The 2-aminotetralin system as a structural base for new dopamine- and melatonin-receptor agents
Copinga, Swier

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CHAPTER 5

8-METHOXY-2-AMIDOTETRALINS:
NONINDOLIC MELATONIN-RECEPTOR AGONISTS*

5.1 INTRODUCTION

Considerable effort has been devoted to understand the mechanism of action of
melatonin (1, N-acetyl-5-methoxytryptamine) since the isolation and chemical
identification of this hormone by Lerner and colleagues in the late 1950's [1,2].
Nowadays, melatonin is known to play a key role in the transduction of photoperiodic
information and to modulate a variety of endocrinological, neurophysiological, and
behavioural functions in vertebrates (see 4.2). However, the understanding of the target
sites, which mediate the various actions of melatonin, has been hampered by the lack of
the necessary tools to study and manipulate these sites. A great step forward in this area
of melatonin research has been made by the recent introduction of 2-[125I]iodo-
melatonin, a high affinity radioligand for melatonin binding sites [3-7], and the
development of quantitative in vitro melatonin receptor-linked bioassays [8-14]. These
tools have led to the localization of specific melatonin receptors in discrete regions of
vertebrate brain, retinas, and pituitary, and a preliminary pharmacological charac-
terization of these melatonin receptors by the use of melatonin and simple analogues of
this hormone (for reviews, see ref. 15-20; see 4.4). Additionally, these tools have
permitted the investigation of the structure-activity-relationships of known melatonin-
receptor agents and the development of novel melatonin-receptor agonists and
antagonists (see 4.5).

Currently, most of the known melatonin-receptor agonists are derivatives of
melatonin itself, as shown in Chart 5.1 [5,7,9,18,21-29]. They all contain as essential
moieties the amide function and the 5-methoxyindole ring system. Recently, however,
potent melatonin-receptor agonists with a naphthalene nucleus instead of an indole
nucleus were described (see 4.5) [30,31]. On the other hand, derivatives of melatonin
lacking the 5-methoxy group, as shown in Chart 5.1, appear to be melatonin-receptor
partial agonists or antagonists [9,32-36]. On the basis of these findings Dubocovich
concluded that the N-acetyl group of melatonin is primarily responsible for its affinity
at melatonin receptors and the 5-methoxy group of melatonin for its intrinsic activity at
these receptors [9,15].

Obviously, to characterize melatonin receptors further and to elucidate the mode of
action of melatonin better, melatonin-receptor agents from other chemical classes than
melatonin are needed as pharmacological tools. In addition, these agents may give a

* This chapter is partially based on:
beneficial impulse to the development of therapeutic agents, which act specifically through melatonin receptors. The therapeutic value of these agents will almost certainly include the synchronization of disrupted circadian rhythms (see 4.3). In view of the need just mentioned, we initiated a study with the goal to develop melatonin-receptor agonists and antagonists from another chemical class than melatonin itself.

5.2 8-METHOXY-2-(ACETAMIDO)TETRALIN AND ANALOGUES: POTENTIAL MELATONIN-RECEPTOR AGONISTS

Due to the successful implementation of the rigid analogue approach in the development of agents, which are active at various hormone and neurotransmitter receptors, we applied this strategy to develop compounds, which are active at melatonin-receptors but belong to another chemical class than melatonin itself. The rigid analogue approach involves the creation of active, conformationally restricted, i.e. (semi-)rigid, analogues of biologically active substances, which possess a high degree of conformational freedom. Subsequently, these rigid analogues are used to gain more information about the mode of action of the biologically active substances [37].
Particularly, (semi-)rigid compounds, incorporating the essential moieties of the flexible melatonin structure [38] in such a way that they can be superimposed on these moieties of melatonin, when it adopts a low-energy conformation, may be valuable melatonin-receptor agents. By examining the structure-activity-relationships of these (semi-)rigid compounds, the restrictions and requirements of the active site of a melatonin receptor can be explored. Consequently, a pharmacophoric model for the mode of interaction of melatonin with its receptor can be deduced. In addition, due to their conformational restraint, these (semi-)rigid compounds may show selectivity for a certain melatonin-receptor subtype, and possibly, may mimic only a few of the many actions of melatonin. Although there is not yet evidence for the existence of melatonin-receptor subtypes on the basis of molecular cloning, according to Dubocovich pharmacological considerations support at least the presence of two subtypes of melatonin binding sites, ML-1 and ML-2 (see 4.4.3) [15,18].

The successful use of the tetralin system as a structural base for the development of dopamine- and serotonin-receptor agonists [39-45] prompted us to take this semi-rigid system as a template for the development of conformationally restricted melatonin-receptor agonists. Especially, the discovery of 8-hydroxy-2-(N,N-di-n-propylamino)-tetralin (8-OH-DPAT, Chart 5.2) as a potent, selective 5-HT\textsubscript{1A}-receptor agonist was of interest to us [44-49]. This finding indicated that it is not always necessary to incorporate all the functional groups of a biologically active substance in a rigid compound to mimic some of its activities. In 8-OH-DPAT, the heterocyclic part of the indole nucleus of serotonin is omitted with retention of serotonin-receptor activity. Keeping this in mind, we prepared, on the basis of the similarities and differences between the chemical structures of serotonin (5-HT) and melatonin (1, ML), 8-methoxy-2-(acetamido)tetralin (18, 8-MeO-AAT)\textsuperscript{*} as a potential melatonin-receptor agonist (Chart 5.2) [50].

\begin{center}
\includegraphics[width=\textwidth]{chart52.png}
\end{center}

\textit{Chart 5.2} 8-Methoxy-2-(acetamido)tetralin (8-MeO-AAT, 18): a potential melatonin-receptor agonist.

\textsuperscript{*} Previously described as intermediate in the synthesis of \alpha\textsubscript{-}adrenergic agents [51].
The distance between the methoxy group and the amide function in 8-methoxy-2-(acetamido)tetralin (18) is comparable to the distance between these moieties in the conformation of melatonin (1), as drawn in Chart 5.2. This particular conformation of melatonin with the ethylamido side chain in a coplanar position and pointing in an upwards direction may be a biologically active conformer of melatonin due to its resemblance to a conformation of serotonin, proposed to be biologically active [45]. Moreover, this serotonin conformer mimics very well the very rigid structure of d-lysergic acid diethylamide (d-LSD), a compound possessing serotonin-receptor activity (Chart 5.3) [45].

![Chart 5.3](image)

Chart 5.3 The proposed biological conformation of serotonin (5-HT) mimicking the structure of d-LSD.

Considering the known structure-activity-relationships of indolic melatonin-receptor agents (see 4.5) and 8-OH-DPAT analogues [47,49,52], we used 8-methoxy-2-(acetamido)tetralin (18) as a starting-point for a series of 2-amidotetralins with potential melatonin-receptor agonist properties [50]. Their synthesis and pharmacological evaluation are described in the next sections. For comparative purposes, a series of nonrigid analogues of 8-methoxy-2-(acetamido)tetralin (18), i.e. phenethylamides, was also prepared.

5.3 SYNTHESIS OF 8-METHOXY-2-(ACETAMIDO)TETRALIN AND ANALOGUES

5.3.1 8-METHOXY-2-(ACETAMIDO)TETRALIN AND METHOXY ANALOGUES

8-Methoxy-2-(acetamido)tetralin (18) [51] and its methoxy analogues 5- (21) [51], 6- (20), and 7-methoxy-2-(acetamido)tetralin (19) were prepared according to known procedures, as outlined in Scheme 5.1. Briefly, the appropriate dihydroxynaphthalenes 22a-22d were methylated and subsequently reduced according to a modification of the method of Cornforth and colleagues to give the corresponding methoxy-2-tetralones 24a-24d upon acidic hydrolysis [53,54]. These tetralones 24a-24d yielded in turn the corresponding methoxy-2-aminotetralins 26a-26d after condensation with benzylamine, a catalytic hydrogenation, and a catalytic debenzylation [40,42,55]. Ultimately, the preparation of the methoxy-2-(acetamido)tetralins 18-21 involved the acetylation of these methoxy-2-aminotetralins 26a-26d utilizing acetic anhydride in the presence of sodium acetate and the biphasic medium water/ethylacetate.
In addition to racemic 8-methoxy-2-(acetamido)tetralin ((±)-18), both optical isomers were prepared by using the pure enantiomers of 8-methoxy-2-amino-tetralin (26a). Acetylation of (2R)-(+) -8-methoxy-2-aminotetralin ((+)-26a) yielded (+)-8-methoxy-2-(acetamido)tetralin ((+)-18), whereas acetylation of (2S)-(−)-8-methoxy-2-aminotetralin ((−)-26a) resulted in (−)-8-methoxy-2-(acetamido)tetralin ((−)-18). Accepting the view that no inversion occurs at C-2 of the tetralin system during this acetylation, (+)- and (−)-8-methoxy-2-(acetamido)tetralin ((+) and (−)-18) possess as absolute configuration the 2R and 2S configuration, respectively. However, these absolute configurations have yet to be experimentally determined by single crystal X-ray analysis.

5.3.2 8-METHOXY-2-AMIDOTETRALINS

Analogues of 8-methoxy-2-(acetamido)tetralin (18), in which the N-acetyl group is replaced by other N-acyl groups (27-38), were prepared by various methods. The preparation of most of these 8-methoxy-2-amidotetralins involved the acylation of the primary amine 26a, as outlined in Scheme 5.2. These acylations were accomplished in three different ways. First, the appropriate anhydride was used in the presence of sodium acetate and the biphasic medium water/ethyl acetate (method A). Second, the appropriate acyl chloride in the presence of sodium hydroxide and the biphasic medium
Method A: Method B: Method C:

Scheme 5.2 Reagents: (a) RCO\(_2\)O, CH\(_3\)COONa; (b) RCOCl, NaOH; (c) RCO\(_2\)O, (C\(_2\)H\(_5\))\(_3\)N; (d) NaI; (e) KF, 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (Kryptofix\textsuperscript{2.2.2}).

<table>
<thead>
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Additionally, 8-methoxy-2-(iodoacetamido)tetralin (35) and 8-methoxy-2-(fluoroacetamido)tetralin (36) were prepared from 8-methoxy-2-(bromoacetamido)tetralin (34) by Finkelsteins halogen exchange reactions, as outlined in Scheme 5.2 [56]. These aliphatic nucleophilic substitution reactions are both equilibrium reactions, which can be shifted to the desired direction. Thus, in the case of the exchange of bromine for iodine, this was done by taking advantage of the fact that sodium iodide, but not sodium bromide is soluble in acetone. When 8-methoxy-2-(bromoacetamido)tetralin (34) was treated with a solution of sodium iodide in acetone, the equilibrium was shifted to the direction of 8-methoxy-2-(iodoacetamido)tetralin (35) by precipitation of sodium bromide [57]. The exchange of bromine for fluorine was executed by the use of potassium fluoride in the presence of 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (Kryptofix\textsuperscript{2.2.2}) as phase transfer catalyst and acetonitrile as solvent. The phase transfer catalyst makes potassium fluoride soluble in acetonitrile by "capturing" the potassium ion, and consequently, increases greatly the reaction rate of the so-called "naked" fluoride ion as a nucleophile [56]. Due to the fact that 8-methoxy-2-(fluoroacetamido)tetralin (36), once formed, has little tendency to react, owing to the extremely poor leaving-group ability of fluorine, the halogen exchange reaction was shifted to the direction of the desired product.
5.3.3 N-SUBSTITUTED 8-METHOXY-2-AMIDOTETRALINS

N-n-Propyl (40,41) and N-benzyl analogues (42,43) of 8-methoxy-2-(acetamido)tetralin (18) and 8-methoxy-2-(propionamido)tetralin (27) were prepared according to known procedures, as outlined in Scheme 5.3. In brief, 8-methoxy-2-tetralone (24a) (see 5.3.1 and Scheme 5.1) was converted to 8-methoxy-2-(N-n-propylamino)tetralin (39) and 8-methoxy-2-(N-benzylamino)tetralin (25a) through condensation with n-propylamine and benzylamine, respectively, followed by catalytic hydrogenation [40, 42,55]. Subsequently, these secondary amines 39 and 25a were acylated utilizing acetic or propionic anhydride in the presence of sodium acetate and the biphasic medium water/ethylacetate (method A) to yield 8-methoxy-2-(N-n-propylacetamido)tetralin (40), 8-methoxy-2-(N-n-propylpropionamido)tetralin (41), 8-methoxy-2-(N-benzylacetamido)tetralin (42) and 8-methoxy-2-(N-benzylpropionamido)tetralin (43).

\[
\begin{align*}
\text{24a} & \quad \xrightarrow{a,b} \quad \text{39} \quad R^1 = n-C_3H_7 \\
\text{25a} & \quad \xrightarrow{c} \quad \text{40} \quad R^1 = n-C_3H_7, \quad R^2 = CH_3 \\
\text{41} & \quad R^1 = n-C_3H_7, \quad R^2 = C_2H_5 \\
\text{42} & \quad R^1 = CH_2Ph, \quad R^2 = CH_3 \\
\text{43} & \quad R^1 = CH_2Ph, \quad R^2 = C_2H_5
\end{align*}
\]

Scheme 5.3 Reagents: (a) R\textsuperscript{1}NH\textsubscript{2}, p-TsOH\cdot H\textsubscript{2}O; (b) PtO\textsubscript{2}, H\textsubscript{2}; (c) (R\textsuperscript{2}CO)\textsubscript{2}O, CH\textsubscript{3}COONa.

5.3.4 2-AMIDOTETRALINS AND 8-SUBSTITUTED 2-(ACETAMIDO)TETRALINS

Analogues of 8-methoxy-2-(acetamido)tetralin (18) with a hydrogen (45-50) or another group at the 8-position (51,53-55) instead of the 8-methoxy group were prepared by various methods. Those analogues, missing the 8-methoxy group, i.e. 2-amidotetralins (45-50), were prepared directly from the commercially available primary amine 2-aminotetralin (44) by the acylation methods A and B, as outlined in Scheme 5.4 (see 5.3.2).

\[
\begin{align*}
\text{44} & \quad \xrightarrow{a \text{ or } b} \quad \text{45-50} \\
\end{align*}
\]

Scheme 5.4 Reagents: (a) (RCO)\textsubscript{2}O, CH\textsubscript{3}COONa; (b) RCOCl, NaOH.

Preparation of 8-hydroxy-2-(acetamido)tetralin (51) involved the demethylation of 8-methoxy-2-(acetamido)tetralin (18) by using boron tribromide in dichloromethane, as outlined in Scheme 5.5 [58]. This 8-hydroxy compound 51 was used as starting material.
for the preparation of 8-benzyloxy-2-(acetamido)tetralin (54) and 8-trifluoromethane-
 sulfonyloxy-2-(acetamido)tetralin (55), as outlined in Scheme 5.5. The conversion to 8-
benzyloxy-2-(acetamido)tetralin (54) was accomplished by alkylation with benzyl 
chloride in the presence of cesium carbonate [59] and the conversion to 8-trifluoro-
methanesulfonyloxy-2-(acetamido)tetralin (55) by triflation with trifluoromethane-
sulfonic anhydride in the presence of pyridine [60].

8-Ethoxy-2-(acetamido)tetralin (53) was prepared from 8-ethoxy-2-aminotetralin 
(52) by acetylation, employing acetic anhydride in the presence of sodium acetate and 
the biphasic medium water/ethylacetate, as outlined in Scheme 5.5. The preparation of 
8-ethoxy-2-aminotetralin (52) involved the same sequence of synthetic steps as the 
preparation of 8-methoxy-2-aminotetralin (26a) (see 5.3.1 and Scheme 5.1). The only 
difference was that the diethylation of 1,7-dihydroxynaphthalene (22a) with diethyl 
sulfate instead of a dimethylation with dimethyl sulfate.

5.3.5 5-/7-SUBSTITUTED 8-METHOXY-2-(ACETAMIDO)TETRALINS

Analogues of 8-methoxy-2-(acetamido)tetralin (18) substituted at the 5-position 
(56-60) or the 7-position (61,62) were prepared by various methods. Four of the five 
5-substituted 8-methoxy-2-(acetamido)tetralins (56-59) were prepared directly from 8-
methoxy-2-(acetamido)tetralin (18), as outlined in Scheme 5.6. The preparation of 5-
chloro-8-methoxy-2-(acetamido)tetralin (56) involved an aromatic electrophilic chlori-
nation by the use of N-chlorosuccinimide in trifluoroacetic acid. Presumably, the 
chlorinating agent trifluoroacetyl hypochlorite is generated under these conditions. How-
However, trying to prepare 5-bromo-8-methoxy-2-(acetamido)tetralin (57) in the same way by the use of N-bromosuccinimide in trifluoroacetic acid always yielded the dibrominated product as a major by-product. Hence, 5-bromo-8-methoxy-2-(acetamido)tetralin (57) was prepared by a different method utilizing the brominating agent 2,4,4,6-tetrabromo-2,5-cyclohexadien-1-one in chloroform [61-63]. The conversion of 8-methoxy-2-(acetamido)tetralin (18) to 5-iodo-8-methoxy-2-(acetamido)tetralin (58) was accomplished by an aromatic electrophilic iodination with the very reactive iodinating reagent trifluoroacetyl hypoiodite, formed in situ from iodine and silver trifluoroacetate [51]. The preparation of 5-nitro-8-methoxy-2-(acetamido)tetralin (59) was first attempted by the use of a mixture of nitric acid with sulfuric acid. However, this attempt gave mainly dinitrated product. Ultimately, aromatic electrophilic nitration at the 5-position of 8-methoxy-2-(acetamido)tetralin (18) was achieved employing sodium nitrate in trifluoroacetic acid [64]. 5-Amino-8-methoxy-2-(acetamido)tetralin (60) was obtained by hydrogenation of 5-nitro-8-methoxy-2-(acetamido)tetralin (59) over palladium on carbon.

5-Bromo-8-methoxy-2-(acetamido)tetralin (57) was used as starting material for the preparation of 7-substituted 8-methoxy-2-(acetamido)tetralins (61,62), as outlined in Scheme 5.7. Nitration, followed by catalytic hydrogenation yielded 7-amino-8-methoxy-2-(acetamido)tetralin (61) [64,65]. This compound 61 was converted to 7-chloro-8-methoxy-2-(acetamido)tetralin (62) by diazotization with sodium nitrite in dilute aqueous hydrochloric acid and subsequent replacement of the diazonium group by chlorine in the presence of copper(I) chloride as catalyst by the method of Sandmeyer [59].

Scheme 5.6 Reagents: (a) N-chlorosuccinimide, CF₃COOH; (b) 2,4,4,6-tetrabromo-2,5-cyclohexadien-1-one, CHCl₃; (c) I₂, CF₃COOAg; (d) NaN₃, CF₃COOH; (e) Pd-on-C (10%), H₂.

Scheme 5.7 Reagents: (a) NaNO₃, CF₃COOH; (b) Pd-on-C (10%), H₂; (c) NaNO₂, HCl; (d) CuCl, HCl.
5.3.6 PHENETHYLAMIDES

As outlined in Scheme 5.8, phenethylamides 67-72, considered to be ring-opened analogues of 8-methoxy-2-(acetamido)tetralin (18) and some corresponding compounds, were prepared from the appropriate phenethylamines 63-66 by acylation method A or B (see 5.3.2).

\[
\begin{array}{ccc}
R^1 & & R^1
\end{array}
\]

Scheme 5.8 Reagents: (a) (RCO)\textsubscript{2}O, CH\textsubscript{3}COONa; (b) RCOCl, NaOH.

5.4 PHARMACOLOGICAL EVALUATION OF 8-METHOXY-2-(ACETAMIDO)TETRALIN AND ANALOGUES

8-Methoxy-2-(acetamido)tetralin (18) and the above-prepared analogues 19-21, 27-38, 40-43, 45-51, 53-62, and 67-72 were evaluated for their in vitro affinities at melatonin receptors (see 4.4.3) by examining their abilities to compete for 2-[\textsuperscript{125}I]iodomelatonin binding to chicken retinal membranes (Tables 5.2 and 5.3). This radioligand binding assay was conducted essentially as reported by Dubocovich and Takahashi [5]. The HCl salt of 8-methoxy-2-aminotetralin (26a) was also tested in this radioligand binding assay (\(K_i > 100000\) nM). Additionally, the in vitro melatonin-receptor affinities of some other analogues of 8-methoxy-2-(acetamido)tetralin (18), whose preparations are not described, i.e. cis-1-methyl-8-methoxy-2-(acetamido)tetralin (73),\* trans-1-methyl-8-methoxy-2-(acetamido)tetralin (74),\* 5-methoxy-3-(acetamido)chromane (75),** and 5-methoxy-3-(propionamido)chromane (76),** were determined in this radioligand binding assay (Table 5.2). For comparative purposes, the abilities of melatonin (1) and the analogous tryptamides 2, 3, 5, 7, 14, and 77*** to compete for 2-[\textsuperscript{125}I]iodomelatonin binding to chicken retinal membranes were also examined (Table 5.1).

Furthermore, some of these amides were evaluated for their in vitro potencies at melatonin receptors by determining their abilities to inhibit the calcium-dependent

\* Prepared by Cor J. Grol.
\** Prepared by Gert Barf.
\*** 77: N-acetyl-5-(trifluoromethanesulfonyloxy)tryptamine. Prepared by Swier Copinga (not described).

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release of $[^3]$H]dopamine from rabbit retina via activation of presynaptic melatonin heteroreceptors (Tables 5.1, 5.2, and 5.3) (see 4.4.3), as described by Dubocovich [8,9,33].

Table 5.1 Pharmacological evaluation of melatonin and analogous tryptamides. \(^a\)Competition for $[^{125}]$iodomelatonin binding to chicken retinal membranes by various concentrations (0.1 nM - 0.1 mM) of the test compounds. \(^b\)K$_i$ values were calculated from IC$_{50}$ values obtained from competition curves by the method of Cheng and Prusoff [81]. Results are mean values of at least three independent determinations in duplicate. \(^c\)Relative affinities of the test compounds to compete for $[^{125}]$iodomelatonin in chicken retina (K$_i$ / K$_{x}$). \(^d\)Inhibition by various concentrations of the test compounds (1 pM - 1 µM) of the calcium-dependent release of $[^3]$H]dopamine from rabbit retina. \(^e\)IC$_{50}$ values were determined graphically from concentration-effect curves. \(^f\)Relative potencies of the test compounds to inhibit calcium-dependent $[^3]$H]dopamine release from rabbit retina (IC$_{50}$ / IC$_{50}$x). \(8\)ML, melatonin (1).

\(^{h}\)See ref. 5,9,34. \(^{i}\)ND, not determined. \(\text{JTF, trifluoromethanesulfonyl} (\text{CF}_3\text{SO}_2)\).

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Table 5.2  Pharmacological evaluation of 2-amidotetralins. For explanations, see Table 5.1. IM, melatonin (1). NE, not effective. ND, not determined. JPh, phenyl. TF, trifluoromethanesulfonyle (CF3SO2). HCl salt.

![Chemical structure of 2-amidotetralins](image)

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Table 5.3 Pharmacological evaluation of phenethylamides. a-f For explanations, see Table 5.1. bML, melatonin (1). hNE, not effective. iND, not determined.

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5.5 DISCUSSION

Evaluation of the melatonin-receptor agonist properties of 8-methoxy-2-(acetamido)tetralin (18) revealed that it competes for 2-[125I]iodomelatonin binding to chicken retinal membranes with a $K_i$ value of 46 nM, and inhibits the calcium-dependent release of $[^3H]$dopamine from rabbit retina with an $IC_{50}$ value of 1.4 nM with a maximal inhibitory effect of 80% at 1 mM [50]. Compared with the properties of melatonin (1) ($K_i = 0.57$ nM, $IC_{50} = 17$ pM with maximal inhibition of 80% at 1 nM [8]), 8-methoxy-2-(acetamido)tetralin (18) displays an 80-fold lower melatonin-receptor affinity and also possesses an 80-fold lower melatonin-receptor potency. However, it reaches the same maximal inhibitory effect as melatonin in the $[^3H]$dopamine release assay. Thus, 8-methoxy-2-(acetamido)tetralin (18) is a melatonin-receptor agonist of moderate potency, displaying still full intrinsic activity.

Evaluation of the melatonin-receptor affinities of both optical isomers of 8-methoxy-2-(acetamido)tetralin (18) revealed that the (-)-enantiomer ((-)18) displays the same melatonin-receptor affinity as the racemic compound ((+)-18), whereas the (+)-enantiomer ((+)18) displays about a 10-fold lower melatonin-receptor affinity than the racemic compound ((±)-18). Hence, we suggest that the melatonin-receptor agonist properties of 8-methoxy-2-(acetamido)tetralin (18) mainly reside in the (-)-enantiomer.

By making slight alterations to the structure of 8-methoxy-2-(acetamido)tetralin (18), we attempted to find good melatonin-receptor agonists in this series of conformationally restricted, nonindolic compounds. The importance of the 8-methoxy substituent at the aromatic nucleus of 8-methoxy-2-(acetamido)tetralin (18) for its melatonin-receptor agonist properties was investigated by changing the position of the methoxy group at the aromatic nucleus, by deleting the methoxy group, and by substituting the 8-methoxy group with other groups.

By changing the position of the methoxy group we obtained analogues without [6-methoxy-2-(acetamido)tetralin, 20], with low [5-methoxy-2(acetamido)tetralin, 21], and with moderate [7-methoxy-2-(acetamido)tetralin, 19] melatonin-receptor affinities and potencies. These results can well be accounted for, when we assume that a biologically active conformation of melatonin is the one in which the flexible side chain is folded in the same way as the flexible side chain in the conformation of serotonin (5-HT), mimicking the very rigid structure of the serotonin-receptor agent d-lysergic acid diethyl-amide (d-LSD) (see Chart 5.3) [45]. Superimposing the acetarnido groups of the methoxy-2-(acetamido)tetralins 18-21 on this conformation of melatonin, as shown in Figure 5.1, makes it clear that the 7-methoxy analogue 19 and the 8-methoxy analogue 18, which have the highest melatonin-receptor affinities and potencies of these four analogues, give also the best fits with regard to the methoxy group.

Removal of the 8-methoxy group led to 2-(acetamido)tetralin (45), an analogue displaying an almost 15-fold lower affinity ($K_i = 660$ nM) than 8-methoxy-2-(acetamido)tetralin (18) in the 2-[125I]iodomelatonin-binding assay and possessing a 37-fold lower
potency ($IC_{50} = 52$ nM) than 8-methoxy-2-(acetamido)tetralin (18) in the $[^{3}H]$-dopamine-release assay. Since 2-(acetamido)tetralin (45) reaches only a maximal inhibition of 50% in the latter assay [50], the intrinsic activity of 2-(acetamido)tetralin (45) is less than 65% of that of melatonin (1) and 8-methoxy-2-(acetamido)tetralin (18).

On the basis of these melatonin-receptor agent properties of 8-methoxy-2-(acetamido)tetralin (18) and 2-(acetamido)tetralin (45), we conclude that for the 2-amidotetralins the methoxy group is more important for melatonin-receptor potency/intrinsic activity than for melatonin-receptor affinity. This conclusion agrees well with that drawn from prior studies concerning indolic melatonin-receptor agents (see Chart 5.1), especially N-acetyltryptamine (15) [5,9,32].

Substitution of the 8-methoxy group of 8-methoxy-2-(acetamido)tetralin (18) with other groups, like hydroxy (51), ethoxy (53), benzyloxy (54) and trifluoromethanesulfonyloxy (55) resulted in analogues, which possess much lower affinities in the 2-$[^{125}I]iodomelatonin-binding assay than the parent compound 8-methoxy-2-(acetamido)tetralin (18). Especially, the total loss of melatonin-receptor affinity of 8-hydroxy-2-(acetamido)tetralin (51) is remarkable. These observations correspond very well with the low melatonin-receptor activities in several assays of melatonin analogues in which the 5-methoxy group is replaced by another group [5,9,21,23,26,32,66]. Hence, we propose that the size and/or the electronic properties of the substituent at the 8-position of the 2-amidotetralins is/are very critical for melatonin-receptor agonist properties, as suggested for the substituent at the 5-position of indolic melatonin-receptor agents (see 4.5.2). In this respect, a methoxy group at the 8-position of the 2-
amidotetralins as well as at the 5-position of the tryptamides plays most likely a role as hydrogen-bond acceptor in the interaction with the mammalian retinal melatonin receptor. Notably, the substitution of the methoxy group with the trifluoromethanesulfonyloxy group in the series of the 2-amidotetralins [8-methoxy-2-(acetamido)tetralin (18) vs. 8-trifluoromethanesulfonyloxy-2-(acetamido)tetralin (55)] has relatively much less influence on the melatonin-receptor affinity than the same substitution executed on melatonin [melatonin (1) vs. N-acetyl-5-(trifluoromethanesulfonyloxy)tryptamine (77)]. An explanation for this difference is yet difficult to give.

The influence of the acetamido group of 8-methoxy-2-(acetamido)tetralin (18) on its melatonin-receptor agonist properties was investigated by deleting its N-acetyl group, giving 8-methoxy-2-aminotetralin (26a), or substituting the methyl group of its N-acetyl group by another alkyl or aryl group.

The HCl salt of 8-methoxy-2-aminotetralin (26a) failed to compete for 2-[125I]-iodomelatonin binding to chicken retinal membranes \( (K_i > 100000 \, \text{nM}) \). Therefore, we conclude that in the series of 8-methoxy-2-amidotetralins, like in the series of the 5-methoxytryptamides, the N-acetyl group is very important for binding to a melatonin receptor [5,9,32].

N-propionyl-5-methoxytryptamine (2) and N-n-butyryl-5-methoxytryptamine (3) are potent melatonin-receptor agonists, whereas 5-methoxytryptamides with longer N-acyl groups are almost inactive at melatonin receptors (see 4.5.2) [21,23,26,67]. Therefore, we substituted the methyl group of the N-acetyl group of 8-methoxy-2-(acetamido)tetralin (18) by an ethyl (27) and a n-propyl group (28). Of these three analogues, the N-propionyl analogue 27 shows the highest potency in the \( [3^H] \)-dopamine-release assay (IC\(_{50} = 0.48 \, \text{nM}; 30\)-fold lower potency than melatonin) and the N-n-butyryl analogue 28 shows the highest affinity in the 2-[125I]-iodomelatonin-binding assay \( (K_i = 3.6 \, \text{nM}; 6\)-fold lower affinity than melatonin). Substitution of the methyl group of the N-acetyl group of 8-methoxy-2-(acetamido)tetralin (18) by a more bulky alkyl group, like iso-propyl (29) or cyclopropyl (30), resulted in analogues, which have moderate melatonin-receptor affinities and potencies. Substitution of the methyl group by an aryl group, like phenyl (31) or benzyl (32), led to analogues without or with very low melatonin-receptor affinities. These results are in good agreement with those found by Frohn and colleagues for 5-methoxytryptamides [23]. They showed that substitution of the methyl group of the N-acetyl group of melatonin by a bulky alkyl group, like tert-butyl, or an aryl group, like phenyl, led to compounds, which could not mimic the activity of melatonin in their in vivo bioassay (see 4.5.2).

Other substitutions involved the replacements of the methyl group of the N-acetyl group of 8-methoxy-2-(acetamido)tetralin (18) by a chloromethyl (33), a bromomethyl (34), an iodomethyl (35), a fluoromethyl (36), a trifluoromethyl (37) and a pentafluoroethyl group (38). Of these analogues, the 8-methoxy-2-(haloacetamido)tetralins 33-35 display higher affinities in the 2-[125I]-iodomelatonin-binding assay than 8-methoxy-2-
(acetamido)tetralin (18). Particularly, 8-methoxy-2-(chloroacetamido)tetralin (33) competes for 2-[\textsuperscript{125}I]iodomelatonin binding to chicken retinal membranes with almost the same $K_i$ value ($K_i = 3.7$ nM; 6.5-fold lower affinity than melatonin) as 8-methoxy-2-(n-butyrylamido)tetralin (28). Additionally, it inhibits the calcium-dependent release of $[^3\text{H}]$dopamine from rabbit retina with an $IC_{50}$ value of 0.063 nM, which is almost the same potency as that of melatonin ($IC_{50} = 0.017$ nM). Possibly, we introduced with this substitution a chemically reactive, electrophilic moiety into the 8-methoxy-2-amidotetralin system, which may form a covalent bond with a nucleophile near the active site of the melatonin binding site, resulting in irreversible attachment. Recently, however, Sugden and colleagues showed that a potent analogue of melatonin possessing the same sort of chemically reactive group, i.e. N-bromoacetyl-5-methoxytryptamine (4), is not an irreversible-binding melatonin-receptor agent [67]. In addition, an irreversible-binding agent acts in general as an antagonist, and not as a potent agonist. Hence, the 8-methoxy-2-(haloacetamido)tetralins 33-35 might be normal melatonin-receptor agonists of high potency. In accordance with this suggestion, a preliminary in vivo study revealed that 8-methoxy-2-(chloroacetamido)tetralin (33) possesses the ability to entrain the rat pineal melatonin production, similar to melatonin (see 4.2) [Drijfhout WJ (1993): personal communication].

Some of the above substitutions were also carried out on 2-(acetamido)tetralin (45). The 2-amidotetralins, which were obtained (46-50), showed the same tendencies in their affinities for the retinal melatonin binding site as the analogous 8-methoxy-2-amidotetralins (27, 28, 31-33).

Based on all these substitutions, we conclude that 8-methoxy-2-amidotetralins as well as 2-amidotetralins require a small, non-branched acyl group, like acetyl, haloacetyl, propionyl or n-butyryl, to display optimal melatonin-receptor agent properties.

To investigate the importance of the hydrogen atom of the secondary amide function of 8-methoxy-2-(acetamido)tetralin (18) and 8-methoxy-2-(propionamido)tetralin (27) for their melatonin-receptor agonist properties, we prepared the N-n-propyl (40,41) and N-benzyl analogues (42,43) of 8-methoxy-2-(acetamido)tetralin (18) and 8-methoxy-2-(propionamido)tetralin (27). These analogues with a tertiary amide function (40-43) show much lower affinities in the 2-[\textsuperscript{125}I]iodo-melatonin-binding assay than the secondary analogues (18,27). These low melatonin-receptor affinities coincide very well with the low melatonin-receptor potency of the N-methyl analogue of melatonin (1) (see 4.5.2) [68]. Based on these results we suggest that a hydrogen bond between the hydrogen atom of a secondary amide function and a hydrogen-bond acceptor of the retinal melatonin binding site could play a role in the interaction of 8-methoxy-2-amidotetralins as well as 5-methoxytryptamides with this melatonin binding site.

In correspondence with the idea behind the development of 6-halogenated analogues (10-14) of melatonin, i.e. the development of melatonin-receptor agonists with greater resistance to metabolism than melatonin itself (see 4.5.2) [22,69,70], we prepared 5-substituted (56-60) and 7-substituted analogues (61,62) of 8-methoxy-2-(acetamido)-
tetralin (18). Most of these analogues display moderate affinities ($100 < K_i < 300$ nM) in the $2\cdot^{125}$Iiodomelatonin-binding assay. Based on the comparison of the melatonin-receptor affinities of the 5-substituted analogues (56-60) of 8-methoxy-2-(acetamido)-tetralin (18), we propose that there is no simple relation between the electronic properties of the substituent at the 5-position and its influence on the melatonin-receptor affinity of a 5-substituted analogue of 8-methoxy-2-(acetamido)-tetralin (18).

The finding that 7-chloro-8-methoxy-2-(acetamido)tetralin (62) shows a lower melatonin-receptor affinity ($K_i = 220$ nM) than 8-methoxy-2-(acetamido)tetralin (18) ($K_i = 46$ nM) is remarkable. Due to the fact that 6-chloromelatonin (11) shows a higher affinity in several 2-$^{125}$Ijodo-melatonin-binding assays than melatonin (1) [see 4.4.3 (Table 4.1)], it was expected that 7-chloro-8-methoxy-2-(acetamido)tetralin (62), which possesses the same aromatic substituents as 6-chloromelatonin in similar positions, would display a higher melatonin-receptor affinity than 8-methoxy-2-(acetamido)-tetralin (18). The reason for this discrepancy is not totally clear. However, it could mention that 8-methoxy-2-(acetamido)tetralin (18) mimics a conformation of melatonin, which differs from the one shown in Figure 5.1.

Another interesting finding is the low affinity of 7-amino-8-methoxy-2-(acetamido)tetralin (61) in the $^{125}$Iiodomelatonin-binding assay, whereas 5-amino-8-methoxy-2-(acetamido)tetralin (60) displays moderate affinity in this binding assay. A possible explanation for this finding is that the amine group of 7-amino-8-methoxy-2-(acetamido)tetralin (61) prevents the possible hydrogen-bond formation between the methoxy group and an hydrogen-bond donor of the retinal melatonin binding site through the formation of an internal hydrogen bond with the methoxy group.

Based on alterations made to the structure of 8-OH-DPAT, which are non-detrimental to its 5-HT$_{1A}$-receptor agonist activity [47,49,52], we prepared the 1-methyl-8-methoxy-2-(acetamido)tetralins, i.e. the cis-isomer (73) as well as the trans-isomer (74), and the 5-methoxy-3-amidochromanes, i.e. 5-methoxy-3-(acetamido)chromane (75) and 5-methoxy-3-(propionamido)chromane (76). *Cis* (73) and *trans*-1-methyl-8-methoxy-2-(acetamido)tetralin (74) appear to be good melatonin-receptor agonists in comparison to 8-methoxy-2-(acetamido)tetralin (18) itself. *Cis*-1-methyl-8-methoxy-2-(acetamido)tetralin (73) shows the highest affinity ($K_i = 2.4$ nM) of the tested analogues of 8-methoxy-2-(acetamido)tetralin (18) in the 2-$^{125}$Iiodomelatonin-binding assay, whereas *trans*-1-methyl-8-methoxy-2-(acetamido)tetralin (74) displays almost the highest potency ($IC_{50} = 0.50$) of the tested analogues of 8-methoxy-2-(acetamido)tetralin (18) in the $[^3]$Hdopamine-release assay. Thus, 1-methyl substitution is nondetrimental to the melatonin-receptor agonist properties of 8-methoxy-2-(acetamido)tetralin (18). A possible explanation for the finding that *cis*-1-methyl-8-methoxy-2-(acetamido)tetralin (73) possesses an almost 20-fold higher melatonin-receptor affinity than 8-methoxy-2-(acetamido)tetralin (18) could be that the *cis*-1-methyl group forces the 2-acetamido group into a certain conformation, which interacts well with the retinal melatonin-binding site.
The replacement of the 4-methylene group of the tetralin system of 8-methoxy-2-(acetamido)tetralin (18) or 8-methoxy-2-(propionamido)tetralin (27) by an oxygen atom resulted in analogues with much lower melatonin-receptor affinities and potencies than the parent compounds. Thus, this alteration to the tetralin system of 8-methoxy-2-amidotetralins has a negative influence on their melatonin-receptor agonist properties.

The importance of the semi-rigidity of 8-methoxy-2-(acetamido)tetralin (18) for its melatonin-receptor agonist properties was investigated by testing the flexible analogue N-acetyl-2-methoxyphenethylamine (68). This phenethylamide 68 shows a 9-fold lower affinity \(K_i = 420 \text{ nM}\) than 8-methoxy-2-(acetamido)tetralin (18) in the 2-[\(^{125}\text{I}\)]iodo-melatonin binding-assay and possesses a 2-fold lower potency \(I_{C_{50}} = 3.0 \text{ nM}\) than 8-methoxy-2-(acetamido)tetralin (18) in the \([\text{H}]\)dopamine-release assay. An explanation for the lower melatonin-receptor affinity and potency of this ring-opened analogue of 8-methoxy-2-(acetamido)tetralin (18) could be that internal hydrogen bonding between the hydrogen atom of the amide function and the oxygen atom of the methoxy group alters the conformation of the side chain in such a way that this compound cannot mimic the active conformation of 8-methoxy-2-(acetamido)tetralin (18). A similar explanation has been proposed by Glennon to account for the lower serotonergic potency of 2-hydroxy-N,N-di-n-propylphenethylamine, a ring-opened analogue of 8-OH-DPAT, compared with that of 8-OH-DPAT [48]. Additionally, internal hydrogen bonding between the hydrogen atom of the amide function and the oxygen atom of the methoxy group of N-acetyl-2-methoxyphenethylamine (68) could prevent the formation of hydrogen bonds between this ring-opened analogue of 8-methoxy-2-(acetamido)tetralin (18) and the melatonin binding site with as result low melatonin-receptor agonist properties. Some of the alterations made to the structure of 8-methoxy-2-(acetamido)tetralin (18) were also applied to the structure of the ring-opened analogue 68, including deleting the methoxy group (67), changing the position of the methoxy group at the aromatic nucleus (71,72) and substituting the methyl group of the N-acetyl group by an ethyl group (69) or a n-propyl group (70). Also in these flexible phenethylamines a well-positioned methoxy group and a small acyl group are structural requirements for optimal melatonin-receptor agonist properties.

In summary, the amide function of 2-amidotetralins must possess a small, non-branched (halo)alkyl group and must be of the secondary amide type in order to allow these compounds to bind with high affinity at the mammalian retinal melatonin receptor. Likewise, the tetralin system of 2-amidotetralins must contain a methoxy group at the 8-position in order to allow these compounds to show maximal agonistic activity at this melatonin receptor. Additionally, some substitutions at the 5- as well as the 7-position of the tetralin system are allowed without losing much of the melatonin-receptor agonist properties of the 8-methoxy-2-amidotetralins. Moreover, a 1-methyl-substitution in the tetralin system gives rise to 8-methoxy-2-amidotetralins possessing better melatonin-receptor agonist properties than the parent compound 8-methoxy-2-(acetamido)tetralin (18).
The series of the 2-amidotetralins constitutes a class of conformationally restricted, nonindolic melatonin-receptor agents, which can be applied as pharmacological tools to further characterize melatonin receptors and to elucidate the mode of action of melatonin better. The parent compound 8-methoxy-2-(acetamido)tetralin (18) and some of its analogues, such as 8-methoxy-2-(propionamido)tetralin (27), 8-methoxy-2-(n-butyrylamido)tetralin (28), 8-methoxy-2-(haloacetamido)tetralins (33: Cl; 34: Br; 35: I), and 1-methyl-8-methoxy-2-(acetamido)tetralins (73: cis; 74: trans), which are melatonin-receptor agonists of moderate to high potency, can probably be used as lead compounds in further structure activity relationship studies and may play a role in the development of therapeutic agents, which interact specifically with melatonin receptors. However, one should keep in mind that almost all of the here presented 2-amidotetralins are racemic mixtures. Thus, to gain more detailed knowledge about the melatonin-receptor agent properties of these 2-amidotetralins, resolution of these 2-amidotetralins into pure enantiomers must be undertaken.

5.7 EXPERIMENTAL SECTION I (CHEMISTRY)

5.7.1 GENERAL ASPECTS

Melting points were determined in open glass capillaries on an Electrothermal digital melting-point apparatus and are uncorrected. IR spectra were recorded on a Philips PU 9706 spectrophotometer or on a Beckman AccuLab 2 spectrophotometer, and only the important absorptions are given. $^1$H NMR spectra were recorded on a 60 MHz Hitachi Perkin-Elmer R-24 B spectrometer, on a 200 MHz Varian Gemini 200 spectrometer, or on a 300 MHz Varian VXR-300 spectrometer. Chemical shifts are reported in $\delta$ units (parts per million) relative to (CH$_3$)$_4$Si as an internal standard or via $\delta$ CDCl$_3$ (7.24) or (CD$_3$)$_2$SO (2.49). $^{13}$C NMR spectra were recorded at 50 MHz on a Varian Gemini 200 spectrometer or at 75 MHz on a Varian VXR-300 spectrometer. Chemical shifts were obtained in $\delta$ units (parts per million) using the solvent as internal standard, related to (CH$_3$)$_4$Si, by using $\delta$ CDCl$_3$ (77.0) or (CD$_3$)$_2$SO (39.7). Chemical-ionisation (CI) mass spectra, using NH$_3$ as reactant gas, were obtained with a Finnegan 3300 system. Elemental analyses for new substances were performed at the Department of Chemistry, University of Groningen. Where elemental analyses are indicated, obtained results were within 0.4% of the theoretical values, except where noted. All mentioned yields are unoptimized.

5.7.2 PREPARATION OF 8-METHOXY-2-(ACETAMIDO)TETRALIN ((\pm), (+), AND (−)) AND METHOXY ANALOGUES

General method for synthesizing Dimethoxynaphthalenes 23a-23d

The method adopted for the synthesis of 1,7-dimethoxynaphthalene (23a) is described. Dimethyl sulfate (40 ml, 0.42 mol) was added at once to a vigorously stirred solution of 1,7-dihydroxynaphthalene (22a) (31.5 g, 0.197 mol) in 2N NaOH (180 ml). The temperature of the reaction mixture raised to 50 °C and the reaction mixture became acidic. Immediately 2N NaOH (135 ml) was added to obtain a basic reaction mixture followed by another amount of dimethyl sulfate (20 ml, 0.21 mol). The basic reaction mixture was stirred for 2 h at 55 °C and subsequently heated at reflux for 2 h. After
cooling, the reaction mixture was extracted with CH₂Cl₂ (3 x 150 ml). The CH₂Cl₂ layer was washed with 2N NaOH (3 x 75 ml) and a saturated aqueous solution of NaCl (1 x 75 ml) and dried over MgSO₄. After removal of the solvent under reduced pressure, the residual oil was purified on an neutral (Merck)/silica gel 60 (Merck) column with CH₂Cl₂ as the eluent to yield 30.1 g (0.160 mol, 81%) of dimethoxynaphthalene 23a as a colourless, viscous oil: bp 88-90 °C (0.01 mbar) [71] bp 123-130 °C (0.4 mmHg), [72] bp 124-127 °C (0.7 mmHg), solid at room temperature. ¹H NMR (60 MHz, CDCl₃) 2 8 (s, 3H, OCH₃), 3.9 (s, 3H, OCH₃), 6.6-7.8 (m, 6H, ArH).

Similarly, the dimethoxynaphthalenes 23b [mp 138-139 °C, EtOH [72] mp 136-137 °C, EtOH; [73] mp 138 °C, EtOH], 23c [mp 151-152 °C, EtOH [74] mp 149-150.5 °C, petroleum ether, [75] mp 153-154 °C], and 23d [mp 58-59 °C, EtOH [72] bp 123-126 °C (0.8 mmHg), solid at room temperature, [76] mp 60-61 °C, petroleum ether] were obtained from the dihydroxynaphthalenes 22b-22d.

**General Method for Synthesizing Methoxy-2-tetralones 24a-24d**

The method adopted for the synthesis of 8-methoxy-2-tetralone (24a) is described.

Dimethoxynaphthalene 23a (27.1 g, 0.144 mol) was added to boiling absolute EtOH (250 ml) under mechanical stirring. Sodium (25 g), cut in little pieces, was added as rapidly as possible (45 min) to the mechanically stirred solution. After addition of another amount of absolute EtOH (70 ml), refluxing was continued until all the sodium had disappeared (1 h). The reaction mixture was cooled to 10 °C and then 2N HCl (470 ml) was added dropwise until pH 6 was obtained (the colour of the reaction mixture changed from white to yellow). More 2N HCl (30 ml) was added and the reaction mixture was refluxed for 30 min. After cooling, the reaction mixture was extracted with Et₂O (125 ml) and the H₂O/EtOH layer was concentrated under reduced pressure until only H₂O remained. This H₂O layer was extracted with Et₂O (3 x 125 ml) and the Et₂O layers were combined. The resulting Et₂O layer was washed with a saturated aqueous solution of NaCl (3 x 75 ml) and dried over MgSO₄. After in vacuo evaporation of the Et₂O, a viscous, brown-orange oil was afforded. The crude oil was purified by vacuum distillation to yield 15.8 g (0.090 mol, 62%) of methoxy-2-tetralone 24a as a light yellow oil, which solidified on standing: bp 104-106 °C (0.02 mbar). Recrystallization from petroleum ether (bp 40-60 °C) gave methoxy-2-tetralone 24a as fine white needles: mp 56.5-57.5 °C [71] mp 58-59 °C, light petroleum; [72] bp 120-123 °C (1.0 mmHg), solid at room temperature; IR (cm⁻¹, neat) 2840 (OCH₃), 1715 (C=O), ¹H NMR (60 MHz, CDCl₃) 2 2.5 (t, 2H, CH₂), 3.0 (t, 2H, CH₂), 3.4 (s, 2H, CH₂), 3.8 (s, 3H, OCH₃), 6.5-7.5 (m, 3H, ArH), MS (CI with NH₃) m/z 177 (M+1), 194 (M+18). Similarly, the methoxy-2-tetralones 24b [mp 26-27 °C, petroleum ether (bp 40-60 °C) [72] bp 130-136 °C (2.3 mmHg), [77] mp 27-28 °C, bp 124-126 °C (1.5 mmHg)], 24c [mp 34-35 °C, petroleum ether (bp 40-60 °C) [53] mp 36 °C, light petroleum], and 24d [bp 112-114 °C (0.01 mbar), solid at room temperature [72] bp 118-124 °C (1.1 mmHg), [78] mp 36-37 °C] were obtained from the dimethoxynaphthalenes 23b-23d.

**General Method for Synthesizing Methoxy-2-(N-benzylamino)tetralins 25a-25d**

The method adopted for the synthesis of 8-methoxy-2-(N-benzylamino)tetralin (25a) is described.

Under an atmosphere of nitrogen, a solution of methoxy-2-tetralone 24a (5.30 g, 30.1 mmol), benzylamine (4.35 ml (4.27g), 39.8 mmol), and p-toluenesulfonic acid monohydrate (0.07 g, 0.4 mmol) in dry benzene (70 ml) was refluxed for 17 h under continuous removal of H₂O using a Dean and Stark apparatus. The benzene and the excess benzylamine were removed under reduced pressure and the residue (in this case a solid emulsion) was dissolved in absolute EtOH (100 ml). After transferring the solution to a Parr hydrogenation flask, PtO₂ (± 50 mg) was added as a catalyst and the mixture was hydrogenated for 2.5 h under a H₂ pressure of 2 atmospheres. The catalyst was filtered off and the solvent was evaporated under reduced pressure to yield methoxy-2-(N-benzylamino)tetralin 25a as a dark brown oil. After converting the crude amine to its HCl salt, the salt was dissolved in EtOH and decolourised with charcoal. Recrystallization (EtOH/H₂O) gave 6.58 g (21.7 mmol, 72%) of the HCl salt.
sult of methoxy-2-(N-benzylamino)tetralin 25a as fine white needles: mp 219-222 °C ([45] 218.5-219.5 °C, MeOH/Et2O); IR (cm⁻¹, KBr) 2850-2300 (NH₂⁺), 775, 750 & 700 (ArH); MS (Cl with NH₃) m/z 268 (M+1) (M: free amine).

Similarly, the HCl salts of the methoxy-2-(N-benzylamino)tetralins 25b-25d were obtained from the methoxy-2-tetralones 24b-24d.

General Method for Synthesizing Methoxy-2-aminotetralins 26a-26d

The method adopted for the synthesis of 8-methoxy-2-aminotetralin (26a) is described.

8-Methoxy-2-(N-benzylamino)tetralin (25a) (2.12 g, 7.94 mmol) was dissolved in absolute EtOH (60 ml), 10% Pd-on-C catalyst (1.6 g) was added, and the solution was deoxygenated in a Parr hydrogenation flask for 1 h at 45 °C under a H₂ pressure of 3 atmospheres. After filtering off the catalyst, the volatiles were removed under reduced pressure to give a dark brown oil, which became solid at room temperature. This solid was taken up in EtOH/Et₂O and precipitated as its HCl salt by Et₂O saturated with dry HCl. After decolourization with charcoal, recrystallization from EtOH/Et₂O yielded 1.17 g (5.48 mmol, 69%) of the HCl salt of methoxy-2-aminotetralin 26a as fine white needles: mp 281-282 °C dec ([51] mp >275 °C, [72] mp 273-275 °C dec; [79] mp 275-278 °C, EtOH/Et₂O); IR (cm⁻¹, KBr) 3160-2420 (NH₃⁺), 2060 (NH₂⁻), 770 & 710 (ArH). MS (Cl with NH₃) m/z 178 (M+1) (M: free amine).

Similarly, the HCl salts of the methoxy-2-aminotetralins 26b-26d were obtained from the methoxy-2-tetralones 24b-24d.

General Method for Synthesizing Methoxy-2-(acetamido)tetralins 18-21 (Acyclation Method A)

The method adopted for the synthesis of 8-methoxy-2-(acetamido)tetralin (18) is described.

Acetic anhydride (0.84 ml, 8.9 mmol) was added dropwise at room temperature to a well stirred mixture of 8-methoxy-2-aminotetralin hydrochloride (26a·HCl) (0.30 g, 1.4 mmol), NaOAc (0.65 g), EtOAc (15 ml), and H₂O (5 ml). After 3 h of stirring and diluting the mixture with H₂O (10 ml), the phases were separated and the H₂O layer was extracted twice with EtOAc (15 ml). Subsequently the EtOAc layers were combined and washed with saturated aqueous solutions of NaHCO₃ (3 x 20 ml) and NaCl (1 x 20 ml) and then dried over MgSO₄. Evaporation of the solvent under reduced pressure yielded a white solid. Recrystallization from acetone/hexane gave a yield of 0.24 g (1.1 mmol, 79%) of 8-methoxy-2-(acetamido)tetralin (18) as fine white needles: mp 156-157 °C; IR (cm⁻¹, KBr) 3230 (NH), 2850 (OCH₃), 1640 (C=O: amide I), 1570, 1520, 1450, 1390 (C=O: amide II); 1H NMR (300 MHz, CDCl₃) δ 1.73 (m, 1H, CHCN), 1.97 (s, 3H, COCH₃), 2.02 (m, 1H, CHCN), 2.44 (dd, 1H, ArCH₂), 2.85 (m, 2H, ArCH₂), 3.05 (dd, 1H, ArCH₂), J = 17.4 Hz, 5.3 Hz), 3.79 (s, 3H, OCH₃), 4.26 (m, 1H, CH₃N), 5.48 (bs, 1H, NH), 6.66 (d, 1H, ArH, J = 8.1 Hz), 7.71 (t, 1H, ArH), 7.11 (t, 1H, ArH). 13C NMR (75 MHz, CDCl₃) δ 23.52 (COCH₃), 27.06, 28.05, 29.64 (C-1, C-3, C-4), 44.19 (C-2), 55.12 (OCH₃), 106.86, 120.83, 126.34 (C-5, C-6, C-7), 122.83, 136.76, 157.33 (C-4a, C-8, C-8a), 169.32 (COCH₃). MS (Cl with NH₃) m/z 220 (M+1), 237 (M+18), Anal. (C₁₂H₁₇NO₂) C, H, N.

Similarly, 7-methoxy-2-(acetamido)tetralin (19) [yield: 51%, mp 105-108 °C, acetone/hexane; Anal. (C₁₂H₁₇NO₂) C, H, N], 6-methoxy-2-(acetamido)tetralin (20) [yield: 64%, mp 115-116 °C, acetone/hexane; Anal. (C₁₃H₁₇NO₂) C (calcd 71.21, found 70.70), H, N], and 5-methoxy-2-(acetamido)tetralin (21) [yield: 62%, mp 158-159 °C, acetone/hexane; Anal. (C₁₃H₁₇NO₂) C, H, N] were obtained from the HCl salts of the methoxy-2-tetralons 26b-26d.

And likewise, (+)-8-methoxy-2-(acetamido)tetralin ((+)-18) [mp 155-156 °C, acetone/hexane, [α]D = +89° (c = 0.185 g/100 ml, CH₂OH, 25 °C) and (-)-8-methoxy-2-(acetamido)tetralin ((-)-18) [mp 156-157 °C, acetone/hexane; [α]D = -88° (c = 0.452 g/100 ml, CH₂OH, 25 °C)] were prepared from the HCl salts of (2R)(+)-8-methoxy-2-(acetamido)tetralin ((+)-26a) and (2S)(-)-8-methoxy-2-(acetamido)tetralin ((-)-26a), respectively. The enantiomeric purities of these enantiomers were estimated by a direct chiral HPLC technique.
assay using a Chiracel OD column (Daicel Chemical Industries, Tokyo, Japan) as chiral stationary phase and a mixture of hexane-ethanol-diethylamine 95:5:0.1 (v/v/v) as eluent at 15 °C [80]. It was shown that in both enantiomers the presence of the other enantiomer as impurity was much less than 1% [Witte DT, Bruggeman FJ (1993); personal communication].

5.7.3 Preparation of 8-Methoxy-2-Amidotetralins

8-Methoxy-2-amidotetralins 27,28 (Acylation Method A)
Using the appropriate anhydrides, 8-methoxy-2-(propionamido)tetralin (27) [yield: 31%; mp 151-152 °C, acetone/hexane; Anal. C_{14}H_{19}NO_2 C, H, N] and 8-methoxy-2-(n-butyramido)tetralin (28) [yield: 63%; mp 139-140 °C, acetone/hexane; Anal. C_{15}H_{21}NO_2 C, H, N] were obtained from 8-methoxy-2-amidotetralin hydrochloride (26a·HCl) by the same method as used for the synthesis of the methoxy-2-acetamido)tetralins 18-21 from the HCl salts of the methoxy-2-amidotetralins 26a-26d (see 5.7.2).

8-Methoxy-2-amidotetralins 29-34 (Acylation Method B)
The method adopted for the synthesis of 8-methoxy-2-(benzamido)tetralin (31) is described. Benzoyl chloride (0.55 ml, 4.7 mmol) was added dropwise at room temperature to a rigorously stirred mixture of 8-methoxy-2-aminotetralin hydrochloride (26a·HCl) (0.40 g, 1.9 mmol), CH_2Cl_2 (20 ml) and 10% NaOH (12 ml). After 3 h of stirring, the reaction mixture was poured into H_2O (50 ml) and the phases were separated. Subsequently the H_2O layer was extracted with CH_2Cl_2 (2 x 20 ml) and the combined organic layers were washed with a saturated aqueous solution of NaHCO_3 (1 x 20 ml) and H_2O (1 x 20 ml), dried over MgSO_4 and then evaporated under reduced pressure to yield a light yellow solid. Recrystallization from acetone/hexane yielded 0.45 g (1.6 mmol, 84%) of 8-methoxy-2-(benzamido)tetralin 31 as white crystals: mp 173-175 °C; IR (cm\(^{-1}\), KBr) 3310 (NH), 2850 (C=O), 1700 (C=O: amide I), 1580 (Ar), 1530 (C=O: amide II). 1H NMR (300 MHz, CDCl_3) \(\delta\) 8.86 (m, 1H, CHCN), 2.15 (m, 1H, CHCN), 2.57 (dd, 1H, ArCH_2, J = 17.2 Hz, 8.1 Hz), 2.93 (m, 2H, ArCH_2), 3.20 (dd, 1H, ArCH_2, J = 17.2 Hz, 5.5 Hz), 3.82 (s, 3H, OCH_3), 4.49 (m, 1H, CHmN), 6.12 (bs, 1H, NH), 6.69 (d, 1H, ArH, J = 8.1 Hz), 6.76 (d, 1H, ArH, J = 7.7 Hz), 7.13 (t, 1H, ArH), 7.45 (m, 3H, ArH), 7.76 (m, 2H, ArH); MS (Cl with NH_3) \(m/z\) = 282 (M+1), Anal. C_{18}H_{19}NO_2 C, H, N.

Similarly, 8-methoxy-2-(i-butyramido)tetralin 29 [yield: 71%; mp 167-168 °C, acetone/hexane; Anal. C_{15}H_{21}NO_2 C, H, N], 8-methoxy-2-(cyclopropane-carboxamido)tetralin 30 [yield: 83%; mp 185-187 °C, acetone/hexane; Anal. C_{14}H_{19}NO_2 C, H, N], 8-methoxy-2-(phenylacetamido)tetralin 32 [yield: 61%; mp 154-156 °C, acetone/hexane; Anal. C_{15}H_{21}NO_2 C, H, N], 8-methoxy-2-(chloroacetamido)tetralin 33 [yield: 63%; mp 134-136 °C, acetone/hexane; Anal. C_{15}H_{16}NO_2Cl C, H, N], and 8-methoxy-2-(bromoacetamido)tetralin 34 [yield: 52%; mp 140-142 °C] were obtained from 8-methoxy-2-amidotetralin hydrochloride (26a·HCl).

8-Methoxy-2-amidotetralins 37,38 (Acylation Method C)
The method adopted for the synthesis of 8-methoxy-2-(pentafluoropropionamido)tetralin (38) is described. Pentafluoropropionic acid chloride (0.69 ml, 3.5 mmol) was added dropwise at 0 °C to a stirred mixture of 8-methoxy-2-amidotetralin hydrochloride (26a·HCl) (0.75 g, 3.5 mmol), CH_2Cl_2 (15 ml) and Et_3N (1.0 ml, 7.2 mmol). After stirring for 3 h at this temperature, the reaction mixture was washed successively with H_2O (1 x 10 ml), a 5% aqueous solution of citric acid (2 x 10 ml), a 5% aqueous solution of NaHCO_3 (2 x 10 ml), and finally H_2O (1 x 10 ml). After drying the organic layer over MgSO_4, the solvent was evaporated under reduced pressure to yield a brownish green solid. Recrystallization from toluene yielded 0.43 g (1.3 mmol, 38%) of 8-methoxy-2-(pentafluoropropionamido)tetralin (38) as a fine white powder: mp 139-142 °C; IR (cm\(^{-1}\), KBr) 3340 (NH), 2850 (OCH_3), 1700 (C=O: amide I), 1550 (C=O: amide II). 1H NMR (200 MHz, CDCl_3) \(\delta\) 1.85 (m, 1H, CHCN), 2.07 (m, 1H, CHCN), 2.53 (dd, 1H, CH=CH), 3.82 (s, 3H, OCH_3), 4.49 (m, 1H, CHmN), 6.12 (bs, 1H, NH), 6.69 (d, 1H, ArH, J = 8.1 Hz), 6.76 (d, 1H, ArH, J = 7.7 Hz), 7.13 (t, 1H, ArH), 7.45 (m, 3H, ArH), 7.76 (m, 2H, ArH); MS (Cl with NH_3) \(m/z\) = 334 (M+1), Anal. C_{14}H_{19}NO_2 C, H, N.
3.83 (s, 3H, \( \text{OCH}_3 \)), 2.90 (m, 2H, \( \text{ArCH}_2 \)), 3.16 (dd, 1H, \( \text{ArCHN} \), \( J = 17.1 \text{ Hz}, 5.6 \text{ Hz} \)), 3.83 (s, 3H, \text{OCH}_3), 4.35 (m, 1H, \( \text{CH}_2\text{N} \)), 6.42 (bs, 1H, NH), 6.70 (d, 1H, ArH, \( J = 8.9 \text{ Hz} \)), 6.74 (d, 1H, ArH, \( J = 8.7 \text{ Hz} \)), 7.15 (t, 1H, ArH); MS (CI with \text{NH}_3) \( m/z \) 341 (M+18).

Similarly, 8-methoxy-2-(trifluoroacetamido)tetralin (37) [yield: 30%; mp 135-136 °C, acetone/hexane] was obtained from 8-methoxy-2-aminotetralin hydrochloride (26a-HCl).

### 8-Methoxy-2-(iodoacetamido)tetralin (35)

Sodium iodide (0.48 g, 3.2 mmol) was added at room temperature to a stirred solution of 8-methoxy-2-(bromoacetamido)tetralin (34) (0.32 g, 1.1 mmol) in acetone (50 ml). After 2 h of stirring, the reaction mixture was filtered. Evaporation of the organic solvent under reduced pressure yielded a white solid. Recrystallization from acetone/hexane yielded 0.31 g (0.9 mmol, 84%) of 8-methoxy-2-(iodoacetamido)tetralin (35) as white crystals; mp 168-170 °C; IR (cm\(^{-1}\), KBr) 3280 (NH), 2835 (OCH\(_3\)), 1640 (C=O: amide I), 1545 (C=O: amide II); \(^1\text{H} \) NMR (200 MHz, CDC\(_3\) \( \delta \) 1.79 (m, 1H, CHCN), 2.02 (m, 1H, CHCN), 2.48 (dd, 1H, ArCH\(_2\), \( J = 17.1 \text{ Hz}, 8.1 \text{ Hz} \)), 2.89 (m, 2H, ArCH\(_2\)), 3.09 (dd, 1H, ArCH\(_2\), \( J = 17.3 \text{ Hz}, 6.2 \text{ Hz} \)), 3.70 (s, 2H, COCH\(_2\)I), 3.82 (s, 3H, OCH\(_3\)), 4.25 (m, 1H, CH\(_2\)N), 6.05 (bs, 1H, NH), 6.68 (d, 1H, ArH, \( J = 8.1 \text{ Hz} \)), 6.74 (d, 1H, ArH, \( J = 7.6 \text{ Hz} \)), 7.13 (t, 1H, ArH); MS (CI with \text{NH}_3) \( m/z \) 364 (M+1), 363 (M+18); Anal. (C\(_{13}\)H\(_{16}\)NO\(_2\)I) \text{C, H, N.}

### 8-Methoxy-2-(fluoroacetamido)tetralin (36)

Anhydrous potassium fluoride (0.14 g, 2.4 mmol) was added to a stirred mixture of 8-methoxy-2-(bromoacetamido)tetralin (34) (0.15 g, 0.5 mmol), Kryptofix\(_{2.2.2}\) (0.25 g) and CH\(_2\)CN (7.5 ml). After stirring for 1.5 h at 65 °C and cooling of the reaction mixture, the solvent was evaporated under reduced pressure and the residue was dissolved in CH\(_2\)Cl\(_2\) (20 ml). This organic layer was washed with \text{MeOH}, after evaporation of the solvent under reduced pressure, the residue was purified by column chromatography on silica gel 60 (Merck) using a mixture of CH\(_2\)Cl\(_2\) and \text{MeOH} (15/1) as the eluent. Evaporation of the solvent under reduced pressure yielded 0.08 g (0.34, 67%) of 8-methoxy-2-(fluoroacetamido)tetralin (36) as a white powder; mp 149-150 °C; IR (cm\(^{-1}\), KBr) 3240 (NH), 2860 (OCH\(_3\)), 1650 (C=O: amide I), 1540 (C=O: amide II); \(^1\text{H} \) NMR (200 MHz, CDC\(_3\) \( \delta \) 1.78 (m, 1H, CHCN), 2.06 (m, 1H, CHCN), 2.50 (dd, 1H, ArCH\(_2\), \( J = 17.2 \text{ Hz}, 8.4 \text{ Hz} \)), 2.91 (m, 2H, ArCH\(_2\)), 3.14 (dd, 1H, ArCH\(_2\), \( J = 17.1 \text{ Hz}, 5.6 \text{ Hz} \)), 3.82 (s, 3H, OCH\(_3\)), 4.35 (m, 1H, CH\(_2\)N), 4.81 (d, 2H, COCH\(_2\)F, \( J_{HF} = 47.4 \text{ Hz} \)), 6.32 (bs, 1H, NH), 6.69 (d, 1H, ArH, \( J = 8.1 \text{ Hz} \)), 6.74 (d, 1H, ArH, \( J = 7.7 \text{ Hz} \)), 7.13 (t, 1H, ArH); MS (CI with \text{NH}_3) \( m/z \) 238 (M+1), 255 (M+18).

### 5.7.4 Preparation of N-Substituted 8-Methoxy-2-Amidotetralins

**8-Methoxy-2-(N-propylamino)tetralin (39)**

The secondary amine 39 was prepared from tetralone 24a (0.86 g, 4.9 mmol) by essentially the same procedure as described for the preparation of 8-methoxy-2-(N-benzylamino)tetralin (25a) from tetralone 24a (see 5.7.2). The yield was 0.56 g (2.2 mmol, 45%) of the \text{HCl} salt of 8-methoxy-2-(N-propylamino)tetralin (39) as fine white platelets; mp 189-190 °C, EtOH/Et\(_2\)O (641) 193-194 °C, EtOH/Et\(_2\)O (72) 189-190 °C; IR (cm\(^{-1}\), KBr) 2900-2550, 2520, 2440 (NH\(_2^+\)), MS (CI with \text{NH}_3) \( m/z \) 220 (M+1) (M: free amine).

**8-Methoxy-2-(N-alkylamido)tetralins 40-43 (Acylation Method A)**

Using the appropriate anhydrides, the 8-methoxy-2-(N-alkylamido)tetralins 40-43 were prepared from 8-methoxy-2-(N-propylamino)tetralin hydrochloride (39-HCl) and 8-methoxy-2-(N-benzylamino)tetralin hydrochloride (25a-HCl) by essentially the same procedure as described for the preparation of the methoxy-2-(acetamido)tetralins 18-21 from the \text{HCl} salts of the methoxy-2-aminotetralins 26a-26d (see 172).
5.7.2. The major difference was that after evaporation of the solvent under reduced pressure the 8-methoxy-2-(N-alkylamido)tetralins 40-43 were obtained as oils instead of solids. Therefore, purification was performed by column chromatography on silica gel 60 (Merck) using a mixture of CH$_2$Cl$_2$ and MeOH (25:1) as the eluent instead of by recrystallization. 8-Methoxy-2-(N-n-propylacetamido)tetralin (40): IR (cm$^{-1}$, NaCl) 1645 (C=O: amide); MS (CI with NH$_3$) m/z 262 (M+1).

8-Methoxy-2-(N-n-propylpropionamido)tetralin (41): IR (cm$^{-1}$, NaCl) 1645 (C=O: amide); MS (CI with NH$_3$) m/z 276 (M+1).

8-Methoxy-2-(N-benzylacetamid0)-tetralin (42): IR (cm$^{-1}$, NaCl) 1650 (C=O: amide); MS (CI with NH$_3$) m/z 310 (M+1).

8-Methoxy-2-(N-benzylpropionamido)tetralin (43): IR (cm$^{-1}$, NaCl) 1650 (C=O: amide); MS (CI with NH$_3$) m/z 324 (M+1).

5.7.5 Preparation of 2-Amidotetralins and 8-Substituted 2-(Acetamido)Tetralins

2-Amidotetralins 45-47 (Acylation Method A)
Using the appropriate anhydrides, 2-(acetamid0)tetralin (45) [yield: 65%; mp 109-111 °C, acetone/hexane; Anal. (C$_{12}$H$_{15}$NO) C, H, N], 2-(propionamido)tetralin (46) [yield: 86%; mp 99-101 °C, acetone/hexane; Anal. (C$_{13}$H$_{17}$NO) C, H, N], and 2-(n-butyramido)tetralin (47) [yield: 81%; mp 81-82 °C, acetone/hexane; Anal. (C$_{14}$H$_{19}$NO) C, H, N] were obtained from 2-aminotetralin hydrochloride (44.HCl) by the same method as used for the synthesis of the 8-methoxy-2-(acetarnido)tetralins 18-21 from the HCI salts of the methoxy-2-aminotetralins 26a-26d (see 5.7.2).

2-Amidotetralins 48-50 (Acylation Method B)
Using the appropriate acid chlorides, 2-(benzamido)tetralin (48) [yield: 69%; mp 154-156 °C, acetone/hexane; Anal. (C$_{17}$H$_{17}$NO) C, H, N], 2-(phenylacetamido)tetralin (49) [yield: 72%; mp 119-121 °C, acetone/hexane; Anal. (C$_{18}$H$_{19}$NO) C, H, N], and 2-(chloroacetamido)tetralin (50) [yield: 56%; mp 131-133 °C, acetone/hexane; Anal. (C$_{12}$H$_{14}$NOC$l$) C (calcd 64.43, found 63.91), H, N] were obtained from 2-aminotetralin hydrochloride (44HCI) by the same method as used for the synthesis of the 8-methoxy-2-(acetamido)tetralins 18-21 from the HCI salts of the methoxy-2-aminotetralins 26a-26d (see 5.7.3).

8-Hydroxy-2-(acetamido)tetralin (51)
Under an atmosphere of nitrogen, an excess of a 1 M solution of BBr$_3$ in CH$_2$Cl$_2$ (14 ml, 14 mmol) was added via a syringe to a stirred solution of 8-methoxy-2-(acetamido)tetralin (18) (1.49 g, 6.8 mmol) in CH$_2$Cl$_2$ (50 ml), cooled at -50 °C. After stirring for 1 h at a temperature between -50 and -40 °C, the temperature of the reaction mixture was allowed to rise to room temperature and the reaction mixture was stirred for another 18 h. After addition of MeOH (20 ml) and stirring for 15 min, the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel 60 (Merck) using a mixture of CH$_2$Cl$_2$ and MeOH (40:1) as the eluent. After recrystallization (CH$_2$Cl$_2$/acetone), the yield was 0.78 g (3.8 mmol, 56%) of 8-hydroxy-2-(acetamido)tetralin (51) as light pink crystals: mp 176-177 °C; IR (cm$^{-1}$, KBr) 3360 (NH), 3500-3100 (OH), 1680 (C=O: amide I), 1550 (C=O: amide II); MS (CI with NH$_3$) m/z 206 (M+1), Anal. (C$_{12}$H$_{15}$NO$_2$) C, H, N.

8-Ethoxy-2-(acetamido)tetralin (53) (Acylation Method A)
8-Ethoxy-2-(acetamido)tetralin (53) was prepared from 8-ethoxy-2-aminotetralin hydrochloride (52.HCl) (1.20 g, 5.3 mmol) by essentially the same procedure as described for the preparation of the methoxy-2-(acetamido)tetralins 18-21 from the HCI salts of the methoxy-2-aminotetralins 26a-26d (see 5.7.2). The only difference was that the recrystallization was done from acetone/Et$_2$O instead of acetone/hexane. The yield was 0.91 g (3.9 mmol, 74%) of 8-ethoxy-2-(acetamido)tetralin (53) as a fine crystalline product: mp 164-167 °C; IR (cm$^{-1}$, KBr) 3320 (NH), 1650 (C=O: amide I), 1550 (C=O: amide II); $^1$H NMR
(200 MHz, CDCl₃) δ 1.42 (t, 3H, OCCH₃), 1.75 (m, 1H, CHCN), 2.00 (s, 3H, COCH₃), 2.04 (m, 1H, CHCN), 2.47 (dd, 1H, ArCHₓN, J = 16.7 Hz, 7.7 Hz), 2.87 (m, 2H, ArCH₂), 3.09 (dd, 1H, ArCH₂Q, J = 16.8 Hz, 5.7 Hz), 4.02 (q, 2H, OCH₂C), 4.29 (m, 1H, CHₓN), 5.49 (bd, 1H, NH), 6.66 (d, 1H, ArH, J = 8.1 Hz), 6.72 (d, 1H, ArH, J = 7.3 Hz), 7.10 (t, 1H, ArH), MS (Cl with NH₃) m/z 234 (M+1), Anal. (C₁₄H₁₈N₂O₂) C, H, N.

8-Benzyl-2-(acetamido)tetrinal (54)
Benzyl chloride (0.92 ml, 8 mmol) was added to a stirred mixture of 8-hydroxy-2-(acetamido)tetrinal (51) (0.16 g, 0.78 mmol), Cs₂CO₃ (0.28 g, 0.86 mmol) and DMF (80 ml). After stirring for 48 h at a temperature between 70 and 80 °C, the reaction mixture was cooled and evaporation under reduced pressure was executed. After boiling up the residue with acetone (50 ml) and filtration of this mixture, the volatiles of the filtrate were evaporated under reduced pressure. The resulting residue was mixed with hexane (20 ml), and, after evaporation under reduced pressure of this hexane, a solid was yielded. Recrystallization from Et₂O yielded 0.20 g (0.68 mmol, 87%) of 8-benzyl-2-(acetamido)tetrinal (54) as a fine white needles: mp 142-144 °C, IR (cm⁻¹, KBr) 3360 (NH), 1650 (C=O: amide I), 1545 (C=O: amide II); ¹H NMR (200 MHz, CDCl₃) δ 1.76 (m, 1H, CHCN), 1.99 (s, 3H, COCH₃), 2.05 (m, 1H, CHCN), 2.53 (dd, 1H, ArCHₓN, J = 17.2 Hz, 8.1 Hz), 2.87 (m, 2H, ArCH₂), 3.14 (dd, 1H, ArCH₂Q, J = 17.3 Hz, 5.3 Hz), 4.30 (m, 1H, CHₓN), 5.06 (s, 2H, OCH₂Ph), 5.64 (bd, 1H, NH), 6.74 (d, 2H, ArH, J = 8.1 Hz), 7.11 (t, 1H, ArH), 7.40 (m, 5H, Ph), MS (Cl with NH₃) m/z 296 (M+1), 313 (M+18); Anal. (C₁₉H₂₆N₂O₂) C, H, N.

8-Trifluoromethanesulfonyloxy-2-(acetamido)tetrinal (55)
Trifluoromethanesulfonic anhydride (2.0 ml, 11 mmol) was added to a stirred solution of 8-hydroxy-2-(acetamido)tetrinal (51) (0.22 g, 1.1 mmol) in pyridine (10 ml), cooled at −20 °C. After stirring for 1 h at a temperature between −20 and −10 °C, the reaction mixture was allowed to warm gradually to room temperature and was stirred for another 18 h. After addition of H₂O (10 ml), the reaction mixture was extracted with CH₂Cl₂ (3 x 10 ml). The combined organic layers were washed with a 5% aqueous solution of NaHCO₃ (2 x 10 ml), a 5% aqueous solution of NaHCO₃ (2 x 10 ml), and a saturated aqueous solution of NaCl (2 x 10 ml). After drying over MgSO₄, the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel 60 (Merck) using a mixture of CH₂Cl₂ and MeOH (10/1) as the eluent. Recrystallization from acetone/Et₂O yielded 0.10 g (0.3 mmol, 27%) of 8-trifluoromethanesulfonyloxy-2-(acetamido)tetrinal (55) as light yellow crystals: IR (cm⁻¹, KBr) 3320 (NH), 1640 (C=O: amide I), 1535 (C=O: amide II); ¹H NMR (200 MHz, CDCl₃) δ 1.74 (m, 1H, CHCN), 2.01 (s, 3H, COCH₃), 2.09 (m, 1H, CHCN), 2.61 (dd, 1H, ArCHₓN, J = 16.7 Hz, 8.6 Hz), 2.94 (m, 2H, ArCH₂), 3.17 (dd, 1H, ArCH₂Q, J = 16.9 Hz, 5.3 Hz), 4.29 (m, 1H, CHₓN), 5.68 (bd, 1H, NH), 7.07-7.27 (m, 3H, ArH), MS (Cl with NH₃) 338 (M+1), 355 (M+18).

5.7.6 PREPARATION OF 5-/7-SUBSTITUTED 8-METHOXY-2-(ACETAMIDO)TETRALINS

5-Chloro-8-methoxy-2-(acetamido)tetrinal (56)
Under an atmosphere of nitrogen, a stirred solution of 8-methoxy-2-(acetamido)tetrinal (18) (2.47 g, 11.3 mmol) in CF₃COOH (50 ml) at 0 °C was treated with N-chlorosuccinimide (1.51 g, 11.3 mmol). The reaction mixture was allowed to warm gradually to room temperature. After 1 h the reaction mixture was poured over ice (50 g); the aqueous solution was made basic with a 25% aqueous solution of NaOH and extracted with CH₂Cl₂ (3 x 100 ml). The combined organic layers were washed with a saturated aqueous solution of NaCl (3 x 75 ml) and H₂O (1 x 75 ml), dried over MgSO₄ and then evaporated under reduced pressure to yield a light yellow solid. Recrystallization from acetone/Et₂O yielded 2.28 g (9.0 mmol, 80%) of 5-chloro-8-methoxy-2-(acetamido)tetrinal (56) as a white solid: mp 183-185 °C; IR
(cm⁻¹, KBr) 3320 (NH), 1650 (C=O: amide I), 1555 (C=O: amide II); ¹H NMR (300 MHz, CDCl₃) δ 1.72 (m, 1H, CHCN), 1.95 (m, 3H, COCH₂), 2.04 (dd, 1H, CHCN), 2.43 (dd, 1H, ArCH₂, J = 17.2 Hz, 8.4 Hz), 2.80 (m, 2H, ArCH₂), 3.03 (dd, 1H, ArCH₂, J = 17.6 Hz, 5.5 Hz), 3.76 (s, 3H, OCH₃), 4.15 (m, 1H, CH₂CN₂), 6.64 (d, 1H, ArH, J = 8.4 Hz), 6.90 (bd, 1H, NH), 7.12 (d, 1H, ArH, J = 8.8 Hz); ¹³C NMR (75 MHz, CDCl₃) δ 22.19 (COCH₂), 25.07, 27.03, 29.27 (C-1, C-3, C-4), 43.59 (C-2), 54.53 (OCH₂), 107.48, 125.77 (C-6, C-7), 105.29, 129.98 (C-10), 130.23, 135.67, 155.33 (C-4a, C-5, C-8, C-8a), 168.94 (COCH₂), MS (CI with NH₃) m/z 254 (M[Cl=35]+1), 256 (M[Cl=37]+1), 271 (M[Cl=39]+1), 273 (M[Cl=37]+1); Anal. (C₁₃H₁₆NO₂Cl) C, H, N.

5-Bromo-8-methoxy-2-(acetamido)tetra2in (57)
Under an atmosphere of nitrogen, 2,4,4,6-tetrabromo-2,5-cyclohexadien-1-one (6.22 g, 15.2 mmol) in CHCl₃ (30 ml) was added dropwise to a stirred solution of 8-methoxy-2-(acetamido)tetra2in (18) (3.30 g, 15.0 mmol) in CHCl₃ (60 ml). After stirring for 20 h, followed by addition of MeOH (10 ml), the reaction mixture was washed successively with 4N NaOH (3 x 25 ml), a saturated aqueous solution of NaCl (2 x 25 ml), and finally H₂O (1 x 25 ml). After drying over MgSO₄ and evaporation of the volatiles, recrystallization from acetone (twice) yielded 3.43 g (15.5 mmol, 77%) of 5-bromo-8-methoxy-2-(acetamido)tetra2in (57) as a white solid: mp 202-203 °C; IR (cm⁻¹, KBr) 3320 (NH), 1650 (C=O: amide II); ¹H NMR (300 MHz, CDCl₃) δ 1.77 (m, 1H, CHCN), 1.98 (s, 3H, COCH₂), 2.03 (m, 1H, CHCN), 2.47 (dd, 1H, ArCH₂, J = 17.2 Hz, 7.7 Hz), 2.81 (m, 2H, ArCH₂), 3.05 (dd, 1H, ArCH₂, J = 17.6 Hz, 5.1 Hz), 3.78 (s, 3H, OCH₂), 4.23 (m, 1H, CH₂CN₂), 5.51 (bs, 1H, NH), 6.58 (d, 1H, ArH, J = 8.7 Hz), 7.37 (d, 1H, ArH, J = 8.8 Hz); ¹³C NMR (75 MHz, CDCl₃) δ 23.39 (COCH₂), 27.87, 28.27, 29.88 (C-1, C-3, C-4), 44.24 (C-2), 55.30 (OCH₂), 108.67, 129.98 (C-6, C-7), 115.69, 125.33, 135.67, 156.56 (C-4a, C-5, C-8, C-8a), 169.51 (COCH₂), MS (CI with NH₃) m/z 298 (M[Br=79]+1), 300 (M[Br=81]+1), 315 (M[Br=79]+18), 317 (M[Br=81]+18); Anal. (C₁₃H₁₆NO₂Br) C, H, N.

5-Iodo-8-methoxy-2-(acetamido)tetra2in (58)
A solution of I₂ (0.76 g, 3.0 mmol) in CH₂Cl₂ (40 ml) was added dropwise to a stirred mixture of 8-methoxy-2-(acetamido)tetra2in (18) (0.66 g, 3.0 mmol), CF₃COOA₄ (0.67 g, 3.0 mmol), and CH₂Cl₂ (25 ml). After stirring for 1 h at room temperature and evaporation of the volatiles under reduced pressure, the residue was boiled up with acetone. After collection of the precipitate by suction filtration, recrystallization from EtOH/H₂O yielded 0.69 g (2.0 mmol, 66%) of 5-iodo-8-methoxy-2-(acetamido)tetra2in (58) as fine white needles: mp 213-215 °C (51) mp 202-203 °C (EtOH); IR (cm⁻¹, KBr) 3340 (NH), 1650 (C=O: amide II); ¹H NMR (200 MHz, CD₂SO) δ 1.79 (s, 3H, COCH₂), 3.73 (s, 3H, OCH₃), 6.61 (d, 1H, ArH, J = 8.5 Hz), 7.62 (d, 1H, ArH, J = 9.0 Hz), 7.89 (bd, 1H, NH); ¹³C NMR (50 MHz, CD₂SO) δ 22.68 (COCH₂), 28.35, 29.52, 34.07 (C-1, C-3, C-4), 44.03 (C-2), 55.42 (OCH₂), 110.20, 136.30 (C-6, C-7), 91.07, 125.69, 136.17, 157.19 (C-4a, C-5, C-8, C-8a), 168.71 (COCH₂), MS (CI with NH₃) 346 (M+1); Anal. (C₁₃H₁₆NO₂I) C, H, N.

5-Nitro-8-methoxy-2-(acetamido)tetra2in (59)
Under an atmosphere of nitrogen, NaNO₃ (1.25 g, 14.7 mmol) was added to a stirred solution of 8-methoxy-2-(acetamido)tetra2in (18) (3.30 g, 15.0 mmol) in CF₃COOH (60 ml), cooled at 0 °C. The reaction mixture was allowed to warm gradually to room temperature. After 1.5 h of stirring at room temperature, the reaction mixture was poured over ice (60 g), made basic with a 25% aqueous solution of NH₄OH, and extracted with CH₂Cl₂ (3 x 120 ml). The combined organic layers were dried over MgSO₄ and evaporated under reduced pressure to yield a brown solid. Purification by column chromatography on silica gel 60 (Merek) using a mixture of CH₂Cl₂ and acetone (7:3) as the eluent yielded 1.24 g (4.7 mmol, 31%) of 5-nitro-8-methoxy-2-(acetamido)tetra2in (59) as a light yellow solid: mp 201-203 °C дек;
5-Amino-8-methoxy-2-(acetamido)tetralin (60)

5-Nitro-8-methoxy-2-(acetamido)tetralin (59) (0.18 g, 0.7 mmol) was dissolved in absolute EtOH (50 ml), 10% Pd-on-C catalyst (0.1 g) was added, and the solution was hydrogenated in a Parr hydrogenation flask for 3 h at room temperature under a H2 pressure of 2 atmospheres. After filtering off the catalyst and removal of the volatiles under reduced pressure, the residue was purified by column chromatography on silica gel 60 (Merck) using a mixture of CH2Cl2 and MeOH (12:1) as the eluent. The yield was 0.12 g (0.5 mmol, 75%) of 5-amino-8-methoxy-2-(acetamido)tetralin (60) as a light pink solid: mp 161-164 °C dec; IR (cm⁻¹, KBr) 3310 (NH), 2840 (OCH3), 1635 (C=O: amide I), 1550 (amide II), 1520 (C=O: amide I), 1320 (NO2); MS (CI with NH3) m/z 282 (M+1), 280 (M+18).

7-Chloro-8-Methoxy-2-(acetamido)tetralin (62)

NaN3 (0.09, 1.3 mmol) was added to a stirred solution of 7-amino-8-methoxy-2-(acetamido)tetralin hydrochloride (61-HCl) (0.32 g, 1.2 mmol) in a mixture of 36% HCl (2.5 ml) and H2O (2.5 ml), while the temperature of the reaction mixture was kept at 0 °C. After stirring for 15 min at 0 °C, a dark green solution of CuCl (0.15 g, 1.5 mmol) in 36% HCl (0.5 ml) was added to the stirred dark brown reaction mixture. The reaction mixture was allowed to warm gradually to room temperature. After cooling to room temperature, the reaction mixture was poured into ice water; the aqueous solution was made basic with 25% aqueous Na2CO3 solution (60 ml), and the mixture was allowed to warm gradually to room temperature. After stirring for 2 h at room temperature, the bulk of the solvent was removed under reduced pressure and the residue was partitioned between EtOAc (50 ml) and H2O (50 ml). The EtOAc layer was washed with a saturated aqueous solution of NaHCO3 (3 x 20 ml), 4N NaOH (3 x 20 ml), and finally H2O (1 x 20 ml). After drying over MgSO4 and evaporation of the solvent under reduced pressure, the residue was dissolved in absolute EtOH (125 ml). After addition of 10% Pd-on-C catalyst (1.0 g), the mixture was hydrogenated in a Parr hydrogenation flask for 18 h at room temperature under a H2 pressure of 3 atmospheres. After filtering off the catalyst and evaporation of the solvent under reduced pressure, the residue was taken up in EtOH/Et2O and precipitated as its HCl salt by Et2O saturated with dry HCl. Suction filtration yielded 7-chloro-8-methoxy-2-(acetamido)tetralin hydrochloride (61-HCl) as a white solid: mp > 210 °C dec.
solution of NH₄OH and extracted with CH₂Cl₂ (3 x 25 ml). The combined orange organic layers were washed with a saturated aqueous solution of NaCl (3 x 25 ml) and dried over MgSO₄. After removal of the volatiles under reduced pressure, the residue was purified by column chromatography on silica gel 60 (Merck) using a mixture of CH₂Cl₂ and MeOH (10/1) as the eluent. Recrystallization from acetone/hexane yielded 0.15 g (0.6 mmol, 50%) of 7-chloro-8-methoxy-2-(acetamido)tetralin (62) as a light yellow solid: mp 172-173 °C; IR (cm⁻¹, KBr) 3310 (NH), 1640 (C=O; amide I), 1550 (C=O; amide II); ¹H NMR (200 MHz, CDCl₃) δ 1.72 (m, 1H, CHCN), 1.98 (s, 3H, COCH₃), 2.03 (m, 1H, CH₂), 3.15 (dd, 1H, ArCH₂, J = 17.4 Hz, 5.5 Hz), 3.82 (s, 3H, OCH₃), 4.26 (m, 1H, CH=N), 5.55 (bs, 1H, NH), 6.82 (d, 1H, ArH, J = 8.4 Hz), 7.15 (d, 1H, ArH, J = 8.8 Hz); ¹³C NMR (50 MHz, CDCl₃) δ 23.55 (COCH₃), 27.04, 28.15, 30.24 (C-1, C-3, C-4), 44.75 (C-2), 59.98 (OCH₃), 125.19, 127.70 (C-5, C-6), 129.76, 135.95, 155.91 (C-4a, C-7, C-8, C-8a); MS (CI with NH₃) m/z 254 (M[CI=35]+1), 256 (M[Cl=37]+1), 271 (M[Cl=35]+18), 273 (M[Cl=37]+18); Anal. (C₁₃H₁₆N₂Cl) C, H, N.

5.7.7 Preparation of Phenethylamides

Phenethylamides 67, 69 (Acylation Method B)
Using the appropriate acid chlorides, N-acetyl-phenethylamine (67) [yield: 65%; mp 52-54 °C, toluene; Anal. (C₁₀H₁₃NO) C, H, N], and N-propionyl-2-methoxyphenethylamine (69) [yield: 26%; mp 47-49 °C, acetone/hexane; Anal. (C₁₂H₁₇N₂O₂) C, H, N] were obtained from phenethylamine hydrochloride (63.HCl) and 2-methoxyphenethylamine (64), respectively, by the same method as used for the synthesis of the 8-methoxy-2-(acetamido)tetralins 29-34 from 8-methoxy-2-aminotetralin hydrochloride (26a.HCl) (see 5.7.3).

Phenethylamides 68, 70-72 (Acylation Method A)
Using the appropriate anhydrides, N-acetyl-2-methoxyphenethylamine (68) [yield: 55%; mp 77-78 °C, toluene; Anal. (C₁₁H₁₅N₂O₂) C, H, N], N-n-butyryl-2-methoxyphenethylamine (70) [yield: 46%; mp 45-47 °C, acetone/hexane; Anal. (C₁₃H₁₇NO₂) C, H, N], N-acetyl-3-methoxyphenethylamine (71) [yield: 69%; a light yellow oil which could not be crystallized; Anal. (C₁₁H₁₅NO₂) C, H, N], and N-acetyl-4-methoxyphenethylamine (72) [yield: 77%; mp 85-86 °C, toluene; Anal. (C₁₁H₁₅NO₂) C, H, N] were obtained from the methoxyphenethylamines 64-66 by the same method as used for the synthesis of the methoxy-2-(acetamido)tetralins 18-21 from the HCl salts of the methoxy-2-aminotetralins 26a-26d (see 5.7.2).

5.8 Experimental Section II (Pharmacology)

5.8.1 Determination of Melatonin-Receptor Affinities by Competition for 2-[¹²⁵I]Iodomelatonin Binding to Chicken Retinal Membranes

Tissue Preparation
Chickens (4-6 weeks old) maintained in a controlled lighting regime (14 h light/10 h dark) were decapitated during the light phase. Retinas were dissected free of pigment epithelium and homogenized in ice-cold buffer containing 50 mM Tris-HCl (pH 7.5 at 25 °C) and 0.1% ascorbic acid with a Brinkmann Polytron PT-5 at settings 5 for 10 sec. The homogenate was centrifuged at 50000g for 10 min at 4 °C. The obtained pellet was rehomogenized and centrifuged for a second time. The final pellet was resuspended by homogenization at a concentration of 500 μg protein/ml and aliquots frozen until use.
2-\[^{125}\text{I}\]iodomelatonin Binding Assay

For competition experiments 2-\[^{125}\text{I}\]iodomelatonin (synthesized by Takahashi, Nikaido and Dubocovich by a modification of the method of Vaakkuri et al. [3,4]; specific activity: 1800-2175 Ci/mmol; stable for 60 days; purity >95%) was diluted in Tris-\(\text{HCl}\) buffer containing 0.01% bovine serum albumin, and the compounds to be tested were dissolved in 1 mM \(\text{HCl}\) containing 0.1% bovine serum albumin. Binding (in duplicate) was initiated by addition of 220 \(\mu\)l of chicken retinal membrane suspension to tubes containing 20 \(\mu\)l of appropriate test compound concentrations or vehicle, and 20 \(\mu\)l of 2-\[^{125}\text{I}\]iodomelatonin dilution (final concentration: \(\pm\) 50 pM (30-60 pM)). The tubes were incubated at 25 °C for 1 h in the dark. Reactions were terminated by addition of 5 ml of ice-cold Tris-\(\text{HCl}\) buffer, and the contents were immediately filtered over glass-fiber filters (Schleicher & Schuell no. 30) soaked in 0.5% (v/v) polyethyleneimine solution. Each filter was washed twice with 5 ml of the ice-cold buffer. Radioactivity was determined in a gamma counter. Non-specific binding was defined as binding in the presence of 3 \(\mu\)M 6-chloromelatonin (donated by Clemens, Eli Lilly Laboratories, Indianapolis, USA). Specific binding of 2-\[^{125}\text{I}\]iodomelatonin was calculated by subtracting non-specific binding from total binding and expressed as fmol/mg of protein. \(K_i\) values of the test compounds were calculated from IC\(_{50}\) values by the method of Cheng and Prusoff [81].

5.8.2 DETERMINATION OF INHIBITION OF CALCIUM-DEPENDENT RELEASE OF \[^{3}\text{H}\]dopamine FROM RABBIT RETINA

Tissue Preparation

Albino rabbits (2.5-3.5 kg) maintained on a 14 to 10 h light-dark cycle were killed by decapitation during the light phase. All experimental procedures were carried out in the light. Rabbit eyes were enucleated carefully. The remaining eye cup was everted and placed in a vial containing 5 ml of Krebs’ solution containing 1.3 mM CaCl\(_2\) and gassed with 95% \(\text{O}_2\)-5% \(\text{CO}_2\). The rabbit retina was detached by gentle shaking during which the retina fragmented into several pieces. These pieces were used in the \[^{3}\text{H}\]dopamine release experiments.

\[^{3}\text{H}\]Dopamine Release Experiments

Retinal pieces were incubated for 20 min at 37 °C in the presence of 0.1 \(\mu\)M \[^{3}\text{H}\]dopamine (specific activity: \(\pm\) 30 Ci/mmol, New England Nuclear, USA). Thereafter, the pieces of retina were washed in 5 ml of Krebs’ solution at 37 °C. The tissue from each retina was divided into four approximately equal portions and placed in four individual cylindrical plastic tubes with a thin nylon mesh on the bottom. These plastic tubes were then transferred to individual glass superfusion chambers containing platinum electrodes 30 mm apart. The tissue samples were superfused with Krebs’ solution, prewarmed at 37 °C, at a rate of 1 ml/min until the spontaneous outflow of radioactivity occurred levelled off (about 60 min). Tritium release, i.e. release of \[^{3}\text{H}\]dopamine, was elicited by field stimulation at 3 Hz for 2 min (20 mA, 2 msec duration). Field stimulations were applied in each experiment at either 60 (S\(_1\)) or 100 (S\(_2\)) min after the end of the incubation with \[^{3}\text{H}\]dopamine. In all experiments S-sulpiride (0.1 \(\mu\)M) was added to the superfusion medium 40 min before S\(_1\) and maintained until the end of the experiment. Melatonin, or a test compound, was added to the perfusion medium 20 min after S\(_1\) and was present throughout the remainder of the experiment. Four-minute samples of the superfusate were collected before, during and after the period of stimulation. The outflow of radioactivity in each sample was expressed as the percentage of the total tissue radioactivity present at the beginning of each sample collection [9]. The overflow of transmitter, also termed “stimulation-evoked release of \[^{3}\text{H}\]dopamine” or “calcium-dependent release of \[^{3}\text{H}\]dopamine”, was the percentage of total tissue radioactivity released above the spontaneous levels during and after the period of stimulation [9]. Results are expressed as the ratio S\(_2\)/S\(_1\) obtained between the percentage of total tissue radioactivity released above spontaneous levels during the second (S\(_2\)) and first (S\(_1\)) periods of field stimulation within the same experiment. The ratio S\(_2\)/S\(_1\)
obtained in the absence of a test compound is approximately one. The IC₅₀ value of a test compound is the concentration of the test compound required to get 50% inhibition of the maximal inhibition of the stimulation-evoked release of [³H]dopamine, that can be reached with the test compound. The IC₅₀ values were determined graphically from concentration-effect curves.

5.9 REFERENCES


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