
<th>ID $R_1$</th>
<th>$R_1$</th>
<th>ID $R_2$</th>
<th>$R_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>-H</td>
<td>01</td>
<td>-CH$_2$CH$_2$CH$_3$</td>
</tr>
<tr>
<td>b</td>
<td>-CH$_3$</td>
<td>02</td>
<td>-CH$_2$CH$_3$</td>
</tr>
<tr>
<td>c</td>
<td>-CH$_3$CH$_3$</td>
<td>03</td>
<td>-CH$_3$</td>
</tr>
<tr>
<td>d</td>
<td>-SO$_2$-CH$_3$</td>
<td>04</td>
<td>-CH$_3$</td>
</tr>
<tr>
<td>e</td>
<td>-SO$_2$-C$_6$H$_5$</td>
<td>05</td>
<td>-H</td>
</tr>
<tr>
<td>f</td>
<td>-SO$_2$-C$_6$H$_4$-CH$_3$</td>
<td>06</td>
<td>-CH$_2$CH$_2$C$_6$H$_5$</td>
</tr>
<tr>
<td>g</td>
<td>-SO$_2$-2-thiophene</td>
<td>07</td>
<td>-CH$_2$CH$_2$-2-thiophene</td>
</tr>
<tr>
<td>h</td>
<td>-SO$_2$-CF$_3$</td>
<td>08</td>
<td>-CH$_2$CCH</td>
</tr>
<tr>
<td></td>
<td></td>
<td>09</td>
<td>-CH$_2$CHCH$_2$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>-CH$_2$(CH$_3$)$_2$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11</td>
<td>-CH$_2$C$_6$H$_5$</td>
</tr>
</tbody>
</table>

Principal Properties

In Quantitative Structure-Activity Relationships (QSAR), Principal Properties (PPs) are frequently used for the description of series of compounds (see Chapter 1). A PP is a score vector ($t$) obtained from a Principal Component Analysis of $X$ ($I \times J$), containing the J physicochemical parameters (columns) characterizing the I compounds (rows). The PPs are linear combinations of the descriptors in $X$ and all PPs are chosen orthogonal, i.e., each PP does not correlate with any of the other PPs. Optimally, each PP represents clearly interpretable features in the molecules, like steric (e.g., size) or electrostatic (e.g., charge) properties. In PCA, each subsequently extracted PP accounts for less variation in $X$ and, consequently, the variation accounted for by the first PP is more significant for the description of $X$, as compared with the following PPs.

Prior to PCA, in this investigation, each column was mean-centered and scaled to have unit standard deviation, often referred to as auto-scaling (see Chapter 2).
Table 3.2 The physicochemical descriptors used for the characterization of the compounds in Table 3.1.

<table>
<thead>
<tr>
<th>descriptor</th>
<th>short</th>
<th>source</th>
</tr>
</thead>
<tbody>
<tr>
<td>heat of formation</td>
<td>hofo</td>
<td>a</td>
</tr>
<tr>
<td>electronic energy</td>
<td>elec</td>
<td>a</td>
</tr>
<tr>
<td>Core-Core repulsion</td>
<td>coco</td>
<td>a</td>
</tr>
<tr>
<td>dipole-moment</td>
<td>dipo</td>
<td>a</td>
</tr>
<tr>
<td>ionization-potential</td>
<td>iopo</td>
<td>a</td>
</tr>
<tr>
<td>homo</td>
<td>homo</td>
<td>a</td>
</tr>
<tr>
<td>lumo</td>
<td>lumo</td>
<td>a</td>
</tr>
<tr>
<td>$\pi R_2$</td>
<td>piNs</td>
<td>b</td>
</tr>
<tr>
<td>$\pi R_1$</td>
<td>piOs</td>
<td>b</td>
</tr>
<tr>
<td>electrostatic potential (-1)</td>
<td>pom1</td>
<td>c</td>
</tr>
<tr>
<td>electrostatic potential (0)</td>
<td>pot0</td>
<td>c</td>
</tr>
<tr>
<td>electrostatic potential (1)</td>
<td>pop1</td>
<td>c</td>
</tr>
<tr>
<td>point charge on N</td>
<td>chaN</td>
<td>a</td>
</tr>
<tr>
<td>point charge on O</td>
<td>chaO</td>
<td>a</td>
</tr>
<tr>
<td>charge on phenyl C in C-O</td>
<td>chCO</td>
<td>a</td>
</tr>
<tr>
<td>molecular weight</td>
<td>mowe</td>
<td>a</td>
</tr>
<tr>
<td>van der Waals volume</td>
<td>vdWv</td>
<td>c</td>
</tr>
<tr>
<td>$pK_{a1}$</td>
<td>$pK_{a1}$</td>
<td>d</td>
</tr>
<tr>
<td>logP</td>
<td>logP</td>
<td>d</td>
</tr>
</tbody>
</table>

*a* from Mopac AM1 single point calculations$^{13,14}$; 
*b* tabulated in literature$^{16}$; 
*c* calculated in SYBYL 6.1$^{14}$; 
*d* calculated in Pallas 1.2$^{15}$

Experimental Design

It is assumed that the descriptors in Table 3.2, *i.e.*, combinations of several descriptors, contain the specific information necessary to distinguish between the ligand-receptor interactions for the different dopamine receptor subtypes. An experimental design in the descriptor space will make it possible to select a few of the most diverse compounds. If the choice stands to select between two compounds, the compound more easily synthesized is selected. Eventually, a compound that portrays selectivity for a receptor subtype, will provide information about which descriptors that are responsible for the selectivity. In order to simplify the interpretation, the selection is performed following a factorial design$^{22}$ protocol.

An initial PCA of $X$ ($88 \times 19$), with two components accounting for 66 % of the variation, clearly divided the 88 compounds in two clusters (Figure 3.1(a)). One cluster contains all compounds with $R_1$ being a H atom, an OMe group and an OEt group corresponding to the compounds in Table 3.1 with ID $R_1$ being a, b and c, respectively. The remaining compounds, *i.e.*, ‘the sulfon esters’, form the second cluster. From Figure 3.1(a) it is also clear, the compounds with a triflate group at the 7 position (ID $R_1 = h$) form a subcluster within the sulfon ester group. Accordingly, compounds were selected from each cluster separately.
First, since compound 1 (N-n-propyl-7-OTf-OHB[f]Q; h02 in Table 3.1) displays affinity (i.e., $D_2 = 200$ and $D_3 = 21$ nM) for dopamine receptors only one additional triflate compound was synthesized. Hence, compound h01 (16) was selected arbitrarily.

![Score and Loading plots from PCA](image)

**Figure 3.1** Score (a) and Loading (b) plots from the PCA of all the computer generated compounds ($88 \times 19$). The selected compounds are high-lighted in (a) with bold face characters.
Second, from the sulfon ester cluster, including compounds with ID R₁ being d, e, f and g (thus, triflates not included), nine compounds were selected. The selection was performed by means of a factorial design in the three first PPs, accounting for 77 % of the variation in the descriptor matrix (44 × 19).

Third, from the cluster without sulfon esters, including compounds with ID R₁ being a, b and c, five compounds were selected. Again, the selection was performed by means of a factorial design in the first two PPs, accounting for 64 % of the variation in the descriptor matrix (33 × 19).

All the selected compounds are high-lighted with bold face characters in Figure 3.1(a) and listed in Table 3.3. The decision to synthesize compound 20 (Table 3.3) was taken at a later stage in the investigation (see below).

Table 3.3 The in vitro receptor binding results from the synthesized compounds. All binding results are reported as Kᵢ (nM) values.

<table>
<thead>
<tr>
<th>Compd</th>
<th>R1</th>
<th>R2</th>
<th>D₂</th>
<th>D₃</th>
<th>D₃:2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SO₂CF₃</td>
<td>-CH₂CH₂CH₃</td>
<td>200</td>
<td>21</td>
<td>ND</td>
</tr>
<tr>
<td>1-(-)</td>
<td>SO₂CF₃</td>
<td>-CH₂CH₂CH₃</td>
<td>56</td>
<td>19</td>
<td>&gt;3300</td>
</tr>
<tr>
<td>1-(+)</td>
<td>SO₂CF₃</td>
<td>-CH₂CH₂CH₃</td>
<td>240</td>
<td>120</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>2</td>
<td>-H</td>
<td>-CH₂CH₂CH₃</td>
<td>61</td>
<td>6.0</td>
<td>26</td>
</tr>
<tr>
<td>3ₐ</td>
<td>-H</td>
<td>-CH₂CH₂C₆H₆</td>
<td>10</td>
<td>3.0</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>-CH₃</td>
<td>-CH₃</td>
<td>&gt;5900</td>
<td>800</td>
<td>540</td>
</tr>
<tr>
<td>5</td>
<td>-CH₃</td>
<td>-CH₂C₆H₅</td>
<td>&gt;5900</td>
<td>820</td>
<td>2000</td>
</tr>
<tr>
<td>6ₗ</td>
<td>-H</td>
<td>-CH₂CH₂CH₂CH₃</td>
<td>29</td>
<td>4.0</td>
<td>50</td>
</tr>
<tr>
<td>7</td>
<td>SO₂CH₃</td>
<td>-CH₂CCH</td>
<td>5100</td>
<td>1800</td>
<td>&gt;3300</td>
</tr>
<tr>
<td>8</td>
<td>SO₂C₆H₅</td>
<td>-CH₂C₆H₅</td>
<td>1600</td>
<td>720</td>
<td>3300</td>
</tr>
<tr>
<td>9</td>
<td>SO₂CH₃</td>
<td>-CH₂CH₃</td>
<td>800</td>
<td>70</td>
<td>330</td>
</tr>
<tr>
<td>10</td>
<td>SO₂C₆H₅</td>
<td>-CH₂CH₂-2-thiophene</td>
<td>350</td>
<td>140</td>
<td>&gt;3300</td>
</tr>
<tr>
<td>11</td>
<td>SO₂CH₃</td>
<td>-H</td>
<td>5700</td>
<td>380</td>
<td>&gt;3300</td>
</tr>
<tr>
<td>12</td>
<td>SO₂C₆H₄C₆H₅</td>
<td>-CH₂CH₂C₆H₆</td>
<td>&gt;5900</td>
<td>&gt;3000</td>
<td>&gt;3300</td>
</tr>
<tr>
<td>13ₙ</td>
<td>SO₂-2-thiophene</td>
<td>-H</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>14</td>
<td>SO₂C₆H₄C₆H₅</td>
<td>-CH₂CH₂CH₃</td>
<td>220</td>
<td>37</td>
<td>&gt;3300</td>
</tr>
<tr>
<td>15</td>
<td>SO₂-2-thiophene</td>
<td>-CH₂CHCH₂</td>
<td>190</td>
<td>47</td>
<td>3300</td>
</tr>
<tr>
<td>16</td>
<td>SO₂CF₃</td>
<td>-CH₂CH₂CH₂CH₃</td>
<td>180</td>
<td>29</td>
<td>&gt;3300</td>
</tr>
<tr>
<td>20</td>
<td>-H</td>
<td>-CH₂CCH</td>
<td>15</td>
<td>13</td>
<td>730</td>
</tr>
</tbody>
</table>

ₐ Reference 23; ₗ Reference 5; ₙ Not synthesized
3.3 Chemistry

The syntheses of cis- and trans-OHB[f]Q have been discussed in a number of publications\textsuperscript{2,4,5,8,12,24} during the last two decades. However, some initial synthetic points need to be mentioned also here.

The OHB[f]Q skeleton may be obtained via the intermediate compound 25 by reduction of the enamide in two steps to obtain 26. Alternatively, 25 is alkylated at the 4-N position before the two reduction steps are carried out. Wikström et al.\textsuperscript{5} chose for the latter route, hence, they benzylated 25 followed by reduction of the enamide with Pd/C under H\textsubscript{2} yielding a 1:1 mixture of cis and trans lactam. After reduction of the lactam with LiAlH\textsubscript{4} the resulting cis and trans isomers were separated by means of chromatography on a silica column. Cannon et al.\textsuperscript{24} preferred the former route, since they managed to selectively reduce 25 with triethylsilane in trifluoroacetic acid, and obtain pure trans isomer as the product. The trans-26 was obtained by a subsequent reduction with LiAlH\textsubscript{4}.

The majority of the compounds in Table 3.3 were synthesized in three consecutive reaction steps (Scheme 3.1), starting from trans-26: a) alkylation of the 4-nitrogen, b) demethylation of the 7-OMe followed by c) sulfon ester formation at the 7-O position.

\begin{center}
\textbf{Scheme 1} (a) alkylation; (b) demethylation; (c) sulfon ester formation of compound trans-26
\end{center}

The alkylation was performed with the alkylating reagent, in refluxing acetonitrile, using Cs\textsubscript{2}CO\textsubscript{3} as the base. Since the intermediates were stable in acids, the demethylation was performed by refluxing the alkylated trans-26 in 48 \% HBr solution. Occasionally, the demethylation was performed using 1 M BBr\textsubscript{3} solution in dichloromethane cooled at -60 °C. The sulfon esters were synthesized using either of two methods: in a two phase system with dichloromethane and 8 \% NaOH with a proper catalyst or in dry dichloromethane, using triethylamine as the base.
For the preparation of trans-5 used for the separation of the enantiomers (see below), the route
described by Wikström et al.5 was used.

3.4 In Vitro Pharmacology

All the compounds synthesized were tested in three different in vitro receptor binding assays
(Table 3.3), i.e., dopamine D2L, D3 and D4,2, using [3H]-spiperone as the radioligand, performed as
described in the Experimental Section.

3.5 Results and Discussion

Quantitative Structure-Activity Relationships (QSAR)

From a PCA of the matrix containing both the descriptors and the response variables, the first
two PCs account for 66 % of the variation (Figure 3.2). The resemblance between the loading plots
in Figure 3.1(b) and Figure 3.2(b), confirm that the selection of molecules were carried out properly.
The response variables are highly correlated and cluster close to the center of the loading plot
(Figure 3.2(b)), indicating a relative low association with the other descriptors. The response
variable, pD4, is placed a bit further to the left from the center of the plot, as compared with pD2 and
pD3, indicating that pD4 is more inversely correlated with the size related descriptors, i.e., vDWv,
pop1, coco and mowe. Consequently, the cluster of compounds in the upper left quadrant in Figure
3.2(a), do have affinity for the dopamine D4 receptor, while the larger compounds to the right in the
same plot, totally lack affinity for the dopamine D4 receptor. The compounds with affinities for the
dopamine D4 receptor do not have a sulfon ester group at the 7 position.
Figure 3.2 Score (a) and Loading (b) plots from a PCA of the descriptors from the synthesized and tested compounds in Table 3.3. In (b), pD2, pD3 and pD4 represent the $-\log(K_i (nM))$ values of the receptor affinities from the respective receptors.
Figure 3.3 The affinities for the three dopamine receptors from the selected compounds (Table 3.3). Numbers 1–12 correspond to the compound numbers in Table 3.3; numbers 13–15 correspond to compounds 14–16 and number 16 corresponds to compound 20.

The affinities for the three receptors are highly correlated and selectivity for any of the three receptors is not observed for any of the compounds (Figure 3.3). However, the compounds with significant high affinity for the dopamine receptors, e.g., compounds 2, 3 and 6, all have a hydroxy group at the 7 position. Therefore, one additional hydroxy compound was synthesized in order to investigate the influence of different 4-N substituents on the receptor affinity. Accordingly, compound 20 (a08 in Table 3.1) was synthesized, and compared with all the other hydroxy compounds in Table 3.4. The pKa and logP were found to possess some association with the receptor affinity in less significant PCs than displayed in Figure 3.2, and were for reason of comparison appended to Table 3.4.

<table>
<thead>
<tr>
<th>Compd</th>
<th>N-substituent</th>
<th>D&lt;sub&gt;2&lt;/sub&gt; (nM)</th>
<th>D&lt;sub&gt;3&lt;/sub&gt; (nM)</th>
<th>D&lt;sub&gt;4&lt;/sub&gt; (nM)</th>
<th>pKa&lt;sup&gt;a&lt;/sup&gt;</th>
<th>logP&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>-CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>29</td>
<td>4.0</td>
<td>49</td>
<td>9.8</td>
<td>4.1</td>
</tr>
<tr>
<td>3</td>
<td>-CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;</td>
<td>10</td>
<td>3.0</td>
<td>30</td>
<td>8.4</td>
<td>4.7</td>
</tr>
<tr>
<td>2</td>
<td>-CH&lt;sub&gt;2&lt;/sub&gt;CHCH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>61</td>
<td>6.0</td>
<td>26</td>
<td>9.7</td>
<td>3.2</td>
</tr>
<tr>
<td>20</td>
<td>-CH&lt;sub&gt;2&lt;/sub&gt;CCH</td>
<td>15</td>
<td>13</td>
<td>730</td>
<td>6.1</td>
<td>2.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> calculated with Pallas 1.2<sup>15</sup>

### Structure-Activity Relationships

From Table 3.4 it can be concluded that the N substituent may be as large as a phenylethyl group (e.g., compound 3) without detrimentally affecting the receptor affinities, confirming the conclusions drawn by Wikström et al., from in vivo biochemical experiments. This is also in agreement with the extended McDermed<sup>25</sup> model (see Chapter 1) as presented by Liljefors et al.<sup>11,26</sup>

The N-propargyl compound (20) has low affinity for the dopamine D<sub>4</sub> receptor (K<sub>i</sub> = 730 nM), which is difficult to comprehend since the N-allyl compound (2) is much more potent (K<sub>i</sub> = 26 nM). The most likely interaction point for the protonated nitrogen atom with the target receptor, is an aspartic acid residue on helix three (i.e., Asp<sub>114</sub>; Table 1.3), which is preserved in all the D<sub>2</sub>-...
like dopamine receptors. Interaction with the aspartic acid residue requires a protonated nitrogen atom since the aspartic acid residue (pKₐ = 4.4) is readily ionized at physiological pH. The pKₐ for 20 and 2 were calculated to be 6.1 and 9.7, respectively, suggesting that 20 is less protonated than 2 at pH 7.4, i.e., the pH used for the *in vitro* receptor binding experiments.

Dijkstra *et al.*\(^{29}\) rationalized the low dopamine D₂ receptor affinity (competition with \(^{3}H\)-N0437) of compound PD128907 with the low measured pKₐ value (6.1), indicating that only two percent of the compound is protonated at the nitrogen atom at pH 7.4. Today, PD128907 is one of the most selective D₃ agonist known. Thus, the significance of the pKₐ value for the explanation of the difference between compounds 2 and 20 is not clear, since both compounds have affinity for the D₂ and D₃ receptors.

An alternative explanation to the lower D₄ affinity of 20 may be the *N*-propargyl group, which is less flexible as compared to the *N*-allyl, *N*-phenylethyl and *N*-butyl groups of compounds 2, 3 and 6 (Table 3.4), respectively.

It is known from literature\(^{30,31}\) that a triflate group increases the lipophilicity and has significant electrostatic influence. One effect induced by the triflate group is increased oral bioavailability,\(^{31,32}\) as compared with hydroxy or methoxy substituents. An explanation for this, as suggested by Sonesson *et al.*,\(^{31}\) may be that the electron-withdrawing effect of the triflate group results in a decrease of the aromatic hydroxylation in, e.g., the liver (cytochrome P450). The three phenyl hydrogens were in general shifted downfield, for compounds with a sulfon ester group attached to the phenyl ring, as compared to compounds without a sulfon ester group (e.g., OMe and OH groups). The three ‘aromatic’ hydrogens were always found in a range clearly above 7 ppm for the sulfon esters, while for the compounds without a sulfon ester group the range was ≤ 7 ppm. In that respect, no apparent difference between the triflate group and the structurally related mesylate group was observed.

The descriptors included in this investigation did not provide any clues as to why the 7-triflates, *i.e.*, compounds 1 and 16, have affinity for the D₃ receptor while the structurally related substituent, the mesylate group, was not present in any potent compounds. Actually, the affinity for the dopamine D₃ receptor of the *N*-propargyl-7-hydroxy compound, 20 (Kᵢ = 13 nM), was reduced significantly to 1800 nM, after mesylation (compound 7). To date, no explanation to why the triflate and the mesylate groups affect the *in vitro* and *in vivo* experiments differently, has been reported.

### Chemistry

The *trans* geometry of 5 could be determined with NMR-spectrometry, since the difference in chemical shift between the *N*-benzyl methylene protons\(^{3,4}\) was large (J = 215 Hz) and centered around δ 3.75, whereas the corresponding difference from the *cis*-5 isomer could not be observed (singlet, δ 3.71). Additionally, the *trans* isomer was confirmed with single crystal X-ray analysis of *trans*-19 (Figure 3.4), which crystallized in the triclinic P-1 space group with two molecules per unit cell (a = 8.233 Å; b = 9.423 Å; c = 11.480 Å; α = 103.55°; β = 98.62°; γ = 108.73°).
Several attempts to separate the (+)- and the (−)-enantiomer of trans-26 with fractional crystallization using (−)-dibenzyoyl-L-tartaric acid were performed without success. In the following attempt, OMe-mandeloyl chloride, (S)-camphanic chloride and (+)-chlocyphos chloride were subsequently coupled to the nitrogen atom, but the separations of the diastereomers using thin layer chromatography were not sufficient. Eventually, the enantiomers of trans-5 were successfully separated using semi-preparative HPLC.

3.6 Conclusions

Experimental design as a tool for rational drug design is effective provided that the descriptors used reflects the variations in the response variable. In the present investigation, the selected compounds were considered proper representatives of the large population of compounds from which they were selected. However, the response variables, i.e., the affinities for the dopamine D_{2L}, D_{3} and D_{4.2} receptor subtypes, were found to correlate poorly with the descriptors, which complicated further QSAR analysis.

The compounds with high affinity for the three receptor subtypes, all had a hydroxy group attached at the seven position. A sulfon ester group at the seven position, however, suppressed the affinity for the D_{4.2} receptor. It was also concluded that the N substituent may be as large as a phenylethyl group without detrimentally affect the ligand-receptor interaction. More difficult to rationalize is the low affinity for the D_{4.2} receptor of compound 20 (N-propargyl-7-hydroxy-OHB[f][Q]). One speculative explanation is that the somewhat rigid N-propargyl group may interfere in the ligand-D_{4.2}-receptor interaction. In addition, the significance of the low pK_{a} value (calculated to be 6.1) of compound 20 is hard to estimate, but may be a contributing factor to the low D_{4.2} affinity.
3.7 Experimental Section

: Computational Chemistry

All the compounds in Table 3.1 were built in SYBYL by adding the proper substituents to a low energy conformation of trans-(4aS,10bS)-OHB[ff]Q (i.e., the enantiomer found active by Wikström et al.) Each molecule was energy minimized in SYBYL, using the Tripos molecular mechanics force field. The 4-N was not protonated in any of the calculations. All settings were used default and all minimization iterations converged properly.

In order to generate some of the physicochemical descriptors in Table 3.2, Mopac AM1 single point calculations with the keywords MULLIK, AM1, T=3600 and 1SCF activated, were performed.

: Chemistry

General Remarks. NMR spectra were recorded at 200 or 300 MHz using a Varian Gemini 200 spectrometer. 1H NMR chemical shifts are given in δ units (ppm) relative to the solvents and converted to the TMS scale using δ (CDCl₃) = 7.26 and δ (CD₃OD) = 3.30. 13C NMR chemical shifts are given in δ units (ppm) relative to the solvents and converted to the TMS scale using δ (CDCl₃) = 76.91 and δ (CD₃OD) = 49.50. The splitting patterns are designated as follows: s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), m (multiplet). Multiplets are given as the range from the first to the last peak, respectively. FT-IR spectra were obtained on a ATI-Mattson spectrometer. Elemental analyses were performed at Parke-Davis (Ann Arbor, MI) and were within 0.4 % of calculated percentages, if not stated otherwise. High resolution mass spectrometric analyses were performed at the Department of Chemistry at the University of Groningen. GC/MS mass spectra were recorded on a Unicam Automass 150 GC/MS system 1. Melting points were determined on an Electrothermal digital melting point apparatus and are uncorrected. Specific optical rotations were measured in methanol at RT on a Perkin Elmer 241 polarimeter. For flash chromatography, silica gel 60 (0.040–0.063 mm, E. Merck, No. 9385) was used. All reagents used were commercially available and used without further purification.

Alkylation at the 4-N position. To a mixture of trans-26 (1 eq.), dry acetonitrile and Cs₂CO₃ (approx. 3 eq.) an alkylating reagent (1.2 eq.) was added. The mixture was refluxed a couple of hours until the reaction was completed as indicated by GC or TLC. The reaction was quenched by adding water and EtOAc. The organic layer was separated and the water layer was extracted three times with EtOAc. The combined organic layers was washed once with brine, dried over Na₂SO₄, filtered and evaporated leaving an oil. The HCl salt was prepared and recrystallized.

Demethylation of the 7-methoxy group. The N-alkyl-7-methoxy-OHB[ff]Q was refluxed under N₂ in 48 % HBr solution for two hours. The HBr was evaporated and the remaining N-alkyl-7-hydroxy-OHB[ff]Q×HBr was recrystallized from an appropriate solvent.
trans-N-(n-Propyl)-7-[[trifluoromethyl]sulfonyl]oxy]-OHB[\text{f}]Q (1). Procedure as for (–)-1 (below). mp 242–245 °C; \textsuperscript{1}H NMR (CDCl\textsubscript{3}) \( \delta \) 7.33 (d, \( J=7.79 \), 1H), 7.23 (t, \( J=7.79 \), 1H), 7.10 (d, \( J=8.12 \), 1H), 3.09–2.98 (m, 2H), 2.89–2.10 (m, 8H), 1.89–1.76 (m, 2H), 1.70–1.43 (m, 3H), 1.37–1.16 (m, 1H), 0.91 (t, \( J=7.36 \), 3H); IR (KBr) 1144 (\(-SO_2O\)) cm\textsuperscript{-1}; Anal. (C\textsubscript{17}H\textsubscript{22}NO\textsubscript{3}SF\textsubscript{3}×HCl) C, N, H

(–)-trans-(4a\textsubscript{S},10b\textsubscript{S})-N-(n-Propyl)-7-[[trifluoromethyl]sulfonyl]oxy]-OHB[\text{f}]Q ((–)-1).

trans-(4a\textsubscript{S},10b\textsubscript{S})-N-Propyl-7-hydroxy-OHB[\text{f}]Q\timesHBr (64 mg, 0.20 mmol), 99 % N-phenyltrifluoro-methane sulfonimide (111 mg, 0.29 mmol) and tetra-butyl-ammonium-hydrogen sulfate (a small spoonful) were suspended in CH\textsubscript{2}Cl\textsubscript{2} (5 mL) and layered with 8 % NaOH (4 mL). The mixture was stirred vigorously for 19 hours and then quenched with water. The organic layer was separated and the water layer was extracted three times with CH\textsubscript{2}Cl\textsubscript{2}. The combined organic layers was washed once with 10 % NaHCO\textsubscript{3}, dried over MgSO\textsubscript{4} and evaporated yielding a brownish oil (116 mg) that crystallized at RT. The product was purified with flash chromatography (gradient from pure CH\textsubscript{2}Cl\textsubscript{2} to CH\textsubscript{2}Cl\textsubscript{2}/MeOH 30:1) and the HCl-salt was prepared. Recrystallization from aceton/ether yielded white crystals (46 mg, 57 %). mp 232–233 °C; base \([\alpha]\)\textsubscript{19} = -46.5° (MeOH c = 0.91); \textsuperscript{1}H NMR (CDCl\textsubscript{3}) \( \delta \) 7.33 (d, \( J=6.93 \), 1H), 7.26 (t, \( J=7.77 \), 1H), 7.12 (d, \( J=8.97 \), 1H), 3.25–3.17 (m, 1H), 3.11–3.01 (m, 1H), 2.91–2.63 (m, 4H), 2.58–2.33 (m, 4H), 2.00–1.88 (m, 2H), 1.79–1.55 (m, 3H), 1.44–1.26 (m, 1H), 0.95 (t, \( J=7.34 \)); \textsuperscript{13}C NMR (CDCl\textsubscript{3}) \( \delta \) 148, 142, 129, 127.3, 125.7, 118.8, 115.5, 63.1, 54.8, 52.9, 41.5, 29.0, 25.0, 24.4, 23.2, 17.6, 11.7; FTIR (KBr) 1142 (\(-SO_2O\)) cm\textsuperscript{-1}

(+)-trans-(4a\textsubscript{R},10b\textsubscript{R})-N-(n-Propyl)-[[trifluoromethyl]sulfonyl]oxy]-OHB[\text{f}]Q ((+)-1).

trans-N-Allyl-7-hydroxy-OHB[\text{f}]Q (2). Trans-18 (170 mg, 0.67 mmol) was dissolved in CH\textsubscript{2}Cl\textsubscript{2} (4 mL) and added to a cooled (-60 °C) 1M solution of BBr\textsubscript{3} (3.1 mL, 3.1 mmol) and dry CH\textsubscript{2}Cl\textsubscript{2} (5 mL). The mixture was stirred at RT over day and boiled in MeOH (5 mL) for 15 minutes. The solvents were evaporated leaving a white solid which was triturated from ethanol. The white solid was filtered, washed with diethylether and dried (120 mg, 75 %). mp 257–260 °C; \textsuperscript{1}H NMR (CD\textsubscript{3}OD) \( \delta \) 6.92–6.87 (m, 1H), 6.73 (d, \( J=7.82 \), 1H), 6.55 (t, \( J=7.45 \), 1H), 5.97–5.89 (m, 1H), 5.28–5.20 (m, 2H), 4.93 (s, 2H), 3.53 (d, \( J=13.44 \), 1H), 3.22–2.34 (m, 8H), 2.11–1.94 (m, 2H), 1.72–1.54 (m, 3H), 1.47–1.35 (m, 1H); \textsuperscript{13}C NMR (CDCl\textsubscript{3}) \( \delta \) 147.8, 141.1, 128.7, 127.5, 125.7, 121.9, 63.3, 54.4, 52.8, 40.7, 28.6, 24.4, 23.6, 23.1, 17.3, 11.5; FTIR (KBr) 1204 (C-F), 3205 (\(-OH\)) cm\textsuperscript{-1}; MS (EIPI) 243; Anal: C\textsubscript{16}H\textsubscript{21}NO\timesHBr\times\textfrac{1}{2}H\textsubscript{2}O) C, H, N

trans-N-Phenylethyl-7-hydroxy-OHB[\text{f}]Q (3). This compound has previously been characterized by Froimowitz,\textsuperscript{23} mp 261–265 °C (lit.\textsuperscript{23} 284–285); \textsuperscript{1}H NMR (CD\textsubscript{3}OD) \( \delta \) 7.4–6.8 (m, 8H), 3.2–2.2 (m, 6H), 2.1–1.5 (m, 5H), 1.4–0.8 (m, 5H); FTIR (KBr) 3205 (\(-OH\)) cm\textsuperscript{-1}

trans-N-Methyl-7-methoxy-OHB[\text{f}]Q (4). This compound was prepared from trans-26 (160 mg, 0.74 mmol) following the general alkylation procedure above with methyl-iodide (70 \mu L, 1.1
mmol) leaving a colorless oil (230 mg, 135%). $^1$H NMR (CDCl$_3$) $\delta$ 7.23 (t, J=7.0, 1H), 6.91 (d, J=8.0, 1H), 6.74 (d, J=8.0, 1H), 4.00 (d, J=12.0, 1H), 3.74 (s, 3H), 3.63 (d, J=11, 1H), 3.51 (s, 2H), 3.17 (s, 3H), 3.11–2.99 (m, 1H), 2.78–2.65 (m, 1H), 2.63–2.36 (m, 2H), 2.22–1.92 (m, 2H), 1.79–1.46 (m, 2H); $^{13}$C NMR (CDCl$_3$) $\delta$ 182.0, 161.8, 152.6, 148.5, 143.3, 133.2, 98.7, 90.1, 79.7, 68.9, 62.1, 53.0, 48.2, 47.6, 45.7; FTIR (KBr) 2936, 2835, 2589, 1585, 1464 cm$^{-1}$; MS (EIPI) 231; Anal: (C$_{15}$H$_{21}$NO$\times$HCl) C, N, H (0.65%)

**trans-N-Benzyl-7-methoxy-OHB[Q]** (5). N-Benzyl-7-methoxy-OHB[Q]-3-on (23.78 g, 74.0 mmol) and LiAlH$_4$ (6.6 g, 0.95 mol) were mixed in THF (300 mL). The reaction was followed on GC which indicated an instant reaction. The reaction was quenched by consecutively adding H$_2$O (6 ml), NaOH (2 M, 6 mL) and H$_2$O (18 mL). The mixture was filtered, dried over MgSO$_4$, again filtered and the solvents were evaporated leaving a crude oil of cis and trans (22.40 g, 98%). The cis and trans isomers were separated with gradient flash chromatography on a silica column starting with ether/petroleumether (ratio 5:1 with 0.1 % TEA) ending with pure ether yielding cis (3.36 g, 15 %), trans (4.87 g, 21 %) and a mixture of cis and trans (8.07 g, 36 %).

**cis-5.** mp 216–221 °C; $^1$H NMR (CDCl$_3$) $\delta$ 7.34–7.17 (m, 5H), 7.11 (t, J=7.69, 1H), 6.89 (d, J=8.06, 1H), 6.66 (d, J=8.06, 1H), 4.11 (d, J=13.18, 1 benzyl-H), 3.78 (s, 3H), 3.39 (d, J=13.19, 1 benzyl-H), 2.98–2.89 (m, 2H), 2.70–2.55 (m, 2H), 2.51–2.39 (m, 2H), 2.17–1.99 (m, 2H), 1.75–1.64 (m, 2H), 1.62–1.47 (m, 1H), 1.25–1.09 (m, 1H); $^{13}$C NMR (CDCl$_3$) $\delta$ 156.9, 143.0, 139.5, 128.6, 128.0, 126.6, 126.1, 124.8, 117.6, 107.0, 63.6, 56.9, 55.1, 53.0, 42.3, 29.6, 26.8, 25.1, 23.0; FTIR (KBr) 3008, 2952, 1583, 1463 cm$^{-1}$; Anal (C$_{21}$H$_{25}$NO$\times$HCl$\times$½H$_2$O) C, H, N

**Enantiomeric separation of trans-N-Benzyl-7-methoxy-OHB[Q]** (5). The separation of the enantiomers was performed by means of semi-preparative HPLC on a Chiralcel OD (250 x 10 mm) column. A stock solution of the HCl salt of the racemic 5 and ethanol (100 mg/mL) was prepared. Each time 100 µL was injected on the column. The mobile phase was used was ethanol mixed with diethylamine (0.1 %), in order to minimize the peak-tailing, at a flow rate of 1.5 mL per minute. The eluent was monitored with a UV-detector (270 nm). R$_S$: 2.02; $\alpha$: 1.24. (Separation on a analytical Chiralcel OD column, flow rate: 0.5 mL/min; mobile phase: EtOH (gradient grade); R$_S$ = 1.65; $\alpha$ = 1.62)

(-)-trans-(4aR,10bR)-N-Benzyl-7-methoxy-OHB[Q] ((-)-5). The (–)-enantiomer was the least retained one. 106 mg of the pure enantiomer was obtained.

(+)-trans-(4aS,10bS)-N-Benzyl-7-methoxy-OHB[Q] ((+)-5). The (+)-enantiomer was the most retained one. 128 mg of the pure enantiomer was obtained.
trans-N-(n-Butyl)-7-hydroxy-OHB[f]Q (6). Previously characterized by Wikström et al.\textsuperscript{3} mp 270–275 °C (lit: 277–279 °C); HR-MS Calcd (Obsd) for C\textsubscript{17}H\textsubscript{25}NO 259.194 (259.195)

trans-N-(1-Prop-2-ynyl)-7-[(methane)sulfonyl]oxy]-OHB[f]Q (7). Trans-20 (60 mg, 0.25 mmol) was dissolved in dry CH\textsubscript{2}Cl\textsubscript{2} (5 mL), a small amount of TEA (4 drops) was added followed by CH\textsubscript{3}SO\textsubscript{2}Cl (30 µL, 0.37 mmol). The mixture was stirred under N\textsubscript{2} at RT over night. The reaction was quenched by adding 10 % NaHCO\textsubscript{3} (3 mL). The organic layer was separated and the water layer was extracted three times with CH\textsubscript{2}Cl\textsubscript{2}. The combined organic layers was washed once with brine, dried over Na\textsubscript{2}SO\textsubscript{4}, filtered and evaporated leaving a crude oil (80 mg, 0.25 mmol). The oil was converted into the HCl salt. mp 225–227 °C; \textsuperscript{1}H NMR (CDCl\textsubscript{3}) δ 7.30–7.20 (m, 3H), 4.31 (d, J=17, 1H), 3.83 (d, J=17, 1H), 3.44 (m, 2H), 3.21–3.06 (m, 5H), 2.9–2.8 (m, 1H), 2.71–2.23 (m, 4H), 2.20–2.00 (m, 2H), 1.46–1.28 (m, 2H); \textsuperscript{13}C NMR (CDCl\textsubscript{3}) δ 146.7, 138.5, 128.4, 127.4, 124.6, 120.2, 79.7, 70.4, 63.0, 53.1, 42.4, 39.0, 38.2, 27.4, 22.5, 22.2; FTIR (KBr) 1173 (s, -SO\textsubscript{2}O-) cm\textsuperscript{-1}; HR-MS Calcd (Obsd) for C\textsubscript{17}H\textsubscript{21}NO\textsubscript{3}S 319.124 (319.125)

trans-N-Benzyl-7-[(phenyl)sulfonyl]oxy]-OHB[f]Q (8). The HBr salt of trans-N-Benzyl-7-hydroxy-OHB[f]Q (70 mg, 0.19 mmol) was suspended in CH\textsubscript{2}Cl\textsubscript{2} (4 mL) and layered with 10 % NaOH (4 mL). A catalytic amount of Bu\textsubscript{4}NH\textsubscript{4}HSO\textsubscript{4} was added. Subsequently, benzenesulfonyl chloride solved in CH\textsubscript{2}Cl\textsubscript{2} (1.5 mL) was added dropwise. The mixture was stirred at RT for 30 hours, water was added and the organic layer was separated. The water layer was extracted with CH\textsubscript{2}Cl\textsubscript{2}, the combined organic layers was dried over Na\textsubscript{2}SO\textsubscript{4}, filtered and evaporated yielding a brownish oil. The oil was purified by flash chromatography on a silica column leaving a colorless oil (120 mg, 0.28 mmol). The HCl salt was prepared. mp 257–258 °C; \textsuperscript{1}H NMR (CDCl\textsubscript{3}) δ 7.90 (d, J=7.32, 2H), 7.69 (t, J=7.69, 1H), 7.55 (t, J=7.69, 2H), 7.31 (s, 3H), 7.27–7.19 (m, 3H), 7.09 (t, J=8.06, 1H), 6.83 (d, J=8.06, 1H), 4.10 (d, J=13.92, 1H), 3.32 (d, J=13.55, 1H), 2.85 (m, 2H), 2.63–2.60 (m, 2H), 2.30 (m, 2H), 2.08–2.01 (m, 2H), 1.73–1.71 (m, 2H), 1.26–1.21 (m, 2H); \textsuperscript{13}C NMR (CDCl\textsubscript{3}) δ 147.4, 142.5, 139.0, 136.2, 134.2, 130.0, 129.0, 128.1, 128.7, 126.8, 126.3, 126.1, 124.3, 119.2, 63.4, 57.2, 53.1, 42.2, 29.4, 26.5, 25.0, 23.2; FTIR (KBr) (s, -SO\textsubscript{2}O-) cm\textsuperscript{-1}; MS (EIPI) 433; Anal: (C\textsubscript{26}H\textsubscript{27}NO\textsubscript{3}S×HCl×½ H\textsubscript{2}O) C, H, N

trans-N-Ethyl-7-[(methane)sulfonyl]oxy]-OHB[f]Q (9). Triethylamine and methanesulfonyl chloride (0.013 mL, 0.17 mmol) were added to a suspension of trans-21 (60 mg, 0.19 mmol) in CH\textsubscript{2}Cl\textsubscript{2} and dioxan. The mixture was stirred at RT over night and quenched with 10 % NaOH. The organic layer was separated, the basic water layer was extracted once with CH\textsubscript{2}Cl\textsubscript{2} and the organic layers were combined. Small solids in the organic layer were formed but GC and TLC indicated no product and were consequently filtered off. The mother liquor was dried over Na\textsubscript{2}SO\textsubscript{4}, filtered and evaporated leaving a solid (50 mg) which still contained starting-material. The reaction was repeated (mesyl chloride 0.015 mL) followed by the same work up procedure. The obtained product was purified on a silica column. mp 221–225 °C; \textsuperscript{1}H NMR δ 7.23–7.11 (m, 3H), 3.19 (s, 3H), 3.18–2.93 (m, 2H), 2.91–2.58 (m, 3H), 2.48–2.43 (m, 2H), 2.36–2.23 (m, 2H), 2.18–2.10 (m, 2H), 1.86–1.80 (m, 2H), 1.58–1.52 (m, 1H), 1.03 (t, J=7.14, 3H); \textsuperscript{13}C NMR (CDCl\textsubscript{3}) δ 147, 142.5, 139.0, 136.2, 134.2, 130.0, 129.0, 128.1, 127.7, 126.8, 126.3, 126.1, 124.3, 119.2, 63.4, 57.2, 53.1, 42.2, 29.4, 26.5, 25.0, 23.2; FTIR (KBr) (s, -SO\textsubscript{2}O-) cm\textsuperscript{-1}; MS (EIPI) 433; Anal: (C\textsubscript{25}H\textsubscript{27}NO\textsubscript{3}S×HCl×½ H\textsubscript{2}O) C, H, N
trans-N-[2-(2-Thienyl)-ethyl]-7-[[phenyl)sulfonyl]oxy]-OHB[f]Q (10). Trans-N-[2-(2-Thienyl)-ethyl]-7-hydroxy-OHB[f]Q (24 mg, 0.07 mmol), TEA and dry CH₂Cl₂ were mixed before benzenesulfonyl chloride (30 µL, 0.24 mmol) was added. The mixture was stirred at RT for 5 hours. The reaction was quenched with 10 % Na₂CO₃ and the organic layer was separated. The water layer was extracted two times with CH₂Cl₂ and the combined organic layers was washed with brine, dried over Na₂SO₄, filtered and evaporated leaving a crude oil (63 mg, 0.14 mmol). mp 190–199 °C; ¹H NMR (CDCl₃) δ 8.0–7.5 (m, 5H), 7.3–7.0 (m, 3H), 6.9–6.7 (m, 3H), 3.8–3.3 (m, 5H), 3.1–2.5 (m, 5H), 2.4–2.0 (m, 2H), 1.5–1.3 (m, 4H); MS (EIPI) m/z (rel. intensity, 170 eV) 454 (M+, 100), 356 (13), 313 (37), 216 (18), 185 (4); MS (CI+) m/z (rel. intensity) 454 (M+, 100), 344 (6), 314 (26), 160 (8) for C₂₅H₂₇NO₃S₂

trans-7-[[Methyl)sulfonyl]oxy]-OHB[f]Q (11). Trans-26 (147 mg, 0.7 mmol) was suspended in dioxan (8 mL) followed by addition of NaHCO₃ (4 mL half saturated in H₂O) and FMOC-chloride (300 mg, 1.2 mmol) solved in dioxan (5 mL). The suspension was left stirring over night at RT, quenched with water (5 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers was dried over Na₂SO₄, filtered and evaporated yielding an yellow oil which was purified by flash chromatography leaving a white solid (310 mg, 100 %).

The obtained trans-N-FMOC-7-methoxy-OHB[f]Q (310 mg, 0.7 mmol) was added to ethylmercaptane (5 mL) together with AlCl₃ (330 mg, 2.5 mmol). The mixture was stirred at RT for 3.5 hours and quenched with ice water. The reaction mixture was extracted with chloroform (3 × 15 mL) and ethylacetat (2 × 10 mL), the combined organic layers was dried over Na₂SO₄, filtered and evaporated leaving a white solid (211 mg, 71 %).

The intermediate, trans-N-FMOC-7-hydroxy-OHB[f]Q (103 mg, 0.24 mmol) was, without further purification, mixed with triethylamine (a few drops) and dry dichlorometane (9 mL) before methanesulfonyl chloride (32 µL, 0.4 mmol) was added. The mixture was stirred at RT for 2.5 hours and quenched with 10 % Na₂CO₃. The basic water layer was extracted with dichloromethane (3 × 10 mL). The combined organic layers was washed once with brine, dried over Na₂SO₄, filtered and evaporated leaving a white solid (110 mg, 91 %).

The final product, trans-11, was obtained from trans-N-FMOC-7-[[methyl)sulfonyl]oxy]-OHB[f]Q (110 mg, 0.22 mmol) which was stirred in a 25 % piperidine/CH₂Cl₂ solution for 15 minutes. The solvents were evaporated and the remaining solids were purified by flash chromatography. mp 270–271 °C; ¹H NMR (CDCl₃) δ 7.31 (m, 3H), 3.48 (d, J=12.7), 3.3 (s, 3H), 3.25–3.09 (m, 2H), 3.04–2.86 (m, 2H), 2.68 (d, J=12.1), 2.28–2.09 (m, 2H), 2.01–1.84 (m, 2H), 1.56–1.48 (m, 1H); ¹³C NMR (CDCl₃) δ 141.9, 129.7, 126.6, 124.2, 119.0, 63.1, 58.2, 46.4, 43.1, 38.1, 29.4, 26.5, 23.2, 147; FTIR (KBr) 2934 (s), 2532, 2362 (m), 1334, 1170 (s, -SO₂O-) cm⁻¹; MS (EIPI) 281; Anal: (C₁₄H₁₉NO₃S×HCl×½ H₂O) C, H, N

trans-N-Phenylethyl-7-[[4-toluoyl)sulfonyl]oxy]-OHB[f]Q (12). Trans-3 (31 mg, 0.08 mmol), TEA and dry CH₂Cl₂ were mixed before p-toluensulfonyl chloride (32 mg, 0.17 mmol) was
added. The mixture was stirred at RT for 5 hours. The reaction was quenched with 10 % Na₂CO₃ and the organic layer was separated. The water layer was extracted two times with CH₂Cl₂ and the combined organic layers was washed with brine, dried over Na₂SO₄, filtered and evaporated leaving a crude oil which was converted to the HCl salt. Recrystallization from ethanol yielded brown crystals. mp 290 °C dec.; MS (EIPI) m/z (rel. intensity, 170 eV) 462 (M+, 11), 239 (100), 102 (76) for C₂₈H₃₁NO₃S 

*trans*-N-(α-Propyl)-7-[(4-toluoylsulfonyl)oxy]-OHB[Q (14). To a solution of dioxan (8 mL), *trans*-17 (60 mg, 0.18 mmol) and a few TEA drops, p-toluensulfonyl chloride (90 mg, 0.47 mmol) was added. The reaction was complete after one nights stirring as indicated by GC/MS. Dichloromethane and 10 % NaHCO₃ was added. The water layer was extracted three times with dichloromethane, and the combined organic layers was dried over Na₂SO₄, filtered and evaporated yielding a brownish oil. After purification by flash chromatography on a silica-column (MeOH/CH₂Cl₂ 1:15) the HCl salt was prepared yielding an oil (80 mg, 100 %) which crystallized while standing. The solid was recrystallized from ethanol. mp 217–219 °C; ¹H NMR (CDCl₃) δ 7.74 (d, J=8.30, 2H), 7.32 (d, J=8.06, 2H), 7.15 (t, J=8.30, 1H), 7.07 (d, 7.81, 1H), 6.80 (d, J=7.81, 1H), 3.07 (d, J=11.47, 1H), 2.83–2.51 (m, 4H), 2.45 (s, 3H), 2.40–2.21 (m, 2H), 2.19–2.07 (m, 2H), 1.87–1.78 (m, 2H), 1.60–1.41 (m, 3H), 1.27–1.19 (m, 2H), 0.88 (t, J=7.33, 3H); ¹³C NMR (CDCl₃) δ 147.4, 145.2, 141.8, 133.1, 129.9, 129.6 (2C), 128.2 (2C), 126.3, 124.1, 119.3, 62.6, 54.7, 52.6, 41.5, 29.1, 25.2, 24.7, 23.0, 21.5, 17.4, 11.7; FTIR (KBr) 1187 (-SO₂O-) cm⁻¹; MS (EIPI) 399; Anal: (C₂₃H₂₉NO₃S × HCl × 1¼ H₂O) C, H (0.5%), N 

*trans*-N-Allyl-7-[(2-thienylsulfonyl)oxy]-OHB[Q (15). *Trans*-2 (80 mg, 0.33 mmol), TEA and dry CH₂Cl₂ were mixed before 2-thienyl-sulfonyl chloride (100 mg, 0.55 mmol) was added. The mixture was stirred at RT for 5 hours. The reaction was quenched with 10 % Na₂CO₃ and the organic layer was separated. The water layer was extracted three times with dichloromethane, and the combined organic layers was dried over Na₂SO₄, filtered and evaporated yielding a brownish oil. After purification by flash chromatography on a silica-column (MeOH/CH₂Cl₂ 1:15) the HCl salt was prepared yielding an oil (80 mg, 100 %) which crystallized while standing. The solid was recrystallized from ethanol. mp 217–219 °C; ¹H NMR (CDCl₃) δ 7.76–7.73 (m, 1H), 7.62–7.60 (m, 1H), 7.23–7.22 (m, 1H), 6.90–6.83 (m, 1H), 6.03–5.84 (m, 1H), 5.46 (s, 1H), 5.39 (d, J=4.64, 1H), 3.78–3.53 (m, 2H), 3.37 (d, J=9.83, 1H), 3.26–3.08 (m, 1H), 2.91–2.43 (m, 5H), 2.35–2.10 (m, 2H), 2.01–1.80 (m, 2H), 1.41 (dd, (d, J=11.79), (d, J=13.76)); ¹³C NMR (CDCl₃) δ 147.3, 139.6, 135.1, 134.8, 129.1, 127.7, 127.0, 126.4, 124.5, 124.1, 119.8, 63.4, 54.7, 52.1, 39.7, 27.8, 23.5, 22.4; FTIR (KBr) 3070, 2928, 2508, 1354, 1182 (-SO₂O-) cm⁻¹; Anal: (C₁₉H₂₃NO₃S₂×HCl) C, H, N 

*trans*-N-(α-Butyl)-7-[(trifluoromethane)sulfonyl]oxy]-OHB[Q (16). *Trans*-6 (90 mg, 0.35 mmol) was mixed with CH₂Cl₂ (4 mL), 99 % N-phenyl-trifluoro-methane sulfonimide (210 mg, 0.59 mmol), Bu₄NH₂HSO₄ (catalytic amount) and layered with 8 % NaOH (4 mL). The mixture was stirred vigorously under N₂ over night and quenched with 10 % HCl. An attempt to perform an acid/base extraction failed because the product was too lipophilic and remained in the organic layer even after extraction with acid. Therefore, the water layer was extracted with CH₂Cl₂. The combined
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organic layers was dried over Na₂SO₄, filtered and evaporated. The product was purified with flash chromatography. mp 217 °C; 1H NMR (CDCl₃) δ 7.31–7.24 (m, 2H), 7.17–7.13 (m, 1H), 3.74 (t, J=10.01, 1H), 3.58–3.46 (m, 1H), 3.24–3.09 (m, 3H), 2.88–2.75 (m, 3H), 2.63 (d, J=11.72, 2H), 2.40–2.38 (m, 2H), 2.07–2.03 (m, 1H), 1.83–1.24 (m, 5H), 0.99 (t, J=7.20, 3H); FTIR (KBr) 2966, 2416, 1411, 1209(-SO₂O-) cm⁻¹; HR-MS Calcd (Obsd) for C₁₈H₂₄NO₃SF₃ 391.143 (391.142).

**trans-N-Allyl-7-methoxy-OHB[f]Q (18).** This compound was prepared from trans-26 (180 mg, 0.83 mmol) following the general alkylation reaction (above) with allyl-bromide (86 µL, 1 mmol) leaving a yellowish oil (220 mg, 0.86 mmol). mp 200–209 °C; 1H NMR (CDCl₃) δ 7.15 (t, J=7.94, 1H), 6.93 (d, J=7.81, 1H), 6.69 (d, J=7.81, 1H), 5.93–5.89 (m, 1H), 5.24–5.15 (m, 2H), 3.80 (s, 3H), 3.54 (d, J=14.16, 1H), 3.21–2.89 (m, 3H), 2.67 (m, 3H), 2.28–2.08 (m, 2H), 1.84–1.77 (m, 2H), 1.57–1.51 (m, 2H), 1.3–1.1 (m, 1H); 13C NMR (CDCl₃) δ 156.7, 140.6, 134.4, 126.1, 124.7, 117.63, 117.59, 106.9, 62.9, 56.1, 55.0, 52.9, 42.4, 29.5, 25.3, 22.8; MS (EIPI) 257 ; HR-MS Calcd (Obsd) for C₁₇H₂₃NO 257.178 (257.180)

**trans-N-(1-Prop-2-ynyl)-7-methoxy-OHB[f]Q (19).** This compound was prepared from trans-26 (190 mg, 0.88 mmol) following the general alkylation procedure above with propargyl chloride (100 µL, 1.38 mmol) leaving a yellow oil (230 mg, 0.9 mmol). mp 213–222 °C; 1H NMR (CDCl₃) δ 7.17 (t, J=7.94, 1H), 6.93 (d, J=8.05, 1H), 6.71 (d, J=8.05, 1H), 3.81 (d, J=17.58, 1H), 3.42 (d, J=17.58, 1H), 2.95–2.87 (m, 2H), 2.64–2.29 (m, 6H), 1.88–1.81 (m, 2H), 1.45–1.10 (m, 3H); 13C NMR (CDCl₃) δ 154.1, 139.5, 125.8, 122.2, 116.3, 111.3, 61.7, 41.8, 41.7, 29.2, 24.9, 24.8, 22.2; FTIR (KBr) 3194 (s, -OH), 2930 (s), 2556 (s), 1585 (s), 1466 (s), 1272 (s) cm⁻¹; MS (EIPI) 241; Anal: (C₁₇H₂₃NO×HCl) C, H, N

**trans-N-(1-Prop-2-ynyl)-7-hydroxy-OHB[f]Q (20).** A mixture of 1M BBr₃ (1.9 mL) and dry CH₂Cl₂ (3 mL) were cooled to -60°C before a solution of trans-19 (100 mg, 0.39 mmol) and CH₂Cl₂ (3.6 mL) was added dropwise. After addition, the temperature was allowed to reach RT and was left stirring overnight. The reaction was cooled to 0°C before MeOH (4 mL) was added, followed by 15 minutes refluxing. The solvents were evaporated the HCl salt was prepared. mp 251–252 °C; 1H NMR (CDCl₃) δ 6.94 (t, J=7.82, 1H), 6.84 (d, J=7.81, 1H), 6.58 (d, J=7.81, 1H), 5.93–5.89 (m, 1H), 5.24–5.15 (m, 2H), 3.81 (d, J=17.58, 1H), 3.40 (d, J=17.58, 1H), 3.34 (s, 1H), 2.95–2.87 (m, 2H), 2.64–2.29 (m, 6H), 1.88–1.81 (m, 2H), 1.45–1.10 (m, 2H); 13C NMR (CDCl₃) δ 154.1, 139.5, 125.8, 122.2, 116.3, 111.3, 61.7, 52.7, 41.8, 41.7, 29.2, 24.9, 24.8, 22.2; FTIR (KBr) 3194 (s, -OH), 2930 (s), 2556 (s), 1585 (s), 1466 (s), 1272 (s) cm⁻¹; MS (EIPI) 241; HR-MS Calcd (Obsd) for C₁₆H₁₉NO 241.147 (241.147)

**trans-N-Ethyl-7-hydroxy-OHB[f]Q (21).** The intermediate trans-N-Ethyl-7-methoxy-OHB[f]Q was prepared from trans-25 (310 mg, 1.4 mmol) following the general alkylation procedure (above) with iodoethane (190 µL, 2.4 mmol) leaving a white solid (310 mg, 89 %). 1H NMR (CDCl₃) δ 7.13 (t, J=8.06, 1H), 6.90 (d, J=8.05, 1H), 6.68 (d, J=8.06, 1H), 3.79 (s, 3H), 3.08–2.92 (m, 3H), 2.81–2.32 (m, 6H), 1.87–1.83 (m, 2H), 1.48–1.41 (m, 3H), 1.07 (t, J=7.14, 3H);
13C NMR (CDCl₃) δ 156.8, 140.7, 126.2, 124.8, 117.8, 107.1, 62.4, 55.2, 52.1, 46.6, 42.5, 29.8, 25.9, 25.5, 23.1, 9.3; MS (EIPI) 245

Trans-N-Ethyl-7-methoxy-OHB[f]Q (220 mg, 0.9 mmol) was used without further purification to prepare trans-21, following the general demethylation procedure (above) yielding a white/brownish solid (270 mg, 96%). The solid was recrystallized from MeOH. mp 300–302 °C; ¹H NMR (CD₃OD) δ 7.03 (t, J=7.33, 1H), 6.85 (d, 1H), 6.65 (d, J=7.8, 1H), 3.47–3.57 (m, 2H), 3.16–3.30 (m, 6H), 3.03 (m, 2H), 2.60–2.66 (m, 2H), 2.10 (m, 2H), 1.80 (m, 1H), 1.33–1.37 (m, 3H); FTIR (KBr) 3192, 2924, 2743, 2679, 1585, 1468, 1273 cm⁻¹; HR-MS Calcd (Obsd) for C₁₅H₂₁NO 231.162 (231.162)

In vitro Pharmacology

Cell Culture. CHO K1 cells stably transfected with the genes of wild type D₂L, D₃, and D₄.2 receptors were grown and harvested as previously described.34-36

Radioligand Binding. CHO K1 cells expressing the human DA D₂L, D₃, and D₄.2 receptors were removed by replacement of growth medium with PBS-EDTA (0.02 % EDTA in phosphate buffered saline). After swelling for 5-10 min, the cells were scraped from the flasks, and centrifuged at about 1000 x g for 5 min. The cells were then resuspended in 50 mM Tris-HCl binding buffer pH 7.4 at room temperature (50 mM Tris-HCl, 1 mM EDTA, 1.5 mM CaCl₂, 5 mM KCl, 120 mM NaCl and 5 mM MgCl₂). The membranes were pelleted by centrifugation at 20,000 × g at 4°C for 20 min. The supernatant fluid was removed and the pellets were resuspended and homogenized with a Brinkman Polytron (setting 5 for 15 sec) in the binding buffer and 1 mL aliquots stored at -80°C until used in the binding assay.

Binding assays were carried out in duplicate in 1.4 mL microtubes (Marsh Biomedical Products, Inc.). Each tube received 50 µL of competing drug or binding buffer, 50 µL of [³H]spiperone (final concentration was 0.2 nM for D₂L and D₄.2 and 0.5 nM for D₃) and 0.4 mL membranes (15–30 µg protein) to give a final volume of 0.5 mL. After 60 min incubation at 25°C, the incubations were terminated by rapid filtration over GF/B filters presoaked in 0.5 % polyethylenimine and washed rapidly with 3 × 1 mL ice-cold buffer. Filters were put in scintillation vials, 4 mL of Beckman Ready Gel Scintillation fluid was added and the radioactive content determined by liquid scintillation spectrophotometry. Non-specific binding was defined in presence of 1 µM haloperidol. Data for IC₅₀ values were analyzed using the iterative nonlinear least square curve-fitting program LIGAND. The dissociation constant, Kᵢ, was derived from the concentration, C, for 50 % inhibition of binding, using Kᵢ = C/(1 + C* / Kᵣ) where C* was the concentration of [³H]spiperone and the Kᵣ was 0.116 nM, 0.152 nM and 0.093 nM for DA D₂L, D₃ and D₄.2 receptors, respectively.37 Experimental compounds were made up as stock solutions in dimethyl sulfoxide (DMSO). The final concentration of 0.1 % DMSO in the incubation mixture had no effect on specific binding.

3.8 References


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