The medicinal chemistry of arylpiperazines with potential antidepressant efficacy

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6 RESOLUTION AND *IN VITRO* AND *IN VIVO* PHARMACOLOGICAL EVALUATION OF THE ENANTIOMERS OF 6-MEO-MIANSERIN *

ABSTRACT

The optically pure enantiomers of 6-methoxymianserin (**6.3**) were obtained by means of chiral HPLC over a cellulose-based OD column. Both enantiomers showed high affinity for the 5-HT_{2A} and 5-HT_{2C} receptor subtypes and no affinity for the 5-HT_3 receptor or NA and 5-HT reuptake sites. The (*S*)-(+)-enantiomer showed high selectivity for the 5-HT_2 receptors. The (*R*)-(-)-enantiomer, however, also showed good affinity for the D_2 receptor and a favorable 'Meltzer ratio' (5-HT_2/D_2) over the atypical neuroleptic clozapine (**6.6**). The absolute configuration was determined indirectly, via *O*-demethylation of (-)-6-methoxymianserin, triflation and subsequent reduction to (*R*)-(-)-mianserin (**6.1**). *In vivo*, in a microdialysis study in the ventral hippocampus of rats, 2.5 mg/kg sc. of either (-)- or (+)-6-methoxy-mianserin caused a rapid and long-lasting increase in the levels of NA, DOPAC (not significant for the (+)-enantiomer), 5-HT (transient) and 5-HIAA (significant for the (-)-enantiomer).

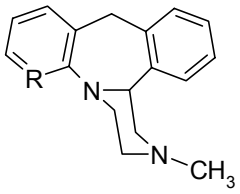
* A modified version of this chapter by Mensonides-Harsema, M.M.; Cremers, T.I.F.H.; Moltzen, E.K.; Arnt, J.; Wikström, H.V., has been accepted by the *J. Med. Chem*.

6.1 INTRODUCTION

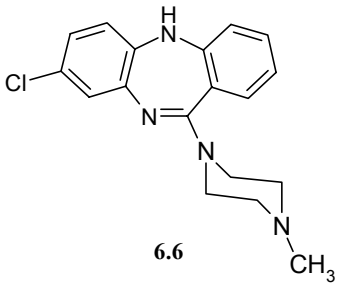
In Chapter 5, the synthesis of several analogues of mianserin (Tolvon®, **6.1**) and mirtazapine (6-azamianserin, Remeron®, **6.2**) is described. The lead compound of this series, 6-methoxymianserin (**6.3**) has been evaluated in a microdialysis study. *In vivo*, 6-methoxymianserin (5 mg/kg sc.) elicits a long-lasting increase of noradrenaline (NA) and dihydroxyphenylacetic acid (DOPAC) levels and a transient increase in the serotonin (5-HT) release. Similar effects on NA, DOPAC and 5-HT release have been reported for mirtazapine.[1] Administration of mianserin, however, only elevates the NA and DOPAC release, but does not effect the 5-HT levels.[1]

The α_2-blocking effect and the affinities for the 5-HT_{2A} and 5-HT_{2C} receptors of mianserin reside in the (*S*)-(+)-enantiomer.[2, 3] The (*R*)-(-)-enantiomer shows selectivity for the 5-HT_3 receptor. For mirtazapine, the same stereoselectivity at the α_2-adrenoceptors is found. The stereoselectivity for the serotonergic receptors, however, is considerably less profound.[4, 5] Neither mianserin nor mirtazapine show stereoselectivity for the histamine H_1 receptor, and both compounds have negligible affinity for the dopamine D_2 receptors.[6, 7]

In this chapter the direct enantiomeric separation of 6-methoxymianserin, 6-hydroxymianserin (**6.4**) and 6-trifluoromethylsulfonyloxymianserin (**6.5**) by means of chiral HPLC is reported. The absolute configuration of the (+)- and (-)-enantiomers of 6-methoxymianserin is determined and the *in vitro* binding profile of both enantiomers is assessed. The effects of these two enantiomers (2.5 mg/kg *sc.*) in the rat ventral hippocampus on the release of NA, DOPAC, 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were measured. Furthermore, an attempt is made to directly synthesize the different enantiomers starting from commercially available (*S*)-(+)- or (*R*)-(-)-styrene oxide.



6.1, R = CH
6.2, R = N
6.3, R = $COCH_3$



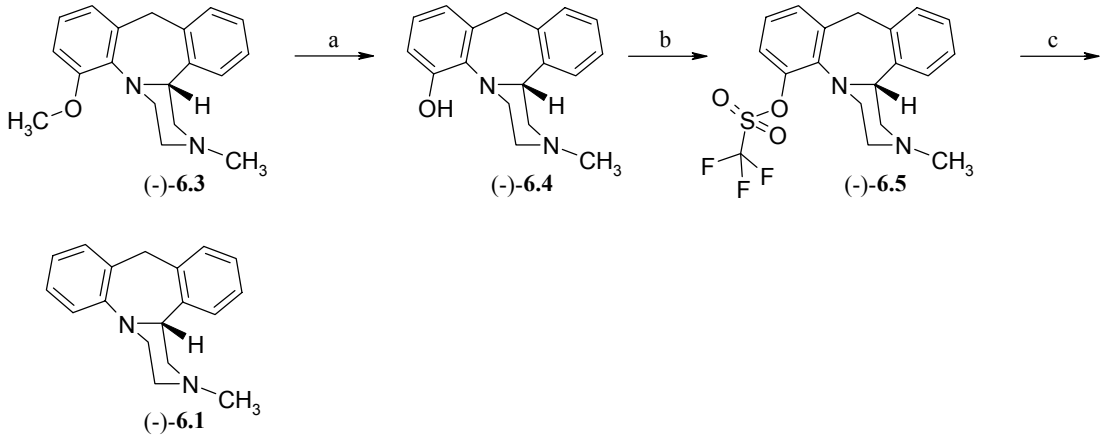
6.6

Chart 6.1. Chemical structure of mianserin (**6.1**), mirtazapine (**6.2**), 6-methoxymianserin (**6.3**) and clozapine (**6.6**).

6.2 CHEMISTRY

SYNTHESIS OF MIANSERIN FROM (-)-6-METHOXYMIANSERIN .

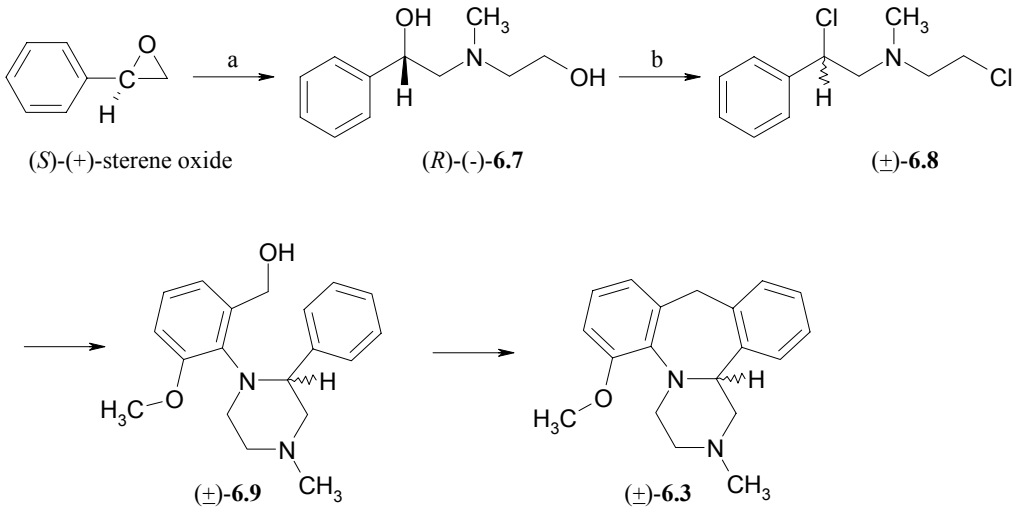
O-demethylation of the (-)-enantiomer 6-methoxymianserin was achieved upon treatment with 6 equivalents of $AlCl_3$ in freshly distilled, refluxing benzene, providing (-)-6-hydroxymianserin (**6.4**). Triflation of (-)-6-hydroxymianserin was accomplished with trifluoromethanesulfonyl anhydride in CH_2Cl_2 in the presence of triethyl amine, yielding (-)-6-trifluoromethylsulfonyloxymianserin (**6.5**). The triflate substituent was removed by reduction using Pd(OAc) in the presence of PPh_3 in refluxing CH_2Cl_2, yielding (*R*)-(-)-mianserin (Scheme 6.1).[8]



SCHEME 6.1 Reagents and conditions: (a) $AlCl_3$, benzene, reflux; (b) trifluoromethane-sulfonyl anhydride, CH_2Cl_2, TEA, -78 ^oC to room temperature; (c) $Pd(OAc)_2$, PPh_3, TEA, HCOOH, MeOH, reflux.

SYNTHESIS OF 6-METHOXYMIANSERIN FROM (*R*)-STYRENE OXIDE.

2-Methylaminoethanol and (*R*)-styrene oxide were reacted at 130 ^oC, yielding (*R*)-β-hydroxy-*N*-methyl-*N*-hydroxyethylphenyl ethylamine (**6.7**), which was converted to the chlorinated phenylethyl amine **6.8** with thionyl chloride in refluxing chloroform. This substitution reaction, however, is not stereoselective. The optical rotation of the intermediates **6.8** and **6.9** and the final product 6-methoxymianserin is zero (Scheme 6.2). Replacement of the solvent CH_2Cl_2 by pyridine also leads to the loss of optical activity of **6.8**, as is the case when the reaction is carried out with $POCl_3$ in pyridine/pentane.[9 - 12]



SCHEME 6.2 Reagents and conditions: (a) 2-methylaminoethanol, 130 °C; (b) $SOCl_2$, CH_2Cl_2, 0 °C to reflux or $SOCl_2$, pyridine, 0 °C to reflux or $POCl_3$, pyridine, pentane, -20 °C to reflux.

6.3 RESOLUTION AND OPTICAL ROTATION

The enantiomers of mianserin, 6-methoxymianserin, 6-hydroxymianserin and 6-trifluoromethylsulfonoxymianserin were separated over a semi-preparative, chiral (cellulose-based) stationary phase The chromatographic data were calculated as follows: the selectivity $\alpha = k_2'/k_1'$, the capacity factor of the least and of the most retained enantiomers (k_1' and k_2', respectively) with $k_1' = (t_1-t_0)/t_0$ and the dead time $t_0 = t_{solvent\ front} - t_{injection}$ (Table 6.1 and Figure 6.1).[13] The optical rotation of the individual enantiomers of compounds 6-methoxymianserin and 6-trifluoromethyl-sulfonoxymianserin were determined according to the following equation:

$$[\alpha]_D^{20} = 100.\alpha / l.c,$$

where α is the rotation measured, l is the length of the cuvette (in dm) and c is the concentration of the individual enantiomers (in MeOH). The enantiomeric purity of the respective enantiomers were determined from the HPLC data of their combined fractions.

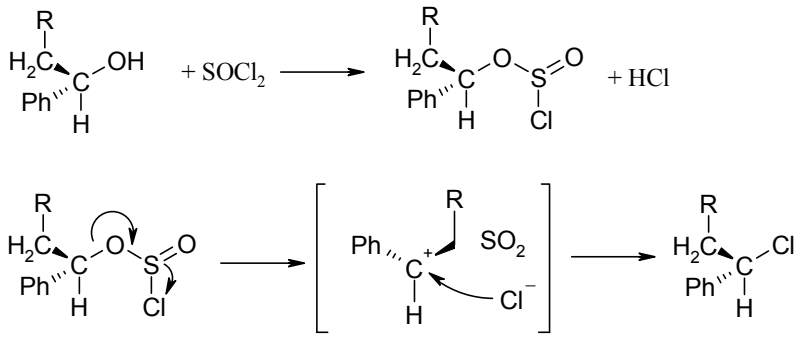
6.4 PHARMACOLOGY

The ability of 6-methoxymianserin, 6-trifluoromethylsulfonyloxymianserin and their enantiomers to displace the respective radioligands at the dopamine D_2, D_3, D_4, serotonin 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_3, noradrenaline α_1, α_2, and histamine H_1 and H_2 receptors and at the 5-HT and NA re-uptake sites were determined (Table 6.3).[14-23] The extracellular levels of NA, DOPAC, 5-HT and 5-HIAA in the ventral hippocampus were measured by *in vivo* microdialysis after

administration of 5 mg/kg *sc.* of the racemate of 6-methoxymianserin or 2.5 mg/kg *sc.* of either of the enantiomers, respectively. Levels were measured every 15 min. for a period of 2.5 h after administration of the test compound. Before administration of test compounds, four consecutive samples are collected (base-line). For clarity, the deviations in the extracellular levels are presented as area under the curves (figure 6.2).[24]

6.5 RESULTS AND DISCUSSION

The stereochemical course of conversion to haloalkanes is normally inversion for the S_N2 reaction of the secondary alcohols (inter molecular). However, when optically active 2-octanol is reacted with thionyl chloride in ether, the configuration is retained. When the solvent is pyridine, the stereochemical pathway is conversion. The initial intermediate formed in the thionyl chloride reaction is an alkyl chlorosulfite, to create a better leaving group. In ether, most of the HCl generated during chlorosulfite formation is not present as dissociated ions. Chloride for the substitution step comes from decomposition of the chlorosulfite. As the sulfur dioxide departs, the chloride is oriented at the same side of the reacting carbon atom. In the nonpolar ether, charge separation is unfavorable and the chloride and carbocation are attracted to each other as an ion pair, that is, a positive and negative ion held close to each other. Collapse of the ion pair to product takes place with retention of configuration. This uncommon reaction in which part of the leaving group attacks the substrate, detaching itself from the rest of the leaving group in the process, is termed substitution nucleophilic internal (S_Ni). The phenomenon is similar to the neighboring-group mechanism, which consists essentially of two S_N2 substitutions. In the first step the neighboring group acts as a nucleophile, causing an inversion. In the second step an external nucleophile displaces the neighboring group by a backside attack, so the net result is retention of configuration (anchimeric assistance). When pyridine is added to the reaction, $ROSON^+C_5H_5$ supposedly is formed before anything further can take place. The Cl^- freed in this process now attacks from the rear, which leads to inversion of the configuration (Scheme 6.3).



SCHEME 6.3 Intra-molecular substitution via the S_Ni mechanism.[9, 10]

However, when the carbon at the β-position of the secondary alcohol is saturated, as is the case for the phenyl substituent, again both reaction mechanisms take place. Thus, it is explained that the enantioselective synthesis of 6-methoxymianserin from optically pure styrene oxide, under the conditions tested, proved to be unsuccessful.

Table 6.1. The selectivity (α) and capacity factors (k') of mianserin (**6.1**), 6-methoxy-mianserin (**6.3**), 6-hydroxymianserin (**6.4**) and 6-trifluoromethyl-sulfonyloxymianserin (**6.5**).

Test compound	α	k_1'	$[\alpha]_D^{20}$	EE (%)	k_2'	$[\alpha]_D^{20}$	EE (%)
6.1	1.38	0.72			1.00		
6.3	1.63	0.94	-403.0	>99.5	1.53	+407.6	>99.7
6.4	1.49	2.31			3.44		
6.5	3.11	0.37	-184.4	>99.6	1.15	+181.0	>99.9

Under the conditions chosen,[13] reasonable quantities, 50 mg/mL, of the racemic mixture of 6-methoxymianserin could be base-line separated in a short time (Figure 6.1A). Both the selectivity α and the capacity factor of the second eluted enantiomer k_2' are improved, as compared to mianserin. 6-Hydroxymianserin exhibited a decrease in selectivity and considerable tailing, which is due to the increased interactions with the supporting silica gel. In contrast, 6-trifluoromethylsulfonyloxy-mianserin showed a strongly improved separation on the cellulose selector compared to 6-methoxymianserin. Injection of 140 mg/mL of the racemic compound (14 mg/injection) still resulted in baseline separation on the semi-preparative column (Figure 6.1B). For each compound the (-)-enantiomer eluted first. The optical rotation values of the (-)- and (+)-enantiomers and the enantiomeric purity (ee) of the different enantiomers of 6-methoxymianserin and 6-trifluoromethylsulfonoxylmianserin, which were determined with HPLC for the combined fractions collected of the different enantiomers.

Because no suitable crystals for X-ray crystallography could be obtained, the absolute configuration was determined indirectly, via the synthesis of mianserin from 6-methoxymianserin. *O*-Demethylation of 6-methoxymianserin and subsequent triflation and reduction to mianserin, proved that the (-)-enantiomer of 6-methoxy-mianserin has the (*R*)-configuration. The product of the synthesis presented in Scheme 6.1 elutes at the same time as does the (*R*)-(-)-enantiomer of mianserin (Figure 6.3).

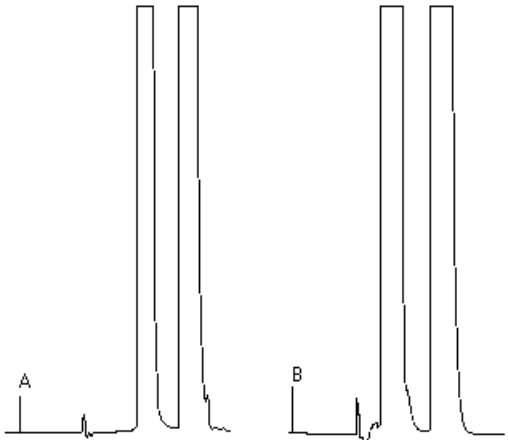


Figure 6.1. Semi-preparative separation on Ciralcel OD (250 x 10 mm I.D.) of racemic 6-methoxymianserin (A; 5 mg/injection) and 6-trifluoromethyl-sulfonoxylmianserin (B; 14 mg/injection)

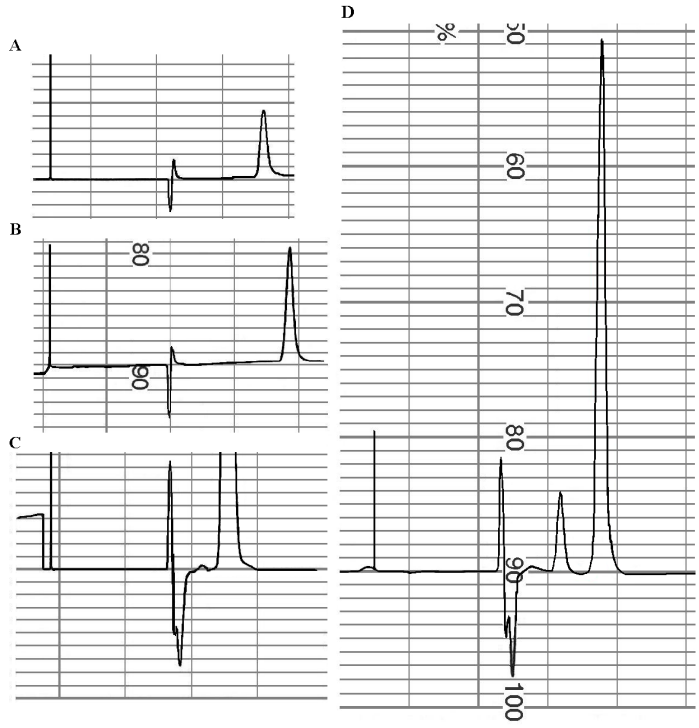


Figure 6.3. Elution of (*R*)-(-)-mianserin (A), (*S*)-(+)-mianserin (B), (-)-6-trifluoromethylsulfonyloxymianserin (C), reaction mixture after reduction of (-)-6-trifluoromethylsulfonoxylmianserin to mianserin (D) on Ciralcel OD (250 x 10 mm I.D.).

Neither 6-trifluormethanesulfonyloxymianserin nor its enantiomers showed submicromolar affinity at any of the receptors tested (data not shown). The binding study of 6-methoxymianserin showed that it has high affinity for the 5-HT_{2A} and 5-HT_{2C} receptors, which is found in both enantiomers, and no affinity for the 5-HT_3 receptor. From literature it is known that both mianserin and mirtazapine have high affinity for the 5-HT_3 receptor.[6, 7] It is therefore remarkable, that

6-methoxymianserin has no affinity for this receptor subtype. The selectivity of the (-)- and (+)- enantiomers of 6-methoxymianserin for both adrenergic receptors, is ca. two times for the α_2 over the α_1 adrenoceptor. 6-Methoxymianserin shows little stereoselectivity for the adrenoceptors and no stereoselectivity for the histamine H_1 receptor. Like mirtazapine, 6-methoxy-mianserin is devoid of affinity for the NA reuptake site.

Table 6.2. *In vitro* binding profiles of mianserin (**6.1**), mirtazapine (**6.2**), and (±)-, (-)- and (+)-6-methoxymianserin (**6.3**), (pK_i's), and clozapine (**6.4**) (IC_{50}'s) in nM.*

Receptor subtype	**Test compounds**					
	6.1	**6.2**	**6.3**	(-)-**6.3**	(+)-**6.3**	**6.6** [a]
D_2	6.0	5.8	7.6	7.7	6.3	260
D_3	nt [b]	< 7.7	6.6	7.0	5.4	450
D_4	nt	< 7.6	7.0	7.3	6.8	52
5-HT_{1A}	6.3	< 7.7	6.5	6.6	6.1	nt
5-HT_{1D}	nt	nt	nt	7.1	5.8	nt
5-HT_{2A}	8.8	8.7	9.2	9.1	9.3	12
5-HT_{2C}	8.9	8.2	9.7	9.4	9.8	11
5-HT_3	7.1	nt	5.5	5.8	<5.3	nt
α_1	7.6	6.0	7.1	7.1	6.7	9.2
α_2	nt	7.3	nt	7.4	7.1	nt
NA uptake[c]	nt	260	1800	1200	2800	nt
5-HT uptake[c]	2900	>100	2900	1600	>10,000	nt
H_1	8.3	8.3	nt	7.8	7.8	23
H_2	nt	nt	7.3	7.4	7.2	nt

[a] data from reference 25; [b] nt = not tested; [c] IC_{50} values

Besides some affinity for the histamine receptor, the (*S*)-(+)-enantiomer of 6-methoxy-mianserin shows high selectivity for the 5-HT_2 receptors (> 100 fold over the H_1 receptor). The (*R*)-(-)-enantiomer of 6-methoxy-mianserin, on the other hand, also showed reasonable affinity for the 5-HT_{1B} receptor subtype and the dopamine receptors. It should be noted, that the 'Meltzer ratio' (5-HT_{2A}/D_2 affinity), a marker which is used as to classify atypical neuroleptics like clozapine, is highly favorable.[25, 26] Neuroleptics, which are used to treat schizophrenia are generally potent dopamine receptor antagonists, especially at the D_2 receptor. The typical antipsychotics, however, have a high propensity to induce acute or chronic motor disturbances (extrapyramidal side effects, EPS). The atypical nature of clozapine may be accounted for with the Meltzer ratio (46×10^{-3}). The fact that the affinity for both the 5-HT_2 and the D_2 receptor of the (*R*)-(-)-enantiomer of 6-methoxymianserin is about a factor 10 higher, plus an improvement in the ratio by a factor 2 (21×10^{-3}), indicate that this enantiomer might be promising as an atypical antipsychotic. It would, however, be

* The receptorbinding assays were run in the laboratories of Lundbeck A/S in Copenhagen, Denmark

necessary to assess whether (*R*)-(-)-6-methoxymianserin acts as a D_2 antagonist in a functional assay, *e.g.* by performing a microdialysis study in the rat striatum.

The microdialysis study in the ventral hippocampus of a freely moving rat showed that, administration of 6-methoxymianserin (5.0 mg/kg *sc*.) caused an increase in NA levels up to 100% and in DOPAC levels up to 60% above base-line. Furthermore, it induced a concurrent, transient increase of 5-HT levels up to 100% above base-line, with stabilization at 40% to 50% during the further course of the experiment. Administration of (-)- or (+)-6-methoxymianserin (2.5 mg/kg *sc*.) also caused an increase in NA levels up to 100%. The DOPAC levels were elevated up to 60% and 30% (not significant) above base-line for (-)- and (+)-6-methoxymianserin, respectively. Both enantiomers induced a transient, concurrent increase of the 5-HT release up to 100% above base-line. The 5-HIAA release was elevated up to 20% after the administration of the racemic mixture or either of the enantiomers. The increase, however, was only significant for the (-)-enantiomer. The area under the curves of NA, DOPAC, 5-HT and 5-HIAA release of 6-methoxymianserin and its enantiomers are presented in Figure 6.2.

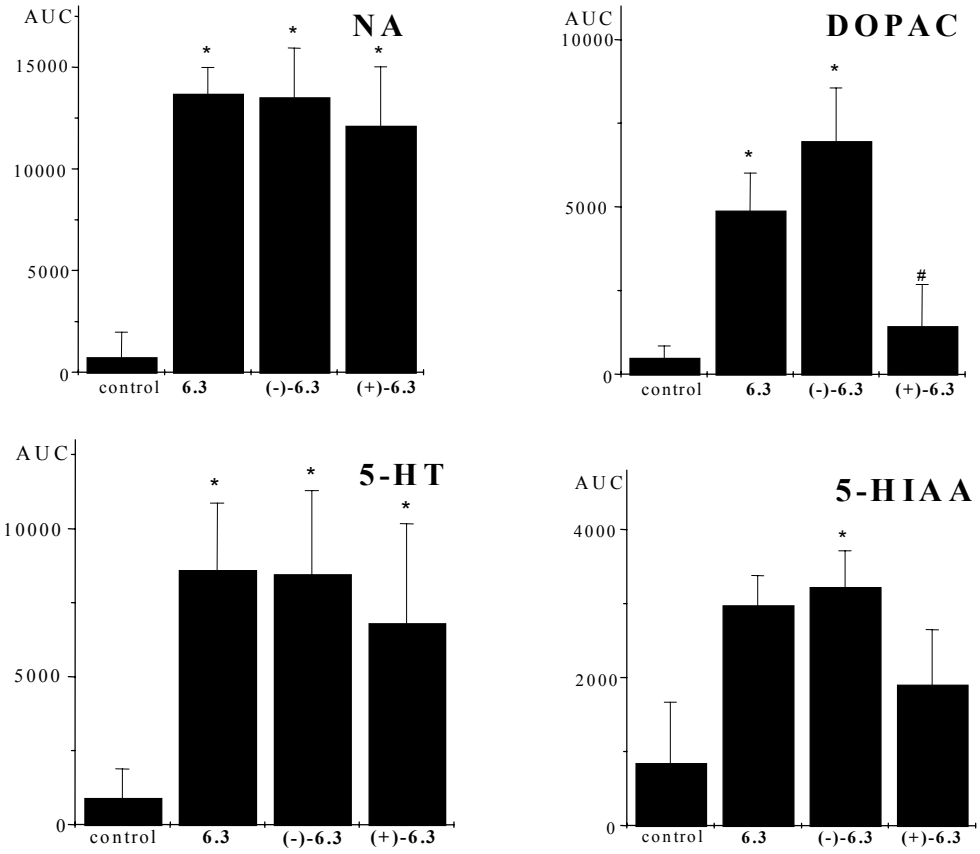


Figure 6.2. Effects of 6-methoxy-mianserin (5 mg/kg, n = 4) and its enantiomers (2.5 mg/kg, n = 6) on NA and 5-HT transmission. Results are expressed as AUC and refer to cumulative percentage above base-line. Statistics: Mann-Whitney Rank Sum: * $p < 0.05$ as compared to control, # $p < 0.05$ as compared to (*R*)-(-)- 6-methoxy-mianserin.

The *in vivo* microdialysis study with the enantiomers of 6-methoxymianserin clearly shows, that the (*R*)-(-)-enantiomer is more potent than the (*S*)-(+)-enantiomer. This is especially profound for the release of DOPAC. It is difficult to account for the difference in potency from the *in vitro* binding results. To resolve the question whether the increased DOPAC levels originate from the increased noradrenergic or dopaminergic activity, another NA metabolite, 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), could be used as a marker for noradrenergic activity in the ventral hippocampus in a microdialysis study.[27 - 30] If the increase in DOPAC in the ventral hippocampus is due to increased dopaminergic activity via D_2 receptors, then this would raise the question why mianserin and mirtazapine, both devoid of D_2 affinity, are able to induce significant elevation of DOPAC levels after their administration.

To summarize, the enantiomers of 6-methoxy-, 6-hydroxy- and 6-trifluromethyl-sulfonyloxymianserin have been directly separated by means of chiral HPLC. Using conditions described before, it was found that both the selectivity α and the capacity factor of the second eluted enantiomer are the most favorable for 6-trifluoromethyl-sulfonyloxymianserin.[13] The absolute configuration of the (-)-enantiomer was determined indirectly via the synthesis of (-)-mianserin from the (-)-enantiomer of 6-methoxymianserin and was found to also be of the (*R*)-configuration. *In vitro* binding studies showed high affinity for the 5-HT_2, reasonable affinity for both adrenoceptors (selectivity ca. 2.5) and the histamine receptors and no affinity for the 5-HT_3 receptor or NA re-uptake sites. Furthermore, it was established that the (*S*)-(+)-enantiomer shows high selectivity for the 5-HT_2 receptors, while the (*R*)-(-)-enantiomer also showed good affinity for the dopamine receptors and a favorable 'Meltzer ratio' (D_2/5-HT_{2A}).[25, 26] *In vivo* functional studies are necessary to asses whether (*R*)-(-)-6-methoxymianserin is a D_2 agonist or antagonist. The microdialysis study showed a similar profile for both the racemic mixture and the different enantiomers of 6-methoxy-mianserin, with the (*R*)-(-)-enantiomer being the most potent. The fact that the (*S*)-(+)-enantiomer is not able to significantly increase the DOPAC release throws suspicion on the assumption that this metabolite stems from noradrenergic activation. It would therefore, be recommendable to use a different metabolite marker for NA activity, *e.g.* 3-methoxy-4-hydroxyphenylethylene glycol (MHPG).[27 - 30]

The fact that both enantiomers contribute to an increase in NA and 5-HT, encourages further research in the possible antidepressant properties of 6-methoxy-mianserin. Furthermore, the (*R*)-(-)-enantiomer of 6-methoxymianserin might even have potential as an atypical antipsychotic.

6.6 EXPERIMENTAL

CHEMISTRY For general remarks see Chapter 2, Section 2.5. For details of the synthesis of compounds **6.3** – **6.5, 6.7 - 6.9**, see Chapter 5, Section 5.6.

Mianserin (6.1) To a suspension of **6.5** (50 mg, 0.121 mmol), triphenylphosphine (10 mol%), triethyl amine (10 mol%) and $Pd(OAc)_2$ (10 mol%) in MeOH (5 mL) is refluxed for 48 h. The reaction mixture is filtrated and the solvent removed *in vacuo*. The residue is purified by flash chromatography (CH_2Cl_2 with 3% ethanol), yielding 10 mg (31 %) of **6.1** as a colorless oil. MS (EIPI) *m/z* 264 (M^+).

HPLC The semi-preparative LC system consisted of an ISCO 2300 HPLC pump, equipped with a Rheodyne injection valve and a 100 µL injection loop. For the detection an ISCO V4 Absorbance detector with 5 µL flowcell (pathway 5 mm) and a Kipp and Zonen BD 40 two channel recorder were used (Beun de Ronde Amsterdam, NL and Delft, NL).
The semi-preparative chiral stationary phase was a Chiralcel OD column (Daicel Chemical Industry, Ltd., 25 cm x 1.0 cm I.D. 10 µm particle size silica gel). The mobile phase consisted of 95 % *n*-hexane (HPLC grade, Labscan, Dublin, Ireland) and 5 % ethanol (LiChrosolv grade, Merck, Darmstadt, Germany). To the mobile phase was added 0.1 % triethyl amine (analytical grade, Janssen Pharmaceuticals, Beerse, Belgium), to prevent peak tailing. The flow-rate was 2.5 mL/min and the detector was set at λ 250 nm. The samples were dissolved in ethanol. Analyses were carried out at room temperature.

BIOLOGICAL ASSAYS

ANIMALS. For the microdialysis experiments male Wistar rats (280-350 g) were used. The animals were housed in groups (four rats per cage) in a temperature- and humidity-controlled colony room (20 $\pm$ 2 °C; 50-60 %) on a natural day-night cycle (light period 6:30 - 18:30 h) until surgery. Food and water were available *ad libitum* at all times. Testing was done between 10:00 h and 18:00 h during the light phase of the day-night cycle. Procedures were conducted in accordance with guidelines published in the NIH Guide for the Care and Use of Laboratory Animals and protocols were approved by the Groningen University Institutional Animal Care and Use Committee.

RECEPTOR BINDING. [14 - 23] Affinities of compounds were determined using competition binding assays to determine IC_{50} values at the various receptors. The affinity for the D_2 receptor subtype was measured as the displacement of [^{3}H]-spiperone (rat corpus striatum), for the D_3 receptor subtype as the displacement of [^{3}H]-spiperone at human cloned D_3 receptors expressed in CHO-cells and for the D_4 receptor subtype as the displacement of [^{3}H]YM-09151-2 at human cloned $D_{4.2}$ receptors expressed in CHO-cells. The affinity for the 5-HT_{1A} receptor subtype was measured as the displacement of [^{3}H]-5-CT from human cloned 5-HT_{1A} receptors expressed in HeLa-cells. The affinity for the 5-HT_{1B} receptor subtype was determined indirectly, as the displacement of [^{3}H]-5-CT from human 5-HT_{1A} receptors in synaptasomes. The cloned receptor was expressed in HeLa-cells. The affinity for the 5-HT_{2A} receptor subtype was determined from the displacement of [^{3}H]-ketanserin (rat cortex) and for the 5-HT_{2C} receptors as the displacement of [^{3}H]-mesulergine from rat cloned 5-HT_{2C} receptors expressed in NIH/3T3 cells. Affinity for the 5-HT_3 receptor subtype was determined from the displacement of [^{3}H]-LY-278584 from (rat brain). The affinity for the different adrenoceptor subtypes was determined from the displacement of [^{3}H]-prazosin (α_1; rat brain tissue) and [^{3}H]-RX-821002 (α_2; rat brain tissue). Inhibition of 5-HT and NA uptake into rat brain synaptosomes was determined as previously described by K.P. Bøgesø (NA uptake 2.68; 5-HT uptake 2.04). The affinity for the H_1 receptor subtype was measured from the displacement of [^{3}H]-pyrilamine in guinea pig lung tissue and for the H_2 receptor subtype from the displacement of [^{3}H]-APT from guinea pig striatum.

MICRODIALYSIS. [30] For procedures see Chapter 5, Section 5.6. Data are presented as area under the curve. Statistical analysis was performed with Mann-Whitney Rank Sum test. The significance level was set at $p<0.05$.

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