3 1,2,3,4-Tetrahydroisoquinoline derivatives as potential new 5-HT₇ receptor agonists

3.1 Introduction

3.1.1 Apomorphine as template for ligands interacting at 5-HT receptors

The rigid chemical structure of apomorphine (1, Figure 3.1) has shown its use as a template for compounds interacting at both dopaminergic and serotonergic systems. Excellent examples of this kind of pharmacologically active compounds arise from the expanding number of ligands based on the 2-aminotetralin skeleton (Figure 3.1)\textsuperscript{12,20,26,35-39,50,51,66,77,84}.

Figure 3.1: Chemical structure of apomorphine. The 2-aminotetralin (left) and tetrahydroisoquinoline subunits (right) are highlighted with thick lines.

However, it has been demonstrated that apomorphine (\textit{R}-enantiomer) is a poor ligand for 5-HT receptors\textsuperscript{9}. Many of its physiological effects are related to dopaminergic receptors, since it contains the extended conformation of dopamine\textsuperscript{59,71,74}. When it was shown, however, that (\textit{R})-11-hydroxy-10-methyl-aporphine possesses both high affinity and efficacy for the 5-HT\textsubscript{1A} receptor, many aporphine derivatives were synthesized and evaluated for their affinity for members of the family of serotonin receptors\textsuperscript{15-19,31-33}. In many cases this radical change in selectivity from dopaminergic to serotonergic receptors originates from the introduction of a steric group at the 10-position of the apomorphine nucleus, suggesting the presence of a (small) lipophilic pocket at the binding site of the serotonin receptor subtype. Although only the (\textit{R})-enantiomer of most compounds was tested, in one particular case (10-methyl-11-hydroxy-N-methylaporphine) the stereochemistry appeared to be crucial in discriminating between agonism and antagonism. Several examples, however, are available that show that stereochemistry is a less important determinant for efficacy. Recent studies have indicated that (\textit{R})-aporphine analogues with aryl substituents at the 11-position exhibit interesting and diverse pharmacological profiles as well\textsuperscript{48,49}. Continuation of this particular research project on serotonergic (\textit{R})-aporphine derivatives resulted in a series of (\textit{S})-2-dimethylaminotetralins with various aryl substituents at the 5-position showing a
possibly even more interesting pharmacological profile. These series of compounds have been discussed extensively in Chapter 1, Sections 1.4.1 and 1.4.4.

3.1.2 1,2,3,4-Tetrahydroisoquinolines

Yet another interesting framework that can be derived directly from the chemical structure of apomorphine is the 1,2,3,4-tetrahydroisoquinoline skeleton (THIQ, Figure 3.1, right). The structure is frequently used in drugs interacting at a number of biological systems. Furthermore, its presence is abundant among various naturally occurring alkaloids. In the central nervous system, various endogenous and non-endogenous THIQs have been identified as well, and often their presence is suggested to be related with Parkinson's disease (e.g. N-methylsalolinol, and alcoholism. In many cases, these derivatives are formed by the metabolism of dopamine and alcohol by in vivo Pictet-Spengler cyclization reactions. The neurotoxic effect of these compounds is closely related to that of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) due to MAO-A and MAO-B mediated conversions of the THIQ moiety into a N-methylisoquinolinium ion (NMIQ+). As a result analogous to MPP+, blockade of normal mitochondrial functioning by NMIQ+ causes cell death.

Figure 3.2: Chemical structures of referred compounds.
In contrast to the neurotoxic effects of THIQs, several reports discuss the possibility of THIQs showing neuroprotective qualities as well\(^4,58,63\). It has been hypothesized that the neuroprotective effect of smoking with respect to Parkinson’s disease can be attributed to the formation of cyano-THIQs, which appear to be potent inhibitors of MAO-A and MAO-B (e.g. 1-cyano-THIQ, \(4\))\(^45,82\). Additionally, endogenous formation of these cyano-THIQs would decrease the levels of neurotoxic THIQs and consequently prevent induction of Parkinson’s disease.

Other highly diverse examples of functionalities of THIQ-based ligands can be found in the inhibition of the \(N\)-methyl-D-aspartate (NMDA) subtype of the glutamate receptor\(^64\), inhibition of the \(\alpha_2\)-adrenergic receptor\(^28,78\), and inhibition of phenylethanolamine \(N\)-methyltransferase (PNMT), which catalyzes the conversion of the neurotransmitter norepinephrine to epinephrine\(^28,29,72\). More closely related to the nature of our own research, and other projects within our medicinal chemistry group is the development of 8-amino-2-methyl-4-fenyl-1,2,3,4-tetrahydroisoquinoline (Nomifensine, \(5\)) as an antidepressant\(^24,40,67\). Despite its withdrawal from the market in 1986 due to serious side effects (acute hemolytic anemia, renal failure), this dopamine-mimetic compound and reuptake inhibitor of noradrenaline and dopamine was an important lead for further development of novel drugs interacting with the dopamine \(D_1\) receptor\(^69,70\). Additionally and highly relevant, it was found that the chiral 5-substituted 2-aminotetralin nucleus of dopamine \(D_3\) antagonists could be replaced by non-chiral 7-substituted THIQs (e.g. \(6a, 6b\))\(^8,13,14,68,79\). The THIQ-analogues not only showed increased affinity and selectivity for the \(D_3\) receptor they also were metabolized less rapidly as their 2-aminotetralin derivatives. Therefore, the THIQ nucleus was seriously considered to be an interesting target in replacing the 2-aminotetralin nucleus of, among other things, the 5-HT\(_7\) active ligand (\(R\))-8-OH-DPAT. Recently, a similar rationale of replacing aryl-piperazine moieties by the THIQ nucleus has been studied thoroughly also, and resulted in potent inhibitors of subtypes of the extensive family of serotonergic receptors (e.g. the THIQ analogue of buspirone, \(7\))\(^10,11,53,54\).

Intensive SAR studies on THIQs performed by this research group revealed that the ionization constant of compounds of this kind (\(pK_a = 9.30\)) is similar to those reported for simple 1-arylpiperazines (\(pK_a = 7.94 - 9.14\)), but significantly lower than that of piperidine (\(pK_a = 10.86\)). With respect to the lipophilicity of these compounds, reported log\(P\) values are also comparable to many 1-arylpiperazines. However, the results of this study also suggested that the crucial distance between the aromatic ring center and the basic nitrogen atom in THIQs is too short (3.77 Å) to form a bioactive complex of THIQ with 5-HT\(_{1A}\) receptors. According to the preliminary pharmacophore model described in Chapter 2, the optimal distance between the centroid of the aromatic ring and the protonatable nitrogen atom for 5-HT\(_7\) agonism should be in the range of 5.2 – 6.4 Å. As a consequence, affinity for the 5-HT\(_7\) receptor of unsubstituted THIQs was expected to be low. Nevertheless, it was hypothesized that an increase of this parameter could be achieved by elongation of the distance between centroid and positively charged nitrogen by introduction of a flexible ethylamine side chain at the chemically readily accessible 2-position of the THIQ skeleton.
Introduction of a hydrogen bond accepting moiety at different positions of the aromatic ring could be provided by selecting the appropriate phenyl ethylamine as a building block for the Pictet-Spengler and Friedel-Crafts cyclization reaction at the onset of the synthesis of THIQ-based 5-HT$_7$ receptor ligands, as depicted in Scheme 3.1 and Scheme 3.2, respectively.

### 3.2 Molecular computations of THIQs

#### 3.2.1 Conformational analysis and pharmacophore fitting

Based on the pharmacophore identification procedure described in the Chapter 2, it was suggested that N-substituted THIQs could comply with the minimal requirements of the preliminary pharmacophore model for 5-HT$_7$ receptor agonism and therefore could be a valuable basis for further exploration and refinement of this model. As compared to the related, yet chiral, 2-aminotetralins, achiral THIQs have the advantage that separation of enantiomers is no longer a necessity. Full conformational analysis of simple THIQs with an ethylamine side chain at the 2-position, followed by the previously described assignment of extension vectors to the functional groups and subsequent calculation of the best fit, indicated that low energy conformations of these ligands could indeed fulfill the structural requirements of the initially determined pharmacophore model. It furthermore appeared that there was a significant difference in best fits when THIQs with hydrogen bond accepting moieties at the 6- or 7-position were compared.

![Stereo view of the fitting of 6-substituted THIQ's relative to ligands used for determination of preliminary pharmacophore model. Non-essential hydrogen atoms have been omitted.](image)

Complying with the three individual items of the preliminary pharmacophore model (i.e. the hydrogen bond accepting moiety, the aromatic ring, and the positively charged nitrogen atom of the ethylamine side chain), the THIQs with a methoxy group at the 7-position (Figure 3.4) adopted an orientation of the THIQ nucleus equiplanar relative to the heterocyclic nuclei of indoles and
ergolines, while those possessing this functionality at the 6-position (Figure 3.3) fitted only when adopting an orientation of the THIQ nucleus perpendicular to the heterocyclic nuclei of the remaining ligands.

![Stereo view of the fitting of 7-substituted THIQ's relative to ligands used for determination of preliminary pharmacophore model. Non-essential hydrogen atoms have been omitted.](image)

The differences in binding modes between the potential new 5-HT₇ receptor agonists based on the THIQ nucleus and the tryptamines were acknowledged at this stage of our research project. As a result, evaluation of the pharmacological data of this class of ligands could present valuable information about the interior of the receptor binding site and contribute to a more detailed pharmacophore model for 5-HT₇ receptor agonism.

### 3.2.2 Physicochemical properties of THIQs

LogP calculations (Table 3.1) of simple THIQs equipped with an ethylamine side chain at the 2-position revealed lipophilicities comparable to those of simple arylpiperazines, tryptamines, and 2-aminotetralins (LogP = 0.8 – 2.2). These data suggest that 2-ethylamino-THIQs, like drugs of the previously mentioned classes that had proven to interact with other members of the family of serotonin receptors, could be candidates for 5-HT₇ receptor ligands. Moreover, calculations of pKₐ values (Table 3.1) of compounds 13a - 13e and 23 indicated that this value is significantly higher for the ethylamine nitrogen (pKₐ = 8.58) than for the nitrogen atom of the heterocyclic ring of the THIQ nucleus (pKₐ = 4.31). However, the pKₐ value of the ethylamine nitrogen is close to the value calculated for serotonin its ethylamine nitrogen (pKₐ = 10.18).
3.3 Synthesis of ligands

3.3.1 6-Substituted 1,2,3,4-tetrahydroisoquinolines

The synthesis route leading to 6-substituted 1,2,3,4-tetrahydroisoquinolines is outlined in Scheme 3.1. The 1,2,3,4-tetrahydroisoquinoline skeleton with an electron donating substituent at the 6-position is easily accessible through Pictet-Spengler cyclization of the appropriately substituted phenethylamines with formaldehyde under strong acidic conditions. 3-Methoxyphenethylamine (9) is commercially available; however, we did obtain it in excellent yield by facile reduction of the benzonitril precursor 8. Substitution of the nitrogen at the 2-position was achieved by alkylation under alkaline conditions with bromo-acetonitril, followed by reduction of the cyano group, and further functionalization of the primary amine under reductive alkylation conditions with the appropriate aldehydes.

Scheme 3.1: Synthesis of 6-substituted-THIQ derivatives. Reagents and conditions: (a) LiAlH₄/AlCl₃, diethyl ether. (b) Formaldehyde/HCl. (c) Bromoacetonitril, K₂CO₃, KI, acetone. (d) LiAlH₄, THF. (e) Aldehyde, NaCNBH₃, ethanol, [H⁺]. (f) 2-Chloroacetyl chloride, K₂CO₃, CH₂Cl₂. (g) Amine, K₂CO₃, KI, acetonitril, DMF. (h) 2-Chloroethyl-piperidine, K₂CO₃, KI, DMF. (i) HBr, Δ.
As an alternative route to these ligands, functionalization of the nitrogen of the THIQ-skeleton occurred through acetylation with 2-chloroacetyl chloride. This provided us with a method for the introduction of primary or secondary amines that were not accessible through the reductive alkylation procedure discussed earlier. Subsequent reduction with LiAlH₄ of the acetamide intermediate obtained after nucleophilic substitution of the halogen by the appropriate amine yielded the target compounds in moderate yields. Under the applied conditions cleavage of the acetamide instead of reduction was major cause of the lower yields. Later on, it was ascertained that this drawback could be reduced significantly by the use of an equimolar mixture of LiAlH₄ and AlCl₃ for the reduction of these acetamides.

In a few cases (13c, 13d), the final products were obtained directly by substitution of the THIQ secondary amino group by alkyl halides under alkaline conditions. This method is the most direct of all, and generally leads to target compounds in good yields. However, this approach is applicable only for those cases were the appropriate alkyl halides are available.

In order to investigate the effect on 5-HT₇ receptor binding of replacement of the methoxy group (hydrogen bond acceptor) by a hydrogen bond donating hydroxyl moiety, dealkylation of the methoxy group (13d) was accomplished by refluxing precursor 13c in HBr. Functional group transformation of this kind proceeded with good yields and without formation of by products.

### 3.3.2 7-Substituted 1,2,3,4-tetrahydroisoquinolines

Synthesis of 1,2,3,4-tetrahydroisoquinolines with a substituent at the 7-position was accomplished starting from commercially available 4-methoxy-phenethylamine. Since Pictet-Spengler cyclization of phenethylamines bearing an electron donating substituent (ortho- / para-director) at the 4-position generally proceeds with poor yields, the THIQ-skeleton was synthesized in a 3-step process via the carbamate as outlined in Scheme 3.2. The tetrahydroisoquinolinone skeleton is formed by a Friedel-Crafts ring closure in polyphosphoric acid (PPA). Careful control of the reaction temperature appeared to be crucial in this reaction step, since formation of the intermediate carbocation requires sufficiently high temperatures, while overheating of the reaction mixture predominantly induces hydrolysis of the carbamate. Once formed, further functionalization of the THIQ-skeleton after reduction of the isoquinolinone intermediate 18 was generally achieved in the same way as used for the 6-substituted-THIQ derivatives.
3.4 Pharmacology

Compounds 12, 13a-e, 19, 21, 22a, and 25 were evaluated for their ability to compete for \[^{3}H\]5-CT binding to cloned rat 5-HT\(_7\) receptors expressed in HEK-293 cells at the laboratories of Pfizer and former SmithKline-Beecham. The results are summarized in Table 3.1.

3.5 Results and discussion

The design and synthesis of THIQ-based ligands as possible targets to investigate the 5-HT\(_7\) receptor binding capacities was profoundly guided by molecular modeling studies as described in Section 3.2. Based on the preliminary pharmacophore model for 5-HT\(_7\) receptor
agonism, as described in Chapter 2, it was hypothesized that THIQs equipped with an ethylamine side chain at the 2-position should be able to adopt a low energy conformation that would comply with the minimal requirements of our model. It therefore appeared to be an interesting class of ligands that could contribute to further analyze the 5-HT7 receptor pharmacophore and investigate the boundaries and molecular interactions of ligands at the binding site of the receptor.

The poor binding data of compounds 12, 13a-e, 19, 21, 22a, and 25, as listed in Table 3.1, unambiguously demonstrate the inability of these series of compounds to compete for [3H]5-CT binding to cloned 5-HT7 receptors, which is in contrast with preliminary molecular modeling computations. The reason for this lack of receptor affinity might arise from the geometrical data of the supposed binding conformations of these ligands. The lack of binding affinity of compounds 19 and 25, both missing the ethylamine side chain at the 2 position, was consistent with our expectations because of the small distance between the protonatable nitrogen atom of the six-membered heterocyclic ring and the centroid of the aromatic ring10,11,53,54. In case of the THIQs with an ethylamine side chain at the 2-position, the average distance between the positively charged nitrogen atom of the ethylamine side chain and the centroid of the six-membered aromatic ring differs only 0.42 Å when the 6-substituted THIQs were compared to the average value derived from the ligands used to determine the preliminary pharmacophore model (Table 2.2). The contrast is larger for the distance between the hydrogen bond accepting moiety and the positively charged nitrogen atom of the ethylamine side chain (1.75 Å). As for the 7-substituted analogues, the differences compared to the preliminary pharmacophore model are even more pronounced (1.76 Å and 3.18 Å, respectively). The binding data of both 12 and 21 clearly indicate that the core

![Chemical Structure](image)

**Table 3.1: Calculated physical properties and experimental 5-HT7 receptor binding affinities of compounds 12, 13a-e, 19, 21, 22a and 25.** \(\Delta E\): calculated energy difference Global minimum – fitted conformation. Not determined abbreviated as n.d.

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<th>Compound</th>
<th>R1, R2</th>
<th>R3</th>
<th>Centroid O (Å)</th>
<th>Centroid N* (Å)</th>
<th>Centroid N* (Å)</th>
<th>(\Delta E) (kJ/mol)</th>
<th>(\text{log}P) (calc)</th>
<th>(pK_a) N (calc)</th>
<th>(pK_a) N (calc)</th>
<th>(K_i) (nM)</th>
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<td>8.39</td>
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<td>8.40</td>
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<td>4.65</td>
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structure of 2-ethylamino-THIQs lacks affinity for the 5-HT\textsubscript{7} receptor. Alterations of log\textit{P} values by attaching simple alkyl substituents at the terminal primary nitrogen atom have no positive effect on binding affinity at all (Table 3.1). Apparently, the different binding modes of the 6- and 7-substituted THIQs (orientations respectively perpendicular to, and equiplanar with the ligands used to derive the preliminary pharmacophore model) as calculated by APOLLO, both share specific features that impede repulsive interactions with the binding site of the receptor. The differences in conformational energies of the fitted conformations, relative to the conformational energies of the global minima, are well within the range of 50 kJ/mol in case of the 6-substituted THIQs ($\Delta E = 6.42$ kJ/mol). Even more, in case of the 7-substituted analogues, APOLLO was able to find good fits with all the other ligands of the preliminary pharmacophore model using the global minimum conformations of these ligands (21, 22a).

Since all ligands used to derive the preliminary pharmacophore model are orientated in an extremely equiplanar mode with no substituents oriented out of this plane, it was hypothesized that ligands, that have to adopt a non-equiplanar orientation (12, 13a – 13e) in order to comply with the requirements of the pharmacophore model, as well as ligands that possess atoms and/or substituents out of this plane (21, 22a, 24a), might experience considerable steric repulsions of amino acid residues at the binding site of the receptor. Also, both the 6- and 7-substituted THIQs adopt a different orientation of the ethylamine side chain as compared to the other ligands used to derive the preliminary pharmacophore model in order to bind to the mutual interaction points mimicking the putative receptor binding site. This discrepancy is likely to be an additional cause of loss of affinity for the 5-HT\textsubscript{7} receptor, suggesting potentially severe steric interactions of amino acid residues located at the binding site of the receptor with the ethylamine side chain of these ligands.

As a consequence of the above results, no further efforts were made to synthesize THIQ-based ligands as potential agonists for the 5-HT\textsubscript{7} receptor. Instead, the development of potentially 5-HT\textsubscript{7} receptor agonists and further characterization of its pharmacophore model for agonism was mainly focused on indole- and aminotetralin-based ligands. These structures show more similarities with the endogenous ligand and high affinity agonists like (+)-LSD and 5-CT. Furthermore, they are characterized by a flat aromatic ring structure free of (groups of) atoms oriented out of this plane possibly causing steric interactions with amino acid residues of the binding site of the receptor.

### 3.6 Conclusions

Evaluation of the pharmacological data determined for two series of 6- and 7-substituted 1,2,3,4-tetrahydrosioquinoline derivatives with ethylamine side chains at the 2-position revealed that these ligands show no affinity for the 5-HT\textsubscript{7} receptor. These results were not in line with the expectations based on our molecular modeling computations (Sections 3.2.1 and 3.5). These
computations indicated that the ligands are able to adopt low energy conformations that are capable of matching the prerequisites of the preliminary pharmacophore model for 5-HT\textsubscript{7} receptor agonism described in Chapter 2. It is hypothesized that ligands, that have to adopt a non-equiplanar orientation in order to comply with the requirements of the pharmacophore model, as well as ligands that possess atoms and/or substituents out of this plane, might experience considerable steric repulsions of amino acid residues at the binding site of the receptor. Additionally, the different orientation of the ethylamine side chain may also be responsible for this observed loss of affinity for the receptor. Severe steric interactions of amino acid residues located at the binding site of the receptor are likely to be the actual cause of this inactivity.

The lack of affinity of these THIQs provides additional information on the interior of the 5-HT\textsubscript{7} receptor binding site, and will prove its use in further development of the 5-HT\textsubscript{7} receptor pharmacophore model and, as a consequence, future design of 5-HT\textsubscript{7} receptor agonists.

### 3.7 Experimental section

#### 3.7.1 Molecular computations

**Conformational analysis**

Calculations of physical properties (Log\textsubscript{P}, pK\textsubscript{a}) were performed with ChemDraw\textsuperscript{3} and Pallas\textsuperscript{1}. All molecular computations were performed on a Silicon Graphics O2 workstation running IRIX 6.3 or a Silicon Graphics IRIS Indigo XS/4000 workstation running IRIX 5.3. For conformational analyses, ligands were sketched with the correct stereochemistry, when relevant, in their protonated, positively charged state from standard fragments in MacroModel\textsuperscript{2}. Structures were minimized with default options prior to full conformational analysis. Conformational analyses were performed using the Monte Carlo Multiple Minimum (MCMM) search protocol\textsuperscript{21} with a minimum of 1500 steps via the SUMM option\textsuperscript{27}. Minimizations were performed using the Truncated Newton Conjugate Gradient (TNCG) minimization method within the MM3\textsuperscript{*} force field\textsuperscript{6,46,47} with simulation of a distance dependent water continuum\textsuperscript{80} as implemented in MacroModel. Per step, 250 iterations were performed until the gradient reached the value of 0.01 kcal \cdot Å\textsuperscript{-1} \cdot mol\textsuperscript{-1}. All conformations within a range of 50 kJ/mol above the global minimum were considered to be relevant. In case of non-chiral ligands, mirror images were preserved using the NANT option. Additional information needed for subsequent calculations with the pharmacophore identifying software program APOLLO was obtained through incorporation of DEBG options 3 and 28. Torsion bonds of n-propyl chains were fixed in their anti-staggered conformation to reduce the number of low energy conformations. Molecular dimensions and atomic distances were determined by manual selection of the appropriate atomic features in the Analyze mode.

**Pharmacophore identification**

The VECADD module of APOLLO\textsuperscript{76} was used to define extension vectors and centroids to all low energy conformations of all ligands as calculated by MacroModel. Extension vectors were allocated to the lone-pairs of the oxygen atoms of substituents (if present) of the six-membered aromatic ring present in all ligands, and the protonated, positively charged nitrogen atom also present in all ligands. A centroid was defined for the six-membered aromatic ring of the indoles and ergoline-based ligands. The RMSFIT module of APOLLO subsequently identified the conformations of the ligands that exhibited the best overall least square fit with respect to the specified extension vectors and centroids. This calculation was performed in an energy dependent manor, while all fitting points were considered equally important. In case multiple
solutions were calculated, the matches were ranked with respect to conformational energies and root mean square deviations. The best fit was extracted from the RMSFIT output file using the MMDFIT module of APOLLO.

3.7.2 Chemistry

General remarks
Syringes were carried out with commercially available chemicals that were used as received. THF, toluene and diethyl ether were distilled from sodium prior to use for chemical reactions (not for extractions). Generally, target compounds were purified by means of chromatography on silica gel (Merck 60) and eluted with various mixtures of solvents. Unless stated otherwise, all target compounds that were evaluated for their pharmacological profiles were converted in the HCl salts by treatment of the free base dissolved in ether or ethanol with 1 equivalent of HCl (1N solution in ether). The purity of the target compounds was established using various techniques. IR spectra were recorded on an ATI-Mattson Genesis Series FT-IR spectrophotometer. NMR data were recorded using Varian Gemini-200 and Varian VXR-300 spectrometers (\(^{1}\)H-NMR at 200 or 300 MHz, \(^{13}\)C-NMR at 50 or 75 MHz). Chemical shifts are denoted in \(\delta\) units (ppm) relative to the solvent (\(^{1}\)H-NMR peaks: 7.26 for CDCl\(_3\) and 3.30 for CD\(_3\)OD. \(^{13}\)C-NMR peaks: 76.91 for CDCl\(_3\) and 49.50 for CD\(_3\)OD). The following abbreviations are used to indicate peak multiplicities and characteristics: b (broad), s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), m (multiplet). Electron impact (EI\(^{+}\)) mass spectra were recorded on a Unicam Automass mass spectrometer in conjunction with a gas chromatograph. TLC analyses were carried out on aluminum plates (Merck) precoated with silica gel 60 F\(_{254}\) (0.2 mm). Visualization of spots was performed with UV light and a commonly used alkaline KMnO\(_4\) spray solution or Silica coated I\(_2\). Elemental analyses (C, H, N) for novel target compounds were performed at the Chemistry Department of the University of Groningen.

2-(3-Methoxy-phenyl)-ethylamine (9)
In a 3-necked flask under nitrogen, LiAlH\(_4\) (0.78 g, 20 mmol) was suspended in dry ether (25 mL) at rt. A solution of AlCl\(_3\) (2.71 g, 20 mmol) in dry ether (30 mL) was added rapidly. After 10 min, 3-methoxy-benzylcyanide (3.00 g, 20 mmol), dissolved in dry ether (40 mL), was added dropwise. The suspension was then stirred at rt for 2 hours. The reaction was carefully quenched with water (40 mL) and the organic phase was separated. The water layer was made alkaline (4N NaOH, pH >10), diluted, and extracted with CH\(_2\)Cl\(_2\) (5 x 25 mL). The combined organic layers were dried (Na\(_2\)SO\(_4\)), filtered, and concentrated in vacuo to yield the 9 as a colorless oil (2.86 g, 19 mmol). \(^{1}\)H-NMR (200 MHz, CDCl\(_3\)), \(\delta\) (ppm): 1.55 (br, 2H), 2.64 – 2.70 (m, 2H), 2.82-3.00 (m, 2H), 3.73 (s, 3H), 6.70 – 6.76 (m, 3H), 7.13-7.17 (m, 1H). \(^{13}\)C-NMR (50 MHz, CDCl\(_3\)), \(\delta\) (ppm): 33.1, 40.5, 54.8, 112.3, 114.2, 120.7, 129.7, 137.7, 159.8. IR (neat, cm\(^{-1}\)): 3368, 2937, 2835, 1609, 1507, 1488, 1454, 1260, 1152, 1045, 780, 696. MS (EI\(^{+}\)): 151, 122 (100%), 91.

6-Methoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (10)
3-Methoxy-phenethylamine (9) (2.86 g, 19 mmol) is stirred at rt. Formaldehyde (1.54 g, 37 % solution in water, 19 mmol) was added dropwise. The mixture was heated at 65 °C for 1 hr, then cooled to rt, and HCl (20%, 25 mL) was added. The clear solution was heated again to 65 °C for 1 hr, after which the volatiles were removed under reduced pressure. This yielded 4.54 g of a white solid, which was crystallized from ethanol to yield 3.62 g (18 mmol) of the HCl salt of 6-methoxy-1,2,3,4-tetrahydroisoquinoline (10). \(^{1}\)H-NMR (200 MHz, CD\(_2\)OD), \(\delta\) (ppm): 3.10 (t, \(J = 6.35\) Hz, 2H), 3.47 (t, \(J = 6.35\) Hz, 2H), 3.78 (s, 3H), 4.28 (s, 2H), 6.81-6.87 (m, 2H), 7.13 (d, \(J = 8.30\) Hz, 1H). \(^{13}\)C-NMR (50 MHz, CD\(_2\)OD), \(\delta\) (ppm): 24.6, 41.1, 43.8, 54.2, 112.9, 113.1, 119.5, 127.6, 132.2, 159.3. IR (KBr, cm\(^{-1}\)): 2926, 2973, 2938, 2835, 1609, 1589, 1507, 1451, 1407, 1331, 1244, 1162, 1115, 1029, 811. MS (EI\(^{+}\), free base): 162 (100%), 134, 91.
(6-Methoxy-3,4-dihydro-1H-isoquinolin-2-yl)-acetonitril (11)

In a 3-necked flask equipped with a reflux condenser, 10 (2.00 g, 10 mmol) was suspended in acetone (100 mL). K$_2$CO$_3$ (2.38 g, 20 mmol) and KI (catalytic amount) were added at rt. Bromoacetonitril (1.80 g, 15 mmol) was added dropwise through a syringe and heating was applied to a gentle reflux until TLC showed total consumption of the starting material. The suspension was evaporated to dryness under reduced pressure, and portioned between water and CH$_2$Cl$_2$. The water layer was further extracted with CH$_2$Cl$_2$ and the combined organic fractions were dried, filtered, and concentrated in vacuo to yield the crude product as a semi-solid (1.70 g). Crystallization from diisopropyl ether yielded pure 11 as colorless platelets (1.50 g, mp = 103 ºC). 1H-NMR (200 MHz, CDCl$_3$), δ (ppm): 1.73 (br, 2H), 2.52-2.28 (m, 2H), 3.65-3.72 (m, 2H), 3.78 (s, 3H), 6.65-6.75 (m, 2H), 6.96 (d, 1H). 13C-NMR (50 MHz, CDCl$_3$), δ (ppm): 29.0, 45.7, 49.4, 53.5, 55.0, 112.2, 113.1, 114.7, 125.3, 127.3, 134.1, 158.1. IR (KBr, cm$^{-1}$): 3005, 2962, 2818, 2228, 1610, 1501, 1458, 1418, 1386, 1320, 1243, 1162, 1135, 1032, 940, 907, 856, 818. MS (EI$^+$): 201 (100%), 175, 134, 91.

2-(6-Methoxy-3,4-dihydro-1H-isoquinolin-2-yl)-ethylamine di-hydrochloride (12)

In a 3-necked flask under nitrogen, LiAlH$_4$ (0.41 g, 11 mmol) was suspended in dry THF (100 mL) at rt. After 10 min, (6-methoxy-3,4-dihydro-1H-isoquinolin-2-yl)-acetonitril (11, 2.00 g, 10 mmol in THF (10 mL), was added dropwise through a dropping funnel. The suspension was then stirred at rt for 90 min. The reaction was carefully stopped by dropwise addition of water (2 mL) and NaOH (4N, 0.5 mL). The salts were removed by filtration over Celite and washed with ether. The organic layer was dried (Na$_2$SO$_4$), filtered, and concentrated in vacuo to yield the product as an oil (1.92g, 9.3 mmol). Salt formation in ethanol (excess of 1N HCl/diethyl ether) gave the pure product 12 as a white solid. 1H-NMR (200 MHz, CDCl$_3$), δ (ppm): 1.73 (br, 2H), 2.52-2.28 (m, 2H), 2.66-2.72 (m, 2H), 2.85 (t, J = 6.1 Hz, 4H), 3.55 (s, 2H), 3.74 (s, 3H), 6.61-6.70 (m, 2H), 6.91 (d, J = 8.3 Hz, 1H). 13C-NMR (50 MHz, CDCl$_3$), δ (ppm): 28.0, 37.6, 49.5, 53.7, 54.1, 59.4, 110.5, 111.6, 125.5, 125.9, 134.0, 156.4. IR (free base, neat, cm$^{-1}$): 3367, 3287, 2924, 2853, 1613, 1503, 1454, 1257, 1158, 1038. MS (EI$^+$): 206, 176 (100%), 162, 91.

[2-(6-Methoxy-3,4-dihydro-1H-isoquinolin-2-yl)-ethyl]-dimethyl-amine di-hydrochloride (13a)

2-(6-Methoxy-3,4-dihydro-1H-isoquinolin-2-yl)-ethylamine (12, 0.50 g, 2.43 mmol) was dissolved in ethanol (25 mL) in a 3-necked flask under nitrogen. A 37 % solution of formaldehyde in water (0.40 g, 4.9 mmol) was added dropwise, followed by the addition of NaCNBH$_3$ (0.15 g, 2.43 mmol). The pH was adjusted (pH 5) by addition of acetic acid. After stirring at rt overnight, water (25 mL) was added, and the ethanol was evaporated in vacuo. The water layer was made alkaline (NaOH, 4N), and extracted with diethyl ether (4 x 20 mL). The combined organic layers were washed (brine), dried (Na$_2$SO$_4$), filtered, and concentrated in vacuo to yield the product as a colorless oil (0.56 g). The free base of 13a was converted in its double HCl salt by addition of HCl (6 mL, 1N in diethyl ether) to a solution of the free base in absolute ethanol. 1H-NMR (free base, 200 MHz, CDCl$_3$), δ (ppm): 2.31 (s, 6H), 2.58-2.89 (m, 8H), 3.60 (s, 2H), 3.76 (s, 3H), 6.62-6.71 (m, 2H), 6.93 (d, J = 8.3 Hz, 1H). 13C-NMR (50 MHz, CDCl$_3$), δ (ppm): 27.6, 44.1, 46.6, 49.8, 53.7, 54.3, 55.3, 110.6, 111.6, 125.0, 126.0, 133.6, 156.5. IR (free base, neat, cm$^{-1}$): 2945, 2458, 1618, 1515, 1457, 1318, 1282, 1258, 1165, 1036. MS (EI$^+$): 234, 176 (100%), 162, 91, 58.

6-Methoxy-2-(2-pyrrolidin-1-yl-ethyl)-1,2,3,4-tetrahydro-isoquinoline di-hydrochloride (13b)

In a 3-necked flask with reflux condenser, 12 (free base, 0.24 g, 1.16 mmol) was dissolved in CHCl$_3$ (15 mL). Succinic anhydride (0.17 g, 1.70 mmol) was added and the mixture was heated to a gentle reflux for 4 hr. The solvent was evaporated in vacuo to yield 0.51 g of a brown oil. Contaminants were removed by chromatography (Silica, gradient CH$_2$Cl$_2$/CH$_3$OH). The colorless oil of 1-[2-(6-methoxy-3,4-dihydro-1H-isoquinolin-2-yl)-ethyl]-pyrrolidine-2,5-dione thus obtained (0.13 g, 0.45 mmol), was dissolved in THF (5 mL) and added dropwise to a stirred suspension of LiAlH$_4$ (0.76 g, 2.0 mmol) in THF (25 mL) under nitrogen. Stirring for 2 hr at rt was followed by careful decomposition of LiAlH$_4$ with adequate amounts of water and NaOH (4N). The salts were removed by filtration and the clear solution was evaporated in vacuo to yield 0.51 g of a brown oil. The clear solution was obtained (0.13 g, 0.45 mmol), was dissolved in THF (5 mL) and added dropwise to a stirred suspension of LiAlH$_4$ (0.76 g, 2.0 mmol) in THF (25 mL) under nitrogen. Stirring for 2 hr at rt was followed by careful decomposition of LiAlH$_4$ with adequate amounts of water and NaOH (4N).
concentrated under reduced pressure. The pure free base of 13b was obtained after chromatography (Silica, gradient CH2Cl2/CH3OH), which was converted in its double hydrochloride salt by treatment of the free base dissolved in diethyl ether with an excess of HCl (1N solution in diethyl ether). 1H-NMR (200 MHz, CDCl3), δ (ppm): 1.77-1.82 (m, 4H), 2.58-2.87 (m, 12H), 3.60 (s, 2H), 3.75 (s, 3H), 6.62-6.70 (m, 2H), 6.91 (d, J = 8.3 Hz, 1H). 13C-NMR (50 MHz, CDCl3), δ (ppm): 23.1, 29.1, 51.1, 53.8, 54.4, 55.0, 55.7, 57.1, 111.8, 113.0, 126.8, 127.3, 135.2, 157.8. IR (KBr, cm−1): 2940, 2459, 1612, 1513, 1458, 1318, 1276, 1255, 1163, 1036. MS (EI+): 260, 176 (100%), 162, 84.

6-Methoxy-2-(2-piperidin-1-yl-ethyl)-1,2,3,4-tetrahydro-isoquinoline di-hydrochloride (13c)

6-Methoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (10, 0.20 g, 1.00 mmol) was suspended in DMF (25 mL) under nitrogen atmosphere. K2CO3 (0.41 g, 3.00 mmol), 1-(2-chloroethyl)-piperidine hydrochloride (0.19 g, 1.10 mmol) and KI (catalytic amount) were added at rt. The suspension was heated at an oil bath at 50 ºC overnight. The salts were removed by filtration, and the clear solution was diluted with water (75 mL). Extraction (CH2Cl2, 3 x 20 mL) of the neutral aqueous phase and subsequent washing of the combined organic layers (water, 20 mL) was followed by drying (Na2SO4), filtration, and evaporation of volatiles in vacuo. This yielded 0.28 g of an yellow oil, which was purified by chromatography (Silica, gradient CH2Cl2/CH3OH). The pure compound was converted in the double hydrochloride salt by addition of an excess of HCl (1N in diethyl ether) to a solution of the free base of 13c in 2-propanol. 1H-NMR (200 MHz, CDCl3), δ (ppm): 1.37-1.43 (m, 2H), 1.43-1.60 (m, 4H), 2.39-2.86 (m, 12H), 3.53 (s, 2H), 3.68 (s, 3H), 6.54-6.64 (m, 2H), 6.85 (d, J = 8.3 Hz, 1H). 13C-NMR (50 MHz, CDCl3), δ (ppm): 23.9, 25.5, 29.0, 51.1, 54.9, 55.4, 56.7, 111.8, 112.9, 126.8, 127.3, 135.1, 157.8. IR (KBr, cm−1): 2942, 2598, 1760, 1620, 98 (100%), 70, 55.

2-(2-Piperidin-1-yl-ethyl)-1,2,3,4-tetrahydro-isoquinolin-6-ol di-hydrochloride (13d)

6-Methoxy-2-(2-piperidin-1-yl-ethyl)-1,2,3,4-tetrahydroisoquinoline dihydrochloride (13c, 0.14 g, 0.45 mmol) was suspended in HBr (5 mL, 48 % in H2O). The mixture was refluxed for 2 hr. After cooling to rt, the brown solution was poured into water (20 mL) and neutralized with NaHCO3 (saturated aqueous solution). The water layer was extracted with CH2Cl2 (4 x 20 mL), and the combined organic layers were dried (Na2SO4), filtered and evaporated. This yielded the pure compound as a slightly yellow oil (90 mg). The free base of 13d was converted in the double hydrochloride salt by adding an excess of HCl (1N in diethyl ether) to the compound dissolved in 2-propanol. 1H-NMR (free base, 200 MHz, CD2OD) δ (ppm): 1.51-1.80 (m, 7H), 2.45-2.88 (m, 12H), 3.58 (s, 2H), 6.52-6.57 (m, 2H), 6.85 (d, J = 7.6 Hz, 1H). 13C-NMR (50 MHz, CD2OD), δ (ppm): 21.8, 23.2, 26.7, 49.5, 52.5, 52.8, 53.8, 53.9, 111.5, 112.7, 123.1, 125.6, 133.1, 154.0. IR (hydrochloride, KBr, cm−1): 3215, 2942, 2546, 1615, 1508, 1450, 1287, 1224, 1156, 1116, 1046, 1008, 968, 907. MS (free base, EI+): 274, 176 (100%), 162, 98.

[2-(6-Methoxy-3,4-dihydro-1H-isoquinolin-2-yl)-ethyl]-dipropyl-amine di-hydrochloride (13e)

In a 3-necked flask with reflux condenser, crude 2-chloro-1-(6-methoxy-3,4-dihydro-1H-isoquinolin-2-yl)-ethanone (14, 0.12 g, 0.51 mmol) was dissolved in DMF (10 mL) with K2CO3 (0.36 g, 2.61 mmol) and dipropylamine (0.12 g, 1.41 mmol). The mixture was stirred at 60 ºC for 3 hr, then quenched with water (50 mL), and extracted with CH2Cl2 (3 x 25 mL). The combined organic layers were washed (NaHCO3, saturated aqueous solution), dried (Na2SO4), and filtered. Volatiles were removed under reduced pressure to yield a yellow oil (15e, 0.15 g). MS (EI+): 304, 203, 161, 114 (100%), 86. This oil was dissolved in THF (2 mL) without further purification, and added to a suspension of LiAlH4 (19.0 mg, 0.50 mmol) in THF (5 mL) under nitrogen. The gray suspension was stirred at rt for 2 hr. The excess of LiAlH4 was decomposed by careful addition of water. Na2SO4 was added and after 15 min, the salts were filtered and washed thoroughly (diethyl ether). Evaporation of the volatiles yielded 13e as a yellow oil (0.13 g), which was purified by column chromatography (Silica, gradient CH2Cl2/CH3OH) and converted in the double hydrochloride salt by treatment of the free base dissolved in diethyl ether with HCl (excess, 1N in diethyl ether). 1H-NMR (200 MHz, CDCl3) δ (ppm): 0.87 (t, J = 7.3
2,3,4-Tetrahydroisoquinoline derivatives as potential new 5-HT<sub>3</sub> receptor agonists

Chapter 3

7-Methoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (10, 2.02 g, 10.1 mmol) and K<sub>2</sub>CO<sub>3</sub> (2.89 g, 20.9 mmol) were suspended in CH<sub>2</sub>Cl<sub>2</sub> (100 mL) under nitrogen. 2-Chloroacetyl chloride (1.15 g, 10.2 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was added dropwise at rt. After 2 hr, the suspension was filtered, and the organic phase was washed (NaHCO<sub>3</sub>, sat.). Drying (Na<sub>2</sub>SO<sub>4</sub>), filtration, and concentration in vacuo yielded an off-white solid (2.08 g). Purification by crystallization from ethyl acetate/hexane yielded pure 14 as colorless crystals. <sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>) δ (ppm): 2.73-2.96 (m, 2H), 3.50-3.67 (m, 2H), 3.84 (s, 3H), 6.63 (br, 1H), 6.96 (d, J<sub>a</sub> = 2.2 Hz, 2H), 7.03 (d, J<sub>b</sub> = 8.3 Hz, 1H), 7.07 (d, J<sub>b</sub> = 8.3 Hz, 1H). <sup>13</sup>C-NMR (50 MHz, CDCl<sub>3</sub>) δ (ppm): 28.1, 39.7, 42.3, 45.8, 53.6, 111.3, 111.7, 126.1. IR (neat, cm<sup>-1</sup>): 2965, 2837, 1698, 1534, 1514, 1269, 1195, 1138, 1048, 1005, 915, 823, 755. MS (EI<sup>+</sup>): 209, 134, 121 (100%), 91, 59.

7-Methoxy-3,4-dihydro-2H-isoquinolin-1-one (17)

Polyphosphoric acid (PPA, 70 g) was stirred in a beaker at 140 °C. To this, 2-chloroacetyl chloride (1.15 g, 10.2 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was added dropwise at rt. After 2 hr, the suspension was filtered, and the organic phase was washed (NaHCO<sub>3</sub>, sat.). Drying (Na<sub>2</sub>SO<sub>4</sub>), filtration, and concentration in vacuo yielded an off-white solid (2.08 g). Purification by crystallization from ethyl acetate/hexane yielded pure 14 as colorless crystals. <sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>) δ (ppm): 2.72-2.98 (m, 2H), 3.51-3.67 (m, 2H), 3.84 (s, 3H), 6.63 (br, 1H), 6.96 (d, J<sub>a</sub> = 2.2 Hz, 2H), 7.03 (d, J<sub>b</sub> = 8.3 Hz, 1H), 7.07 (d, J<sub>b</sub> = 8.3 Hz, 1H). <sup>13</sup>C-NMR (50 MHz, CDCl<sub>3</sub>) δ (ppm): 28.1, 39.7, 42.3, 45.8, 53.6, 111.3, 111.7, 126.1. IR (neat, cm<sup>-1</sup>): 2965, 2837, 1698, 1534, 1514, 1269, 1195, 1138, 1048, 1005, 915, 823, 755. MS (EI<sup>+</sup>): 209, 134, 121 (100%), 91, 59.

7-Methoxy-3,4-dihydro-2H-isoquinolin-1-one (18)

Polyphosphoric acid (PPA, 70 g) was stirred in a beaker at 140 °C. To this, 17 (5.00 g, 23.9 mmol) was added portionwise. After 20 min, heating was removed and ice (ca 500 g) was added portionwise to decompose the PPA. The acidic water layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (6 x 80 mL). The combined organic layers were washed with NaHCO<sub>3</sub> (100 mL, saturated aqueous solution, 50 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and the solvent was removed under reduced pressure. This yielded 18 as a colorless oil (1.52 g, 8.59 mmol) that crystallized upon standing and was chemically pure according to NMR. mp 111-113 °C. <sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>) δ (ppm): 2.89-2.96 (m, 2H), 3.52-3.58 (m, 2H), 3.84 (s, 3H), 6.80 (br, 1H), 7.01 (dd, J<sub>a</sub> = 2.7 Hz, J<sub>b</sub> = 8.3 Hz, 1H), 7.12 (d, J<sub>a</sub> = 8.3 Hz, 1H), 7.58 (d, J<sub>a</sub> = 2.7 Hz, 1H). <sup>13</sup>C-NMR (50 MHz, CDCl<sub>3</sub>) δ (ppm): 25.9, 38.9, 54.0, 109.6, 118.3, 126.9, 129.5, 157.2, 165.0. IR (KBr, cm<sup>-1</sup>): 3198, 3066, 2955, 2903, 2863, 2837, 1698, 1534, 1514, 1269, 1195, 1138, 1048, 1005, 915, 823, 755. MS (EI<sup>+</sup>): 177 (100%), 148, 120, 105, 91, 77.

7-Methoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (19)

In a 3-necked flask with reflux condenser and dropping funnel under nitrogen, LiAlH<sub>4</sub> (0.65 g, 17.0 mmol) was suspended in THF (25 mL). At 0 °C, a solution of 18 (1.25 g, 7.06 mmol) in THF (25 mL) was added dropwise. Heating to reflux was applied for 1 hr. At rt, the excess of LiAlH<sub>4</sub> was destroyed with appropriate amounts of water (0.3 mL), NaOH (1N, 0.3 mL), and water (1mL). The suspension was diluted with CH<sub>2</sub>Cl<sub>2</sub> (50 mL), and Na<sub>2</sub>SO<sub>4</sub> was added as drying agent. Salts were removed by filtration over Celite, and the solvents were removed in vacuo to yield the free base of 19 as a colorless

Hz, 6H), 1.42-1.60 (m, 4H), 2.49 (t, J = 7.8 Hz, 4H), 2.64-2.79 (m, 6H), 2.83-2.89 (m, 2H), 3.59 (s, 2H), 3.75 (s, 3H), 6.61 (s, 1H), 6.66 (dd, J<sub>a</sub> = 2.2 Hz, J<sub>b</sub> = 8.3 Hz, 1H), 6.91 (d, J = 8.3 Hz, 1H). <sup>13</sup>C-NMR (50 MHz, CDCl<sub>3</sub>) δ (ppm): 11.6, 19.6, 29.1, 51.1, 55.0, 55.7, 55.8, 65.3, 111.9, 113.0, 126.7, 127.3, 135.1, 157.8. IR (neat, cm<sup>-1</sup>): 2944, 2460, 1617, 1515, 1456, 1319, 1280, 1258, 1165, 1034. MS (EI<sup>+</sup>): 290, 218, 176 (100%), 147, 114.
oil (1.00 g, 6.13 mmol) that was chemically pure according to NMR. The free base was converted in the hydrochloride salt by treatment of the free base dissolved in ether with an excess of HCl (2.5 N solution in diethyl ether). \(^1\)H-NMR (200 MHz, CDCl\(_3\)) \(\delta\) (ppm): 2.60-2.75 (m, 2H), 3.03-3.15 (m, 2H), 3.72 (s, 3H), 3.92 (s, 2H), 6.51 (s, 1H), 6.66-6.69 (m, 1H), 6.96 (d, \(J = 7.8\) Hz, 1H). \(^13\)C-NMR (50 MHz, CDCl\(_3\)) \(\delta\) (ppm): 26.8, 42.6, 47.0, 53.7, 109.3, 110.9, 125.3, 128.6, 135.3, 156.1. IR (KBr, cm\(^{-1}\)): 3305, 2995, 2924, 2833, 1609, 1503, 1464, 1430, 1311, 1270, 1250, 1217, 1157, 1110, 1038, 841, 800, 714. MS (El\(^+\)): 163, 134 (100%), 119, 104, 91, 77, 65, 51.

7-Methoxy-2-methyl-1,2,3,4-tetrahydro-isoquinoline hydrochloride (25)

7-Methoxy-1,2,3,4-tetrahydro-isoquinoline (19) (free base, 0.11 g, 0.55 mmol) was suspended in ethanol (10 mL). Formaldehyde (0.04 mL, 37% solution in water), and NaCNBH\(_3\) (0.05 g, 0.79 mmol) were added. AcOH was added to adjust the pH to slightly acidic (pH = 5). The mixture was stirred at rt overnight. The solvent was evaporated, and the residue was portioned between HCl (1N) and ether. The organic phase was washed (brine), dried (Na\(_2\)SO\(_4\)), filtered, and the solvent was removed under reduced pressure to yield the pure free base of 25 as a colorless oil (85 mg), which was converted in its hydrochloride salt by treatment of the free base with an excess of HCl (2.5N solution in diethyl ether). \(^1\)H-NMR (free base, 300 MHz, CDCl\(_3\)) \(\delta\) (ppm): 2.38 (s, 3H), 2.59-2.63 (m, 2H), 2.79 (t, \(J = 5.9\) Hz, 2H), 3.49 (s, 2H), 3.70 (s, 3H), 6.49 (d, \(J = 2.6\) Hz, 1H), 6.65 (dd, \(J_a = 2.9\) Hz, \(J_b = 5.5\) Hz, 1H), 6.95 (d, \(J = 8.4\) Hz, 1H). \(^13\)C-NMR (75 MHz, CDCl\(_3\)) \(\delta\) (ppm): 25.8, 34.5, 50.6, 52.7, 55.6, 108.6, 109.9, 110.3, 123.4, 127.0, 133.2, 155.0. IR (neat, cm\(^{-1}\)): 3300, 2991, 2924, 1604, 1503, 1466, 1428, 1310, 1270, 1251, 1219, 1155, 1110, 1030, 871, 711. MS (El\(^+\)): 177, 161, 146, 134 (100%), 119, 104, 91, 77, 65, 51.

(7-Methoxy-3,4-di-hydro-1H-isoquinolin-2-yl)-acetonitrile (20)

In a 3-necked flask with reflux condenser, 19 (0.50 g, 2.50 mmol) was suspended in acetonitril (15 mL) under a nitrogen. K\(_2\)CO\(_3\) (0.70 g, 5.07 mmol) and bromoacetonitril (0.30 g, 2.50 mmol) were added, and the mixture was warmed on an oil bath to 70 °C for 1 hr. The reaction was allowed to cool to rt, and the solvent was removed under reduced pressure. The residue was portioned between HCl (1N) and ether. The organic phase was washed (brine), dried (Na\(_2\)SO\(_4\)), filtered, and the solvent was removed under reduced pressure to yield 0.50 g of a slightly yellow semi solid. Purification by column chromatography (Silica, CH\(_2\)Cl\(_2\)) yielded pure 20 as a colorless oil, which crystallized upon standing (0.40 g, 1.98 mmol). Mp: 70-72 °C. \(^1\)H-NMR (CDCl\(_3\)) \(\delta\) (ppm): 2.38-2.50 (m, 4H), 2.68-2.89 (m, 6H), 3.59 (s, 2H), 3.74 (s, 3H), 6.62 (d, \(J = 8.3\) Hz). \(^13\)C-NMR (CDCl\(_3\)) \(\delta\) (ppm): 28.5, 42.6, 47.0, 53.7, 109.3, 110.9, 125.3, 128.6, 135.3, 156.2. IR (KBr, cm\(^{-1}\)): 3303, 2995, 2924, 2833, 1609, 1503, 1464, 1430, 1311, 1270, 1250, 1217, 1157, 1110, 1038, 841, 800, 714. MS (El\(^+\)): 163, 134 (100%), 119, 104, 91, 77, 65, 51.

2-(7-Methoxy-3,4-di-hydro-1H-isoquinolin-2-yl)-ethylamine di-hydrochloride (21)

LiAlH\(_4\) (63 mg, 1.65 mmol) was suspended in THF (10 mL) under nitrogen. Compound 20 (0.30 g, 1.48 mmol), dissolved in THF (5 mL), was added dropwise at rt. After 1 hr, the excess of LiAlH\(_4\) was decomposed with a requisite amount of water (1 eq), NaOH (1N, 1 eq) and water (4 eq). Na\(_2\)SO\(_4\) was added, after which the salts were removed by filtration, washed extensively with CH\(_2\)Cl\(_2\), and the clear solution was concentrated in vacuo to yield a colorless oil (0.30 g, 1.48 mmol). This was the pure free base of 21 according to NMR. The free base was converted in the double hydrochloride salt by addition of an excess of HCl (1N in diethyl ether) to a solution of the compound in 2-propanol. \(^1\)H-NMR (free base, 200 MHz, CDCl\(_3\)) \(\delta\) (ppm): 1.74 (br, 2H), 2.56 (t, \(J = 6.1\) Hz, 2H), 2.68-2.89 (m, 6H), 3.59 (s, 2H), 3.74 (s, 3H), 6.54 (d, \(J = 2.7\) Hz, 1H), 6.67-6.72 (m, 1H), 6.99 (d, \(J = 8.3\) Hz, 1H). \(^13\)C-NMR (50 MHz, CDCl\(_3\)) \(\delta\) (ppm): 26.8, 37.6, 49.7, 53.7, 54.9, 59.2, 109.7, 111.0, 125.0, 128.0, 134.3, 156.0. IR (KBr, hydrochloride, cm\(^{-1}\)): 3503, 3400, 2931, 2665, 2558, 1613, 1508, 1458, 1329, 1255, 1235, 1164, 1026, 835. MS (El\(^+\)): 206, 176 (100%), 162, 135, 91.

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Chapter 3 1,2,3,4-Tetrahydroisoquinoline derivatives as potential new 5-HT\(_{1}\) receptor agonists
[2-(7-Methoxy-3,4-dihydro-1H-isooquinolin-2-yl)-ethyl]-dimethyl-amine di-hydrochloride (22a)

Under a nitrogen atmosphere, 21 (free base, 0.13 g, 0.62 mmol), formaldehyde (37% in water, 0.15 mL, 1.85 mmol), and NaCNBH3 (40.0 mg, 0.62 mmol) were dissolved in ethanol (10 mL, absolute). Acetic acid was used to adjust the pH (pH = 4). After 4 hr stirring at rt, the reaction was quenched with brine (50 mL) and made alkaline (1N NaOH, pH = 10). The water layer was extracted (ethyl acetate, 3 x 15 mL), and the combined organic layers were dried (Na2SO4), filtered, and evaporated to yield the free base of 22a as a slightly orange oil (0.14g), which was converted in the hydrochloride salt by addition of an excess of HCl (1N in diethyl ether) to a solution of the free base in 2-propanol. 1H-NMR (200 MHz, CDCl3) δ (ppm): 2.20 (s, 6H), 2.32-2.73 (m, 8H), 3.53 (s, 2H), 3.65 (s, 3H), 6.44 (d, J = 2.7 Hz, 1H), 6.60 (dd, Jα = 2.7 Hz, Jβ = 8.3 Hz, 1H), 6.89 (d, J = 8.5 Hz, 1H). 13C-NMR (50 MHz, CDCl3, δ (ppm): 26.1, 43.9, 49.9, 53.6, 53.9, 54.9, 109.6, 111.3, 124.3, 128.0, 133.4, 156.1. IR (free base, neat, cm−1): 2944, 2460, 1617, 1515, 1456, 1319, 1280, 1258, 1165, 1034. MS (EI+): 234, 176 (100%), 91, 58.

2-Chloro-1-(7-methoxy-3,4-dihydro-1H-isooquinolin-2-yl)-ethanone (23)

7-Methoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (19, 0.50 g, 2.51 mmol) and K2CO3 (0.73 g, 5.02 mmol) were suspended in acetonitril (15 mL) under nitrogen. 2-Chloroacetyl chloride (0.29 g, 2.51 mmol), dissolved in acetonitril (2 mL), was added dropwise. The reaction was heated to 50 °C on a water bath for 2 hr. The suspension was filtered and washed extensively with CH2Cl2 (80 mL). The organic fraction was washed with NaOH (1N, 15 mL) and water (15 mL); then dried (Na2SO4), filtered, and concentrated in vacuo to yield a semi solid (0.57 g), which was purified by chromatography (Silica, gradient CH2Cl2/CH3OH) to yield pure 23 as a colorless solid. 1H-NMR (200 MHz, CDCl3, δ (ppm): 2.76-2.91 (m, 2H), 3.67-3.83 (m, 2H), 3.76 (s, 3H), 4.14 (s, 2H), 4.66, (d, J = 2.7 Hz, 1H), 6.62-6.68 (m, 1.5 H), 6.88-6.96 (m, 1H). 13C-NMR (50 MHz, CDCl3, δ (ppm): 27.0, 39.7, 42.6, 43.4, 53.8, 109.7, 111.6, 124.5, 127.9, 132.1, 156.2, 164.0. IR (free base, neat, cm−1): 2997, 2941, 2909, 1654, 1610, 1508, 1438, 1405, 1264, 1218, 1190, 1041, 953, 811. MS (EI+): 239, 204 (100%), 162, 134, 91.

2-Dimethylamino-1-(7-methoxy-3,4-dihydro-1H-isooquinolin-2-yl)-ethanone (24a)

In a 3-necked flask under nitrogen, 23 (0.28 g, 1.17 mmol) was dissolved in acetonitril. K2CO3 (0.40 g, 2.93 mmol), KI (catalytic amount), and dimethylamine hydrochloride (0.19 g, 2.34 mmol) were added, and the reaction was stirred for 2 hr at 50 °C. The suspension was cooled to rt, diluted with CH2Cl2 (50 mL), and filtered. The mother liquor was washed (NaHCO3, saturated aqueous solution, 10 mL), dried (Na2SO4), and filtered. Evaporation of volatiles yielded a colorless oil (0.17 g) that crystallized upon standing. Crystallization (CH3OH) yielded pure 24a as colorless crystals. 1H-NMR (200 MHz, CDCl3, δ (ppm): 2.60-2.82 (m, 2H), 3.51 (s, 6H), 3.64 (s, 3H), 3.65 (d, J = 2.7 Hz, 2H), 4.50 (s, 2H), 4.94 (s, 2H), 6.52 (d, J = 2.2 Hz, 0.5 H), 6.62-6.68 (m, 1.5 H), 6.88-6.96 (m, 1H). 13C-NMR (50 MHz, CDCl3, δ (ppm): 26.7, 38.8, 41.7, 42.7, 51.5, 53.8, 109.7, 111.6, 124.5, 127.8, 131.7, 156.7, 161.5. MS (EI+): 248, 203, 161, 134, 91, 58 (100 %).

3.7.3 Pharmacology

5-HT7 Receptor Binding Assay

Binding assays on membranes from HEK cells expressing rat 5-HT7 (r5-HT7 receptor obtained from Dr. David Sibley) receptors were performed according to standard procedures. The cell paste was homogenized in 50 mM Tris HCl buffer (pH = 7.4) containing 2.0 mM MgCl2 using a hand-held Polytron (setting 6 for 10 seconds) and centrifuged at 40,000 g for ten minutes. The pellet was resuspended in 50 mM Tris HCl buffer (pH = 7.4) containing EDTA (0.5 mM), MgSO4 (10 mM), CaCl2 (2 mM), pargyline (10 μM), and ascorbic acid (0.1%). Incubations were initiated by the addition of membranes (20 μg protein per well) to 96-well plates containing test drugs and [3H]5-CT (0.3 nM, final volume of 250 μl). Non-specific binding was determined by radioligand binding in the presence of a saturating concentration of 5-HT (10 μM). After a two-hour incubation period at room temperature, assay samples were rapidly filtered through Whatman
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GF/B filters and rinsed with ice-cold Tris buffer (50 mM, pH = 7.4) using a Skatron harvester (Molecular Devices). Membrane bound [^3H]5-CT levels were determined by liquid scintillation counting of the filters in BetaScint. The IC_{50} value (concentration at which 50% inhibition of specific binding occurs) was calculated by linear regression of the log concentration-response data. K_i values were calculated according to the Cheng-Prusoff equation, K_i = IC_{50}/(1 + (L/K_d)), where L is the concentration of the radioligand used in the experiment and the K_d value is the dissociation constant for the radioligand (determined previously by saturation analysis).
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