

## CHAPTER 9

### SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF A SERIES OF DOPAMINERGIC AND SEROTONERGIC 8-CHLORO-5-HYDROXY-2-(DIALKYLAMINO)-TETRALINS

**Summary:** A series of 8-chloro-5-hydroxy-2-(dialkylamino)tetralins (**5b**, **6b**, **7b**) were prepared in a one step reaction using a hexachlorocyclohexadienone as a regioselective chlorinating agent. The reaction proceeds in a highly selective but unexpected manner. In *in vitro* and *in vivo* tests of dopaminergic activity, chlorination was found to diminish the DA agonist activity. The effect of chlorination was most remarkable in the brain microdialysis of **5b**, where the activity is changed from DA receptor agonist to an antagonist. In addition, we have evaluated the ability of these compounds to displace [<sup>3</sup>H]-8-OHDPAT from 5-HT<sub>1A</sub> binding sites. All of the chlorinated compounds display affinity for this receptor.

#### 9.1 Introduction

Until recently, the classification of dopamine (DA) receptors was based on their association with the enzyme adenylyl cyclase [1]. DA receptors positively linked to this enzyme were termed D<sub>1</sub>, whereas those either negatively coupled or not associated to cAMP generation were designated D<sub>2</sub>. Sokoloff et al. [2] have recently identified a novel dopamine receptor, which they called the D<sub>3</sub> receptor. Although this finding is exciting, the functional significance of the D<sub>3</sub> receptor is as yet unknown. This study will focus only on D<sub>1</sub> and D<sub>2</sub> DA receptors.

From our research on CNS active compounds, particularly on DA and 5-HT<sub>1A</sub>, we have several times encountered a close relationship between the structure activity (SAR) of these agents. This article will be focussed on the structural class of the 2-aminotetralins.

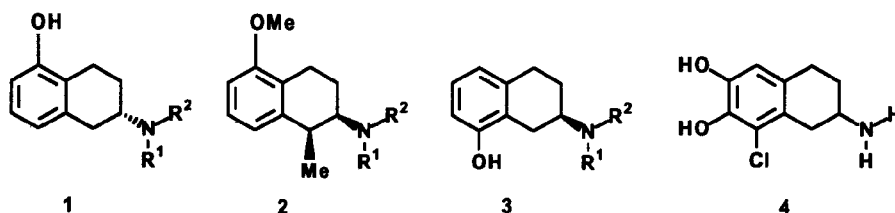
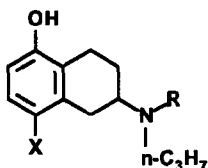


Figure 9.1. Chemical structures of various classes of 2-aminotetralins

The aminotetralins are semi-rigid structures that lock the phenylethylamine side chain in the extended, presumed bioactive, conformation. The tetralin template could be modified to give selective ligands to other monoamino receptors as well as being effective probes to explore the relative similarities and differences in the topology of central neurotransmitter receptors. In practice, the modification of the tetralin structure has led to the discovery of selective dopamine (DA) D<sub>2</sub> receptor agonists (1), e.g. 5-hydroxy-2-(di-n-propylamino)tetralin (5-OH-DPAT) [3]; putative DA autoreceptor antagonists (2) e.g. *cis*-(1S,2R)-5-methoxy-1-methyl-2-(di-n-propylamino)tetralin ((+)-UH 232) which has a 3-4 times higher affinity for D<sub>3</sub> than for D<sub>2</sub> DA receptors [2,4]; and 5-HT<sub>1A</sub> agonists (3), e.g. 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) [5]. The recent finding that 7-OH-DPAT has a 70 times preference for the D<sub>3</sub> over the D<sub>2</sub> DA receptor is intriguing and will lead to even more interest in these 2-aminotetralins. Weinstock et al. [6] have found that, in contrast to benzazepines, replacement of either the 6- or 7-hydroxyl group of ADTN with a halogen did not change the activity from a dopamine receptor agonist to an antagonist. However, their data suggest that 8-chloro- (4), but not 5-chloro substitution of ADTN results in a relatively selective agonist for the D<sub>1</sub> subpopulation of dopamine receptors. The 5-chloro substitution produced a relatively high degree of D<sub>2</sub> receptor potency. In order to explore the influence of 8-chloro substitution on the structure activity relationship of a series of potent D-2 agonists, the 8-chloro analogues **5b**, **6b** and **7b** of the 5-hydroxy-2-aminotetralins **5a** (5-OH-DPAT), **6a** (N-0434) and **7a** (N-0437) [7] have been synthesized. The regioselective synthesis and pharmacological evaluation of these monohydroxylated 2-aminotetralins are the subject of this paper.

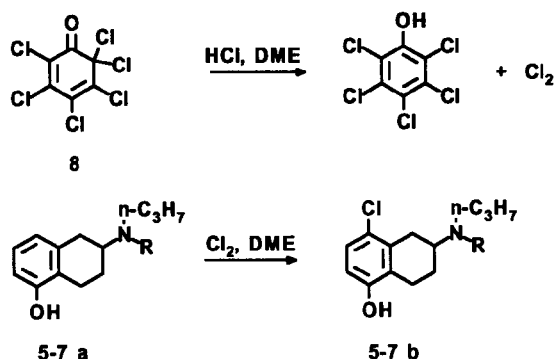


- 5a. X = H, R = n-propyl;      5b. X = Cl, R = n-propyl  
 6a. X = H, R = phenylethyl;    6b. X = Cl, R = phenylethyl  
 7a. X = H, R = thienylethyl;    7b. X = Cl, R = thienylethyl

## 9.2 Chemistry.

The presence of the non-chlorinated aminotetralins in our lab prompted us to look for a single step reaction for the regioselective production of the desired compounds. Most attempts to achieve site-selectivity in aromatic chlorination have been directed towards controlling the approach of the chlorinating agent to the substrate. These attempts have been made with bulky [8] or micellar chlorinating agents [9], complexes between substrate and chlorinating agent etc. [10]. Recently, Lindsay

Smith et al. [11] have shown that remarkable selectivity can be obtained by using N-chloroamines. A certain number of reagents for selectivity control have a cyclohexadienic structure [10]. As the aromatization energy of cyclohexadienes acts as the driving force, reactions can be performed under extremely mild and selective conditions. The regioselectivity of the aromatic substitution can also be controlled by use of an appropriate solvent and of cyclohexadienone in particular. Guy et al. [10] believe that the selectivity and their dramatic response to experimental conditions can only be explained by the formation of an intermediate complex formed as a result of non-covalent, donor-acceptor and hydrogen-bonding interactions between reagents and substrate. Surprisingly, it was possible to obtain the para-chlorinated products **5b**, **6b** and **7b** with the ortho-chlorinating agent i.e. 2,3,4,5,6,6-hexachloro-2,4-cyclohexadiene-1-one **8** (Scheme 9.1)



**Scheme 9.1.** Synthesis of the p-chlorinated aminotetralins

The mechanism suggested by Guy et al. [10] is not valid for the reaction circumstances that we have used. We suggest that the hexachlorocyclohexadienone slowly reacts with the hydrochloric acid to give a low concentration of chlorine. The function of the hexachlorohexadienone is therefore to provide a source of Cl<sub>2</sub> in a low steady-state concentration. The HCl will be liberated again in step 2. Evidence for this reaction mechanism is shown in chapter 8 [24]. The chlorinations with the enantiomers of **5a**, **6a** and **7a** were carried out as described for the racemic compounds.

### 9.3 Results and Discussion

The dopaminergic affinity of the racemic compounds was measured *in vitro* by determining the ability of the test compounds to displace [<sup>3</sup>H]-SCH 23390 and [<sup>3</sup>H]-spiperone from calf striatum membrane homogenates as a measure of the affinity for

D<sub>1</sub> and D<sub>2</sub> binding activity, respectively. The results are shown in Table 9.1. As can be seen from the table, all compounds display affinity for both the D<sub>1</sub> and the D<sub>2</sub> receptor. However, the chlorinated compounds have lower affinity than their non-chlorinated counterparts. Moreover, introduction of a chloro atom in the 8-position did not result in compounds with a higher selectivity for either of these receptor subtypes. This is in contrast with results found for the 8-chlorinated ADTN derivatives, which resulted in compounds with a higher selectivity for the D<sub>1</sub> receptor [6]. However, the selective D<sub>1</sub> affinity in this study was based on comparison of K<sub>i</sub> values for the displacement of a D<sub>1</sub> agonist ([<sup>3</sup>H]-fenoldopam) with those for the displacement of a D<sub>2</sub> antagonist ([<sup>3</sup>H]-Spiperone). Murray and Waddington [18] concluded that this method might lead to an inappropriate conclusion of selectivity for D<sub>1</sub> receptors.

**Table 9.1.** Affinity of various aminotetralins as measured by their ability to displace *in vitro* [<sup>3</sup>H]-SCH 23390, [<sup>3</sup>H]-Spiperone from striatal membranes and [<sup>3</sup>H]-8-OH-DPAT from cortical and hippocampal membranes. The values are expressed in nM ± SEM for 3-4 separate determinations performed in triplicate. Each displacing compound was used at concentrations ranging from 10<sup>-11</sup> to 10<sup>-4</sup> M. <sup>a</sup> I is inactive [5]. NT is not tested.

Compound	K <sub>i</sub> (nM)		
	[ <sup>3</sup> H]-Spiperone	[ <sup>3</sup> H]-SCH 23390	[ <sup>3</sup> H]-8-OH-DPAT
±-5a	63 ± 12	650 ± 158	1a
±-5b	122 ± 57	1230 ± 161	44
(R)-(+)-5b	NT	NT	26
(S)-(-)-5b	NT	NT	35
(±)-6a	15 ± 5	462 ± 51	
(±)-6b	75 ± 8	750 ± 74	46
(R)-(+)-6b	40 ± 9	NT	
(S)-(-)-6b	65 ± 38	NT	120
(±)-7a	21 ± 1	460 ± 89	
(±)-7b	52 ± 5	1540 ± 182	58
(R)-(+)-7b	NT	NT	37
(S)-(-)-7b	NT	NT	39
(R)-8-OH-DPAT			4

The affinities of **5b**, **6b**, and **7b** and their enantiomers for 5HT<sub>1A</sub> receptors were also evaluated. All the compounds showed a pronounced affinity for this receptor. Consequently, 8-chlorination gave a dramatic shift from no affinity for the 5-HT<sub>1A</sub>

receptor to compounds with a considerable affinity for this receptor, whereas the binding affinity for the D<sub>1</sub> and D<sub>2</sub> receptor decreased. The binding studies further demonstrated no significant affinity differences between the enantiomers of the chlorinated compounds. With the concentrations applied in the 6-hydroxydopamine model (5-10 μmol/kg), no 5-HT syndrome was observed. Of particular interest is, that the affinity for the (R)-enantiomer is a little higher than that for the (S)-enantiomer.

In an attempt to find an explanation for this pharmacological shift, we performed semi-empirical calculations with the MOPAC-package program PM3, accessible through the SYBYL program from Tripos. The results of the computational model are not as dramatic as the pharmacological results, but it emphasizes, that in the highest occupied molecular-orbital (HOMO) state, the charge on the chlorine atom differs from the charge on the hydrogen atom. In addition, it is polarizable and can participate in a H bond from e.g. serine in the receptor.

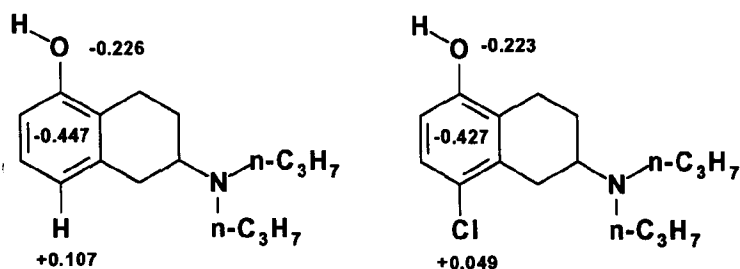


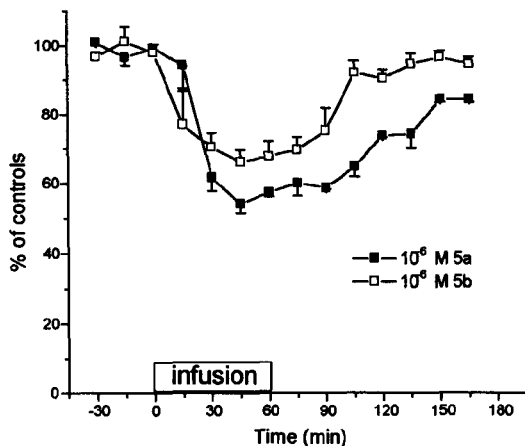
Figure 9.2 MOPAC charges of 5-OH-DPAT and 8-Cl-5-OH-DPAT. In the aromatic ring the total charge of the  $\pi$ -electron system is given.

There seems to be a delicate balance between the 5-HT<sub>1A</sub> and DA receptor. In this case H-bond acceptance by the chlorine atom is probably sufficient for inducing 5-HT<sub>1A</sub> receptor interaction.

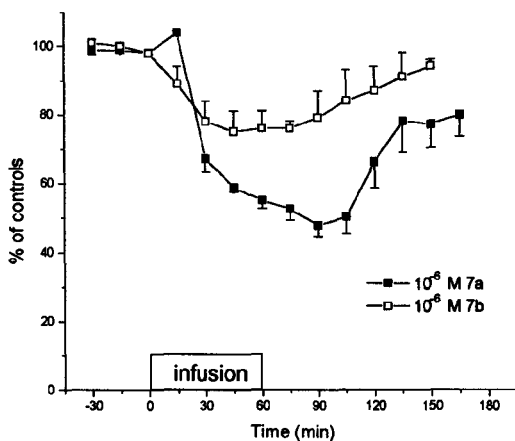
The compounds were also tested for their pharmacological action on DA D<sub>2</sub> autoreceptors *in vivo*, by measuring DA release by on-line brain microdialysis in freely moving rats during administration [12-14]. An advantage with brain microdialysis is that the drug studied can be applied locally in the area from which the release is being measured, thus providing useful information clarifying its intracerebral site of action. The compounds (10<sup>-6</sup> M) were infused via the dialysis membrane in the striatum during 60 min.

Agonists of dopamine receptors, with activation at the D<sub>2</sub> receptor subtype reduce the *in vivo* release, as well as its turnover rate. On the other hand D<sub>1</sub> and D<sub>2</sub> specific antagonists of DA receptors increase the *in vivo* release of DA. Local

application of racemic **5a** and **5b** induced a 50% and 30% decrease in DA release, respectively (Fig. 9.3). The onset of the decrease was immediate after the start of the infusion.



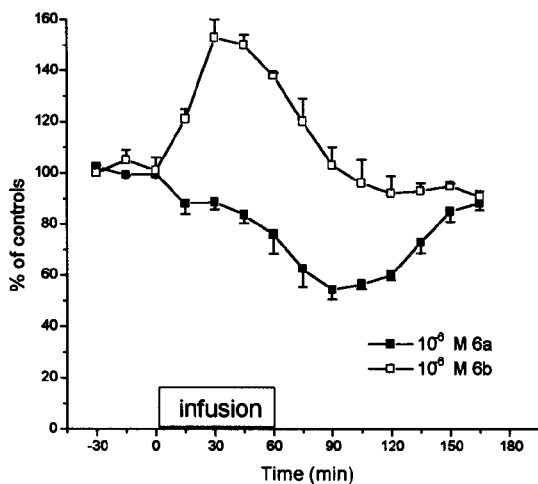
**Figure 9.3** Effect of a  $10^{-6}$  M infusion of 5-OH-DPAT (**5a**) and ( $\pm$ )-8-Cl-5-OH-DPAT (**5b**) on DA release from the striatum. The compounds were dissolved in Ringer solution. The results are the mean  $\pm$  SEM of data obtained from 3-4 rats.



**Figure 9.4.** Effect of a  $10^{-6}$  M infusion of N-0437 (**7a**) and ( $\pm$ )-8-Cl-N-0437 (**7b**) on DA release from the striatum. The compounds were dissolved in Ringer solution. The results are the mean  $\pm$  SEM of data obtained from 3-4 rats.

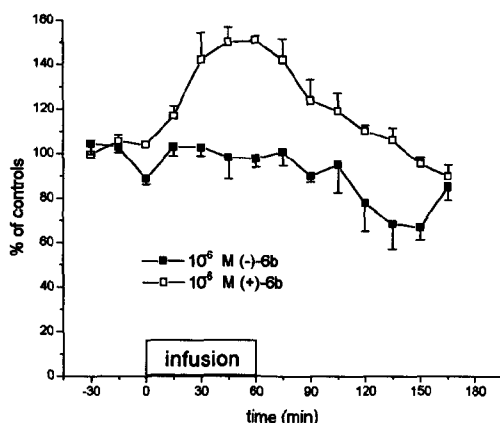
N-0437 (**7a**) and racemic 8-Cl-N-0437 (**7b**) infusion induced a decrease in DA release of 50% and 25%, respectively (Fig 9.4). The decreases induced by the chlorinated analogues **5b** and **7b** was less pronounced than those of the non-chlorinated compounds **7a** and **7a**.

A  $10^{-6}$  M infusion of N-0434 (**6a**) also gave, as expected, a 45% decrease of the DA release (Fig 9.5). However, a striking effect was obtained with the chlorinated analogue ((±)-8-Cl-N-0434, **6b**). This compound induced, immediately after infusion an increase of 50% in DA release. Thus, infusion of racemic **6b** resulted in antagonistic effects on receptors mediating DA release. Timmerman et al. [13,14] have demonstrated that the (+)-enantiomer of **7a** displayed a weak antagonistic action in this model. However, in contrast to racemic 8-Cl-N-0434 (**6b**) in the racemic mixture of N-0437 (**7a**) the agonistic effect predominates over the antagonistic effect. Because of the resemblance with N-0437, we have also prepared the enantiomers of 8-Cl-N-0434.

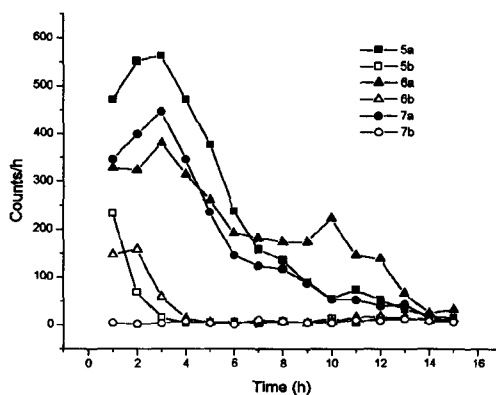


**Figure 9.5.** Effect of  $10^{-6}$  M infusion of N-0434 (**6a**) and (±)-8-Cl-N-0434 (**6b**) on DA release from the striatum. The compounds were dissolved in Ringer solution. The results are the mean  $\pm$  SEM of data obtained from 3-4 rats.

The testing of enantiomers of **6b** (Fig 9.6) showed that the antagonistic activity resided in the (R)-(+)-enantiomer. Infusion of  $10^{-6}$  M S-(-)-**6b** did not influence DA and DOPAC levels (results not shown). This is surprising because S-(-)-**6b** has a  $K_i$  of 65 nM at the  $D_2$  receptor and this must result in a biochemical effect.



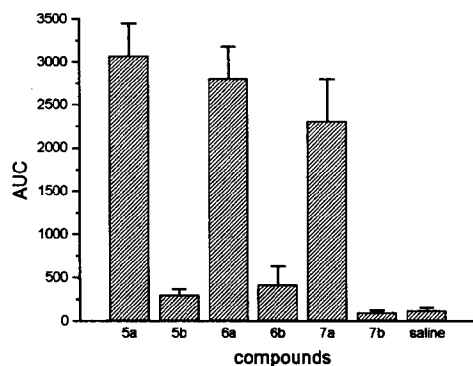
**Figure 9.6.** Effect of a  $10^{-6}$  M infusion of the enantiomers of 6b on DA release from the striatum. The compounds were dissolved in Ringer solution. The results are the mean  $\pm$  SEM of data obtained from 3-4 rats.



**Figure 9.7.** Contralateral turning behaviour of unilateral 6-OHDA-lesioned rats as a result of i.p. administration (on  $t = 0$ ) of  $5 \mu\text{mol/kg}$  of the test compounds. Each value (mean;  $n = 6$ ) are the sum of all contralateral turns during a time interval of 1 h. For clarity the SEM have been left out of this graph (see also figure 9.7).

The animal model most commonly used to investigate the central effects of DA agonists on motor function is turning behaviour in rats with unilateral 6-hydroxydopamine (6-OHDA) induced lesions of the nigrostriatal pathway. In this

model, both D<sub>1</sub>- and D<sub>2</sub>-agonists produce contralateral rotational behaviour, presumably by acting on striatal receptors rendered supersensitive by the lesion [15]. The resulting contralateral turning was used to measure the post-synaptic activity of the test compounds (Fig 9.7).

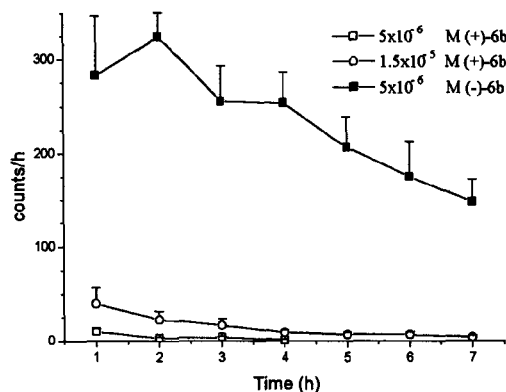


**Figure 9.8.** The area under the curve (AUC) from the time interval 1-15 h. of vehicle and the aminotetralins after i.p. administration. Error bars are  $\pm$  SEM (n = 6).

By calculating the area under the curve (AUC) of the time-effect curves, a significantly lower total activity was found for the halogenated compounds during the measured time interval 1-15 h (Fig.9.8). A surprising result was the complete inability of 8-chloro-N-0437 (**7b**) to induce contralateral turning, since this compound possessed, although lower than N-0437, a high affinity for the D<sub>2</sub> receptor and was active in the brain microdialysis model.

Although on a low level, racemic **6b** still induced contralateral turning. Separate administration of both enantiomers (Fig 9.9) revealed that only the (S)-(-)-enantiomer demonstrated some post-synaptic stimulation

An explanation of this decreased *in vivo* activity could be the metabolism. Introduction of a chlorine atom in the 8-position dramatically increases the glucuronidation speed in human and rat liver microsomes [16]. Through this first-pass effect, the concentration of the drug in the striatum will be presumably very low after i.p. administration. It is known that especially the thiophene derivatives are very sensitive to glucuronidation [17].



**Figure 9.9.** Contralateral turning behaviour of unilateral 6-OHDA-lesioned rats as a result of i.p. administration (on  $t = 0$ ) of 5 and 15  $\mu\text{mol/kg}$  of the enantiomers of **3b**. Each value (mean  $\pm$  SEM;  $n = 6$ ) is the summation of all contralateral turns during a time interval of 1 h.

In summary, the regioselective para chlorination of the hydroxylated aminotetralins is mild, fast and non-destructive towards the phenol and amine functions, and it is facile to perform, as compared to other routes described in the literature. The non-chlorinated compounds are DA agonists with selectivity for the  $D_2$  receptor. Substitution of chlorine in the 8-position resulted in compounds with a decreased affinity for the  $D_1$  and  $D_2$  receptor. The results of our study of the 8-chlorinated 5-hydroxyaminotetralins, in contrast to the 8-chlorinated ADTN derivatives, have not resulted in compounds with a higher affinity for the  $D_1$  receptor. Chlorination resulted in compounds with a greatly decreased agonistic activity in the test involving post-synaptic receptors. The effect of chlorination was most remarkable in the brain dialysis of racemic **6b**. In this model the activity of the compound is changed from a dopamine receptor agonist to an antagonist. Thus in contrast to most other 5-OH-aminotetralins, the effects of the (+)-enantiomer predominate over those of the (-)-enantiomer in this racemate. Most surprising was the dramatical shift from affinity for the DA receptor into affinity for the  $5\text{-HT}_{1A}$  receptor, indicating that small modifications within these molecules yield compounds which show affinities for another G-protein coupled receptor. The potential biochemical and physiological profile of some of these  $5\text{-HT}_{1A}$  receptor agents described should be further elucidated.

## 9.4 Experimental section

Melting points were determined with an Electrothermal digital melting point apparatus and are uncorrected. IR, NMR, and MS data were determined for all compounds and were evaluated as consistent with the indicated structures. These data are presented only where required for structural assignment. Infrared spectra were recorded on a Philips spectrophotometer, and only the important absorptions are given. The 300 MHz NMR spectra were recorded on a Varian XL-300 instrument. The chemical ionization (CI) mass spectra were obtained on a Finnegan 3300 system. For purity tests, TLC was performed on fluorescent silicagel plates developed in at least two different systems. For all the compounds, only one spot (visualized by UV light and I<sub>2</sub> vapor) was obtained. Specific rotations were determined at 20 °C on a Perkin Elmer 241 polarimeter. Concentrations (c) for specific rotations are reported in g/100 mL. Elemental analysis were performed in the Department of Chemistry, University of Groningen. Where elemental analysis are indicated results obtained were within 0.4% of theoretical values.

### **8-Chloro-5-hydroxy-2-(di-n-propylamino)tetralin (5b).**

2.15 g (7.1 mmol) 2,3,4,5,6,6-hexachloro-2,4-cyclohexadien-1-one was added to a stirred suspension of 1.55 g (5.5 mmol) of **5a.HCl** in 300 mL dry DME. Stirring was continued at room temperature for 2 h under nitrogen. The mixture was diluted with 200 mL ether and the solid was collected by filtration. The resulting hydrochloride was recrystallized from MeCN-Et<sub>2</sub>O to give 1.5 g (86.3%) of crystals, mp 204-205 °C. MS (CI with NH<sub>3</sub>) m/e 282 (M+1), 284 (M+3); NMR (DMSO-d<sub>6</sub>) <sup>13</sup>C-NMR (300 MHz, D<sub>2</sub>O) 153.80, 131.83, 126.70, 124.62, 122.49, 113.48, 58.91, 51.80, 51.46, 27.57, 22.99, 21.78, 17.89, 11.15; <sup>1</sup>H-NMR (300 MHz) δ 7.19 (d, 1H, J = 1.92 Hz), 6.83 (d, 1H, J = 1.92 Hz); Anal. (C<sub>16</sub>H<sub>24</sub>NC10.HCl), C, H, N, Cl.

### **(R)-(+)-8-Chloro-5-hydroxy-2-(di-n-propylamino)tetralin (R)-(+)-5b.**

The procedure described above for the synthesis of (±)-**5b** was followed for the enantiomers. All spectral data for (R)-(+)-**5b** were identical with those described above for (±)-**5b**. The resulting HCl salt was recrystallized from MeCN-Et<sub>2</sub>O to give in 83.1 % yield (R)-(+)-**5b**: mp 216-219 °C; [α] = + 75.9° (c = 0.1, MeOH).

### **(S)-(-)-8-Chloro-5-hydroxy-2-(di-n-propylamino)tetralin (S)-(-)-5b.**

The procedure described above for the synthesis of (±)-**5b** was followed for the enantiomers. All spectral data were for (S)-(-)-**5b** were identical with those described above for (±)-**5b**. The resulting HCl salt was recrystallized from MeCN-Et<sub>2</sub>O to give in

75 % yield (S)-(-)-**5b**: mp 218-220 °C;  $[\alpha] = -74.8^\circ$  (c = 0.1, MeOH).

**8-Chloro-5-hydroxy-2-(N-n-propyl-N-phenylethylamino)tetralin (6b).**

By using of the procedure described for **5b**, the phenol **6a** gave the chlorinated compound **5b.HCl** in a 86.3% yield after crystallization from ethanol-ether: mp 180-182 °C; IR (KBr) 3200  $\text{cm}^{-1}$  (OH), 2500 ( $\text{NH}^+$ ); MS (CI with  $\text{NH}_3$ ) m/e 344 (M+1), 346 (M+3); NMR ( $\text{DMSO-d}_6$ )  $\delta$  9.08 (s, 1H, OH), 6.6-7.4 (m, 7H, ArH), 0.9 (t, 3H,  $\text{CH}_3$ ). Anal. ( $\text{C}_{17}\text{H}_{26}\text{NClO.HCl}$ ) C, H, N.

**(R)-(+)-8-Chloro-5-hydroxy-2-(N-n-propyl-N-phenylethylamino)tetralin (+)-6b**

This enantiomer was obtained from (+)-**5a** according to the procedure described above for the preparation of the racemic **5b**. The resulting HCl salt was recrystallized from MeCN-Et<sub>2</sub>O to give in 75.1 % yield a compound having the same characteristics of racemic **6b** in TLC, IR, MS and NMR; mp 152-153 °C;  $[\alpha]_{589} = +57.9^\circ$  (c = 0.1, MeOH).

**(S)-(-)-8-chloro-5-hydroxy-2-(N-n-propyl-N-phenylethylamino)tetralin (-)-6b**

This enantiomer was obtained from (-)-**5a** according to the procedure described above for the preparation of the racemic **6b**. The resulting HCl salt was recrystallized from MeCN-Et<sub>2</sub>O to give in 72.8 % yield a compound having the same characteristics as (+)-**6b** and the racemic **6b** in TLC, IR, MS and NMR; mp 153-155 °C;  $[\alpha]_{589} = -57.1^\circ$  (c = 0.1, MeOH)

**8-Chloro-5-hydroxy-2-(N-n-propyl-N-2-thienylethylamino)tetralin (7b).**

The chlorinated compound **7b** was prepared from **7a** according to the method used for the preparation of **5b**. Recrystallization from 2-propanol gave pure **7b.HCl** (yield 82.9%): mp 185-186 °C; NMR ( $\text{D}_2\text{O}$ )  $\delta$  6.69 (d, J = 2.25 Hz, 1H, Ph-H), 7.12 (d, J = 2.25 Hz, 1H, Ph-H); MS (CI with  $\text{NH}_3$ ), m/e 350 (M+1), 352 (M+3). Anal. ( $\text{C}_{19}\text{H}_{24}\text{NClOS.HCl}$ ) C, H, N, Cl, S.

**(R)-(+)-8-Chloro-5-hydroxy-2-(N-n-propyl-N-2-thienylethylamino)tetralin (R)-(+)-7b**

This enantiomer was obtained from (-)-**7a** according to the procedure use described above for the preparation of the racemic **5b**. The resulting HCl salt was recrystallized from MeCN-Et<sub>2</sub>O to give in 68.4 % yield a compound having the same characteristics as (+)-**7b** and the racemic **7b** in TLC, IR, MS and NMR; mp 145-147 °C;  $[\alpha]_{589} = +54.3^\circ$  (c = 0.1, MeOH).

**(S)-(-)-8-Chloro-5-hydroxy-2-(N-n-propyl-N-2-thienylethylamino)tetralin** · (S)-(-)-**(7b)**. These enantiomer was obtained from (-)-**7a** according to the procedure use described above for the preparation of the racemic **5a**. The resulting HCl salt was recrystallized from MeCN-Et<sub>2</sub>O to give in 65.2 % yield a compound having the same characteristics as (+)-**7b** and the racemic **7b** in TLC, IR, MS and NMR; mp 148-150 °C;  $[\alpha]_{589} = -54.7^\circ$  (c = 0.1, MeOH).

## Pharmacology

### Displacement of the Specific Binding of [<sup>3</sup>H]-Spiperone (D<sub>2</sub>) in Calf Striatal Membranes.

This assay which was performed with homogenized and washed membrane preparations from calf striatal tissue, was carried out as described previously [19]. In each experiment the amount of [<sup>3</sup>H]-spiperone bound was determined in the absence (total) and presence (nonspecific) of 10<sup>-6</sup> M (+)-butaclamol, the difference yielding specific [<sup>3</sup>H]-spiperone binding. The ability to compete with [<sup>3</sup>H]-spiperone was tested over a concentration range of 10<sup>-11</sup>-10<sup>-4</sup> M.

### Displacement of the Specific Binding of [<sup>3</sup>H]-SCH 23390 (D<sub>1</sub>) in Calf Striatal Membranes.

The binding of [<sup>3</sup>H]-SCH 23390 was determined using membranes as prepared above [19]. In each experiment the amount [[<sup>3</sup>H]-SCH 23390 bound was determined in the absence (nonspecific) and presence (nonspecific) 10<sup>-6</sup> M cis-(Z)-flupentixol, the difference yielding the specific [<sup>3</sup>H]-SCH 23390 binding. The ability to compete with [<sup>3</sup>H]-SCH 23390 was tested over a concentration range of 10<sup>-10</sup>-10<sup>-4</sup> M.

### Displacement of the Specific Binding of [<sup>3</sup>H]-8-OH-DPAT (5-HT<sub>1A</sub>) in Rat Cortical and Hippocampal Membranes.

The binding of [<sup>3</sup>H]-8-OH-DPAT was determined using membranes as prepared according [24]. In each experiment the amount [<sup>3</sup>H]-8-OH-DPAT bound was determined in the absence (nonspecific) and presence (nonspecific) 10<sup>-6</sup> M 5-HT.HCl, the difference yielding the specific [<sup>3</sup>H]-8-OH-DPAT binding.

### Brain dialysis and Surgery.

On-line brain microdialysis in freely moving animals has been described previously [20, 21]. In brief: rats were anaesthetized with chloral hydrate and placed in a stereotaxic frame. Holes were made in the temporal bones to allow the transstriatal canula to be inserted [17]. The dialysis device was fixed on the head and the rat was given one day

to recover. The experiments were done 24-48 h after implantation of the cannula. The inlet of the dialysis tube was connected to a Braun perfusor VI and the outlet to the injection valve of the HPLC apparatus by means of polyethylene tubing (inner diameter 0.28 mm). The sample valve was held in the load position for 20 min and was switched automatically to the inject position for 15 s. The striata were perfused with a Ringer solution (mM: 147 NaCl, 4 KCl, 3.4 CaCl<sub>2</sub> and 0.02 ascorbic acid) at 6-7 µl/min. The *in vitro* recovery of the probes for DA was approximately 12-15%. DA was assayed by HPLC with electrochemical detection.

#### **Turning behaviour and surgery.**

Rats were anaesthetized with chloral hydrate (400 mg/kg) and placed in a stereotaxic instrument (Kopf). After removing skin and tissue a small hole was drilled into the skull and 6-OHDA (8 µg dissolved in 1 µl 0.9% NaCl containing 3 mg/ml of ascorbic acid) was injected in the left medial forebrain bundle at coordinates A 4.8, L2.2 and V2.4 [22] to destroy the dopaminergic input to the striatum. Successfully denervated animals were selected by testing one week after surgery the response to 2 µmol/kg apomorphine for their frequency of turning. Rotational behaviour was automatically recorded in rotameters according to the method of Ungerstedt and Arbuthnott [15]. Only animals showing more than 100 contralateral turnings per half hour were selected for the experiments. Registration of the turning behaviour started 10 min after injection. The time of every contralateral and ipsilateral turn of a maximum of 5 rats was registered and counted on a microcomputer. For comparison all doses were equimolar i.e. µmol/kg.

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