2j, 103234-05-5; 2k, 133794-96-4; 3, 103233-91-6; 3·HCl, 103247-16-1; 4, 103247-26-3; 5, 103233-92-7; 5·HCl, 103247-17-2; 6, 103247-07-0; 7, 103247-08-1; 7·HCl, 133794-97-5; 8, 103233-89-2; 8·2HCl, 103247-09-2; 9, 103233-90-5; 9·HCl, 103247-11-6; 10, 103247-12-7; 11, 103247-10-5; 12, 133794-98-6; 12·HCl, 133794-99-7; 13, 103247-37-6; 14, 133795-00-3; 15, 103247-28-5; 16, 103247-25-2; 17, 103247-48-9; 18, 103233-65-4; 19, 103247-33-2; 20, 103247-47-8; 21, 103247-57-0; 22, 103247-50-3; 23, 103233-66-5; 24, 103247-53-6; 25, 103247-41-2; 26, 103247-54-7; 27, 103247-43-4; 28, 103247-29-6; 29, 103247-15-0; 30, 103247-55-8; 31, 103247-56-9; 32, 103247-44-5; 33, 103247-49-0; 34, 103247-45-6; 35, 103247-46-7; 36, 103247-14-9; 37, 103247-38-7; 38, 133795-01-4; 39, 103247-13-8; 40, 103247-06-9; 41, 133795-02-5; 42, 103247-22-9; 43, 133795-03-6; 44, 93747-96-7; 45, 103233-69-8; 45·$C_4H_4O_4$, 103233-70-1; 46, 103233-67-6; 46·$^1/_2C_4H_4O_4$, 133795-04-7; 47, 133795-05-8; 48, 133795-06-9; 49, 103233-56-3; 50, 103233-57-4; 51, 103233-58-5; 52, 103233-60-9; 53, 103233-61-0; (L)-54, 103233-62-1; 55, 103233-63-2; 56a, 103233-55-2; 56b, 133795-07-0; chloroacetyl chloride, 79-04-9; 3-chloropropionyl chloride, 625-36-5; 1-(3-chlorophenyl)piperazine, 6640-24-0; 1-(3-methoxyphenyl)piperazine, 16015-71-7; 1-(2,3-dimethylphenyl)piperazine, 1013-22-5; 1-phenylpiperazine, 92-54-6; 1-(3-methylphenyl)piperazine, 41186-03-2; 1-(2,3-dichlorophenyl)piperazine, 41202-77-1; 1-(3,5-dichlorophenyl)piperazine, 55827-50-4; 1-(2-methoxyphenyl)piperazine, 35386-24-4; 1-(4-methoxyphenyl)piperazine, 38212-30-5; 1-(3,4-dimethoxyphenyl)piperazine, 16015-73-9; 1-(2-ethoxyphenyl)piperazine, 13339-01-0; 1-(2-chlorophenyl)piperazine, 39512-50-0; 1-(2-fluorophenyl)piperazine, 1011-15-0; 1-(3-fluorophenyl)piperazine, 3801-89-6; 1-(4-fluorophenyl)piperazine, 2252-63-3; 1-(3-bromophenyl)piperazine, 31197-30-5; 1-(3-trifluoromethylphenyl)-piperazine, 15532-75-9; 1-(4-nitrophenyl)piperazine, 6269-89-2; 1-(2,5-dichlorophenyl)piperazine, 1013-27-0; 1-(3,4-dichlorophenyl)piperazine, 57260-67-0; 1-[(3-chloro-4-methyl)phenyl]-piperazine, 3606-03-9; 1-[(5-chloro-2-methyl)phenyl]piperazine, 76835-20-6; 1-(2,4,6-trimethylphenyl)piperazine, 91904-13-1; decanoyl chloride, 112-13-0; p-toluoyl chloride, 874-60-2; 1-(chloroacetyl)-4-(3-chlorophenyl)piperazine, 70395-06-1; 1-methylpiperazine, 109-01-3; 2-pyrrolidone, 616-45-5; morpholine, 110-91-8; thiomorpholine, 123-90-0; piperidine, 110-89-4; pyrrolidine, 123-75-1; (L)-proline, 147-85-3; imidazole, 288-32-4.

Octoclothepin Enantiomers. A Reinvestigation of Their Biochemical and Pharmacological Activity in Relation to a New Receptor–Interaction Model for Dopamine D-2 Receptor Antagonists

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Octoclothepin (1) was resolved into its *R* and *S* enantiomers via the diastereomeric tartaric acid salts. The enantiomers were shown to be of high optical purity by ^1H NMR with use of the chiral shift reagent (*R*)-(−)-2,2,2-trifluoro-1-(9-anthryl)ethanol. Pharmacological and biochemical testing confirmed that (*S*)-1 is the more potent dopamine (DA) D-2 antagonist both in vitro and in vivo, although the *R* enantiomer still has significant D-2 antagonistic activity. In contrast, both enantiomers were equally active in test models detecting activity at D-1 receptors, serotonin-2 (5-HT_2) receptors and α_1 adrenoceptors. Contrary to a previous prediction, it was found that norepinephrine (NE) uptake inhibition was confined solely to the *S* enantiomer. Overall, (*S*)-1 has a "classical" neuroleptic profile, while the *R* enantiomer has a more "atypical" profile. These pharmacological profiles seem to be in agreement with the reported clinical profiles of the two enantiomers. A previous conformational study was revised in light of the biochemical test results with enantiomers of known optical purity. Their relative D-2 receptor affinity corresponded well with the calculated conformational energy difference between their "active conformations" deduced from a previously reported new D-2 receptor model. Also the high enantioselectivity of (*S*)-1 at the NE uptake site could be explained after a detailed conformational analysis showing strict requirements for the orientation of the piperazine lone-pair direction at the NE uptake site.

We have previously reported extensive conformational analysis and least-squares molecular superimposition studies of the enantiomers of the two neuroleptic compounds octoclothepin (1) and tefludazine (2) (Figure 1).[1] In contrast to previous assumptions we concluded, that the conformation of (*S*)-1 which is responsible for the dopamine D-2 (DA D-2) receptor antagonism is significantly different from the one observed in the crystal.[2] The superimposition of the suggested active conformations of (*S*)-1 and (1*R*,3*S*)-2 (Figure 2) defines the spatial relationships of the pharmacophore elements (the phenyl rings, the nitrogen atom and the nitrogen atom–nitrogen lone pair (or in the protonated case the N–H) vector). These spatial relationships represent in our opinion a contemporary DA D-2 receptor interaction model. D-2 antagonists from various chemical classes (thioxanthenes, phenothiazines, butyrophenones, and benzamides) can be accomodated into the model in low-energy conformations.[3a] Furthermore, Froimowitz has successfully employed this model in a study of dibenzocycloheptene and cyproheptadine derivatives.[3b]

The affinity of (*R*)-1 for the D-2 receptor has been reported by Seeman et al.[4] to be only nine times lower than that of (*S*)-1. Valchář have reported a stereoselectivity ratio of 38 in favor of the *S* enantiomer.[5] We explained this relatively low stereoselectivity by pointing out that also (*R*)-1 could be accommodated in the D-2 model in a conformation with a steric energy that was 2.2 kcal/mol higher than that of the suggested active conformation of (*S*)-1.

In contrast to the rather low stereoselectivity observed in vitro for the enantiomers of 1, they have been reported to show a *high* stereoselectivity in certain in vivo models for neuroleptic activity. These include catelepsy, antagonism of apomorphine-induced gnawing, chewing or agi-

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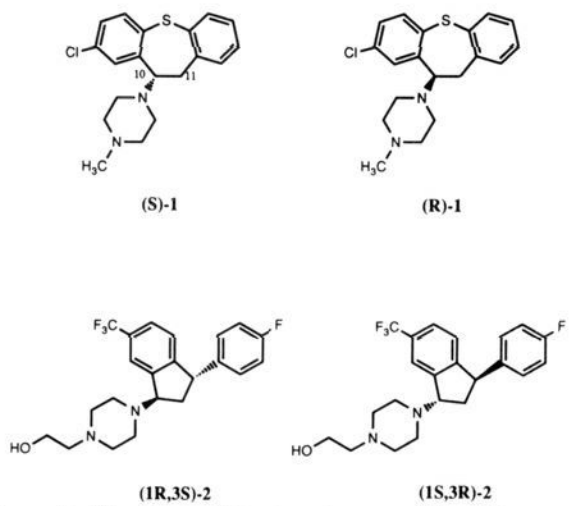

Figure 1. Structures of the stereoisomers of 1 and 2.

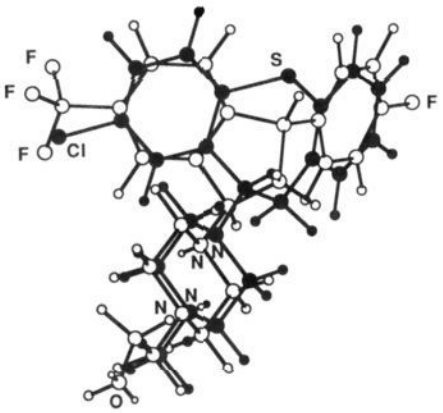

Figure 2. A D-2 model[1] based on least-squares superimposition of the suggested active conformations of (*S*)-1 and (1*R*,3*S*)-2.

tation, antagonism of amphetamine-induced stereotypies in the rat,[6,7] and the ability to increase striatal HVA levels in the rat.[5] However in models for central depressant effects such as inhibition of motility and in the rotarod test no stereoselectivity was observed.[7]

Both 1 and 2 also have a high affinity for serotonin-2 (5-HT_2) receptors and for adrenergic α_1 receptors.[8] Also at these receptor sites the enantiomers of 2 show a high stereoselectivity, again with all activity residing in the 1*R*,3*S* enantiomer.[8] No data is available concerning the affinity of the enantiomers of 1 for these receptor sites. It has, however, been reported that the enantiomers of methiothepin (the corresponding 2-methylthio derivative of 1) display equal, high affinity for the 5-HT_2 receptor site (no determination of optical purity).[9]

Irindalone, a derivative of 2 lacking the "neuroleptic" 6-substituent in the indane ring, is a selective 5-HT_2 antagonist.[10-12] A conformational study and molecular superimposition of irindalone and another selective 5-HT_2 antagonist ketanserin resulted in the conclusion, that the active conformation of irindalone was identical with the D-2 receptor active conformation of (1*R*,3*S*)-2, indicating that 2 probably interacts with D-2 and 5-HT_2 receptors in the same conformation.[10] If this is true, one would predict that the enantiomers of 1 also should have a low stereoselectivity for 5-HT_2 receptors.

In our previous paper we also discussed the potent inhibitory effect of racemic octoclothepin on nonepinephrine (NE) uptake inhibition. The 1*S*,3*R* enantiomer of 2 and related 1-piperazino-3-phenylindans are also potent NE (and DA) uptake inhibitors.[1,8,13,14] The conformational study confirmed an earlier observation, that there was a good correspondance between the conformation of (*S*)-1 found in the crystal and a low-energy conformer of (1*S*,3*R*)-2.[14] However, we also showed that a conformer of (*R*)-1 could be fitted very well on the same conformer of (1*S*,3*R*)-2. Because of the steric energy of the *R* conformer was slightly higher than that of the *S* conformer, we predicted that the enantioselectivity of the enantiomers of 1 with respect to NE-uptake inhibition should be low with the *S* enantiomer as the most potent of the two.

The optical purity have not been accurately established in any of the previously reported biochemical or pharmacological studies, which leaves some uncertainty in the interpretation of the results. We therefore decided to resolve 1 ourselves, try to establish the optical purity of the enantiomers, and perform a number of biochemical and pharmacological experiments with the following objectives: (1) Measure affinity for both DA D-2 and DA D-1 receptors and investigate the DA blocking effect in both general as well as receptor specific in vivo animal models. (2) Measure affinity for 5-HT_2 receptors and α_1 adrenoceptors and investigate any central effects at these receptors. (3) Measure affinity for the NE (and DA and 5-HT) uptake sites in order to test the prediction mentioned above. (4) Revise our original conformational study on the basis of the results obtained.

Chemistry

The preparation of racemic octoclothepin and subsequent resolution was performed essentially as described by Jílek et al.[15] The *R* and *S* enantiomers were obtained from the diastereomeric salts with D-(−)- and L-(+)-tartaric acid, respectively. The enantiomers were finally isolated as crystalline bases. These had melting points which were 20 °C below that reported by Jílek et al. However, as both the CHN analyses and the NMR spectrum was in agreement with the structure of 1, and the optical rotation of the enantiomers was identical with that reported by Jílek et al., we must conclude that there exists at least two different crystal modifications of the enantiomers.

In order to determine the optical purity of the enantiomers we developed a sensitive NMR method using the chiral shift reagent (*R*)-(−)-2,2,2-trifluoro-1-(9-anthryl)-ethanol. This reagent has a remarkable influence on the chemical shifts of the aliphatic protons in the thiepin ring system (Figure 3). The same part of the spectrum for the enantiomers influenced by the chemical shift reagent are shown in Figure 3, too. By measuring the peak heights in

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Table I. Receptor Binding of Octoclothepin (1) and Its Enantiomers[a]

receptor/type	ligand	1	(S)-1	(R)-1
DA/D-1	[^{3}H]SCH	2.2 (1.0–4.7)	2.2 (1.0–4.7)	1.2 (0.56–2.6)
DA/D-2	[^{3}H]SPI	2.4 (1.1–5.4)	1.3 (0.6–2.9)	6.6 (2.9–14.9)
5-HT/5-HT$_2$	[^{3}H]KET	0.57 (0.28–1.2)	0.48 (0.23–0.98)	0.48 (0.23–0.98)
NE/α_1	[^{3}H]PRAZ	0.18 (0.082–0.40)	0.45 (0.20–0.99)	1.4 (0.64–3.1)

[a] Results are expressed as IC_{50} values in nM (logarithmic means). Two full concentration curves were measured by using five concentrations of test drug in triplicate (covering three decades). Ninety five percent confidence intervals are stated in parentheses. Standard deviation ratios were obtained by calculating the variance of repeated measures of ratios between the first and second IC_{50} determination for a series of 100 drugs. If the ratio was greater than 3x sd (99% confidence interval) extra determinations were performed and outliers were discarded. The following 95% confidence ratios (2x sd ratio) were calculated: D-1 2.13; D-2 2.26; 5-HT$_2$ 2.05; α_1 2.20.

Table II. Inhibition of the Accumulation of ^{3}H-Labeled Amines into Rat Brain Synaptosomes in Vitro

[^{3}H]amine	1	(S)-1	(R)-1
NE	0.54 (0.20–1.5)	0.96 (0.36–2.6)	300 (111–804)
DA	5300 (1920–14628)	2300 (833–6348)	2700 (978–7452)
5-HT	190 (93–388)	190 (93–388)	270 (132–550)

[a] See footnote, Table I. The following 95% confidence ratios (2x sd ratio) were calculated: NE uptake 2.68; DA uptake 2.76; 5-HT uptake 2.04.

the double doublet at 2.5 ppm in the normal and the 19 times enlarged spectrum the optical purity of the *S* enantiomer is estimated to be 98.5%. For the *R* enantiomer no contamination with the other enantiomer can be seen in the 18 times enlarged spectrum. Since the signal to noise ratio is 150:1 the optical purity can be estimated to be better than 99.3%.

Results

Details concerning the test methods are described in the Experimental Section. The affinities of 1 and its enantiomers for central D-1 receptors, D-2 receptors, 5-HT$_2$ receptors, and α_1 adrenoceptors are shown in Table I. The ability of the compounds to inhibit the reuptake of tritiated NE, DA, and 5-HT is shown in Table II.

In Table III are presented results with 1 and its enantiomers in a number of in vivo animal models. Antagonism of methyl phenidate induced stereotyped gnawing in mice and induction of catalepsy in rats are test models where both D-2 and D-1 antagonists are active.[16] Inhibition of motility in rats was included, because it was reported that no stereoselectivity was observed with the enantiomers of 1 in models for "central depressant effects".[7] Antagonism of circling behavior induced by either the D-2 agonist pergolide or the D-1 agonist SK&F 38393 in 6-hydroxydopamine (6-OHDA) lesioned rats are models highly selective for D-2 antagonists or D-1 antagonists, respectively.[17] Central 5-HT$_2$-antagonistic activity is assessed by the ability of the compounds to antagonize quipazine-induced head twitches in rats. Finally, central α_1 antagonism is measured as the ability of the compounds to inhibit the increase in the electrically stimulated flexor reflex induced by the selective α_1 agonist St 587.

Like Seeman et al.[4] we find a rather low stereoselectivity at D-2 receptors. We confirm that the *S* enantiomer is the most potent enantiomer, having five times higher affinity for the D-2 receptors than the *R* enantiomer. At D-1 receptors we find that the enantiomers have almost identical affinity, the *R* enantiomer being slightly more potent than the *S* enantiomer. A similar stereoselectivity pattern for D-2 and D-1 receptors can also be observed in the corresponding receptor selective in vivo models (6-OHDA rotation models, Table III). The *S* enantiomer is 18 times more potent than the *R* enantiomer as an inhibitor of the rotations induced by the D-2 agonist pergolide, while the two compounds are almost equipotent as inhibitors of rotations induced by the D-1 agonist SK&F 38393.

We also confirm the stereoselectivity pattern reported by Petcher et al.[6] and by Metyšová and Protiva[7] in test

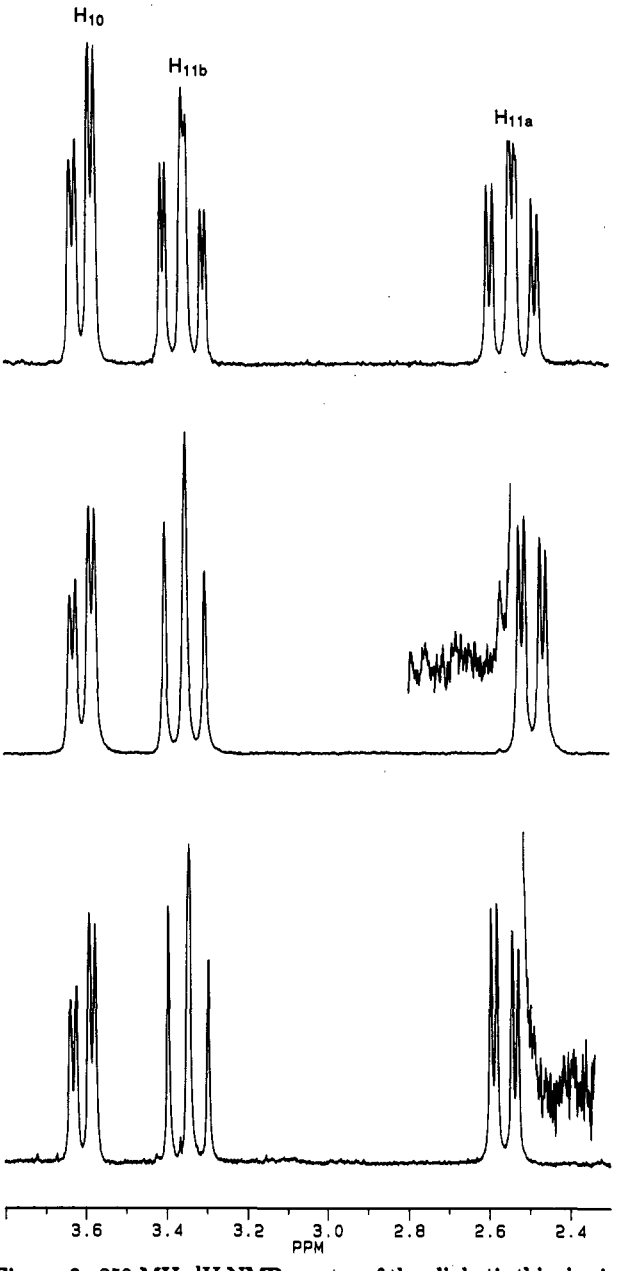

Figure 3. 250-MHz ^{1}H NMR spectra of the aliphatic thiepin ring protons of 1 (top), (*S*)-1 (middle), and (*R*)-1 (bottom) in combination with the chiral shift reagent (*R*)-2,2,2-trifluoro-1-(9-anthryl)ethanol (see Experimental Section for assignment of signals). The enlarged parts correspond to a 19 times enlargement for the *S* enantiomer and an 18 times enlargement for the *R* enantiomer.

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Table III. Activity of 1 and Its Enantiomers in Pharmacological Test Models[a]

test	1	(S)-1	(R)-1
methyl phenidate antagonism, mice	0.19 (0.14–0.27)	0.20 (0.14–0.28)	3.8 (2.1–6.8)
catalepsy, max eff 1–6 h, rats	1.3 (0.39–4.3)	0.65 (0.49–0.87)	24 (16–36)
pergolide antagonism, 6-OHDA-lesioned rats	0.033 (0.012–0.092)	0.017 (0.009–0.034)	0.31 (0.15–0.64)
SK&F 38393 antagonism, 6-OHDA-lesioned rats	0.25 (0.09–0.68)	0.85 (0.37–1.96)	1.9 (0.90–4.0)
inhibition of locomotor motility, rats	0.18 (0.09–0.38)	0.12 (0.06–0.26)	0.46 (0.13–1.6)
quipazine antagonism, rats	0.019 (0.012–0.030)	0.020 (0.013–0.030)	0.012 (0.007–0.021)
flexor reflex St 587 antagonism, rats	0.064 (0.038–0.11)	0.26 (0.10–0.70)	0.095 (0.030–0.30)

[a] Doses are expressed in μmol/kg sc. Ninety five percent confidence intervals are stated in parentheses.

models for neuroleptic and "central depressant" effects. The *S* enantiomer is 19 times more potent than the *R* enantiomer as an antagonist of methyl phenidate induced stereotyped gnawing. In the catalepsy test we find the *S* enantiomer to be twice as potent as the racemate, and almost 40 times as potent as the *R* enantiomer. Finally, we find, like Metyšová and Protiva, that both enantiomers are almost equipotent as inhibitors of locomotor activity.

At 5-HT_2 receptors we find that 1 and its enantiomers have identical, high affinity (as suggested above). This result is also confirmed in vivo (quipazine antagonism) where all three components show a similar, very potent antagonism. The same is true at α_1 adrenoceptors where the three compounds are almost equipotent both as inhibitors of prazosin binding and as antagonists of St 587 induced increase of the flexor reflex.

Finally, we find surprisingly that all the NE-uptake inhibiting activity of 1 resides in the *S* enantiomer (Table II). In contrast both enantiomers show, like the racemate, a weak DA- and 5-HT-uptake inhibition.

Discussion

Petcher et al.[6] summarizes their findings with 1 and its enantiomers in the following statement: "The biological activity of the neuroleptic agent octoclothepin has been shown to be confined to its (+)-enantiomer which has the *S*-configuration." The results obtained with enantiomers of documented high optical purity show that this is certainly not the case, when one includes receptor specific test models and models that detect activity at other receptors than dopamine receptors.

There is no doubt that the *S* enantiomer is the more potent D-2 antagonist, although the *R* enantiomer still has significant activity both in binding experiments and in in vivo models detecting neuroleptic activity (methyl phenidate antagonism and catalepsy) and central D-2 antagonism (inhibition of pergolide rotations). The eudismic ratio in these three models are 18, 37, and 19, respectively. In the SK&F 38393 rotation model the eudismic ratio is 2. Although also D-1 antagonists are able to antagonize stereotypies and induce catalepsy,[16] the effects of the enantiomers in these models are mainly related to their D-2 antagonistic potency. The antistereotypic effect of (*R*)-1 corresponds well with the results reported by Petcher et al. concerning antagonism of amphetamine-induced stereotypies in rats. They report an eudismic ratio of 36 in this model (in favor of the *S* enantiomer).[6]

Although both Petcher et al.[6] and Metyšová and Protiva[7] find that (*R*)-1 is *inactive* in the catalepsy test, a close inspection of their data reveal that these are not necessary in disagreement with ours. Petcher et al. find no induction of catalepsy with (*R*)-1 using a maximal dose of 20 mg/kg sc (corresponding to 58 μmol/kg), but they use a more strict immobility criterion (30-s immobility) than we do (15-s immobility). This is clearly reflected in the ED_{50} value they report for the *S* enantiomer (1.47 mg/kg = 4.3 μmol/kg), which is 6–7 times higher than our ED_{50} for this enantiomer.

Metyšová and Protiva[7] observe induction of catalepsy with (*R*)-1 in 20% of the rats after a dose of 5 mg/kg *iv* (14.5 μmol/kg) while they find it inactive after *oral* administration of 25 mg/kg (72 μmol/kg). However after *oral* administration of (*R*)-1 we find the ED_{50} for inhibition of methyl phenidate induced stereotypies is >29 μmol/kg (in contrast to 3.8 μmol/kg after sc administration).[18] It is therefore very likely that the apparent deviation of the results in the catalepsy test can be explained by the different routes of administration and differences in test criteria.

The lower eudismic ratio (2.2) we observe in motility inhibition is in agreement with the findings of Metyšová and Protiva. A possible explanation of this observation might be that both enantiomers are equally potent as α_1 antagonists. Selective α_1 antagonists such as prazosin are active in this model.[18]

The very potent 5-HT_2 antagonism shown by 1 and *both* enantiomers is interesting especially in relation to the clinical potential of the compounds. An abnormality in the interaction of the dopaminergic and the serotonergic system has been suggested to be involved in the etiology of schizophrenia.[19,20] Thus negative symptoms may be related to decreased dopaminergic activity (high ratio of serotonergic to dopaminergic activity), while the positive symptoms may be related to increased dopaminergic activity (low ratio of serotonergic to dopaminergic activity).[20] Accordingly, the selective 5-HT_2 antagonist ritanserin and the neuroleptic compound risperidone (which is also a potent 5-HT_2 antagonist) have been reported to ameliorate the negative symptoms of schizophrenia.[21,22] "Atypical" neuroleptic compounds such as clozapine (which is known *not* to cause extrapyramidal side-effects in the clinic) and sertindole, have a profile resembling that of (*R*)-1, i.e. weak or no effect in classical neuroleptic test models (catalepsy, antisterotypic effect).[23] Like (*R*)-1 these compounds are potent 5-HT_2 and α_1 antagonists.[23]

Interestingly, both the *S* and the *R* enantiomer of 1 have been tested clinically. Two controlled studies with a duration of 3 and 6 weeks, respectively, were performed.[24-26] In the 3-week study the effect of the enantiomers was

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compared with perphenazine as a reference compound;[25] in the 6-week study the enantiomers were compared with racemic 1 and clozapine.[26] Overall it was found that both enantiomers were clinically effective in treatment of schizophrenia, the *S* enantiomer being more effective than the *R* enantiomer although the difference was not statistically significant. It is interesting that the *R* enantiomer was given in only slightly higher doses than the *S* enantiomer despite their very different D-2 antagonistic activity in vivo in the animal models. Although clinical efficacy still has to be demonstrated with selective D-1 antagonists, it is nevertheless tempting to suggest that the D-1 antagonistic effects of the enantiomers also could contribute to their clinical efficacy. Because both enantiomers are equally effective as D-1 antagonists this might be part of the explanation of why they are effective in the same dose range.

Looking at the individual symptoms according to the FKP rating scale there was no statistical difference between the enantiomers in the control of positive or negative symptoms. It was noted that both enantiomers (and the racemate) were very effective in controlling insomnia.[26] It has recently been demonstrated that selective 5-HT_2 antagonists are able to normalize sleep in humans by increasing the slow-wave sleep.[27,28] It is possible that the sleep normalizing effect of the enantiomers and the racemate is related to their equally potent 5-HT_2 antagonistic effect.

A clear, statistical significant difference was seen in the frequencies of extrapyramidal side effects. The *S* enantiomer had a higher frequency of dyskinetic reactions and akathesia than the *R* enantiomer in accordance with their different cataleptic potential in the animal tests. Clozapine had as expected even less extrapyramidal side effects (no dyskinetic reactions and fewer parkinson symptoms) but the *R* enantiomer was clearly belonging to the group of neuroleptics "less aggresive in this respect".[25,26]

Overall the observed clinical profiles seem to be in good agreement with what one would predict from the pharmacological results we present here. Once more it underlines the importance of investigating the effects of the individual isomers of any racemic compound before any conclusions are drawn or decisions are taken.

The finding that the effect on inhibition of NE uptake was confined to the *S* enantiomer was, as mentioned, surprising and in contradiction to our previous prediction. This problem will be further commented below.

DA Receptor Binding Conformations of (*S*)-1 and (*R*)-1. We have previously reported an extensive conformational analysis of compound 1 using MM2(85) calculations.[1] The corresponding results obtained by MM2(87) are essentially the same. The only significant exception is that the conformer of 1 observed in the crystalline state[2,6] (**2a** in ref 1) is calculated to be the lowest energy one by MM2(87), while the previously calculated lowest energy conformer (**2g** in ref 1) now is calculated to be 0.4 kcal/mol higher in energy than the crystal structure. Rotation about the C–S bonds (C–S–C bridge "flipping"), the C–C bonds in the ethylene bridge, and/or the C–N bond connecting the piperazine ring and the tricyclic system makes a large number of different conformations possible for this compound. Although (*S*)-1 and (*R*)-1 by definition are nonsuperimposable, this conformational flexibility makes it feasible for the enantiomers to adopt very similar 3D-shapes. In particular, it makes it possible for (*R*)-1 to structurally mimic our previously proposed biologically active conformation of (*S*)-1 with respect to DA D-2 receptor binding.[1] This is illustrated in Figure 4. In this figure, (*S*)-1 (top left) is shown in its proposed DA D-2 receptor binding conformation. The *R* enantiomer (bottom left) may adopt, through a "flip" of the C–S–C bridge and a reorientation of its piperazine ring, a conformation (top right) with a 3D-shape extremely similar to that of the proposed biologically active conformation of (*S*)-1. Figure 5 shows a least squares superimposition of (*S*)-1 and (*R*)-1 in these conformations. The rms value for this fit is 0.28 Å when the fitting points described in the Computational Methods section are used. The calculated conformational energy difference (MM2(87)) for (*S*)-1 and (*R*)-1 in these conformations is 1.66 kcal/mol, favoring the *S* enantiomer. This number, calculated by MM2(87), is slightly lower than the number previously calculated by MM2(85)[1] and mentioned in the introduction.

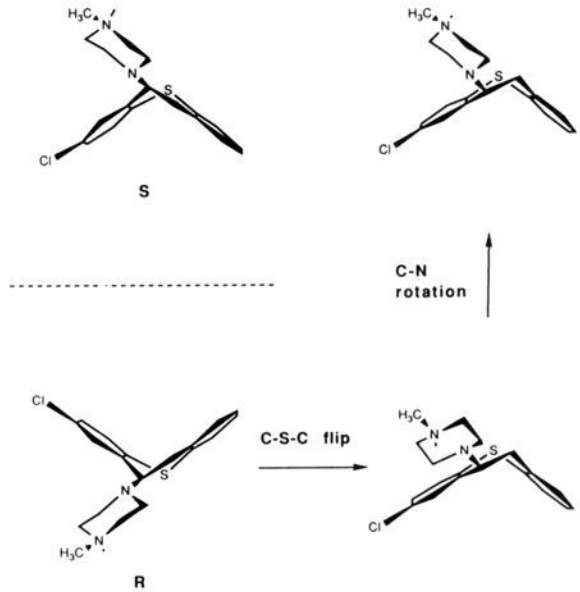

Figure 4. Conformational changes of (*R*)-1 giving a high similarity of the 3D shapes of the proposed DA D-2 receptor binding conformation of (*S*)-1 and (*R*)-1.

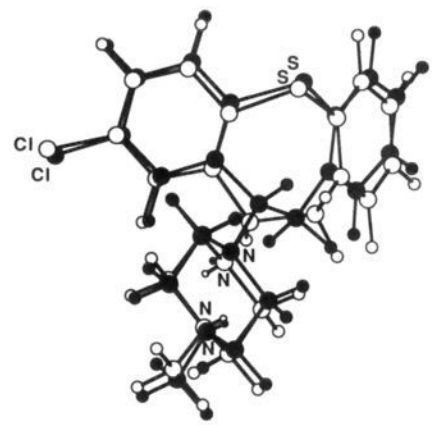

Figure 5. Least-squares superimposition of (*S*)-1 (filled atoms) in its proposed DA D-2 receptor binding conformation and (*R*)-1.

Enantiomers have identical physicochemical and thermodynamical properties such as free energies of solvation, lipophilicity, entropy, etc. Furthermore, the entropies of the conformations of the enantiomers in Figure 5 should be very similar. The very close structural fit displayed in

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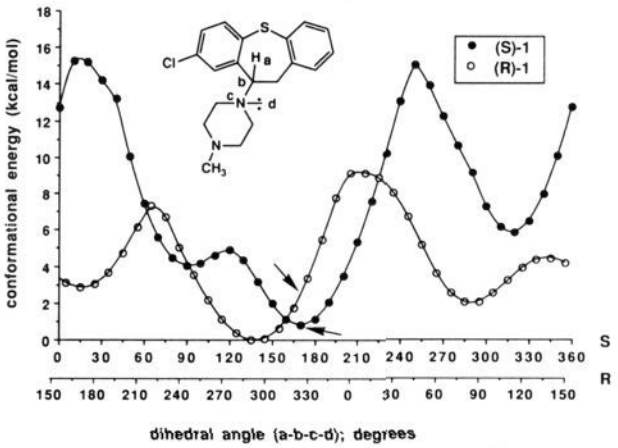

Figure 6. Calculated potential energy curves for simultaneous rotation about the C(seven-membered ring)–N bonds in (*S*)-1 and (*R*)-1 with tricyclic ring system conformations as in Figure 5. The arrows point at the dihedral angle values and the corresponding conformational energies for the proposed receptor binding conformations.

this figure indicates that the interactions between the pharmacophore elements of the enantiomers in these conformations and the corresponding receptor sites should be very similar. Thus, the difference in the free energy of binding of the enantiomers to the DA D-2 receptor should largely be determined by the difference in the conformational energies of their biologically active conformations. This is a particularly advantageous situation from a computational point of view which makes it possible to directly relate differences in calculated conformational energies and observed relative affinities. The calculated conformational energy difference, 1.66 kcal/mol, corresponds to a relative affinity (at 300 K) of about 10 with (*S*)-1 as the highest affinity enantiomer. This is in good agreement with the observed relative affinity of 5.1 for the DA D-2 receptor (Table I). The agreement clearly supports the proposed active conformations of (*S*)-1 and (*R*)-1 on binding to the DA D-2 receptor.

As may be expected from the superimposition in Figure 5, simultaneous rotations about the C(tricyclic ring)–N(piperazine) bonds in (*S*)-1 and (*R*)-1 generate new conformations of these compounds with a preserved high degree of structural similarity. To investigate if other piperazine ring rotamers of (*S*)-1 and (*R*)-1 may have a conformational energy difference corresponding to the observed relative affinity of these compounds, potential energy curves for a simultaneous rotation about the C–N bond were calculated. The result is shown in Figure 6. The potential energy curves in this figure are positioned so that dihedral angles and energy values corresponding to an optimal fit of the N lone pair directions in (*S*)-1 and (*R*)-1 are aligned. The large differences in the potential energy curves for (*S*)-1 and (*R*)-1 are due to the different invertomers of the tricyclic ring systems in the proposed active conformations of these compounds (see Figure 5). The arrows in Figure 6 point at the dihedral angle values and the corresponding conformational energies for the proposed DA D-2 receptor binding conformations.

The potential energy curves in Figure 6 show that low-energy conformations of (*S*)-1 and (*R*)-1 with the same nitrogen atom–nitrogen lone pair direction and with relative energies corresponding to the observed relative affinity for the DA D-2 receptor are only found for a very small range of dihedral angles at 172° and 325° for (*S*)-1 and (*R*)-1, respectively. These correspond to the dihedral angle values in the superimposition in Figure 5. This

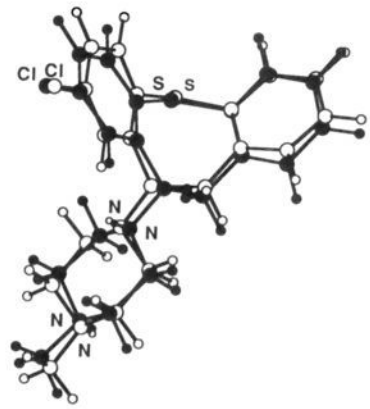

Figure 7. Least-squares superimposition of (*S*)-1 (filled atoms) in its proposed NE-uptake site binding conformation and (*R*)-1.

observation provides further support for the proposed active conformations of (*S*)-1 and (*R*)-1 with respect to DA D-2 receptor binding. Interestingly, a decrease of the dihedral angle a–b–c–d by ca. 10 degrees in each of the enantiomers from the angles indicated by the arrows in Figure 6 results in identical conformational energies for the two enantiomers corresponding to identical affinities for the enantiomers. A further decrease of the dihedral angles makes the *R* enantiomeric lower in energy than the *S* enantiomer which corresponds to a higher affinity for (*R*)-1 than for (*S*)-1. This clearly indicates that the requirements on the orientation of the piperazine ring and thus on the nitrogen atom–nitrogen lone pair (N–H) directions for an optimal binding to the DA D-2 receptor is very strict.

This analysis above suggests a possible explanation for the very similar affinities of (*S*)-1 and (*R*)-1 for the DA D-1 receptor. The enantiomers may in this case bind to the receptor essentially as they bind to the DA D-2 receptor but with a slightly different optimal orientation of the piperazine rings corresponding to dihedral angles in Figure 6 of about 160 and 315 for (*S*)-1 and (*R*)-1, respectively.

Conformational Analysis of (*S*)-1 and (*R*)-1 in Relation to NE-Uptake Inhibition. As mentioned in the introduction we have previously predicted that (*S*)-1 should be the most potent enantiomer with respect to NE-uptake inhibition.[1] The data for uptake inhibition reported in the present work (Table II) show that this prediction indeed was correct. We also predicted the enantioselectivity of 1 to be low. However, this prediction is not correct, (*S*)-1 displays more than 300 times higher affinity than (*R*)-1 for the NE-uptake site (Table II). These predictions were made on the basis of superimpositions of stable low-energy conformations of (1*S*,3*R*)-**2**, (*S*)-1, and (*R*)-1.[1] In order to further investigate the structural basis for the high observed enantioselectivity of 1 with respect to NE-uptake inhibition, we have analyzed the conformational properties of (*S*)-1 and (*R*)-1 in the same way as described above for DA receptor binding. Figure 7 shows a superimposition of (*S*)-1 and (*R*)-1 with (*S*)-1 in the previously proposed conformation for binding to the NE-uptake site.[1] The rms value of this fit is 0.19 Å, indicating a very high similarity of the 3D shapes of the enantiomers in these conformations. Figure 8 shows the calculated potential curves for a simultaneous rotation about the C–N bonds (b–c in Figure 8) in (*S*)-1 and (*R*)-1. The potential curves are aligned as described above and the arrows indicate the dihedral angles and the corresponding conformational energies for the conformations in the superimposition in Figure 7. The calculated energy difference for (*S*)-1 and (*R*)-1 in these conformations is 3.2

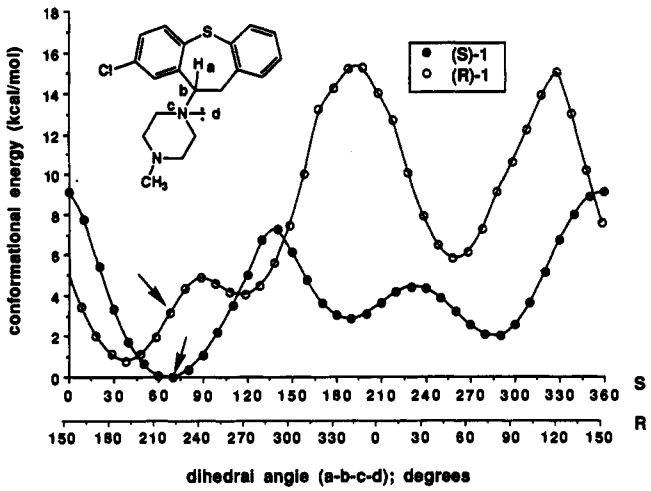

Figure 8. Calculated potential energy curves for simultaneous rotation about the C(seven-membered ring)–N bonds in (*S*)-1 and (*R*)-1 with tricyclic ring system conformations as in Figure 7. The arrows point at the dihedral angle values and the corresponding conformational energies for the proposed NE uptake site binding conformations.

kcal/mol corresponding to an factor of about 200 in relative affinity (at 300 K) for the NE-uptake site, with (*S*)-1 as the most potent enantiomer. This is in good agreement with the observed relative affinity, 312.5 (Table II). The prediction made in our previous study[1] was based on a comparison of the energies for the structurally closely related stable conformations with dihedral angles (a–b–c–d in Figure 5) of 68.8° and 188.4° for (*S*)-1 and (*R*)-1, respectively. Thus, the assumption of a strict requirement on the orientation of the piperazine rings and, thus, the N lone pair (N–H) directions for an optimal binding to the NE-uptake site, rationalizes the observed high enantioselectivity in this case.

The interpretation of the potential energy curves in Figure 8 is not as unambiguous as it was in the case of DA receptor binding, discussed above. The *R* enantiomer may contain a small amount of (*S*)-1 (<0.7%, see Chemistry section). Furthermore, there are several dihedral angle values in the range of 155–340° for (*S*)-1, for which this enantiomer is sufficiently more stable than (*R*)-1 to rationalize the high observed enantioselectivity. However, the corresponding conformations for (1*S*,3*R*)-2 all have conformational energies in excess of 3 kcal/mol.[1] Considering the very high affinity of (*S*)-1 (Table II) and properly substituted (1*S*,3*R*)-2,[4] we find these higher energy rotamers to be less likely candidates for the biologically active conformations of these compounds with respect to NE-uptake inhibition.

Experimental Section

Melting points (uncorrected) were determined on a Büchi SMP-20 apparatus. The ^{1}H NMR spectra were recorded in chloroform-*d*, with Me_4Si used as internal standard at 250.133 MHz on a Bruker AC-250 instrument. (*R*)-(–)-2,2,2-Trifluoro-1-(9-anthryl)ethanol was purchased from Aldrich and used without purification. Spectra for determination of optical purity were recorded for 5-mg samples of octoclothepin enantiomer containing 12 mg of chemical-shift reagent. The signals from the alicyclic protons under influence of the chemical-shift reagent were assigned from the 2-D carbon–proton correlated spectrum. Chemical shift values and coupling constants were measured under influence of chemical shift reagent. Microanalyses (within ±0.4% of theoretical values) were performed by Lundbeck Analytical Department.

Preparation of Octoclothepin Enantiomers ((*R*)-1 and (*S*)-1). To a warm solution of 1 (71 g, 0.21 mol) in ethanol was added D-(–)-tartaric acid (31 g, 0.21 mol). The solution was kept at room temperature overnight, whereupon the crystals were filtered, washed with ethanol, and dried to give 76 g of the D-(–)-tartaric salt, mp 160–190 °C. The salt was recrystallized in a mixture of ethanol (3 L) and methanol (1 L), which was concentrated to a volume of 3 L. After the mixture was cooled to room temperature and filtered there was obtained 37 g, mp 221–223 °C. The filtrate from the first tartrate salt was evaporated in vacuo and converted to the base (15 g). This base was treated in ethanol with 1 equiv of L-(+)-tartaric acid to give 12.5 g of the L-(+)-tartaric salt, mp 228–229 °C (Jílek et al.[15] report a melting point for the L-(+)-tartaric salt recrystallized twice from ethanol of 228–232 °C).

In a similar way was 1 treated with L-(+)-tartaric acid, giving 37 g of L-(+)-tartrate salt, mp 222–224 °C. From the concentrated filtrate from this salt was in a similar way obtained a D-(–)-tartrate salt with a higher melting point (9 g, mp 229–230 °C).

The higher melting tartrate salts were converted in a conventional manner to their respective maleate salts, which were recrystallized once from ethanol. The maleates both melted at 188–189 °C. Optical rotation of *S* enantiomer: $[\alpha]_D^{22}$ +27.9° (*c* 1, MeOH) and of *R* enantiomer: $[\alpha]_D^{22}$ –26.1° (*c* 1, MeOH), Jílek et al. reported melting points of 195–196 °C and 192–195 °C for the maleates of the *S* and *R* enantiomers, respectively, while the optical rotations for these enantiomers were reported to be +35° and –29° ($[\alpha]_D^{20}$, *c* 1, MeOH).

Finally the maleate salts were converted to the bases which crystallized from petroleum ether. There was obtained 0.6 g of the *S* enantiomer, mp 90–92.5 °C, $[\alpha]_D^{22}$ +46.0° (*c*, 1, MeOH) and 0.6 g of the *R* enantiomer, mp 90–92.5 °C, $[\alpha]_D^{22}$ +46.1° (*c* 1, MeOH). ^{1}H NMR data ($CDCl_3$, with shift reagent): *S* enantiomer, δ 2.50 (dd, *J* = 3.6, 12.8 Hz, 1 H, H_{11a}), 3.36 (dd, *J* = 11.7, 12.8 Hz, 1 H, H_{11b}), 3.61 (dd, *J* = 3.6, 11.7 Hz, H_{10}); *R* enantiomer, δ 2.57 (dd, *J* = 3.8, 12.8 Hz, 1 H, H_{11a}), 3.34 (dd, *J* = 11.7, 12.8 Hz, 1 H, H_{11b}), 3.61 (dd, *J* = 3.8, 11.7 Hz, 1 H, H_{10}). Anal ($C_{19}H_{21}ClN_2S$), C, H, N.

As described in the Chemistry Section the optical purity was determined by the NMR method to be about 98.5% for the *S* enantiomer and above 99.3% for the *R* enantiomer.

Jílek et al.[15] reported melting points of 112–114 °C and 110–112 °C for their *S* and *R* enantiomers, respectively, while the corresponding optical rotations were reported to be +50 and –45° (*c* 1, MeOH). As there can be not doubt about the identity and the purity of the enantiomers that we report here, we must conclude that there exist at least two different crystal forms of the bases.

Computational Methods. Conformational energies and energy-optimized geometries were calculated by using the molecular mechanics program MM2(87) developed by Allinger and coworkers.[29,30] In addition to the standard MM2(87) force field a torsional V2 parameter of 15.0 was used for dihedral angles of type 1–2–2–42 (C_{sp3}–C_{sp2}–C_{sp2}–S_{sp2}). As the version of MM2(87) employed in this work does not include monopole–dipole interactions all calculations were, as in our previous work,[1] done on the unprotonated amines with the unshared electron pair represented by a pseudoatom. It is not likely that the conformational energy differences discussed in the present work would be significantly altered in the protonated case.

Potential energy curves were calculated by using the MM2(87) driver option with full energy minimization except for the dihedral angle used as the driving angle and with an angle increment of 10°.

The construction of input structures for MM2(87) and the studies on molecular superimpositions were performed with the molecular modeling program MIMIC[31,32] or the Macintosh II program MacMimic.[33] Fitting points used in the superimposition

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(30) The program is available from the Quantum Chemistry Program Exchange (University of Indiana, Bloomongton, IN 47405) and from Molecular design Ltd (San Leandro, CA 94577).
(31) Liljefors, T. *J. Mol. Graphics* **1983**, *1*, 111.
(32) Von der Lieth, C. V.; Carter, R. E.; Dolata, D. P.; Liljefors, T. *J. Mol. Graphics* **1984**, *2*, 117.
(33) The program is available from Instar Software, IDEON Research Park, S-223 70 Lund, Sweden.

studies were (i) the center of each of the phenyl rings, (ii) the distal nitrogen atom, and (iii) a point 2.8 Å from the distal nitrogen atom in the direction of the nitrogen lone pair. This point is assumed to simulate a receptor site hydrogen bonding with the nitrogen atom.

Pharmacology. Animals. The rats were male Wistar (Mol: Wist) SPF, weighing 170–270 g. The mice were male (NMRI/BOM) SPF, weighing 18–25 g. Rats (five animals/Macrolon III cage) and mice (20 animals/plastic cage) were housed conventionally in animal rooms with automatic control of temperature (21 ± 1 °C), relative humidity (55 ± 5%), air exchanges (16 times/h) and day/night cycle (light on 6 am to 6 pm). They had free access to a commercial pelleted diet (New Rostoc diet, KFK, Aarhus) and tap water.

Drugs. Quipazine dimaleate, SK&F 38393 (2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1*H*-3-benzazapine) hydrobromide, and St 587 (2-[[2-chloro-5-(trifluoromethyl)phenyl]imino]-imidazolidine) hydrochloride were all synthesized at the Department of Medicinal Chemistry, H. Lundbeck A/S. Methyl phenidate was obtained from Ciba-Geigy and pergolide mesylate from Eli Lilly.

Receptor Binding Studies. DA D-1 Receptors. Inhibition of [^{3}H]SCH 23390 binding to DA D-1 receptors in rat striatal membranes was determined as described by Hyttel[34] and Hyttel and Arnt.[35]

DA D-2 Receptors. Inhibition of [^{3}H]spiperone binding to DA D-2 receptors in rat striatal membranes was determined as described by Hyttel.[36,37]

5-HT$_2$ Receptors. Inhibition of [^{3}H]ketanserin binding to 5-HT$_2$ receptors in membranes from rat cortex was determined as described by Hyttel.[37]

α_1 Adrenoceptors. Inhibition of [^{3}H]prazosin binding to α_1 adrenoceptors in membranes from rat brain was determined as described by Hyttel and Larsen,[38] and Skarsfeldt and Hyttel.[39]

Uptake Inhibition in Vitro. Inhibition of DA, NE, and 5-HT uptake in vitro was measured as previously described.[14]

In Vivo Neuropharmacology. Antagonism of Methyl Phenidate Induced Gnawing Behavior in Mice. The experiments were performed as described by Pedersen and Christensen.[40] Test compounds were injected sc 2 h before methyl phenidate (222 μmol/kg = 60 mg/kg, sc), and two mice were placed on corrugated cardboard in each gnawing cage (12 × 25 cm). Six to nine pairs of animals were used per dose. The ability to inhibit methyl phenidate induced gnawing was evaluated after 1 h by insepection of the corrugated cardboard.

Cataleptogenic Effect in Rats. Catalepsy was measured every hour 1–6 h after test drug administration on a vertical wire grid and defined as being present after at least 15-s immobility. The maximum effect between 1–6 h after administration is reported. A total of 8–12 animals were used per dose.

Locomotor Activity in Rats. Inhibition of locomotor activity was measured in photocell cages 2 h after administration of test compound or saline. Four to eight animals were used per dose.

Antagonism of Pergolide SK&F 38393 Induced Circling Behavior in Rats with Unilateral 6-OHDA Lesions. The experiments were performed as described by Arnt and Hyttel.[17] The experiments were done 2–9 months after lesioning when stable contralateral circling response to pergolide (0.05 μmol/kg = 0.02 mg/kg, sc) or SK&F 38393 (4.3 μmol/kg = 1.4 mg/kg, sc) were obtained.[17,41] The test compounds were injected 2 h before administration of pergolide or SK&F 38393. Antagonistic effects were calculated as percent inhibition of control responses for each rat. Four to eight animals were used per dose.

Antagonism of Quipazine-Induced Head Twitches in Rats. The experiments were performed as described by Arnt et al.[12] Test compounds were injected 2 h before quipazine (15 μmol/kg = 6.8 mg/kg, sc). Head twitches were counted 30–40 min after quipazine treatment. The rats were individually placed in perspex boxes (12 × 25 cm) for observation. Four to eight animals were used at each dosage. Inhibition of quipazine-induced head twitches was expressed in percent of the number of head twitches in a parallel control group.

Inhibition of St 587 Induced Flexor Reflex in Pithed Rats. The rats were treated with reserpine (16 μmol/kg = 10 mg/kg, sc) 24 h before the experiment. Nialamide (170 μmol/kg = 50 mg/kg, sc) was injected 1 h before the injection of St 587 (38 μmol/kg = 10 mg/kg, sc). Contractions of the tibial muscle were evoked by electrical stimulations. The test substance was injected iv in the jugular vein in doses which were increased every 20 min with a factor of 2. Three animals were used and the inhibition was measured and expressed as percent inhibition of the predrug level. For further details see Skarsfeldt and Hyttel.[39]

Acknowledgment. We thank the staff of the Research Departments at H.Lundbeck A/S for excellent technical assistance. The computational chemistry work was supported by grants (to T. Liljefors) from the Swedish Science Research Council, which is gratefully acknowledged.

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