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Research paper

New systematically modified vesamicol analogs and their affinity and selectivity for the vesicular acetylcholine transporter – A critical examination of the lead structure

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ABSTRACT

To verify vesamicol as lead structure in the development of radioligands for imaging of VAChT in the brain by PET, we systematically modified this molecule and investigated four different groups of derivatives. Structural changes were conducted in all three ring systems A, B, and C resulting in a library of different vesamicol analogs. Based on their in vitro binding affinity toward VAChT as well as σ_1 and σ_2 receptors, we performed a structure-affinity relationship (SAR) study regarding both affinity and selectivity. The compounds possessed VAChT affinities in the range of 1.32 nM (benzovesamicol) to >10 μM and selectivity factors from 0.1 to 73 regarding σ_1 and σ_2 receptors, respectively. We could confirm the exceptional position of benzovesamicols as most affine VAChT ligands. However, we also observed that most of the compounds with high VAChT affinity demonstrated considerable affinity in particular to the σ_1 receptor. Finally, none of the various vesamicol analogs in all four groups showed an in vitro binding profile suitable for specific VAChT imaging in the brain.

1. Introduction

The vesicular acetylcholine transporter (VAChT) is a transmembrane protein located on the presynaptic vesicular membrane of cholinergic neurons. It is responsible for the accumulation of acetylcholine inside the vesicles and thus plays an important role in cholinergic neurotransmission. In the last decade this transporter was highly regarded as potential target to investigate functional alterations observed in degenerative processes of the cholinergic system [1,2]. Cholinergic deficits in brain are discussed as early pathological feature of the cognitive decline occurring in neurodegenerative disorders such as Alzheimer's Disease (AD) [3–8]. Noninvasive in vivo imaging based on radiolabeled VAChT ligands using PET (positron emission tomography) or SPECT (single-photon emission computed tomography) is regarded as useful tool for studying changes of cholinergic function in normal and diseased brain. By contrast to other potential targets available for imaging of the cholinergic system by PET, VAChT is expressed solely presynaptically and thus might support early diagnostics of AD by exclusive visualization of cholinergic neurons. Imaging of VAChT in human brain was already performed with SPECT [9] as well as very recently with PET [10] ligands both showing advantages and disadvantages. Nevertheless, PET, with fluorine-18 as the currently most widely used radionuclide, is superior regarding its detection efficiency, spatial resolution, and quantification.

The previous development of VAChT radiotracers was based on the single known chemical lead compound vesamicol (*trans*-2-(4-phenylpiperidino)cyclohexanol), that binds with high affinity to an allosteric site of the transporter [11–13]. However, vesamicol also demonstrates substantial affinity to the sigma receptors σ_1 and σ_2, which are distributed in cholinergic brain regions, thereby impairing the possibility of specific in vivo imaging [14,15].

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Therefore, the development of VAChT ligands is focused on structural modifications of vesamicol with the aim to keep or improve the VAChT affinity and to reduce the affinity for sigma receptors. In 1989, Rogers et al. synthesized about 80 vesamicol derivatives and determined their potential to inhibit the [^{3}H]ACh transport into vesicles [16]. These data have been the basis for most of the VAChT ligand developments and as a result, several classes of vesamicol analogs have arisen. Noteworthy are the benzovesamicols with the most potential candidate [^{18}F]FEOBV [17–21] with promising in vivo imaging properties in rodents and monkeys (see Fig. 1). In 2014, the first human study using this tracer has been reported [10]. However, so far the selectivity of this tracer is not sufficiently verified in vivo. Besides FEOBV, some of the recently published carbonyl group containing benzovesamicols [22–25] seem to be appropriate candidates for further in vivo evaluation. Another important class is the group of trozamicols, with [^{18}F]FBT [26] as representative. However, in particular these derivatives suffer from low selectivity [14].

Based on the qualitative structure-activity observations reported by Rogers et al. [16], we started in 2004 with the development of 4-*O*- and 5-*O*-substituted fluorobenzyl ether derivatives of vesamicol with the aim of improving the selectivity [27,28]. In particular, the 4-*O*-derivatives (Fig. 1) showed good VAChT affinities with K_i values in the low nanomolar range; however the affinity for sigma receptors was high as well. Based on the idea of a ring fusion as made with benzovesamicols, structurally related compounds were developed. Two candidates of the class of morpholinovesamicols were ^{18}F-radiolabeled and studied in vivo in rats and pigs [29–31]. In particular, the 4-fluoro-benzoylated derivative [^{18}F]FBMV showed a typical accumulation in VAChT-containing brain regions and a significant reduction of cortical binding after a specific cholinergic lesion. However, to confirm the high in vitro selectivity of this tracer, further studies in vivo have to be performed using appropriate sigma receptor ligands. In parallel to the morpholinovesamicols we developed the azaspirovesamicols [32] and the recently published novel group of pyrrolovesamicols [33]. In particular, the latter showed unexpectedly low in vitro affinities for the VAChT and both classes are not appropriate for in vivo evaluation. The numerous literature data and our own intensive work in developing VAChT ligands demonstrate the difficulty to predict properties of a newly synthesized compound regarding VAChT affinity and selectivity. As for many promising VAChT ligands observed, high VAChT affinity is often accompanied by high sigma receptor binding. However, beside an appropriate target affinity, a high selectivity is essential for the development of a specific VAChT PET tracer. So with our study we wanted to put some light in particular on this fact and we determined the affinity to the σ_1 and σ_2 receptors. Therefore, in this paper we report on the synthesis of a library of mainly novel vesamicol analogs and their binding profile regarding both VAChT and σ_1/σ_2 receptors. Systematic modifications in ring B and C of the vesamicol skeleton were performed and combined with different modifications in ring A (vesamicol can be subdivided into the three rings A (cyclohexanol), B (piperidine), and C (phenyl), see Fig. 1).

The in vitro binding affinities for VAChT and the sigma receptors σ_1 and σ_2 were determined by competitive radioligand binding assays. In particular, for the determination of VAChT affinities different protocols are reported resulting in considerable affinity differences for identical compounds, e.g. vesamicol. Rogers and Parsons et al. used an assay with synaptic vesicles from the electric organ of *Torpedo californica* ($K_{i(vesamicol)}$ = 1.0 nM) [34], whereas cerebral membranes of rats were used by the group of K. Shiba ($K_{i(vesamicol)}$ = 33.9 nM) [35,36] and a cell line transfected with human VAChT by Tu et al. [37]. In our study, we investigated rat brain membranes and a cell line stably transfected with rat VAChT as possible target materials to develop a standardized test setup. We have chosen rat material in order to avoid possible species dependent differences when comparing the VAChT affinity data with the data obtained for the σ_1/σ_2 receptor binding. A few of known vesamicol analogs were re-synthesized to compare their VAChT affinity data reported with the data obtained in our assay.

2. Results and discussion

2.1. Chemistry

A very convenient approach to synthesize vesamicol derivatives is the nucleophilic ring opening of an epoxide precursor using a secondary amine. By varying the epoxide precursor, structural modifications in ring A of the vesamicol skeleton can be realized. Due to an elaborate selection of different amines, ring B and C can be sterically or electronically modified. Therefore, we selected several amines which are shown in Fig. 2. Amines **b**-**d** differ in the constitution of ring B compared to 4-phenylpiperidine (**a**), the amine used for most of the known vesamicol analogs. 4-Phenylpiperazine (**b**) contains a further ionizable nitrogen atom resulting in electronic changes. Considering amine **c**, the typical chair conformation of piperidine is hampered due to the double bond and in amine **d** the piperidine ring is replaced by an open-chain amine. In amines **e**–**g**, the 4-phenylpiperidine skeleton is maintained and different sized groups are additionally substituted at 4-position of the piperidine ring B. Amines **h**–**m** are characterized by spatial and electronic changes in ring C. While in 4-benzylpiperidine (**h**) the space between the aromatic and the piperidine ring is increased, in amine **i** the benzene ring is directly annulated to ring B. 4-(piperidin-4-yl)pyridine (**m**) and 1,4′-

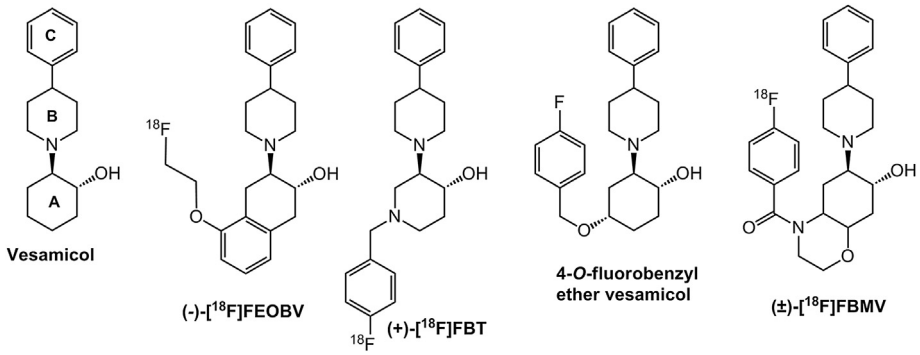


Fig. 1. Vesamicol and selected VAChT ligands.

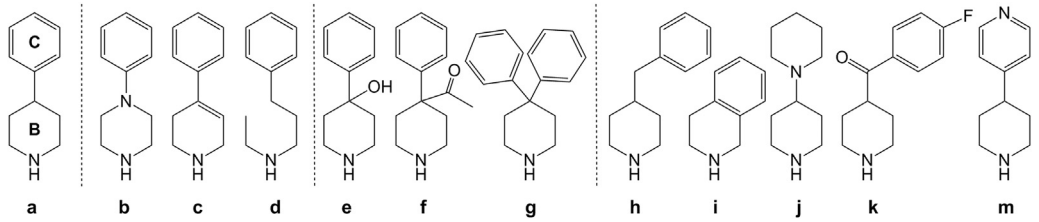


Fig. 2. Structurally modified amines **a**–**m**: **a**: basic structure; **b**–**d**: modifications in ring B; **e**–**g**: modifications between ring B and C; **h**–**m**: modifications in ring C.

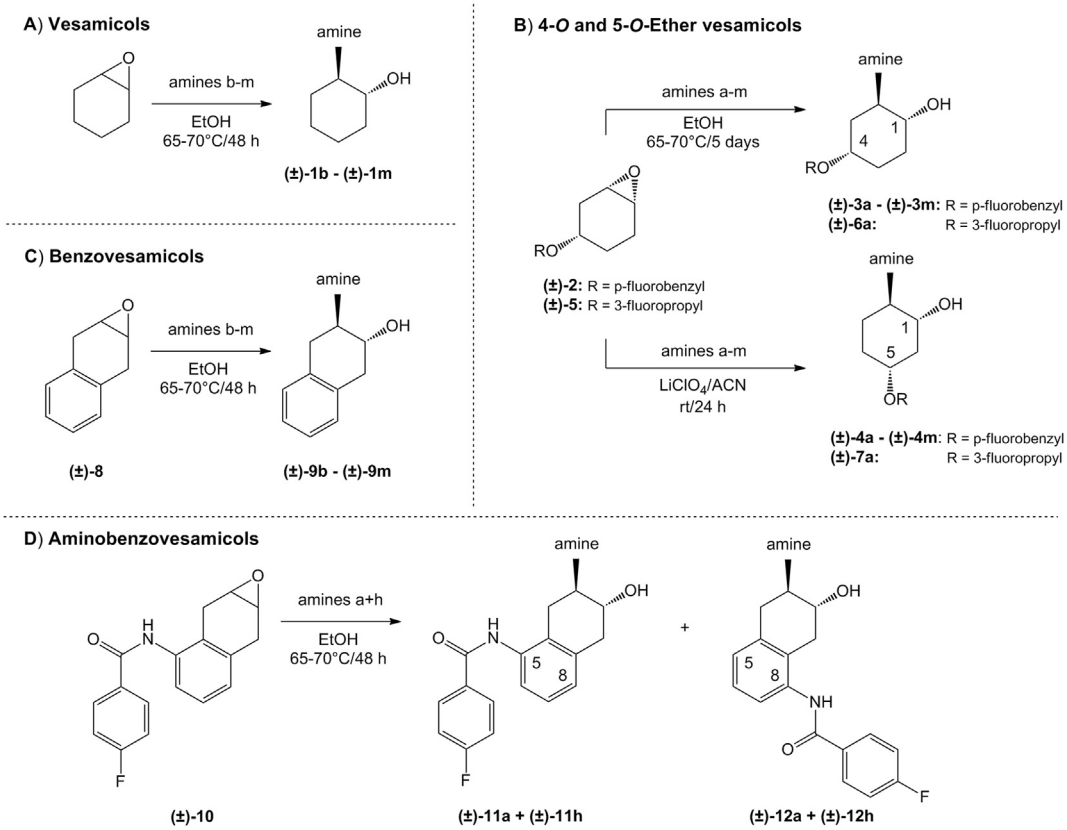
bipiperidine (**j**) contain a nitrogen atom at different positions in ring C, whereas in the latter one ring C is nonaromatic, resulting additionally in structural changes. With the *p*-fluorobenzoyl moiety, amine **k** consists of a carbonyl functionality providing high electron density.

To investigate the influence of these different modifications of ring B and C, the group of vesamicols (**1b**–**1m**) without modifications in ring A were synthesized (A in Scheme 1). The combination of the ring B and C moieties with ring A modifications was achieved by the introduction of (i) a *p*-fluorobenzyl ether functionality (**3a**–**3m** and **4a**–**4m**, B in Scheme 1) and (ii) the fusion of different ring systems (benzovesamicols **9b**–**9m** and aminobenzovesamicols **11a**–**12h**, C and D in Scheme 1).

The racemic vesamicol derivatives **1b**–**1m** were synthesized as shown in Scheme 1(A) by reaction of cyclohexene oxide with the corresponding amine. The synthesis of derivatives **1b**, **1c**, **1h**, **1i**, and **1k** is already described in literature [16,22].

Regioselective nucleophilic ring opening reaction of the epoxide precursor **2** using two different reaction conditions provided the racemic 4-*O*- and 5-*O*- substituted *p*-fluorobenzyl ether derivatives **3a**–**3m** and **4a**–**4m** (B in Scheme 1). The principles of these reactions as well as the synthesis of the epoxide precursor **2** were already described in detail by our group [27] and others [38,39]. Regioisomeric purity of all *p*-fluorobenzyl ether derivatives was confirmed with HPLC. The 3-fluoropropyl ether derivatives **6a** and **7a** were synthesized in the same way as the *p*-fluorobenzyl ether derivatives. Briefly, to get the epoxide precursor **5**, we started with the elimination of water from cyclohexane-1,4-diol to obtain cyclohex-3-enol [40] which was epoxidized diasterioselectively by using *tert*-butyl hydroperoxide and $Mo(CO)_6$ [41]. Subsequent *O*-3-fluoropropylation with 3-fluoropropyl tosylate resulted in the *cis*-epoxide **5** which reacted regioselectively with 4-phenylpiperidine (**a**) to the isomers **6a** and **7a** (reaction conditions as described in B in Scheme 1). To obtain 3-fluoropropyl tosylate, 3-chloropropanol was converted into 3-fluoropropanol [42] and subsequently tosylated with *p*-toluenesulfonyl chloride under basic conditions [43].

For the synthesis of racemic benzovesamicols **9b**–**9m**, **BV** and **FBBV** (C in Fig. 3) 1,4-dihydronaphthalene was epoxidized to get **8**



Scheme 1. Synthesis of A) vesamicols **1b**–**1m**, B) 4-*O*- and 5-*O*-ether vesamicols **3a**–**3m**, **4a**–**4m**, **6a**, and **7a**, C) benzovesamicols **9b**, **9c**, **9e**, **9h**, **9j**, and **9m**, and D) aminobenzovesamicols **11a**, **11h, 12a**, and **12h**.

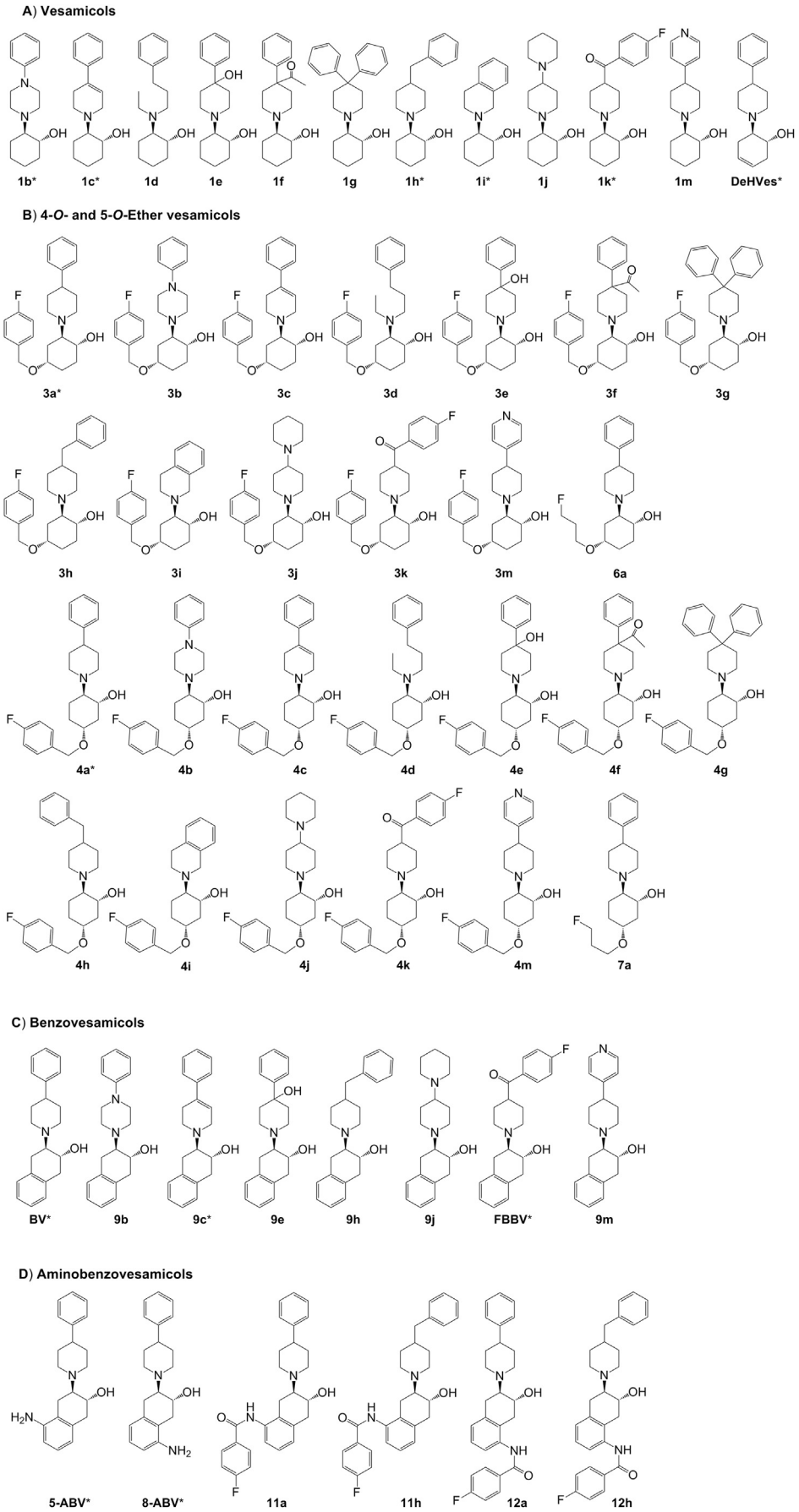


Fig. 3. Molecular structures of synthesized vesamicol derivatives; * means these derivatives are already described by our group or others [16,22,27,37].

as precursor for the reaction with the different amines under conditions as reported earlier [16,37].

The structurally related aminobenzovesamicols **11a**–**12h** (D in Scheme 1) were synthesized on the basis of epoxide **10** which was obtained in three steps starting from naphthalene-1-amine. Briefly, the latter was reduced under Birch conditions using sodium as reducing agent in liquid ammonia and the resulting dihydro product was converted into **10** after N-protection with *p*-fluorobenzoyl chloride and subsequent epoxidation using *m*-chloroperbenzoic acid. The reaction of the racemic epoxide **10** with amines **a** and **h**, as described in D in Scheme 1, resulted in a mixture of both regioisomers with a ratio of ~1:1 in each case. According to the Fürst-Plattner rule, there seems to be no preferred conformer of **10** resulting in a non-regioselective attack of the amine and the equal formation of both products. The resulting pairs of regioisomers were separated using semi-preparative HPLC (see experimental section) and, afterwards, their regioisomeric purity was confirmed by analytical HPLC.

Dehydrovesamicol (**DeHVes**, A in Fig. 3) was synthesized by reaction of 4-phenylpiperidine with the monoepoxide of 1,4-cyclohexadiene as already described [34]. The two regioisomers of aminobenzovesamicol (**5**-/**8-ABV**, D in Fig. 3) were also synthesized according to known synthetic procedures [16].

All synthesized new and reference compounds (shown in Fig. 3) were well characterized using HRMS, ^{1}H, ^{13}C, APT, and two-dimensional NMR. Additionally, ^{19}F NMR spectra were recorded for derivatives containing at least one fluorine atom. Molecular structures are drawn as enantiomers to illustrate the *trans*-position of tertiary amine and hydroxyl group and do not display the actual stereochemistry. All compounds were yielded as racemic mixtures and were used without chiral resolution for biological testing.

2.2. In vitro binding studies

With the aim to develop a standard protocol for the determination of VAChT affinities of the compounds, we investigated rat brain homogenates and neuroendocrine PC12 cells stably transfected with a cDNA of rat VAChT [44] (rVAChT-PC12) as different biological targets and (−)-[^{3}H]vesamicol as radioligand. Briefly, the brain material was prepared by dissection and homogenization of the brain of Sprague Dawley rats (without cerebellum) and stored at −30 °C in a sucrose solution. Crude membranes for the competition assay were obtained after thawing, dilution, removal of cell debris by centrifugation, and homogenization of the pellets in assay buffer. The stably transfected rVAChT-PC12 cells were grown in Dulbecco's modified Eagle Medium (see exp. part), harvested, pelleted and washed, and stored at −30 °C in assay buffer. For binding assay cells were thawed, diluted with test buffer, and homogenized.

When comparing the affinity data of several vesamicol derivatives with these two target systems, considerable differences of K_i values became apparent. For example for (±)-benzovesamicol (**BV**) K_i values of 1.3 nM with rVAChT-PC12 cells and 23.4 nM with rat brain membranes were obtained. Also, for (±)-vesamicol a higher binding affinity with rVAChT-PC12 cells (K_i = 47.4 nM) was observed compared to a K_i = 84.2 nM with rat brain. Since the two VAChT binding targets investigated are of rat origin, comparable affinity data of the vesamicol derivatives should be expected. Differences between brain and rVAChT-PC12 cell homogenates are mainly (i) the much lower concentration of VAChT and (ii) the presence of several other proteins in the brain material, which becomes evident by a higher nonspecific binding of the (−)-[^{3}H]vesamicol of >20% in brain membranes compared to <5% using rVAChT-PC12 cells. Furthermore, it has to be considered that (−)-[^{3}H]vesamicol is not selective due to its high affinity to sigma receptors which are widely distributed in the brain but rarely present in rVAChT-PC12 cells. This was confirmed by in-house experiments demonstrating that the binding of (−)-[^{3}H]vesamicol toward rVAChT-PC12 cells was not significantly affected by the σ_1/σ_2 specific ligand 1,3-di-o-tolylguanidine (DTG) at 50, 100, and 200 nM. The corresponding binding curves obtained with DTG were similar to those without DTG (see Fig. 4). Based on these results, we consistently used PC12 cells stably transfected with rat VAChT and (−)-[^{3}H]vesamicol as standard method for the determination of VAChT binding affinity of the new vesamicol derivatives (data in Table 1).

The affinity of the new compounds for σ_1 and σ_2 receptors was determined, in order to estimate their selectivity toward rat VAChT. Using homogenates obtained from rat cortex and rat liver, respectively, enables a comparison of binding data of the three targets considered within the same species. (+)-[^{3}H]Pentazocine and [^{3}H]DTG were used as radioligands for σ_1 and σ_2, respectively [45]. To block competitive binding of [^{3}H]DTG on σ_1 receptor, the σ_2 receptor assays were performed using 1 μM σ_1 receptor-specific dextrallorphan.

When comparing our VAChT affinity data for vesamicol, **DeHVes**, **BV**, and **5-ABV** with the data obtained by using vesicles of the electric organ of *Torpedo californica* [34], considerable differences become apparent. In general, K_i values obtained with vesicles of the torpedo fish are 20- to 50-fold lower than the herein reported values obtained with rat VAChT-PC12 cells. A particularly strong deviation shows **5-ABV** with a K_i value of 0.013 nM reported for *Torpedo californica* compared to 25.1 nM for rat VAChT in our assay. Also when comparing data obtained with human VAChT transfected PC12 cells slight differences can be observed e.g. for the previously developed **FBBV** with a K_i of 2.7 nM for human VAChT [37] compared to 24.4 nM for rat VAChT. Obviously, the differences between rVAChT and hVAChT transfected cell lines are not as pronounced as observed for vesicles of the torpedo fish. Altogether, these findings show that an interpretation or comparison of in vitro binding data obtained with different species material should be avoided.

To estimate the biological significance of the selectivity of this series of vesamicol analogs, the expression densities of VAChT, σ_1, and σ_2 receptors in brain have to be considered. Regarding VAChT, Custers et al. reported on B_{max} data in rat brain showing values ranging from about 140 (striatum) to 70 fmol/mg tissue (hippocampus) by co-incubation of (−)-[^{3}H]vesamicol and DTG to block σ receptors [14]. Selecting the hippocampus as a region of relatively low VAChT expression, the B_{max} value of vesamicol binding sites is comparable to the B_{max} value of about 50 fmol/mg tissue for the σ_1 receptor sites in rats determined by Bouchard et al. [49]. Besides, although the densities of σ_2 receptors are generally lower than that of σ_1 receptors in rat brain, with a B_{max} of about 20 fmol/mg tissue (hippocampus) this potential binding site also has to be considered [49]. Following the rule of thumb that the binding potential (BP = B_{max}/K_D) should be at least > 2 for a good PET radioligand [50], the K_i value for VAChT binding should be lower than ~35 nM. By comparison, already a K_i value of ~25 nM would be sufficient to fulfill the minimal binding potential criteria for the σ_1 receptor sites in the hippocampus. Moreover, to achieve a good signal to noise ratio in the VAChT imaging process, the ratio of the BPs of the ligand to the σ receptors versus VAChT should be at least 1/100. For the hippocampus this would result in K_i values of >2500 nM and >1000 nM for the σ_1 and σ_2 receptor sites, respectively. In other words, selectivity factors ($K_i(\sigma)/K_i$(VAChT)) which were calculated for each vesamicol analog (Table 1) should be >70 for σ_1 and >30 for σ_2. Based on our in vitro data we have to assume that obviously none of the investigated derivatives fullfils the requirements for a PET radioligand specifically targeting the VAChT in brain. Best selectivity factors regarding σ_1 are in the range of 10–40 and were

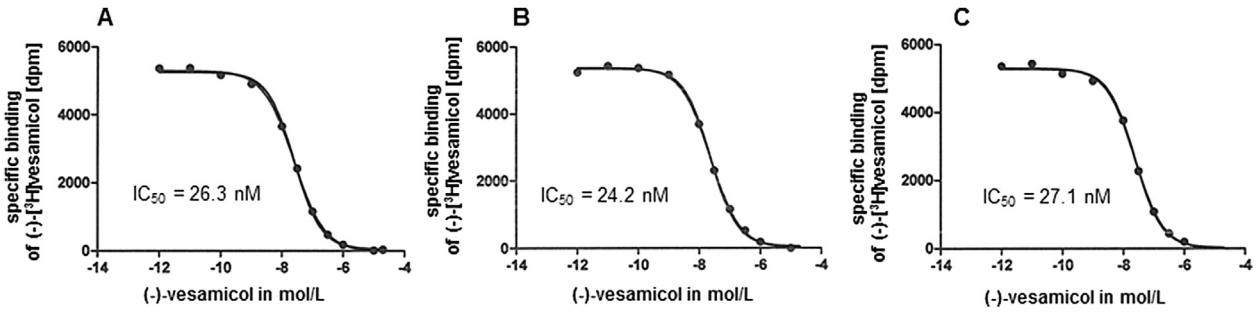


Fig. 4. Concentration-dependent displacement of (−)-[^{3}H]vesamicol by (−)-vesamicol on rat VAChT transfected PC12 cells; A) without DTG, B) with 50 nM DTG, and C) with 200 nM DTG added as σ_1/σ_2 specific blocking agent.

estimated for the aminobenzovesamicol derivative **11a** (41.2), **BV** (19.7), (−)-**FEOBV** (10.7), and **FBBV** (8.8). Although rather low, these values might be sufficient for imaging of the brain regions with high VAChT densities and low σ_1 receptor expression [14,49], such as the striatum. Actually, in vivo studies in rats with (−)-**[^{18}F]FEOBV** [17] and **[^{18}F]FBBV** [37] showed the highest accumulation in striatal regions followed by the cortex and the hippocampus. In contrast to our in vitro findings, the pretreatment of (+)-3-(3-hydroxyphenyl)-N-propyl piperidine ((+)-3-PPP) and haloperidol, two more or less specific σ_1 receptor ligands, did not result in significantly diminished tracer accumulation in these three brain regions, which was interpreted by the authors as high specificity of (−)-**[^{18}F]FEOBV**. For the striatum, this may be plausible, however in particular for brain regions with similar expression densities of VAChT and σ_1 receptors as the hippocampus, the in vivo selectivity of this most promising VAChT PET tracer should be re-investigated by using highly affine and selective brain permeable sigma ligands.

2.3. Structure-affinity considerations

2.3.1. Alternating ring A, B and C moieties

2.3.1.1. Vesamicols. Considering the group of vesamicols (**1c**–**1m**) which differ regarding their structures in ring B or C, we can observe considerably decreased binding affinities to VAChT compared to vesamicol as a reference. As the best derivative within this group, **1c** with a K_i value of 439 nM shows a ten-fold lower affinity for VAChT than vesamicol. The use of the open chain amine in **1d** and the tetrahydroisoquinoline in **1i** results in a complete loss of VAChT affinity. Interestingly, the introduction of a nitrogen atom in the phenyl moiety in **1m** also reduces the VAChT affinity drastically.

For most of the derivatives also a reduction of the σ_1 and σ_2 receptor affinities can be observed. Only for **1c** a higher σ_1 receptor affinity (K_i = 9.7 nM) is found compared to vesamicol.

The introduction of a double bond at 4- and 5-positions in ring A (**DeHVes**) leads to an increase of affinities for all three investigated targets, resulting in a low selectivity.

2.3.1.2. 4-O- and 5-O-Ether vesamicols. The insertion of the *p*-fluorobenzyl ether functionality in the 4-position of the cyclohexanol ring A (derivatives **3a**–**3m**) results in an increase of VAChT affinity compared to the corresponding vesamicol derivatives **1b**–**1m**. **3a**, with a K_i value of 25.1 nM, shows a two-fold higher affinity than vesamicol and is comparable to the known derivatives **FBBV** and **5-ABV**. However, the σ_1 receptor affinity is in the same range as for vesamicol resulting in low selectivity [27]. Besides **3a**, the 4-benzylpiperidine derivative **3h**, with a K_i value of 110 nM, shows the best affinity within this group. Interestingly, the open chain compound **3d** binds with a very high affinity to the σ_1 receptor (K_i = 10.5 nM). The lowest σ_1 affinity is found for **3j** accompanied by a loss in VAChT affinity which is in accordance to **1j** of the vesamicol group. By contrast, the tetrahydroisoquinoline substituted derivative **3i** exhibits similar low affinity for VAChT as **1i** but ten-fold higher affinities for both σ_1 and σ_2 receptor. This finding shows the diversity in the shift of σ receptor affinities when considered as function of VAChT affinities within the group of 4-*O*-ether vesamicols.

When the *p*-fluorobenzyl ether functionality is inserted in 5-position of the cyclohexanol ring A, the VAChT affinities decrease considerably. However, for the open chain derivative **4d** and the diphenyl derivative **4g** an increase of VAChT affinity compared to the corresponding vesamicol derivatives **1d** and **1g** can be observed. By contrast, there is no tendency for the sigma receptor affinities; some derivatives show higher and other lower K_i values compared to the corresponding vesamicols. In this group the 4-benzylpiperine-substituted compound **4h**, with a K_i value of 9.2 nM, shows a σ_1 receptor affinity, which is in the range of other known σ_1 receptor ligands.

2.3.1.3. Benzovesamicols. In the group of benzovesamicols, the affinities toward the transporter increase consistently in comparison to the vesamicol derivatives **1a**–**1m**. Obviously, ring B and C modifications which led to a loss of affinity within the vesamicol group, are well tolerated within the benzovesamicol group. With a K_i of 1.32 nM, the already described (±)-benzovesamicol (**BV**) is the compound with the highest affinity toward VAChT in our study. Besides, although with a relatively high affinity to σ_1 receptor, a selectivity factor of around 20 can be estimated.

The analog **9b**, with the piperazine ring B, demonstrates lowest affinity toward both sigma receptors within this group (K_i values > 460 nM). This observation is comparable to findings of Bando et al. [51] for the iodine derivative DRC140 also bearing the piperazine moiety. The benzovesamicol derivative **9m**, with the substitution of a pyridine ring instead of the phenyl ring C, demonstrates the highest affinity to the σ_1 receptor (K_i = 8.9 nM). Interestingly, when we compare the VAChT affinity of this derivative (K_i = 19.3 nM) to the correspondent vesamicol (**1m**) and benzylether analogs (**3m**, **4m**) all exhibiting micromolar binding affinities for VAChT, the outstanding position of benzovesamicols as VAChT ligands becomes evident. However, it also demonstrates the often observed parallel high affinity to both targets, VAChT and σ_1 receptor.

2.3.1.4. Aminobenzovesamicols. When extending the rigid benzyl ring with a primary amine function in 5-position of **BV** (**5-ABV**), the affinities for both VAChT (K_i = 25.1 nM) and σ receptors (K_i = 119 and 474 nM, resp.) slightly decrease compared to **BV**. Substitution in 8-position (**8-ABV**) results in a loss of affinity for VAChT (K_i = 339 nM), however, accompanied by an increase in affinity toward σ_1 receptor (K_i = 55.3 nM). Rogers et al. also observed the

Table 1
Binding affinity data of vesamicol analogs and reference compounds.

Compound	Selectivity factor				
	K_i (VAChT)[a]	K_i (σ_1)[b]	K_i (σ_2)[c]	K_i (σ_1)/K_i (VAChT)	K_i (σ_2)/K_i (VAChT)
A) Vesamicols					
(±)-**vesamicol**	47.4 ± 6.3 *(1.0 ± 0.50)*[d]	20.4 ± 4.7	112 ± 36	0.4	1.6
(±)-**1b** [16]	838 ± 98	83.9 ± 57.1	695 ± 135	0.1	0.8
(±)-**1c** [16]	439 ± 30	9.7 ± 5.6	158 ± 64	<0.1	0.4
(±)-**1d**	>10 000	115 ± 6	652 ± 171		
(±)-**1e**	1000 ± 108	454 ± 301	1994 ± 548	0.4	1.9
(±)-**1f**	1460 ± 311	22.5 ± 21.4	164 ± 58	<0.1	0.1
(±)-**1g**	6708 ± 904	43.2 ± 25.7	1829 ± 731	<0.1	0.3
(±)-**1h** [16]	647 ± 62	20.1 ± 21.3	108 ± 55	<0.1	0.2
(±)-**1i** [16]	>10 000	54.3 ± 16.3	809 ± 145	/	/
(±)-**1j**	5106 ± 516	>1000	4710 ± 2880	/	/
(±)-**1k** [22]	3239 ± 469 *(423 ± 2)*[d]	60.9 ± 44.9 *(302 ± 25)*[f]	730 ± 210 *(112 ± 8)*[g]	<0.1	0.2
(±)-**1m**	3380 ± 859	>1000	3317 ± 1340	/	/
(±)-**DeHVes**	18.4 ± 2.3 *(0.34 ± 0.04)*[d]	5.8 ± 0.9	77.1 ± 5.9	0.3	4.2
B) 4-*O*- and 5-*O*-Ether vesamicols					
(±)-**3a**	25.1 ± 4.4	16.6 ± 2.7	53.1 ± 7.8	0.7	2.1
(±)-**3b**	697 ± 127	103 ± 24	167 ± 46	0.2	0.24
(±)-**3c**	310 ± 23	29.9 ± 1.8	46.9 ± 6.7	<0.1	0.2
(±)-**3d**	1605 ± 198	10.5 ± 0.01	109 ± 13	/	<0.1
(±)-**3e**	316 ± 29	47.3 ± 9.6	134 ± 2	0.2	0.4
(±)-**3f**	260 ± 15	63.1 ± 3.8	18.8 ± 1.9	0.2	<0.1
(±)-**3g**	603 ± 104	456 ± 22	2363 ± 735	0.8	3.9
(±)-**3h**	110 ± 22	25.2 ± 15.2	61.8 ± 0.2	0.2	0.6
(±)-**3i**	4672 ± 149	59.0 ± 11.1	45.9 ± 0.3	<0.1	<0.1
(±)-**3j**	4789 ± 210	553 ± 5	543 ± 113	0.1	0.1
(±)-**3k**	1026 ± 156	59.4 ± 9.7	640 ± 30	<0.1	0.6
(±)-**3m**	1351 ± 11	26.4 ± 0.9	85.8 ± 28.6	<0.1	<0.1
(±)-**4a**	2387 ± 370	16.8 ± 4.3	67.1 ± 15.6	<0.1	<0.1
(±)-**4b**	6194 ± 752	189 ± 25	1041 ± 510	<0.1	0.2
(±)-**4c**	2825 ± 43	15.9 ± 1.1	55.5 ± 1.3	<0.1	<0.1
(±)-**4d**	1931 ± 192	19.7 ± 1.7	280 ± 21	<0.1	0.1
(±)-**4e**	>10 000	31.0 ± 29.2	254 ± 47	/	/
(±)-**4f**	>10 000	120 ± 5	103 ± 0	/	/
(±)-**4g**	2733 ± 288	736 ± 1	5537 ± 2316	0.3	2.0
(±)-**4h**	1298 ± 170	9.2 ± 0.09	207 ± 6	<0.1	0.2
(±)-**4i**	>10 000	23.1 ± 5.1	45.6 ± 2.4	/	/
(±)-**4j**	>10 000	300 ± 105	374 ± 31	/	/
(±)-**4k**	5843 ± 1392	65.4 ± 14.5	1996 ± 381	<0.1	0.3
(±)-**4m**	>10 000	41.1 ± 3.6	164 ± 51	/	/
(±)-**6a**	47.8 ± 9.6	25.9 ± 6.0	88.2 ± 20.9	0.5	1.8
(±)-**7a**	59.6 ± 5.5	97.6 ± 0.8	717 ± 81	1.6	12.0
C) Benzovesamicols					
(±)-**BV** [16]	1.32 ± 0.25 *(0.055 ± 0.01)*[d]	26.1 ± 4.5	96.4 ± 22.3	19.7	73.0
(±)-**9b**	56.0 ± 18.2	466 ± 67	1275 ± 261	8.3	22.7
(±)-**9c** [16]	7.3 ± 1.3	24 ± 2	166 ± 60	3.3	22.7
(±)-**9e**	11.5 ± 2.5	67 ± 12	549 ± 73	5.8	47.7
(±)-**9h**	23.2 ± 6.7	65 ± 29	60 ± 22	2.8	2.6
(±)-**9j**	112.3 ± 9.3	248 ± 11	161 ± 2	2.2	1.4
(±)-**FBBV** [37]	24.4 ± 2.8 *(2.70 ± 0.40)*[e]	215 ± 132 *(191 ± 57)*[f]	284 ± 6 *(251 ± 39)*[g]	8.8	11.6
(±)-**9m**	19.3 ± 0.9	8.9 ± 0.4	258 ± 57	0.5	13.4
(−)-**FEOBV** [17,46]	19.6 ± 1.1	209 ± 94	n.d.	10.7	n.c.
D) Aminobenzovesamicols					
(±)-**5-ABV** [16]	25.1 ± 0.5 *(0.013 ± 0.001)*[d]	119 ± 6	474 ± 57	4.7	18.9
(±)-**8-ABV** [16]	339 ± 17.6	55.3 ± 27.1	726 ± 388.6	0.2	2.1
(±)-**11a**	156 ± 26	6431 ± 1851	3623 ± 901	41.2	23.2
(±)-**11h**	527 ± 62	1773 ± 51	2079 ± 442	3.4	3.9
(±)-**12a**	188 ± 29	626 ± 396	1381 ± 724	3.3	2.2
(±)-**12h**	>10 000	296 ± 202	2112 ± 41	/	/

K_i values in nM (mean ± S.D., n ≥ 2) were derived from IC_{50} values according to the Cheng-Prusoff equation: $K_i = IC_{50}/(1 + C/K_D)$, where C is the concentration of the radioligand and K_D the dissociation constant of the corresponding radioligand. Italics were used to better differenciate between own and reported data.

[a] (−)-[^{3}H]vesamicol: K_D = 25.6 nM to VAChT on PC12 cells transfected with rat VAChT cDNA.

[b] [^{3}H]pentazocine: K_D = 6.9 nM to σ_1 receptors on rat cortex homogenates.

[c] [^{3}H]DTG with blocking of σ_1 receptors using dextrallorphan: K_D = 29.0 nM to σ_2 receptors on rat liver homogenates.

[d] Reported K_i values obtained with (−)-[^{3}H]vesamicol and vesicles from the electric organ of *Torpedo californica* [22,34,47].

[e] Reported K_i values obtained with (−)-[^{3}H]vesamicol and PC12 cells stably expressing human VAChT [37,48].

[f] Reported K_i values obtained with (+)-[^{3}H]pentazocine and guinea pig brain membrane homogenates [22,37,48].

[g] Reported K_i values obtained with [^{3}H]DTG and (+)-pentazocine and rat liver homogenates [22,37,47,48].

higher potency of **5-ABV** to block the [^{3}H]ACh transport compared to **8-ABV**, however in a less pronounced manner ($IC_{50(5\text{-ABV})}$ = 100 versus $IC_{50(8\text{-ABV})}$ = 300 nM) [16].

The N-substitution of **5-ABV** and **8-ABV** with the *p*-fluorobenzoyl group results in a high diversity in affinity data. It reaches from moderate VAChT affinity (K_i = 156 nM) and relative high

selectivity over σ receptors (>23) for **11a** until complete loss of affinity for **12h** with a K_i for VAChT over 10 μM. As general tendency for the aminobenzovesamicols, we conclude that the substitution of this bulky amide functionality in particular in combination with variations at ring C is obviously not tolerated by the VAChT binding site.

Regarding the σ_1 and σ_2 receptor affinities also a decrease of binding is observed compared to the corresponding vesamicol derivative **1h**, which is more pronounced than the shift observed for VAChT binding.

2.3.2. Alternating ring A and fixed ring B and C moieties

To investigate the influence of changes in ring A on the affinities to VAChT, σ_1, and σ_2 receptors independently from other modifications, derivatives of identical ring B and C were compared. Therefore, the affinities of all derivatives bearing amine **a** (4-phenylpiperidine) were considered in relation to the values of vesamicol to obtain a relative affinity value defined as K_i(target; vesamicol)/K_i(target; compound). To simplify matters, we visualized our findings in a bar graph (see Fig. 5), which shows the relative affinity values in logarithmic scale, whereas vesamicol was set as x-coordinate with a $\log_{(\text{rel. aff.})} = 0$. Thus, all bars above this coordinate represent a higher affinity than vesamicol, whereas bars in the negative range indicate decreased binding affinities toward the correspondent target.

Four of the ten examined compounds (**3a**, **DeHVes**, **BV**, and **5-ABV**) show higher VAChT affinity than vesamicol. All these derivatives are characterized by a substitution or ring fusion in 4-position of ring A of the vesamicol skeleton. Only two of them, **BV** and **5-ABV**, exhibit a favorable selectivity profile compared to vesamicol with more or less decreased affinities for both σ receptors. Interestingly, considering compound **11a**, the substitution of a fluorobenzylamide function at 5-position of the benzovesamicol structure resulted in a 6-fold decrease of VAChT affinity compared to the corresponding **5-ABV**.

The 3-fluoropropyl ether vesamicols **6a** and **7a** show comparable VAChT affinities as vesamicol itself, whereas **7a** with the substituent in 5-position of the cyclohexyl ring A exhibits a better selectivity profile due to lower affinities toward σ receptors. So, it can be stated that alkyl ether chains are able to improve the selectivity by reducing the affinity to the σ receptors, which is in contrast to the corresponding *p*-fluorobenzyl derivative **4a**. Moreover, for this derivative the most intense loss of affinity for VAChT compared to vesamicol was observed, obviously caused by the substitution of a sterically demanding benzyl ether group in 5-position of the cyclohexyl ring A.

In summary, a substitution or ring fusion at the 4-position of ring A of the vesamicol skeleton lead to higher binding affinities toward VAChT but depends in a complex manner of the steric demand of the substituents. This finding is in accordance with the results of our CoMFA study based on ligands taken from the literature [52]. We could demonstrate herein that an increase of steric bulk in the region of cyclohexyl ring A diagonally opposite to the hydroxyl group favors binding to VAChT.

Regarding σ receptor binding, the bar graph also illustrates that the desired decrease of σ_1 and σ_2 receptor affinities is often accompanied by a decrease in VAChT affinity. In the same way structural modifications effecting an increase in VAChT affinity (**3a** and **DeHVes**) led to an increase of affinity to at least one of the sigma receptors. By contrast, a more or less pronounced decrease of σ receptor binding of **BV** and **5-ABV** was observed with increasing VAChT affinity. To explain this behavior, we investigated the 3-dimensional structures of several derivatives and compared them with known pharmacophore models published in particular for ligands of the σ_1 receptor [53,54]. According to the first and most common model reported by Glennon et al. [53], a potent σ_1 receptor ligand should comprise an amino group flanked by two hydrophobic substituents with a distance ranging between 6 and 10 Å for the "Primary" and 2.5–3.9 Å for the "Secondary Hydrophobic Binding site". Generally, vesamicol analogues with the central piperidine ring exhibit this arrangement and are therefore potential ligands for this target. In particular, the derivative **DeHVes** ($K_{i(\sigma 1)} = 5.8$ nM) with distances of 3.6 for the "Primary" and 7.1 Å for "Secondary Hydrophobic Site" corresponds to ligands of this pharmacophore model. Also **BV** shows distances being within the range of the model which is reflected by its binding affinity toward

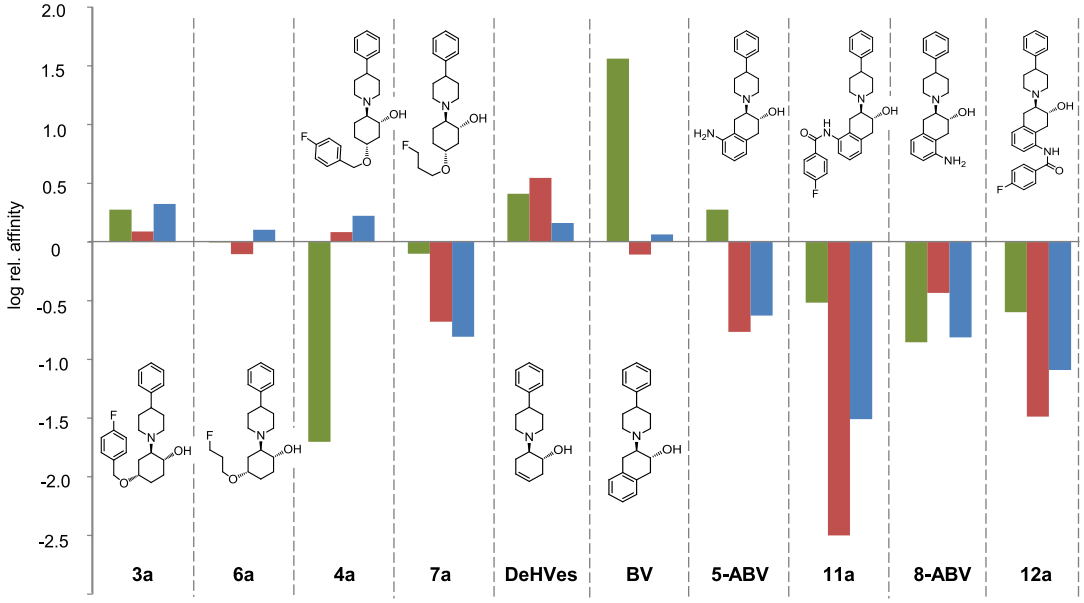


Fig. 5. Relative affinity of all derivatives bearing 4-phenylpiperidine (amine a) in relation to vesamicol (set as x-coordinate); green: VAChT, red: σ_1, blue: σ_2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the σ_1 receptor ($K_{i(\sigma1)} = 26.1$ nM). Interestingly, despite the similar structural behavior, the corresponding **5-ABV** shows significantly decreased binding to this receptor ($K_{i(\sigma1)} = 119$ nM), which could be caused by the polar ionizable amino group at the hydrophobic region. This could also explain the low σ receptor binding of the other aminobenzovesamicol derivatives **11a**, **12a**, and **8-ABV**. Probably, a substitution of ionic or polar functionalities at the annelated benzyl ring of benzovesamicols could be an alternative to achieve improved selectivity of vesamicol based ligands.

3. Conclusion

To our knowledge, for the first time a SAR study was performed which considered the VAChT affinity *and* selectivity of derivatives belonging to four different structural classes of vesamicol. Based on the in vitro binding data obtained with this series of vesamicol analogs systematically modified on the three ring systems (A, B, and C) we can conclude: (i) modifications in ring B and C do not improve the VAChT binding affinity, (ii) modifications in ring A, in particular via ring fusion or insertion of less bulky functionalities in 4-position, may increase VAChT affinity, (iii) the influence of the ring A, B, and C modifications on the affinity to σ_1 and σ_2 receptors is of high diversity without obvious tendency, and (iv) in most cases, an increase of VAChT affinity is accompanied by an increase in σ_1 and/or σ_2 receptor affinity. On the other hand, high sigma receptor affinities do not implicate necessarily high VAChT affinities, indicating that the three target affinities are not completely dependent on each other.

Finally, we have to assume that obviously none of the investigated derivatives fulfill the requirements for a PET radiotracer specifically targeting the VAChT in the brain. Moreover, this structure-affinity-selectivity relationship study illustrates the challenge in the development of VAChT imaging ligands based on vesamicol as lead structure. This is mainly caused by the vesamicol skeletal structure which corresponds to structural requirements of ligands for σ_1 receptors.

4. Materials and methods

4.1. Chemical part

4.1.1. Instruments and materials

Analysis of all final compounds was performed by HR-MS, HPLC, TLC, and NMR spectroscopy.

High-resolution mass spectra were recorded on a FT-ICR APEX II (Bruker Daltonics) using ElectroSpray Ionization (ESI). NMR spectra (^{1}H, ^{13}C, ^{13}C-APT, ^{19}F, H,H-COSY, HSQC, HMBC) were recorded on spectrometers from Varian. Splitting patterns have been designated as follows: s: singlet, d: doublet, bs: broad singlet, bd: broad doublet, bm: broad multiplet, om: overlapping multiplet, m: multiplet, t: triplet, td: triplet of doublet; dt: doublet of triplet.

Analytical thin-layer chromatography (TLC) was performed on silica gel coated plates (Machery-Nagel, ALUGRAM SIL G/UV$_{254}$). The spots were identified using a UV lamp or by dipping into a solution of 2% ninhydrine in EtOH/MeOH 1:1.

The chemical purity of all compounds is >97% and was controlled by HPLC using a 250 × 4.6 mm Reprosil-Pur Basic HD − 5 μm (Dr. Maisch GmbH, Germany) or a LiChrospher 100 CN − 5 μm column (Merck KGaA, Germany). These analytical chromatographic separations were performed on a JASCO LC-2000 system, incorporating a PU-2080*Plus* pump, AS-2055*Plus* auto injector (100 μL sample loop), and a MD-2010 dioden-array-detector (monitoring from 195 to 600 nm). Mixtures of ACN and aqueous 20 mM NH_4OAc were used as eluents.

Chemical names of compounds were generated by ChemDraw Ultra 10.0. Three-dimensional structures were generated by Chem3D Pro12.0 and energy-minimized on MM2 level.

Racemic vesamicol was purchased as hydrochloride from BIOTREND Chemikalien GmbH (Germany). Amines **b**, **c**, and **e**–**m** were purchased from several providers. N-Ethyl-3-phenylpropan-1-amine (**d**) was synthesized according to known procedures [55]. Vesamicol hydrochloride was purchased by BIOTREND Chemikalien GmbH, Germany and cyclohexene oxide by Sigma–Aldrich, Germany.

4.1.2. Syntheses and analytical data

*4.1.2.1. General procedure for vesamicol derivatives (±)-**1b**–(±)-**1m**.* Cyclohexene oxide (10 mmol) and the amine (10.5 mmol) were dissolved in 5–10 mL EtOH and stirred at 65–70 °C for 48 h. The crude product was purified by column chromatography (silica gel 60) using mixtures of EtOAc/*n*-hexane/NH_3. All derivatives were obtained as colorless solids with yields of 50–70%.

(±)-**1b** (±)-2-(4-phenyl-piperazin-1-yl)-cyclohexanol [16] (there are no detailed NMR data in the reference): ^{1}H NMR (300 MHz, $CDCl_3$) δ in ppm: 7.27 (m, 2H), 6.94 (m, 2H), 6.88 (m, 1H), 3.94 (s, 1H, CH–OH), 3.41 (m, 1H, CH–OH), 3.26–3.13 (om, 4H, CH$_2$ piperazine), 2.93 (m, 2H, CH$_2$ piperazine), 2.62 (m, 2H, CH$_2$ piperazine), 2.32 (m, 1H, CH–N), 2.18 (m, 1H, CH$_2$ cyclohexane), 1.87–1.71 (om, 3H, CH$_2$ cyclohexane), 1.30–1.16 (om, 4H, CH$_2$ cyclohexane); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ in ppm: 151.3 (s), 129.0119.8 (s), 116.1 (s), 70.2 (s, CH–N), 68.5 (s, CH–OH), 49.8 and 48.2 (s, CH$_2$ piperazine), 33.1, 25.4, 24.0, and 22.2 (s, CH$_2$ cyclohexane); HRMS (ESI): $m/z = 261.19605$ (M + H$^+$) calc. 261.19614; $m/z = 283.17792$ (M + Na$^+$) calc. 283.17808.

(±)-**1c** (±)-2-(4-phenyl-5,6-dihydropyridin-1(2H)-yl)cyclohexanol [16] (there are no detailed NMR data in the reference): ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 7.40–7.34 (om, 2H), 7.32 (m, 2H), 7.24 (m, 1H), 6.10 (bm, 1H), 3.51–3.41 (om, 2H, CH–OH, CH$_2$–N), 3.22 (m, 1H, CH$_2$–N), 3.00 (m, 1H, CH$_2$–N), 2.62–2.55 (om, 3H, 2 × CH$_2$CPh, CH$_2$–N), 2.36 (m, 1H, CH–N), 2.16 (m, 1H, CH$_2$ cyclohexane), 1.87–1.81 (om, 2H, CH$_2$ cyclohexane), 1.74 (m, 1H, CH$_2$ cyclohexane), 1.35–1.18 (om, 4H, CH$_2$ cyclohexane); ^{13}C NMR (100 MHz, $CDCl_3$) δ in ppm: 140.8 (s), 135.2 (s), 128.4 (s), 127.1 (s), 124.9 (s), 122.4 (s), 69.6 (s, CH–N), 68.9 (s, CH–OH), 48.8 and 45.1 (s, CH$_2$–N), 33.4 (s, CH$_2$ cyclohexane), 28.9 (s, CH$_2$CPh), 25.7, 24.2, and 22.1 (s, CH$_2$ cyclohexane); HRMS (ESI): $m/z = 258.18530$ (M + H$^+$) calc. 258.18524; $m/z = 537.34520$ (2M + Na$^+$) calc. 537.34515.

(±)-**1d** (±)-2-(ethyl(3-phenylpropyl)amino)cyclohexanol: ^{1}H NMR (300 MHz, $CDCl_3$) δ in ppm: 7.28 (m, 2H), 7.20–7.16 (om, 3H), 4.01 (bs, 1H, OH), 3.33 (m, 1H, CH–OH), 2.68–2.54 (om, 4H, 3 × CH$_2$–N, CH$_2$Ph), 2.45–2.28 (om, 3H, CH$_2$–N, CH$_2$Ph, CH–N), 2.15 (m, 1H, CH$_2$ cyclohexane), 1.85–1.71 (om, 5H, 2 × CH$_2$CH$_2$Ph, 3 × CH$_2$ cyclohexane), 1.27–1.12 (om, 4H, CH$_2$ cyclohexane), 1.04 (t, $^3J_{HH} = 7.0$ Hz, 3H, CH$_3$); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ in ppm: 142.3 (s), 128.4 (s), 125.8 (s), 69.2 (s, CH–N), 66.7 (s, CH–OH), 49.3 (s, CH$_2$Ph), 43.8 and 33.8 (s, CH$_2$–N), 33.3 (s, CH$_2$ cyclohexane), 31.0 (s, CH$_2$CH$_2$Ph), 25.8, 24.3, and 22.8 (s, CH$_2$ cyclohexane), 14.8 (s, CH$_3$); HRMS (ESI): $m/z = 262.21652$ (M + H$^+$) calc. 262.21654.

(±)-**1e** (±)-1-(2-hydroxy-cyclohexyl)-4-phenyl-piperidin-4-ol: ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 7.51 (m, 2H), 7.36 (m, 2H), 7.25 (m, 1H), 4.08 (bs, 1H, CH–OH), 3.40 (m, 1H, CH–OH), 3.09 (m, 1H, CH$_2$ pip), 2.70 (m, 1H, CH$_2$ pip), 2.62 (m, 1H, CH$_2$ pip), 2.54 (m, 1H, CH$_2$ pip), 2.26 (m, 1H, CH–N), 2.19–2.16 (om, 2H, CH$_2$ pip), 2.02 (m, 1H, CH$_2$ cyclohexane), 1.92–1.68 (om, 6H, 3 × CH$_2$ cyclohexane, 2 × CH$_2$ pip, OH), 1.30–1.20 (om, 4H, CH$_2$ cyclohexane); ^{13}C NMR (100 MHz, $CDCl_3$) δ in ppm: 148.4 (s), 128.5 (s), 127.2 (s), 124.6 (s), 71.7 (s, PhCOH), 70.7 (s, CH–N), 68.8 (s, CH–OH), 48.6 and 41.2 (s, CH$_2$ pip), 39.4 and 39.1 (s, CH$_2$ pip), 33.4, 25.7, 24.2, and 22.5 (s, CH$_2$

cyclohexane); HRMS (ESI): m/z = 276.19575 ($M + H^+$) calc. 276.1958; m/z = 298.17770 ($M + Na^+$) calc. 298.17775.

(±)-**1f** (±)-1-[1-(2-hydroxy-cyclohexyl)-4-phenyl-piperidin-4-yl]-ethanone: ^{1}H NMR (300 MHz, $CDCl_3$) δ in ppm: 7.36–7.21 (om, 5H), 3.95 (s, OH), 3.33 (m, 1H, CH–OH), 2.83–2.69 (om, 2H, CH_2 pip), 2.58–2.46 (om, 3H, CH_2 pip, 2.30 (m, 1H, CH–N), 2.18–1.93 (om, 4H, 3 × CH_2 pip, 1 × CH_2 cyclohexane), 1.88 (s, 3H, CH_3), 1.73–1.66 (om, 3H, CH_2 cyclohexane), 1.24–1.05 (om, 4H, CH_2 cyclohexane). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ in ppm: 209.5 (s, C=O), 141.8 (s), 128.9 (s), 127.2 (s), 126.4 (s), 70.5 (s, CH–N), 68.7 (s, CH–OH), 55.1 (s, PhCC = O), 48.4 and 43.9 (s, CH_2 pip), 33.8 (s, CH_2 cyclohexane), 33.5 and 33.3 (s, CH_2 pip), 25.7 (s, CH_3), 25.6, 24.1, and 22.4 (s, CH_2 cyclohexane); HRMS (ESI): m/z = 302.21131 ($M + H^+$) calc. 302.21146.

(±)-**1g** (±)-2-(4,4-diphenylpiperidin-1-yl)cyclohexanol: ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 7.27 (bm, 8H), 7.13 (m, 2H), 4.11 (bs, OH), 3.35 (m, 1H, CH–OH), 2.82–2.76 (om, 2H, 2 × CH_2 pip), 2.47–2.44 (om, 6H, 6 × CH_2 pip), 2.20–2.10 (om, 2H, 1 × CH–N, 1 × CH_2 cyclohexane), 1.70–1.60 (om, 3H, 3 × CH_2 cyclohexane), 1.26–1.12 (om, 3H, 3 × CH_2 cyclohexane), 1.06–1.00 (m, 1H, 1 × CH_2 cyclohexane). ^{13}C NMR (100.58 MHz, $CDCl_3$) δ in ppm: 147.7 (s), 128.4 (s), 127.2 (s), 125.8 (s), 70.4 (s, CH–N), 68.7 (s, CH–OH), 45.7 (bs, CH_2 pip), 45.0 (s, CH_2 pip), 37.1 (s, CH_2 pip), 33.4, 25.6, 24.2, and 22.4 (s, CH_2 cyclohexane); HRMS (ESI): m/z = 336.23199 ($M + H^+$) calc. 336.23219.

(±)-**1h** (±)-2-(4-benzyl-piperidin-1-yl)-cyclohexanol [16] (there are no detailed NMR data in the reference): ^{1}H NMR (300 MHz, $CDCl_3$) δ in ppm: 7.27 (m, 2H), 7.20–7.12 (om, 3H), 4.08 (s, OH), 3.33 (m, 1H, CH–OH), 2.79 (m, 1H, CH_2 pip), 2.64–2.48 (om, 4H, 2 × CH_2 pip, CH_2Ph), 2.20–1.99 (om, 3H, CH–N, CH_2 pip, CH_2 cyclohexane), 1.76–1.62 (om, 5H, 2 × CH_2 pip, 3 × CH_2 cyclohexane), 1.51 (m, 1H, CH–CH_2Ph), 1.37–1.09 (om, 6H, 2 × CH_2 pip, 4 × CH_2 cyclohexane). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ in ppm: 140.7 (s), 129.0 (s), 128.1 (s), 125.7 (s), 70.5 (s, CH–N), 68.8 (s, CH–OH), 52.7 (s, CH_2Ph), 45.1 and 43.2 (s, CH_2 pip), 38.3 (s, CH pip), 33.2 and 33.0 (s, CH_2 pip), 32.8 25.6, 24.0, and 22.1 (s, CH_2 cyclohexane); HRMS (ESI): m/z = 274.21640 ($M + H^+$) calc. 274.21654; m/z = 296.19843 ($M + Na^+$) calc. 296.19849.

(±)-**1i** (±)-2-(3,4-dihydro-1H-isoquinolin-2-yl)-cyclohexanol [16] (there are no detailed NMR data in the reference): ^{1}H NMR (300 MHz, $CDCl_3$) δ in ppm: 7.12 (bm, 3H), 7.02 (m, 1H), 3.97 (bs, OH), 3.93 and 3.66 (AB, $^2J_{HH}$ = 15 Hz, 2H, CH_2 pip), 3.49 (m, 1H, CH–OH), 3.04 (m, 1H, CH_2 pip), 2.90 (m, 2H, CH_2 pip), 2.62 (m, 1H, CH_2 pip), 2.41 (m, 1H, CH–N), 2.16 (bm, 1H, CH_2 cyclohexane), 1.91–1.75 (om, 3H, CH_2 cyclohexane), 1.28 (bm, 4H, CH_2 cyclohexane). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ in ppm: 135.4 (s), 134.7 (s), 128.9 (s), 126.6 (s), 126.2 (s), 125.7 (s), 70.1 (s, CH–N), 68.9 (s, CH–OH), 51.7 (s, CH_2 pip), 46.0 (s, CH_2 pip), 33.4 (s, CH_2 cyclohexane), 30.2 (s, CH_2 pip), 25.6, 24.2, and 22.1 (s, CH_2 cyclohexane); HRMS (ESI): m/z = 232.16949 ($M + H^+$) calc. 232.16959; m/z = 485.31355 ($2M + Na^+$) calc. 485.31385.

(±)-**1j** (±)-2-[1,4′]bipiperidinyl-1′-yl-cyclohexanol: ^{1}H NMR (300 MHz, $CDCl_3$) δ in ppm: 3.99 (s, 1H, OH), 3.30 (m, 1H, CH–OH), 2.84 (m, 1H, CH_2 pip), 2.63 (m, 1H, CH–N), 2.69–2.46 (om, 5H, CH_2 pip), 2.18–2.05 (om, 4H, CH–N, 2 × CH_2 pip, 1 × CH_2 cyclohexane), 1.83–1.33 (om, 13H, 10 × CH_2 pip, 3 × CH_2 cyclohexane), 1.25–1.09 (om, 4H, 4 × CH_2 cyclohexane); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ in ppm: 70.2 (s, CH–N), 68.7 (s, CH–OH), 62.8 (s, CH–N), 52.5 (s, CH_2 pip), 50.2 (s, 2 × CH_2 pip), 44.4 (s, CH_2 pip), 33.2 (s, CH_2 cyclohexane), 28.9 and 28.6 (s, CH_2 pip), 26.4 (s, 2 × CH_2 pip), 25.5 and 24.7 (s, CH_2 cyclohexane), 24.0 (s, CH_2 pip), 22.1 (s, CH_2 cyclohexane); HRMS (ESI): m/z = 267.24316 ($M + H^+$) calc. 267.24309; m/z = 289.22501 ($M + Na^+$) calc. 289.22503.

(±)-**1k** (±)-(4-fluoro-phenyl)-[1-(2-hydroxy-cyclohexyl)-piperidin-4-yl]-methanone [22] (reference provides exclusively ^{1}H NMR data): ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 7.95 (m, 2H), 7.13 (m, 2H), 3.94 (bs, OH), 3.38 (m, 1H, CH–OH), 3.21 (bm, 1H, CH-COPhF), 2.95 (m, 1H, CH_2 pip), 2.76–2.70 (om, 2H, CH_2 pip), 2.30–2.21 (om, 2H, CH_2 pip, CH–N), 2.12 (m, 1H, CH_2 cyclohexane), 1.88–1.70 (om, 7H, 4 × CH_2 pip, 3 × CH_2 cyclohexane), 1.30–1.16 (om, 4H, CH_2 cyclohexane); ^{13}C NMR (100 MHz, $CDCl_3$) δ in ppm: 201.0 (s, C=O), 165.7 (d, $^1J_{CF}$ = 245.7 Hz), 132.5 (d, $^4J_{CF}$ = 3.0 Hz), 130.9 (d, $^3J_{CF}$ = 9.2 Hz), 115.8 (d, $^2J_{CF}$ = 21.8 Hz), 70.8 (s, CH–N), 68.7 (s, CH–OH), 51.9 (s, CH_2 pip), 45.1 (s, CH-COPhF), 43.9 (s, CH_2 pip), 33.3 (s, CH_2 cyclohexane), 29.6 and 29.2 (s, CH_2 pip), 25.6, 24.2, and 22.4 (s, CH_2 cyclohexane); ^{19}F NMR (376.3 MHz, $CDCl_3$) δ in ppm: − 105.8 (bs); HRMS (ESI): m/z = 306.18626 ($M + H^+$) calc. 306.18638; m/z = 633.34774 ($2M + Na^+$) calc. 633.34744.

(±)-**1m** (±)-2-(4-(pyridin-4-yl)piperidin-1-yl)cyclohexanol: ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 8.50 (m, 2H), 7.12 (m, 2H), 3.99 (bs, OH), 3.39 (m, 1H, CH–OH), 2.95 (m, 1H, CH_2 pip), 2.79–2.70 (om, 2H, CH_2 pip, CH–N), 2.48 (m, 1H, CH pip), 2.24 (m, 2H, CH_2 pip), 2.12 (m, 1H, CH_2 cyclohexane), 1.90–1.59 (om, 7H, 4 × CH_2 pip, 3 × CH_2 cyclohexane), 1.31–1.16 (om, 4H, CH_2 cyclohexane); ^{13}C NMR (100 MHz, $CDCl_3$) δ in ppm: 154.9 (s), 150.0 (s), 122.4 (s), 70.9 (s, CH–N), 68.7 (s, CH–OH), 53.1 (s, CH_2 pip), 45.3 (s, CH pip), 42.3 (s, CH_2 pip), 33.5 (s, CH_2 cyclohexane), 33.4 and 33.1 (s, CH_2 pip), 25.6, 24.2, and 22.4 (s, CH_2 cyclohexane); HRMS (ESI): m/z = 261.19610 ($M + H^+$) calc. 261.19618; m/z = 543.36722 ($2M + Na^+$) calc. 543.36695.

*4.1.2.2. General procedure for 4-O- and 5-O-p-fluorobenzyl ether vesamicol derivatives (±)-**3a**-(±)-**3m** and (±)-**4a**-(±)-**4m**.* The *cis*-epoxide precursor (±)-**2** was synthesized as already described [27]. In brief, elimination of water from cyclohexane-1,4-diol gave cyclohex-3-enol [40] which was epoxidized diastereoselectively by using *tert*-butyl hydroperoxide and $Mo(CO)_6$ [41] to obtain the racemic epoxy alcohol. Subsequent *O*-*p*-fluorobenzylation resulted in the *cis*-epoxide (±)-**2**.

To synthesize the 4-*O*-*p*-fluorobenzyl ether vesamicol derivatives (±)-**3a**–(±)-**3m**, the amine (1.41 mmol) and (±)-**2** (1.12 mmol) were dissolved in 5 mL EtOH and stirred at 65–70 °C for 5 days. The crude product was purified by column chromatography (silica gel 60) or by crystallization (details described for each compound).

To synthesize the 5-*O*-*p*-fluorobenzyl ether vesamicol derivatives (±)-**4a**–(±)-**4m**, the *cis*-epoxide (±)-**2** (1.12 mmol) and $LiClO_4$ (1.97 mmol) were dissolved in 3 mL CH_3CN and stirred for 10 min. Subsequently, the amine (1.41 mmol) was added and the reaction mixture was stirred at rt for 24 h. Products were purified by column chromatography (silica gel 60) or by crystallization (details described for each compound). The regioisomeric purity was checked by HPLC.

(±)-**3a**, (±)-**3b**, and (±)-**4a** are already described by our group [27,28].

(±)-**3c** (±)-4-(4-fluorobenzyloxy)-2-(4-phenyl-5,6-dihydropyridin-1(2*H*)-yl)cyclohexanol: The product crystallized in the reaction mixture, was isolated and recrystallized from EtOH at 4 °C to give 149 mg (35%) of colorless crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ in ppm: 7.40–7.30 (om, 6H), 7.24 (m, 1H), 7.04 (m, 2H), 6.09 (bm, 1H), 4.51 and 4.43 (AB, $^2J_{HH}$ = 11.9 Hz, 2H, OCH_2Ph), 3.85 (m, 1H, CH–OCH_2Ph), 3.54 (m, 1H, CH–OH), 3.40 (m, 1H, CH_2–N), 3.20 (m, 1H, CH_2–N), 3.00–2.84 (om, 2H, CH_2–N, CH–N), 2.61–2.56 (om, 3H, CH_2–N, 2 × CH_2CPh), 2.13–1.95 (om, 3H, CH_2 cyclohexane), 1.76 (m, 1H, CH_2 cyclohexane), 1.45–1.30 (om, 2H, CH_2 cyclohexane); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ in ppm: 162.3 (d, $^1J_{CF}$ = 245.3 Hz), 140.7 (s), 135.2 (s), 134.8 (d, $^4J_{CF}$ = 3.1 Hz), 129.1 (d, $^3J_{CF}$ = 8.0 Hz), 128.4 (s), 127.1 (s), 124.9 (s), 122.2 (s), 115.2 (d, $^2J_{CF}$ = 21.4 Hz), 73.5 (s, $COCH_2$Ph), 69.3 (s, OCH_2Ph), 68.8 (s, CH–OH), 63.6 (s, CH–N), 48.6 and 44.9 (s, CH_2–N), 28.8 (s, CH_2CPh), 27.8, 27.7, and 26.1 (s, CH_2

cyclohexane); ^{19}F NMR (282.3 MHz, $CDCl_3$) δ in ppm: − 115.6 (m); HRMS (ESI): m/z = 382.21765 (M + H^+) calc. 382.21768; m/z = 785.41022 (2M + Na^+) cal. 785.41004.

(±)-**3d** (±)-2-(ethyl(3-phenylpropyl)amino)-4-(4-fluorobenzyloxy) cyclohexanol: For this derivative 1.5 mmol of *cis*-epoxide (±)-**4** was used and the reaction was performed at 85 °C for 8 days. The crude product was purified by column chromatography starting with EtOAc/*n*-hexane/NH_3 (1/2/0.01) and ending with EtOAc/NH_3 (1/0.01). 120 mg (28%) of (±)-**5d** were obtained as nearly colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ in ppm: 7.30–7.24 (om, 4H), 7.19 (m, 3H), 7.01 (m, 2H), 4.46 and 4.40 (AB, $^2J_{HH}$ = 11.9 Hz, 2H, OCH_2Ph), 3.79 (m, 1H, CH–OCH_2Ph), 3.38 (m, 1H, CH–OH), 2.86 (m, 1H, CH–N), 2.73–2.51 (om, 4H, 3 × CH_2–N, CH_2Ph), 2.37 (bm, 2H, CH_2–N, CH_2Ph), 2.04–1.40 (om, 6H, 2 × CH_2CH_2Ph, 4 × CH_2 cyclohexane), 1.40–1.20 (om, 2H, CH_2 cyclohexane), 1.06 (t, $^3J_{HH}$ = 7.0 Hz, 3H, CH_3); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ in ppm: 162.3 (d, $^1J_{CF}$ = 245.3 Hz), 142.1 (s), 134.8 (d, $^4J_{CF}$ = 3.1 Hz), 129.5 (d, $^3J_{CF}$ = 8.1 Hz), 128.5 (s), 128.4 (s), 125.9 (s), 115.3 (d, $^2J_{CF}$ = 21.4 Hz), 73.5 (s, $COCH_2Ph$), 69.2 (s, OCH_2Ph), 69.0 (s, CH–OH), 60.8 (s, CH–N), 49.2 (s, CH_2Ph), 43.8 and 33.7 (s, CH_2–N), 30.7 (bs, CH_2CH_2Ph), 27.8, 27.7, and 26.9 (s, CH_2 cyclohexane), 14.6 (s, CH_3); ^{19}F NMR (282.3 MHz, $CDCl_3$) δ in ppm: − 115.7 (bs); HRMS (ESI): m/z = 386.24892 (M + H^+) calc. 386.24898.

(±)-**3e** (±)-1-(5-(4-fluorobenzyloxy)-2-hydroxycyclohexyl)-4-phenylpiperidin-4-ol: The crude product was purified by column chromatography using $CHCl_3$/MeOH/NH_3 (10/1/0.1). 239 mg (49%) of colorless hydrochloride of (±)-**3e** were obtained by adding ethanolic 10% HCl to an ethanolic solution of (±)-**3e**. ^{1}H NMR (300 MHz, $CDCl_3$) δ in ppm: 7.52 (m, 2H), 7.39–7.27 (om, 5H), 7.03 (m, 2H), 4.48 and 4.43 (AB, $^2J_{HH}$ = 12.0 Hz, 2H, OCH_2Ph), 4.05 (bs, 1H, CH–OH), 3.83 (m, 1H, CH–OCH_2Ph), 3.49 (m, 1H, CH–OH), 3.06 (m, 1H, CH_2 pip), 2.84–2.55 (om, 4H, 3 × CH_2 pip, CH–N), 2.23–1.91 (om, 5H, 2 × CH_2 pip, 3 × CH_2 cyclohexane), 1.81 (s, 1H, OH), 1.77–1.64 (m, 3H, 2 × CH_2 pip, 1 × CH_2 cyclohexane), 1.42–1.29 (om, 2H, CH_2 cyclohexane); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ in ppm: 162.6 (d, $^1J_{CF}$ = 245.2 Hz), 148.3 (s), 134.8 (d, $^4J_{CF}$ = 3.3 Hz), 129.1 (d, $^3J_{CF}$ = 7.7 Hz), 128.5 (s), 127.2 (s), 124.6 (s), 115.3 (d, $^2J_{CF}$ = 21.4 Hz), 73.4 (s, $COCH_2Ph$), 71.6 (s, OCH_2Ph), 69.2 (s, PhCOH), 68.6 (s, CH–OH), 64.7 (s, CH–N), 48.4 and 40.9 (s, CH_2 pip), 39.2 and 38.9 (s, CH_2 pip), 27.8, 27.7, and 26.4 (s, CH_2 cyclohexane); ^{19}F NMR (282.3 MHz, $CDCl_3$) δ in ppm: − 115.6 (m); HRMS (ESI): m/z = 400.22797 (M + H^+) calc. 400.22825; m/z = 422.21050 (M + Na^+) cal. 422.21019.

(±)-**3f** (±)-1-(1-(5-(4-fluorobenzyloxy)-2-hydroxycyclohexyl)-4-phenylpiperidin-4-yl)ethanone: The crude product was purified by column chromatography using EtOAc/*n*-hexane/NH_3 (1/1/0.01). 159 mg (33%) of colorless hydrochloride of (±)-**3f** were obtained by adding of ethanolic 10% HCl to an ethanolic solution of (±)-**3f**. ^{1}H NMR (300 MHz, $CDCl_3$) δ in ppm: 7.38–7.28 (om, 7H), 7.01 (m, 2H), 4.45 and 4.39 (AB, $^2J_{HH}$ = 11.9 Hz, 2H, OCH_2Ph), 3.92 (bs, 1H, OH), 3.78 (m, 1H, CH–OCH_2Ph), 3.43 (m, 1H, CH–OH), 2.82–2.65 (om, 3H, 2 × CH_2 pip, CH–N), 2.58–2.47 (om, 3H, CH_2 pip), 2.30 (m, 1H, CH_2 pip), 2.13–1.90 (om, 8H, 2 × CH_2 pip, 3 × CH_2 cyclohexane, CH_3), 1.68 (m, 1H, CH_2 cyclohexane), 1.34 (m, 1H, CH_2 cyclohexane), 1.21 (m, 1H, CH_2 cyclohexane); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ in ppm: 209.6 (s, C=O), 162.4 (d, $^1J_{CF}$ = 244.7 Hz), 141.7 (s), 134.8 (d, $^4J_{CF}$ = 3.3 Hz), 129.1 (d, $^3J_{CF}$ = 8.2 Hz), 129.0 (s), 127.3 (s), 126.4 (s), 115.2 (d, $^2J_{CF}$ = 21.4 Hz), 73.3 (s, $COCH_2Ph$), 69.3 (s, OCH_2Ph), 68.5 (s, CH–OH), 64.5 (s, CH–N), 55.1 (s, PhCC = O), 48.2 and 43.8 (s, CH_2 pip), 33,6 and 33.4 (s, CH_2 pip), 27.8, 27.7, and 26.2 (s, CH_2 cyclohexane), 25.8 (s, CH_3); ^{19}F NMR (282.3 MHz, $CDCl_3$) δ in ppm: − 115.6 (m); HRMS (ESI): m/z = 426.24351 (M + H^+) calc. 426.24390; m/z = 448.22606 (M + Na^+) cal. 448.22584.

(±)-**3g** (±)-2-(4,4-diphenylpiperidin-1-yl)-4-(4-fluorobenzyloxy) cyclohexanol: The crude product was purified by column chromatography using $CHCl_3$/MeOH/NH_3 (10/1/0.1). Concentration of the collected fractions to a smaller volume and keeping a few days at 4 °C resulted in the formation of 216 mg (42%) of a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 7.27–7.22 (om, 10H), 7.12 (m, 2H), 6.98 (m, 2H), 4.39 and 4.32 (AB, $^2J_{HH}$ = 11.6 Hz, 2H, OCH_2Ph), 4.04 (bs, OH), 3.71 (bm, 1H, CH–OCH_2Ph), 3.40 (m, 1H, CH–OH), 2.75–2.64 (om, 3H, 2 × CH_2 pip, CH–N), 2.49–2.41 (om, 6H, 6 × CH_2 pip), 1.94–1.82 (om, 3H, 3 × CH_2 cyclohexane), 1.66 (m, 1H, CH_2 cyclohexane), 1.29 (m, 1H, CH_2 cyclohexane), 1.11 (m, 1H, CH_2 cyclohexane); ^{13}C NMR (100 MHz, $CDCl_3$) δ in ppm: 162.3 (d, $^1J_{CF}$ = 244.0 Hz),147.6 (bs), 134.8 (d, $^4J_{CF}$ = 3.0 Hz), 129.1 (d, $^3J_{CF}$ = 7.4 Hz),128.5 (s), 127.3 (s), 125.9 (s), 115.3 (d, $^2J_{CF}$ = 22.1 Hz), 73.5 (s, $COCH_2Ph$), 69.3 (s, OCH_2Ph), 68.5 (s, CH–OH), 64.4 (s, CH–N), 45.5 (bs, C-$(Ph)_2$), 45.0 (s, 2 × CH_2 pip), 37.0 (s, 2 × CH_2 pip), 27.7, 27.6, and 26.3 (s, CH_2 cyclohexane); ^{19}F NMR (376.3 MHz, $CDCl_3$) δ in ppm: − 115.7 (bs); HRMS (ESI): m/z = 460.26428 (M + H^+) calc. 460.26463.

(±)-**3h** (±)-2-(4-benzylpiperidin-1-yl)-4-(4-fluorobenzyloxy) cyclohexanol: The crude product was purified by column chromatography using EtOAc/*n*-hexane/NH_3 (1/1/0.01). By dissolving of the obtained oil in MTBE and keeping at 4 °C for 24 h, 151 mg (34%) of a colorless crystalline solid could be isolated. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 7.31–7.27 (om, 4H), 7.19 (m, 1H), 7.14 (m, 2H), 7.01 (m, 2H), 4.45 and 4.40 (AB, $^2J_{HH}$ = 11.6 Hz, 2H, OCH_2Ph), 4.04 (bs, 1H, OH), 3.79 (m, 1H, CH–OCH_2Ph), 3.42 (m, 1H, CH–OH), 2.82–2.47 (om, 7H, CH–N, 4 × CH_2 pip, CH_2Ph), 2.01–1.92 (om, 4H, 2 × CH_2 pip, 2 × CH_2 cyclohexane), 1.73–1.63 (om, 3H, 1 × CH_2 pip, 2 × CH_2 cyclohexane), 1.51 (m, 1H, CH–CH_2Ph), 1.38–1.20 (om, 3H, 1 × CH_2 pip, 2 × CH_2 cyclohexane); ^{13}C NMR (100.0 MHz, $CDCl_3$) δ in ppm: 162.2 (d, $^1J_{CF}$ = 244.0 Hz),140.7 (s), 134.7 (d, $^4J_{CF}$ = 3.0 Hz), 129.2 (s), 129.1 (d, $^3J_{CF}$ = 8.0 Hz), 128.3 (s), 125.9 (s), 115.3 (d, $^2J_{CF}$ = 21.0 Hz), 73.4 (s, $COCH_2Ph$), 69.3 (s, OCH_2Ph), 68.6 (s, CH–OH), 64.6 (s, CH–N), 52.7 (s, CH_2Ph), 45.2 (s, CH pip), 43.3, 38.4, 33.1, and 32.7 (s, CH_2 pip), 27.8, 27.7, and 26.2 (s, CH_2 cyclohexane); ^{19}F NMR (376.3 MHz, $CDCl_3$) δ in ppm: − 115.6 (bs); HRMS (ESI): m/z = 398.24867 (M + H^+) calc. 398.24898.

(±)-**3i** (±)-2-(3,4-dihydroisoquinolin-2(1*H*)-yl)-4-(4-fluorobenzyloxy)cyclohexanol: The crude product was purified by column chromatography using EtOAc/*n*-hexane/NH_3 (1.5/1/0.01). 126 mg (28%) of colorless hydrochloride of (±)-**3i** were obtained by adding of ethanolic 10% HCl to an ethanolic solution of (±)-**3i**. ^{1}H NMR (300 MHz, $CDCl_3$) δ in ppm: 7.34 (m, 2H), 7.16–7.01 (om, 6H), 4.53 and 4.45 (AB, $^2J_{HH}$ = 12 Hz, 2H, OCH_2Ph), 3.91–3.86 (om, 3H, OH, CH–OCH_2Ph, CH_2 pip), 3.66–3.53 (om, 2H, CH–OH, CH_2 pip), 3.03–2.88 (om, 4H, CH–N, CH_2 pip, 2 × CH_2 pip), 2.60 (m, 1H, CH_2 pip), 2.17–1.96 (om, 3H, CH_2 cyclohexane), 1.78 (m, 1H, CH_2 cyclohexane), 1.46–1.33 (om, 2H, CH_2 cyclohexane). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ in ppm: 162.3 (d, $^1J_{CF}$ = 243.0 Hz),135.1 (s), 134.8 (d, $^4J_{CF}$ = 3.0 Hz),134.6 (s), 129.1 (d, $^3J_{CF}$ = 10.0 Hz),128.8 (s), 126.6 (s), 126.2 (s), 125.7 (s), 115.3 (d, $^2J_{CF}$ = 21.0 Hz), 73.5 (s, $COCH_2Ph$), 69.4 (s, OCH_2Ph), 66.7 (s, CH–OH) 64.1 (s, CH–N), 51.5, 45.6, and 30.1(s, CH_2 pip), 27.7, 27.6, and 26.2 (s, CH_2 cyclohexane); ^{19}F NMR (282.3 MHz, $CDCl_3$) δ in ppm: − 115.5 (m); HRMS (ESI): m/z = 356.20190 (M + H^+) calc. 356.20203; m/z = 733.37859 (2M + Na^+) calc. 733.37874.

(±)-**3j** (±)-2-(1,4′-bipiperidin-1′-yl)-4-(4-fluorobenzyloxy)cyclohexanol: The crude product was purified by column chromatography using $CHCl_3$/MeOH/NH_3 (10/1/0.1). The solvent of collected fractions was removed and the residual oil was dissolved in MTBE. Keeping the MTBE solution a few days at 4 °C, resulted in the formation of 97 mg (22%) of colorless needles. ^{1}H NMR (300 MHz, $CDCl_3$) δ in ppm: 7.27 (m, 2H), 7.00 (m, 2H), 4.45 and 4.39 (AB, $^2J_{HH}$ = 11.9 Hz, 2H, OCH_2Ph), 3.92 (bs, OH), 3.78 (bm, 1H, CH–OCH_2Ph), 3.38 (m, 1H, CH–OH), 2.85 (m, 1H, CH_2 pip), 2.69–2.65 (om, 2H, CH_2 pip, CH–N), 2.55–2.47 (om, 5H, CH_2 pip), 2.19 (m, 1H, CH–N), 2.05–1.79 (om, 6H, 3 × CH_2 pip, 3 × CH_2 cyclohexane), 1.72–1.17 (om, 11H, 8 × CH_2 pip, 3 × CH_2 cyclohexane); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ in ppm: 162.2 (d,

$^1J_{CF}$ = 245.2 Hz), 134.8 (d, $^4J_{CF}$ = 3.3 Hz), 129.0 (d, $^3J_{CF}$ = 8.0 Hz), 115.2 (d, $^2J_{CF}$ = 21.4 Hz), 73.5 (s, COCH$_2$Ph), 69.2 (s, OCH$_2$Ph), 68.6 (s, CH–OH), 64.2 (s, CH–N), 63.0 (s, CH–N), 52.5 (s, CH$_2$ pip), 50.3 (s, 2 × CH$_2$ pip), 44.3 (s, CH$_2$ pip), 28.9 and 28.6 (s, CH$_2$ pip), 27.6 (s, 2 × CH$_2$ cyclohexane), 26.4 (s, 2 × CH$_2$ pip), 26.1 (s, CH$_2$ cyclohexane), 24.9 (s, CH$_2$ pip); HRMS (ESI): m/z = 391.27540 (M + H$^+$) calc. 391.27553.

(±)-**3k** (±)-(1-(5-(4-fluorobenzyloxy)-2-hydroxycyclohexyl)piperidin-4-yl) (4-fluorophenyl)methanone: The crude product was purified by column chromatography using EtOAc/*n*-hexane/NH$_3$ (1/1/0.01). Removal of the solvent led to 197 mg (41%) of a slightly yellow oil. ^{1}H NMR (400 MHz, CDCl$_3$) δ in ppm: 7.95 (m, 2H), 7.29 (m, 2H), 7.11 (m, 2H), 7.02 (m, 2H), 4.48 and 4.40 (AB, $^2J_{HH}$ = 11.6 Hz, 2H, OCH$_2$Ph), 3.82 (bm, 1H, CH–OCH$_2$Ph), 3.44 (m, 1H, CH–OH), 3.21 (bm, 1H, CH-COPhF), 2.95 (m, 1H, CH$_2$ pip), 2.79–2.64 (om, 3H, 2 × CH$_2$ pip, CH–N), 2.23 (bm, 1H, CH$_2$ pip), 2.08 (m, 1H, CH$_2$ cyclohexane), 2.01–1.64 (om, 7H, 4 × CH$_2$ pip, 3 × CH$_2$ cyclohexane), 1.39–1.26 (om, 2H, CH$_2$ cyclohexane); ^{13}C NMR (100 MHz, CDCl$_3$) δ in ppm: 201.0 (s, C=O), 165.7 (d, $^1J_{CF}$ = 253.0 Hz),162.8 (d, $^1J_{CF}$ = 244.0 Hz), 134.8 (d, $^4J_{CF}$ = 3.0 Hz), 132.5 (d, $^4J_{CF}$ = 3.0 Hz), 130.9 (d, $^3J_{CF}$ = 9.0 Hz), 129.1 (d, $^3J_{CF}$ = 8.0 Hz), 115.9 (d, $^2J_{CF}$ = 21.0 Hz), 115.3 (d, $^2J_{CF}$ = 21.0 Hz), 73.4 (s, COCH$_2$Ph), 69.3 (s, OCH$_2$Ph), 68.5 (s, CH–OH), 64.8 (s, CH–N), 51.7 (s, CH$_2$ pip), 45.0 (s, CH-COPhF), 43.8 (s, CH$_2$ pip), 29.5 and 29.2 (s, CH$_2$ pip), 27.7, 27.5, and 26.4 (s, CH$_2$ cyclohexane); ^{19}F NMR (376.3 MHz, CDCl$_3$) δ in ppm: − 115.6 (bs), −105.8 (bs); HRMS (ESI): m/z = 430.21847 (M + H$^+$) calc. 430.21883; m/z = 452.20075 (M + Na$^+$) cal. 452.20077; m/z = 881.41217 (2M + H$^+$) calc. 881.41232.

(±)-**3m** (±)-4-(4-fluorobenzyloxy)-2-(4-(pyridin-4-yl)piperidin-1-yl)cyclohexanol: The crude product was purified by column chromatography using CHCl$_3$/MeOH/NH$_3$ (10/1/0.1). Adding of *n*-hexane to a concentrated CHCl$_3$ solution of (±)-**3m** and keeping a few days at 4 °C resulted in the formation of 220 mg (59%) of a colorless solid. ^{1}H NMR (400 MHz, CDCl$_3$) δ in ppm: 8.50 (m, 2H), 7.30 (m, 2H), 7.12 (m, 2H), 7.01 (m, 2H), 4.48 and 4.41 (AB, $^2J_{HH}$ = 11.9 Hz, 2H, OCH$_2$Ph), 3.82 (bm, 1H, CH–OCH$_2$Ph), 3.44 (m, 1H, CH–OH), 2.95 (m, 1H, CH$_2$ pip), 2.79–2.73 (om, 2H, CH$_2$ pip), 2.67 (m, 1H, CH–N), 2.47 (m, 1H, CH pip), 2.20 (m, 1H, CH$_2$ pip), 2.09 (m, 1H, CH$_2$ cyclohexane), 2.01–1.64 (om, 7H, 4 × CH$_2$ pip, 3 × CH$_2$ cyclohexane), 1.40–1.25 (om, 2H, 2 × CH$_2$ cyclohexane); ^{13}C NMR (100.0 MHz, CDCl$_3$) δ in ppm: 162.2 (d, $^1J_{CF}$ = 245.2 Hz),154.8 (s), 149.9 (s), 134.7 (d, $^4J_{CF}$ = 3.2 Hz), 129.0 (d, $^3J_{CF}$ = 8.1 Hz), 122.3 (s), 115.2 (d, $^2J_{CF}$ = 21.3 Hz), 73.4 (s, COCH$_2$Ph), 69.3 (s, OCH$_2$Ph), 68.6 (s, CH–OH), 64.7 (s, CH–N), 52.9 (s, CH$_2$ pip), 45.2 (s, CH pip), 42.8, 33.4, and 33.1 (s, CH$_2$ pip), 27.7, 27.5, and 26.4 (s, CH$_2$ cyclohexane); ^{19}F NMR (376.3 MHz, CDCl$_3$) δ in ppm: − 115.6 (m); HRMS (ESI): m/z = 385.22834 (M + H$^+$) calc. 385.22858; m/z = 791.43224 (2M + H$^+$) calc. 791.43183.

(±)-**4b** (±)-5-(4-fluorobenzyloxy)-2-(4-phenylpiperazin-1-yl) cyclohexanol: The compound crystallized in the reaction mixture. The white precipitate was isolated and washed with water, isopropanol, and *n*-hexane to obtain 215 mg (49%) of (±)-**4b**. ^{1}H NMR (400 MHz, CDCl$_3$) δ in ppm: 7.33–7.42 (om, 4H), 7.02 (m, 2H), 6.93–6.85 (om, 3H), 4.55 and 4.54 (AB, $^2J_{HH}$ = 11.8 Hz, 2H, OCH$_2$Ph), 3.83 (bs, 1H, CH–OH), 3.48–3.33 (om, 2H, CH–OH, CH–OCH$_2$Ph), 3.26–3.15 (om, 4H, CH$_2$ piperazine), 2.90–2.85 (om, 2H, CH$_2$ piperazine), 2.63–2.54 (om, 3H, 2 × CH$_2$ piperazine, 1 × CH$_2$ cyclohexane), 2.35 (m, 1H, CH–N), 2.17 (m, 1H, CH$_2$ cyclohexane), 1.87 (m, 1H, CH$_2$ cyclohexane), 1.43–1.15 (om, 3H, CH$_2$ cyclohexane); ^{13}C NMR (100 MHz, CDCl$_3$) δ in ppm: 162.4 (d, $^1J_{CF}$ = 244.0 Hz), 151.3 (s), 134.4 (d, $^4J_{CF}$ = 3.0 Hz), 129.4 (d, $^3J_{CF}$ = 8.1 Hz), 129.2 (s), 120.1 (s), 116.3 (s), 115.3 (d, $^2J_{CF}$ = 21.2 Hz), 74.9 (s, COCH$_2$Ph), 69.7 (s, OCH$_2$Ph), 69.6 (s, CH–N), 66.3 (s, CH–OH), 49.8 and 48.5 (s, CH$_2$ piperazine), 38.8, 31.3, and 18.8 (s, CH$_2$ cyclohexane); ^{19}F NMR (376.3 MHz, CDCl$_3$) δ in ppm: − 115.3 (m).

(±)-**4c** (±)-5-(4-fluorobenzyloxy)-2-(4-phenyl-5,6-dihydropyridin-1(*2H*)-yl)cyclohexanol: The compound crystallized in the reaction mixture. The white precipitate was isolated and washed with water, isopropanol, and *n*-hexane to obtain 255 mg (59%) of (±)-**4c**. ^{1}H NMR (400 MHz, CDCl$_3$) δ in ppm: 7.44–7.33 (om, 6H), 7.28 (m, 1H), 7.06 (m, 2H), 6.12 (bm, 1H), 4.59 and 4.54 (AB, $^2J_{HH}$ = 11.8 Hz, 2H, OCH$_2$Ph), 3.55 (m, 1H, CH–OH), 3.48–3.39 (om, 2H, CH–OCH$_2$Ph, CH$_2$–N), 3.25 (m, 1H, CH$_2$–N), 2.99 (m, 1H, CH$_2$–N), 2.67–2.59 (om, 4H, CH$_2$–N, 2 × CH$_2$CPh, CH$_2$ cyclohexane), 2.44 (m, 1H, CH–N), 2.22 (m, 1H, CH$_2$ cyclohexane), 1.91 (m, 1H, CH$_2$ cyclohexane), 1.45–1.22 (om, 3H, CH$_2$ cyclohexane); ^{13}C NMR (100 MHz, CDCl$_3$) δ in ppm: 162.4 (d, $^1J_{CF}$ = 245.3 Hz), 140.7 (s), 135.2 (s), 134.5 (d, $^4J_{CF}$ = 3.0 Hz), 129.4 (d, $^3J_{CF}$ = 8.1 Hz), 128.4 (s), 127.2 (s), 124.9 (s), 122.1 (s), 115.3 (d, $^2J_{CF}$ = 21.4 Hz), 75.0 (s, COCH$_2$Ph), 69.7 (s, OCH$_2$Ph), 68.8 (s, CH–N), 66.5 (s, CH–OH), 48.8 and 45.2 (s, CH$_2$–N), 38.9 (s, CH$_2$ cyclohexane), 31.4 (s, CH$_2$CPh), 28.8 and 18.4 (s, CH$_2$ cyclohexane); ^{19}F NMR (376.3 MHz, CDCl$_3$) δ in ppm: − 115.4 (m); HRMS (ESI): m/z = 382.21778 (M + H$^+$) calc. 382.21768.

(±)-**4d** (±)-2-(ethyl(3-phenylpropyl)amino)-5-(4-fluorobenzyloxy)cyclohexanol: The crude product was purified by column chromatography using EtOAc/*n*-hexane/NH$_3$ (1/1/0.01). 215 mg (41%) of (±)-**4d** were obtained as nearly colorless oil. ^{1}H NMR (300 MHz, CDCl$_3$) δ in ppm: 7.33–7.26 (om, 4H), 7.21–7.18 (om, 3H), 7.03 (m, 2H), 4.50 and 4.48 (AB, $^2J_{HH}$ = 11.8 Hz, 2H, OCH$_2$Ph), 3.39–3.29 (om, 2H, CH–OCH$_2$Ph, CH–OH), 2.73–2.52 (om, 5H, 3 × CH$_2$–N, CH$_2$Ph, CH$_2$ cyclohexane), 2.46–2.32 (om, 3H, CH–N, CH$_2$Ph, CH$_2$–N), 2.13 (m, 1H, CH$_2$ cyclohexane), 1.86–1.74 (om, 3H, 2 × CH$_2$CH$_2$Ph, 1 × CH$_2$ cyclohexane), 1.41–1.12 (om, 3H, CH$_2$ cyclohexane), 1.05 (t, $^3J_{HH}$ = 7.1 Hz, 3H, CH$_3$); ^{13}C NMR (75.4 MHz, CDCl$_3$) δ in ppm: 162.4 (d, $^1J_{CF}$ = 245.0 Hz), 142.0 (s), 134.5 (d, $^4J_{CF}$ = 3.2 Hz), 129.4 (d, $^3J_{CF}$ = 7.8 Hz), 128.5 (s), 128.4 (s), 125.9 (s), 115.3 (d, $^2J_{CF}$ = 21.5 Hz), 76.7 (s, COCH$_2$Ph), 69.6 (s, OCH$_2$Ph), 66.7 (s, CH–OH), 66.1 (s, CH–N), 49.3 (s, CH$_2$Ph), 43.9 (s, CH$_2$–N), 38.7 (s, CH$_2$ cyclohexane), 33.7 (s, CH$_2$–N), 31.6 (s, CH$_2$ cyclohexane), 30.8 (bs, CH$_2$CH$_2$Ph), 19.3 (s, CH$_2$ cyclohexane), 14.6 (s, CH$_3$); ^{19}F NMR (282.3 MHz, CDCl$_3$) δ in ppm: − 115.4 (m); HRMS (ESI): m/z = 386.24899 (M + H$^+$) calc. 386.24898.

(±)-**4e** (±)-1-(4-(4-fluorobenzyloxy)-2-hydroxycyclohexyl)-4-phenylpiperidin-4-ol: The compound crystallized in the reaction mixture. The white precipitate was isolated and washed with water, isopropanol, and MTBE to obtain 292 mg (73%) of (±)-**4e**. ^{1}H NMR (300 MHz, CDCl$_3$) δ in ppm: 7.51 (m, 2H), 7.39–7.27 (om, 5H), 7.03 (m, 2H), 4.54 and 4.49 (AB, $^2J_{HH}$ = 11.8 Hz, 2H, OCH$_2$Ph), 3.89 (bs, 1H, CH–OH), 3.47–3.36 (om, 2H, CH–OH, CH–OCH$_2$Ph), 3.11 (m, 1H, CH$_2$ pip), 2.66–2.52 (om, 4H, 3 × CH$_2$ pip, 1 × CH$_2$ cyclohexane), 2.32 (m, 1H, CH–N), 2.22–2.14 (om, 2H, CH$_2$ pip), 2.08–1.98 (m, 1H, CH$_2$ cyclohexane), 1.91 (m, 1H, CH$_2$ cyclohexane), 1.83–1.76 (om, 2H, CH$_2$ pip), 1.66 (bs, 1H, OH), 1.42–1.15 (om, 3H, CH$_2$ cyclohexane); ^{13}C NMR (75.4 MHz, CDCl$_3$) δ in ppm: 162.4 (d, $^1J_{CF}$ = 245.2 Hz), 148.2 (s), 134.5 (d, $^4J_{CF}$ = 3.3 Hz), 129.4 (d, $^3J_{CF}$ = 8.2 Hz), 128.5 (s), 127.3 (s), 124.6 (s), 115.3 (d, $^2J_{CF}$ = 21.8 Hz), 75.0 (s, COCH$_2$Ph), 71.6 (s, OCH$_2$Ph), 70.0 (s, PhC-OH), 69.6 (s, CH–N), 66.4 (s, CH–OH), 48.8 and 41.0 (s, CH$_2$ pip), 39.2 (s, CH$_2$ cyclohexane), 38.9 and 38.8 (s, CH$_2$ pip), 31.5 (s, CH$_2$ cyclohexane), 18.9 (s, CH$_2$ cyclohexane); ^{19}F NMR (282.3 MHz, CDCl$_3$) δ in ppm: − 115.4 (m); HRMS (ESI): m/z = 400.22822 (M + H$^+$) calc. 400.22825; m/z = 422.21046 (M + Na$^+$) cal. 422.21019.

(±)-**4f** (±)-1-(1-(4-(4-fluorobenzyloxy)-2-hydroxycyclohexyl)-4-phenylpiperidin-4-yl)ethanone: The crude product was purified by column chromatography using EtOAc/*n*-hexane/NH$_3$ (1/1/0.01). By dissolving of the obtained oil in MTBE and cooling to 4 °C for 24 h, a colorless precipitate could be obtained. 176 mg (37%) of (±)-**4f** were isolated as colorless solid. ^{1}H NMR (300 MHz, CDCl$_3$) δ in ppm: 7.37–7.25 (om, 7H), 7.01 (m, 2H), 4.52 and 4.47 (AB,

$^2J_{HH}$ = 11.8 Hz, 2H, OC$\underline{H}_2$Ph), 3.84 (bs, 1H, O$\underline{H}$), 3.42–3.28 (om, 2H, C$\underline{H}$–OH, C$\underline{H}$–OCH$_2$Ph), 2.79–2.70 (om, 2H, C$\underline{H}_2$ pip), 2.62–2.47 (om, 4H, 3 × C$\underline{H}_2$ pip, 1 × C$\underline{H}_2$ cyclohexane), 2.38–2.22 (om, 2H, C$\underline{H}_2$ pip, C$\underline{H}$–N), 2.14–1.94 (om, 3H, 2 × C$\underline{H}_2$ pip, 1 × C$\underline{H}_2$ cyclohexane), 1.89 (s, 3H, C$\underline{H}_3$), 1.76 (m, 1H, C$\underline{H}_2$ cyclohexane), 1.40–1.03 (om, 3H, C$\underline{H}_2$ cyclohexane); ^{13}C NMR (75.4 MHz, CDCl$_3$) δ in ppm: 209.6 (s, C=O), 162.4 (d, $^1J_{CF}$ = 245.4 Hz), 141.6 (s), 134.5 (d, $^4J_{CF}$ = 3.3 Hz), 129.4 (d, $^3J_{CF}$ = 8.2 Hz), 129.0 (s), 127.3 (s), 126.3 (s), 115.3 (d, $^2J_{CF}$ = 21.2 Hz), 74.9 (s, C$\underline{O}$CH$_2$Ph), 69.8 (s, O$\underline{C}$H$_2$Ph), 69.6 (s, C$\underline{H}$–N), 66.3 (s, C$\underline{H}$–OH), 55.1 (s, PhC$\underline{C}$=O), 48.6 and 44.1 (s, C$\underline{H}_2$ pip), 38.9 (s, C$\underline{H}_2$ cyclohexane), 33,6 and 33.4 (s, C$\underline{H}_2$ pip), 31.3 (s, C$\underline{H}_2$ cyclohexane), 25.7 (s, C$\underline{H}_3$), 18.8 (s, C$\underline{H}_2$ cyclohexane); ^{19}F NMR (282.3 MHz, CDCl$_3$) δ in ppm: − 115.4 (m); HRMS (ESI): m/z = 426.24388 (M + H$^+$) calc. 426.24390; m/z = 448.22580 (M + Na$^+$) cal. 448.22584.

(±)-**4g** (±)-2-(4,4-diphenylpiperidin-1-yl)-5-(4-fluorobenzyloxy) cyclohexanol: The crude product was purified by column chromatography using CHCl$_3$/MeOH/NH$_3$ (10/1/0.1). Concentration of the collected fractions to a smaller volume and keeping a few days at 4 °C resulted in the formation of 335 mg (65%) of a colorless solid. ^{1}H NMR (400 MHz, CDCl$_3$) δ in ppm: 7.30–7.24 (om, 10H), 7.15 (m, 2H), 7.01 (m, 2H), 4.51 and 4.46 (AB, $^2J_{HH}$ = 11.6 Hz, 2H, OC$\underline{H}_2$Ph), 3.97 (bs, O$\underline{H}$), 3.41–2.27 (om, 2H, C$\underline{H}$–OCH$_2$Ph, C$\underline{H}$–OH), 2.78 (m, 2H, C$\underline{H}_2$ pip), 2.56–2.48 (om, 7H, 6 × C$\underline{H}_2$ pip, C$\underline{H}_2$ cyclohexane), 2.29 (m, 1H, C$\underline{H}$–N), 2.05 (m, 1H, C$\underline{H}_2$ cyclohexane), 1.63 (m, 1H, C$\underline{H}_2$ cyclohexane), 1.38–1.21 (om, 2H, C$\underline{H}_2$ cyclohexane), 1.04 (m, 1H, C$\underline{H}_2$ cyclohexane); ^{13}C NMR (100 MHz, CDCl$_3$) δ in ppm: 162.4 (d, $^1J_{CF}$ = 246.0 Hz),147.5 (bs), 134.5 (d, $^4J_{CF}$ = 3.0 Hz), 129.3 (d, $^3J_{CF}$ = 8.8 Hz),128.5 (s), 127.3 (s), 125.9 (s), 115.4 (d, $^2J_{CF}$ = 22.1 Hz), 75.0 (s, C$\underline{O}$CH$_2$Ph), 69.8 (s, C$\underline{H}$–N), 69.6 (s, O$\underline{C}$H$_2$Ph), 66.3 (s, C$\underline{H}$–OH), 45.8 (bs, C-(Ph)$_2$), 45.0 (s, 2 × C$\underline{H}_2$ pip), 38.8 (s, C$\underline{H}_2$ cyclohexane), 37.1 (s, 2 × C$\underline{H}_2$ pip), 31.4 and 18.8 (s, C$\underline{H}_2$ cyclohexane); ^{19}F NMR (376.3 MHz, CDCl$_3$) δ in ppm: − 115.5 (m); HRMS (ESI): m/z = 460.26431 (M + H$^+$) calc. 460.26463; m/z = 941.50377 (2M + Na$^+$) calc. 941.50394.

(±)-**4h** (±)-2-(4-benzylpiperidin-1-yl)-5-(4-fluorobenzyloxy) cyclohexanol: After the reaction, the solvent was removed and the residual oil dissolved in CHCl$_3$. This organic phase was washed with small amounts of water, dried with MgSO$_4$ and concentrated to a smaller volume. Adding of MTBE led to the formation of 232 mg (52%) of a white precipitate of (±)-**4h**. ^{1}H NMR (300 MHz, CDCl$_3$) δ in ppm: 7.32–7.27 (om, 4H), 7.21–7.12 (om, 3H), 7.01 (m, 2H), 4.53 and 4.47 (AB, $^2J_{HH}$ = 11.6 Hz, 2H, OC$\underline{H}_2$Ph), 3.94 (bs, 1H, O$\underline{H}$), 3.40–3.30 (om, 2H, C$\underline{H}$–OCH$_2$Ph, C$\underline{H}$–OH), 2.74 (m, 1H, C$\underline{H}_2$ pip), 2.68–2.52 (om, 5H, 2 × C$\underline{H}_2$ pip, C$\underline{H}_2$Ph, 1 × C$\underline{H}_2$ cyclohexane), 2.19 (m, 1H, C$\underline{H}$–N), 2.04–1.99 (om, 2H, C$\underline{H}_2$ pip, C$\underline{H}_2$ cyclohexane), 1.77 (m, 1H, C$\underline{H}_2$ cyclohexane), 1.69–1.62 (om, 2H, 2 × C$\underline{H}_2$ pip), 1.52 (m, 1H, C$\underline{H}$–CH$_2$Ph), 1.36–1.05 (om, 5H, 2 × C$\underline{H}_2$ pip, 3 × C$\underline{H}_2$ cyclohexane); ^{13}C NMR (75.4 MHz, CDCl$_3$) δ in ppm: 162.6 (d, $^1J_{CF}$ = 243.0 Hz),140.7 (s), 134.5 (d, $^4J_{CF}$ = 3.0 Hz), 129.4 (d, $^3J_{CF}$ = 8.0 Hz),129.2 (s), 128.3 (s), 125.9 (s), 115.3 (d, $^2J_{CF}$ = 21.0 Hz), 75.0 (s, C$\underline{O}$CH$_2$Ph), 69.9 (s, O$\underline{C}$H$_2$Ph), 69.6 (s, C$\underline{H}$–N) 66.3 (s, C$\underline{H}$–OH), 53.1 (s, C$\underline{H}_2$Ph), 45.3 and 43.3 (s, C$\underline{H}_2$ pip), 38.9 (s, C$\underline{H}_2$ cyclohexane), 38.4 (s, CH, pip), 33.1 and 32.8 (s, CH$_2$ pip), 31.5 and 18.6 (s, CH$_2$ cyclohexane); ^{19}F NMR (282.3 MHz, CDCl$_3$) δ in ppm: − 115.5 (m); HRMS (ESI): m/z = 398.24880 (M + H$^+$) calc. 398.24898.

(±)-**4i** (±)-2-(3,4-dihydroisoquinolin-2(1*H*)-yl)-5-(4-fluorobenzyloxy)cyclohexanol: The crude product was purified by column chromatography using EtOAc/*n*-hexane/NH$_3$ (1/1/0.01). Adding of *n*-hexane to a concentrated EtOAc solution of (±)-**4i** resulted in the formation of 185 mg (46%) of colorless crystals after a few days at 4 °C. ^{1}H NMR (400 MHz, CDCl$_3$) δ in ppm: 7.32 (m, 2H), 7.16–7.10 (om, 3H), 7.06–7.01 (om, 3H), 4.54 and 4.42 (AB, $^2J_{HH}$ = 11.6 Hz, 2H, OC$\underline{H}_2$Ph), 3.93 and 3.66 (AB, $^2J_{HH}$ = 14.4 Hz, 2H, C$\underline{H}_2$ pip), 3.83 (bs, O$\underline{H}$), 3.53 (m, 1H, C$\underline{H}$–OH), 3.40 (m, 1H, C$\underline{H}$–OCH$_2$Ph), 3.00 (m, 1H, C$\underline{H}_2$ pip), 2.90 (m, 2H, C$\underline{H}_2$ pip), 2.68–2.57 (om, 2H, C$\underline{H}_2$ pip, CH$_2$ cyclohexane), 2.48 (m, 1H, C$\underline{H}$–N), 2.19 (m, 1H, CH$_2$ cyclohexane), 1.91 (m, 1H, C$\underline{H}_2$ cyclohexane), 1.46–1.22 (om, 3H, C$\underline{H}_2$ cyclohexane). ^{13}C NMR (100 MHz, CDCl$_3$) δ in ppm: 162.4 (d, $^1J_{CF}$ = 244.0 Hz),134.9 (s), 134.5 (s), 134.4 (d, $^4J_{CF}$ = 3.0 Hz), 129.4 (d, $^3J_{CF}$ = 8.0 Hz),128.9 (s), 126.6 (s), 126.3 (s), 125.8 (s), 115.3 (d, $^2J_{CF}$ = 17.0 Hz), 75.0 (s, C$\underline{O}$CH$_2$Ph), 69.7 (s, O$\underline{C}$H$_2$Ph), 69.4 (s, C$\underline{H}$–N), 66.6 (s, C$\underline{H}$–OH), 51.7 and 46.2 (s, C$\underline{H}_2$ pip), 38.9 and 31.4 (s, CH$_2$ cyclohexane), 30.0 (s, C$\underline{H}_2$ pip), 18.5 (s, C$\underline{H}_2$ cyclohexane); ^{19}F NMR (376.3 MHz, CDCl$_3$) δ in ppm: − 115.4 (m); HRMS (ESI): m/z = 356.20183 (M + H$^+$) calc. 356.20203.

(±)-**4j** (±)-2-(1,4′-bipiperidin-1′-yl)-5-(4-fluorobenzyloxy)cyclohexanol: The compound crystallized in the reaction mixture. The white precipitate was isolated and washed with water, isopropanol, and MTBE to obtain 153 mg (35%) of (±)-**4j**. ^{1}H NMR (400 MHz, CDCl$_3$) δ in ppm: 7.29 (m, 2H), 7.01 (m, 2H), 4.52 and 4.47 (AB, $^2J_{HH}$ = 11.8 Hz, 2H, OC$\underline{H}_2$Ph), 3.82 (bs, O$\underline{H}$), 3.37–3.30 (om, 2H, C$\underline{H}$–OCH$_2$Ph, C$\underline{H}$–OH), 2.8 (m, 1H, C$\underline{H}_2$ pip), 2.72 (m, 1H, C$\underline{H}_2$ cyclohexane), 2.61–2.48 (om, 6H, 6 × C$\underline{H}_2$ pip), 2.26–2.19 (om, 2H, 2 × C$\underline{H}$–N), 2.13–2.03 (om, 2H, 1 × C$\underline{H}_2$ pip, 1 × C$\underline{H}_2$ cyclohexane), 1.87–1.76 (om, 3H, 2 × C$\underline{H}_2$ pip, 1 × C$\underline{H}_2$ cyclohexane), 1.62–1.56 (om, 5H, 5 × C$\underline{H}_2$ pip), 1.46–1.25 (om, 5H, 3 × C$\underline{H}_2$ pip, 2 × C$\underline{H}_2$ cyclohexane), 1.11 (m, 1H, 1 × C$\underline{H}_2$ cyclohexane); ^{13}C NMR (100 MHz, CDCl$_3$) δ in ppm: 162.3 (d, $^1J_{CF}$ = 245.4 Hz), 134.5 (d, $^4J_{CF}$ = 3.2 Hz), 129.3 (d, $^3J_{CF}$ = 8.1 Hz), 115.3 (d, $^2J_{CF}$ = 21.4 Hz), 75.0 (s, C$\underline{O}$CH$_2$Ph), 69.6 (s, O$\underline{C}$H$_2$Ph, C$\underline{H}$–N), 66.4 (s, C$\underline{H}$–N), 63.0 (s, C$\underline{H}$–OH), 52.8 (s, C$\underline{H}_2$ pip), 50.4 (s, 2 × C$\underline{H}_2$ pip), 44.5 (s, C$\underline{H}_2$ pip), 38.6 and 31.5 (s, C$\underline{H}_2$ cyclohexane), 28.9 and 28.7 (s, C$\underline{H}_2$ pip), 26.4 (s, 2 × C$\underline{H}_2$ pip), 24.8 (s, C$\underline{H}_2$ pip), 18.6 (s, C$\underline{H}_2$ cyclohexane); ^{19}F NMR (376.3 MHz, CDCl$_3$) δ in ppm: − 115.5 (m); HRMS (ESI): m/z = 391.27531 (M + H$^+$) calc. 391.27553.

(±)-**4k** (±)-(1-(4-(4-fluorobenzyloxy)-2-hydroxycyclohexyl) piperidin-4-yl) (4-fluorophenyl)methanone: The crude product was purified by column chromatography using CHCl$_3$/MeOH/NH$_3$ (10/1/0.1). Adding of *n*-hexane to a concentrated CHCl$_3$ solution of (±)-**4k** and keeping a few days at 4 °C resulted in the formation of 323 mg (67%) of a colorless solid. ^{1}H NMR (400 MHz, CDCl$_3$) δ in ppm: 7.95 (m, 2H), 7.29 (m, 2H), 7.13 (m, 2H), 7.01 (m, 2H), 4.53 and 4.48 (AB, $^2J_{HH}$ = 11.6 Hz, 2H, OC$\underline{H}_2$Ph), 3.78 (bs, O$\underline{H}$), 3.43–3.22 (om, 2H, C$\underline{H}$–OCH$_2$Ph, C$\underline{H}$–OH), 3.22 (bm, 1H, C$\underline{H}$-COPhF), 2.87 (m, 1H, C$\underline{H}_2$ pip), 2.79–2.71 (om, 2H, C$\underline{H}_2$ pip), 2.54 (m, 1H, C$\underline{H}_2$ cyclohexane), 2.28–2.24 (om, 2H, C$\underline{H}_2$ pip, C$\underline{H}_2$ cyclohexane), 2.14 (m, 1H, C$\underline{H}_2$ cyclohexane), 1.88–1.70 (om, 5H, 4 × C$\underline{H}_2$ pip, 1 × C$\underline{H}_2$ cyclohexane), 1.39–1.31 (om, 2H, C$\underline{H}_2$ cyclohexane) 1.14 (m, 1H, C$\underline{H}_2$ cyclohexane); ^{13}C NMR (100 MHz, CDCl$_3$) δ in ppm: 200.9 (s, C=O), 165.8 (d, $^1J_{CF}$ = 253.0 Hz),162.4 (d, $^1J_{CF}$ = 244.0 Hz), 134.5 (d, $^4J_{CF}$ = 3.0 Hz), 132.5 (d, $^4J_{CF}$ = 3.0 Hz), 130.9 (d, $^3J_{CF}$ = 9.0 Hz), 129.4 (d, $^3J_{CF}$ = 8.0 Hz), 115.9 (d, $^2J_{CF}$ = 22.0 Hz), 115.3 (d, $^2J_{CF}$ = 21.0 Hz), 74.9 (s, C$\underline{O}$CH$_2$Ph), 70.0 (s, C$\underline{H}$–N), 69.6 (s, O$\underline{C}$H$_2$Ph), 66.3 (s, C$\underline{H}$–OH), 52.1 (s, C$\underline{H}_2$ pip), 45.1 (s, C$\underline{H}$-COPhF), 43.8 (s, C$\underline{H}_2$ pip), 38.8 and 31.8 (s, C$\underline{H}_2$ cyclohexane), 29.6 and 29.2 (s, C$\underline{H}_2$ pip), 18.8 (s, C$\underline{H}_2$ cyclohexane); ^{19}F NMR (376.3 MHz, CDCl$_3$) δ in ppm: − 115.4 (m); HRMS (ESI): m/z = 430.21853 (M + H$^+$) calc. 430.21883; m/z = 452.20076 (M + Na$^+$) cal. 452.20077; m/z = 881.41196 (2M + H$^+$) calc. 881.41232.

(±)-**4m** (±)-5-(4-fluorobenzyloxy)-2-(4-(pyridin-4-yl)piperidin-1-yl)cyclohexanol: The crude product was purified by column chromatography using CHCl$_3$/MeOH/NH$_3$ (10/1/0.1). Adding of *n*-hexane to a concentrated CHCl$_3$ solution of (±)-**4m** and keeping a few days at 4 °C resulted in the formation of 263 mg (61%) of a colorless solid. ^{1}H NMR (400 MHz, CDCl$_3$) δ in ppm: 8.50 (m, 2H), 7.30 (m, 2H), 7.12 (m, 2H), 7.02 (m, 2H), 4.53 and 4.48 (AB, $^2J_{HH}$ = 11.8 Hz, 2H, OC$\underline{H}_2$Ph), 3.85 (bs, 1H, O$\underline{H}$), 3.45–3.32 (om, 2H, C$\underline{H}$–OCH$_2$Ph, C$\underline{H}$–OH), 2.90 (m, 1H, C$\underline{H}_2$ pip), 2.83–2.71 (om, 2H, C$\underline{H}_2$ pip), 2.58–2.46 (om, 2H, C$\underline{H}$ pip, 1 × C$\underline{H}_2$ cyclohexane),

2.33–2.22 (om, 2H, CH–N, CH_2 pip), 2.14 (m, 1H, CH_2 cyclohexane), 1.91–1.77 (om, 4H, CH_2 pip), 1.64 (m, 1H, CH_2 cyclohexane), 1.41–1.28 (om, 2H, CH_2 cyclohexane), 1.17 (m, 1H, CH_2 cyclohexane); ^{13}C NMR (100 MHz, $CDCl_3$) δ in ppm: 162.4 (d, $^1J_{CF}$ = 245.3 Hz),154.7 (s), 150.0 (s), 134.4 (d, $^4J_{CF}$ = 3.2 Hz), 129.4 (d, $^3J_{CF}$ = 8.1 Hz), 122.3 (s), 115.3 (d, $^2J_{CF}$ = 21.4 Hz), 74.9 (s, $COCH_2Ph$), 70.0 (s, CH–N), 69.7 (s, OCH_2Ph), 66.3 (s, CH–OH), 53.4 (s, CH_2 pip), 45.3 (s, CH pip), 42.2 (s, CH_2 pip), 38.9 (s, CH_2 cyclohexane), 33.4 and 33.0 (s, CH_2 pip), 31.5 and 18.8 (s, CH_2 cyclohexane); ^{19}F NMR (376.3 MHz, $CDCl_3$) δ in ppm: − 115.4 (m); HRMS (ESI): *m/z* = 385.22827 (M + H^+) calc. 385.22858; *m/z* = 407.21074 (M + Na^+) cal. 407.21053; *m/z* = 791.43210 (2M + H^+) calc. 791.43183.

(±)-**5** 3-(3-fluoropropoxy)-7-oxabicyclo[4.1.0]heptane: To a solution of the epoxy alcohol 7-oxabicyclo[4.1.0]heptan-3-ol [40,41] (2 g, 17.5 mmol) in 30 mL dried THF, NaH (1.2 g of 60% suspension in mineral oil, 30 mmol) was added and stirred for 90 min. This suspension was added via a cannula to a mixture of 3-fluoropropyl tosylate (4.4 g, 19 mmol) and KI (0.3 g, 1.75 mmol) in 80 mL dried THF at 0 °C, then stirred for 10 min at rt followed by stirring for 6 h at 60 °C. When the reaction was finished, the mixture was concentrated to a smaller volume, treated with water and extracted with MTBE (4 × 20 mL). After drying of the organic phase with Na_2SO_4, the solvent was removed resulting in brown oil which was purified by column chromatography (silica gel, EtOAc/*n*-hexane 2/1) to give 0.75g (25%) of **5** as slightly yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ in ppm: 4.45 (td, $^2J_{HF}$ = 47.1 Hz, $^3J_{HH}$ = 1.5 Hz, 2H, CH_2CH_2F), 3.45 (dt, $^3J_{HH}$ = 1.5 Hz, $^4J_{HF}$ = 0.5 Hz, 2H, $CH_2CH_2CH_2F$), 3.15 (m, 1H, CH–$OCH_2CH_2CH_2F$), 3.02 (om, 2H, 2 × CH–O), 2.35–2.23 (m, 1H, CH_2 cyclohexane), 2.16–2.10 (m, 1H, CH_2 cyclohexane), 1.91–1.63 (om, 4H, 2 × CH_2 cyclohexane, $CH_2CH_2CH_2F$), 1.58–1.53 (m, 1H, CH_2 cyclohexane), 1.31 (m, 1H, CH_2 cyclohexane); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ in ppm: 81.0 (d, $^1J_{CF}$ = 163.7 Hz), 73.8 (s, CH–$OCH_2CH_2CH_2F$), 63.4 (d, $^3J_{CF}$ = 5.6 Hz), 51.8 and 50.8 (s, CH–O), 31.0 (d, $^2J_{CF}$ = 20.4 Hz), 30.7 and 23.7 (s, 3 × CH_2 cyclohexane).

(±)-**6a** 4-(3-fluoropropoxy)-2-(4-phenylpiperidin-1-yl)cyclohexanol: To synthesize the 4-*O*-3-fluoropropyl ether vesamicol derivative (±)-**6a**, 4-phenylpiperidine 370 mg (2.29 mmol) and (±)-**5** (400 mg, 2.29 mmol) were dissolved in 5 mL EtOH and stirred at 65–70 °C for 4 days. The crude product was purified by column chromatography (silica gel 60, EtOAc) and 348 mg (46%) of (±)-**6a** crystallized as colorless needles from a solution of EtOAc/*n*-hexane at 4 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ in ppm: 7.33–7.17 (om, 5H), 4.55 (td, $^2J_{HF}$ = 47.1 Hz, $^3J_{HH}$ = 1.5 Hz, 2H, CH_2CH_2F), 4.0 (bs, OH), 3.72 (m, 1H, CH–$OCH_2CH_2CH_2F$), 3.52–3.41 (om, 3H, CH–OH, O–$CH_2CH_2CH_2F$), 2.95 (m, 1H, CH_2 pip), 2.79–2.67 (om, 3H, CH_2 pip, CH–N), 2.49 (m, 1H, CH pip), 2.18 (m, 1H, CH_2 pip), 2.08 (m, 1H, CH_2 cyclohexane), 2.01–1.56 (om, 9H, 4 × CH_2 pip, 3 × CH_2 cyclohexane, $CH_2CH_2CH_2F$), 1.40–1.22 (om, 2H, 2 × CH_2 cyclohexane); ^{13}C NMR (100.0 MHz, $CDCl_3$) δ in ppm: 146.4 (s), 128.7 (s), 127.1 (s), 126.4 (s), 81.1 (d, $^1J_{CF}$ = 163.7 Hz), 74.07 (s, CH–$OCH_2CH_2CH_2F$), 68.7 (s, CH–OH), 64.8 (s, CH–N), 63.4 (d, $^3J_{CF}$ = 5.7 Hz), 53.5 and 45.6 (s, CH_2 pip), 43.2 (s, CH pip), 34.4, and 34.1 (s, CH_2 pip), 31.4 (d, $^2J_{CF}$ = 19.9 Hz), 27.9, 27.8, and 26.2 (s, CH_2 cyclohexane); ^{19}F NMR (282.3 MHz, $CDCl_3$) δ in ppm: 33.15 (m).

(±)-**7a** 5-(3-fluoropropoxy)-2-(4-phenylpiperidin-1-yl)cyclohexanol: To synthesize the 5-*O*-3-fluoropropyl ether vesamicol derivative (±)-**7a**, the *cis*-epoxide (±)-**5** (487 mg, 2.8 mmol) and $LiClO_4$ (424 mg, 4.0 mmol) were dissolved in 10 mL CH_3CN and stirred for 10 min. Subsequently, 4-phenylpiperidine (496 mg, 3.1 mmol) was added and the reaction mixture was stirred at rt for 24 h. After removal of the solvent, the crude product was purified by column chromatography (silica gel 60, $CHCl_3$/MeOH/NH_3 (10/1/0.1) and 310 mg (33%) of (±)-**7a** crystallized as colorless needles from a solution of ethanol at 4 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ in ppm: 7.35–7.25 (om, 5H), 4.55 (td, $^2J_{HF}$ = 47.1 Hz, $^3J_{HH}$ = 1.5 Hz, 2H, CH_2CH_2F), 4.0 (bs, OH), 3.63 (m, 2H, O–$CH_2CH_2CH_2F$), 3.48 (m, 1H, CH–OH), 3.31 (m, 1H, CH–$OCH_2CH_2CH_2F$), 2.94 (m, 1H, CH_2 pip), 2.83–2.75 (om, 2H, CH_2 pip), 2.58–2.51 (om, 2H, CH pip, CH_2 cyclohexane), 2.36–2.16 (om, 3H, CH_2 pip, CH–N, CH_2 cyclohexane), 2.02–1.66 (om, 7H, 4 × CH_2 pip, 1 × CH_2 cyclohexane, $CH_2CH_2CH_2F$), 1.35–1.17 (om, 3H, CH_2 cyclohexane); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ in ppm: 146.3 (s), 128.7 (s), 127.1 (s), 126.4 (s), 81.2 (d, $^1J_{CF}$ = 163.7 Hz), 76.1 (s, CH–$OCH_2CH_2CH_2F$), 70.2 (s, CH–N), 66.5 (s, CH–OH), 64.1 (d, $^3J_{CF}$ = 5.7 Hz), 53.9 and 45.7 (s, CH_2 pip), 43.1 (s, CH pip), 39.1 (s, CH_2 cyclohexane), 34.5, and 34.1 (s, CH_2 pip), 31.5–31.3 (os, $CH_2CH_2CH_2F$, CH_2 cyclohexane), 18.8 (s, CH_2 cyclohexane); ^{19}F NMR (282.3 MHz, $CDCl_3$) δ in ppm: −44.5 (m).

*4.1.2.3. General procedure for benzovesamicol derivatives (±)-**9b**-(±)-**9m**.* The *cis*-epoxide precursor (±)-**8** was synthesized as already described [16]. The amine (1.5 mmol) and (±)-**8** (1.4 mmol) were dissolved in 5–10 mL EtOH and stirred at 65–70 °C for 48 h. The crude product was purified by column chromatography (silica gel 60) or by crystallization (details described for each compound).

(±)-**9b** (±)-3-(4-phenylpiperazin-1-yl)-1,2,3,4-tetrahydronaphthalen-2-ol: The crude product was purified by column chromatography (silica gel 60, EtOAc/*n*-hexane/NH_3 3:1:0.1) to give 78 mg (19%) of a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 7.32–7.24 (m, 2H), 7.17–7.07 (m, 4H), 6.96 (m, 2H), 6.89 (m, 1H), 4.21 (s, 1H, OH), 3.93 (m, 1H, CH–OH), 3.39–3.21 (m, 5H, piperazine), 3.05–2.79 (m, 6H, CH–N, CH_2 piperazine, cyclohexane), 2.78–2.68 (m, 2H, CH_2 cyclohexane); ^{13}C NMR (101 MHz, $CDCl_3$) δ in ppm: 150.2 (s), 133.4 (s), 132.8 (s), 128.2 (s), 128.1 (s), 128.1 (s), 125.3 (s), 125.1 (s), 119.0 (s), 115.3 (s), 65.2 (s, CH–N), 64.7 (s, CH–OH), 48.8 and 47.2 (s, CH_2 piperazine), 36.8 and 25.2 (s, CH_2 cyclohexane); HRMS (ESI): *m/z* = 309.19615 (M + H^+) calc. 309.19614; *m/z* = 331.17820 (M + Na^+) calc. 331.17808.

(±)-**9c** (±)-3-(4-phenyl-5,6-dihydropyridin-1(2H)-yl)-1,2,3,4-tetrahydronaphthalen-2-ol: The product crystallized in the reaction mixture. The precipitate was washed with water, isopropanol, and MTBE to obtain 79 mg (19%) of a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ in ppm: 7.51–7.06 (om, 9H), 6.15 (s, 1H, CHCPh), 4.31 (bs, 1H, OH), 4.05–3.88 (m, 1H, CH–OH), 3.65–2.54 (m, 11H, 2xCH_2-N, CH_2CPh, CH–N, 2xCH_2 cyclohexane); ^{13}C NMR (75 MHz, $CDCl_3$) δ in ppm: 140.7 (s), 135.3 (s, CPh), 134.8 (s), 134.1 (s), 129.4(s), 129.3 (s), 128.5 (s), 127.3 (s), 126.3 (s), 126.2 (s), 125.0 (s), 122.0 (s,CHCPh), 66.0 (s, CH–OH), 65.5 (s, CH–N), 48.7 and 45.0 (s, CH_2–N), 38.0 (s, CH_2 cyclohexane), 28.8 (s, CH_2CPh), 26.0 (s, CH_2, cyclohexane); HRMS (ESI): *m/z* = 306.18515 (M + H^+) calc. 306.18524.

(±)-**9e** (±)-1-(3-hydroxy-1,2,3,4-tetrahydronaphthalen-2-yl)-4-phenylpiperidin-4-ol: The product crystallized in the reaction mixture. The precipitate was washed with water, isopropanol, and *n*-hexane to obtain 133 mg (30%) of a white solid. ^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ in ppm: 7.56–7.44 (m, 2H), 7.34–7.26 (m, 2H), 7.22–7.17 (m, 1H), 7.13–7.02 (m, 4H), 4.76 (s, 1H, PhCH-OH), 4.45 (bs, 1H, CH–OH), 3.91–3.80 (m, 1H, CH–OH), 3.13–2.51 (om, 9H, 4xCH_2 pip, CH–N), 2.07–1.88 (m, 2H, 2xCH_{eq} pip), 1.69–1.52 (m, 2H, 2xCH_{ax} pip).; ^{13}C NMR (75 MHz, $(CD_3)_2SO$) δ in ppm: 150.9 (s), 136.3 (s), 135.1 (s), 129.4 (s), 129.3 (s), 128.4 (s), 126.8 (s), 126.4 (s), 125.5 (s), 70.8 (s, PhCH-OH), 66.4 (s, CH–N), 66.3 (s, CH–OH), 47.9 and 42.5 (s, CH_2 pip), 39.5 and 39.1 (s, CH_2 pip), 38.5 (s, CH_2 cyclohexane), 27.6 (s, CH_2 cyclohexane); HRMS (ESI): *m/z* = 324.19567 $[M+H]^+$calc 324.19581.

(±)-**9h** (±)-3-(4-benzylpiperidin-1-yl)-1,2,3,4-tetrahydronaphthalen-2-ol: The product crystallized in the reaction mixture, was isolated and recrystallized from MTBE/*n*-hexane at 4 °C to give 65 mg (15%) of colorless crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ in ppm: 7.32–7.22 (m, 5H), 7.19–7.03 (m, 4H), 4.52 (bs, 1H, CH–OH),

4.02–3.86 (m, 1H, CH–OH), 3.35–3.23 (m, 1H, CH_2 cyclohexene), 2.98–2.76 (m, 5H, CH_2 pip, CH_2 cyclohexene, CH–N), 2.72–2.60 (m, 2H, CH_2 pip, CH_2 cyclohexene), 2.58–2.52 (m, 2H, CH_2Ph), 2.30–2.22 (m, 1H, CH_2 pip), 1.92–1.40 (m, 5H, 2xCH_2 pip, CHCH_2Ph); ^{13}C NMR (75 MHz, $CDCl_3$) δ in ppm: 140.5 (s), 135.5 (s), 134.5 (s), 129.3 (s), 128.7 (s), 128.5 (s), 128.4 (s), 127.0 (s), 126.5 (s), 125.8 (s), 66.2 (s, CH–N), 66.0 (s, CH–OH), 47.3 and 42.5 (s, CH_2 pip), 43.4 (s, CH_2Ph), 38.5 (s, CHCH_2Ph), 35.6 (s, CH_2 cyclohexene), 32.5 and 32.4 (s, CH_2 pip), 26.8 (s, CH_2 cyclohexene); HRMS (ESI): m/z = 322.21631 $[M+H]^+$calc 322.21654.

(±)-**9j** (±)-3-([1,4′-bipiperidin]-1′-yl)-1,2,3,4-tetrahydronaphthalen-2-ol: The product crystallized in the reaction mixture, was isolated and recrystallized from EtOAc at 4 °C to give 67 mg (16%) of colorless crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ in ppm 7.17–7.00 (m, 4H), 4.29 (s, 1H, OH), 3.89–3.75 (m, 1H, CH–OH), 3.35–3.23 (m, 1H, CH_2 cyclohexene), 2.96–2.63 (m, 7H, CH_2 cyclohexene, CH_{eq} pip, CH_2 cyclohexene, CH–N cyclohexene, CH_2 pip), 2.57–2.42 (m, 4H, 2xCH_2 pip), 2.34–2.12 (m, 2H, CH_2 pip, CH–N), 1.92–1.82 (m, 2H, CH_2 pip), 1.72–1.34 (m, 8H, 4xCH_2 pip); ^{13}C NMR (101 MHz, $CDCl_3$) δ in ppm 135.1 (s), 134.2 (s), 129.5 (s), 129.4 (s), 126.3 (s), 126.2 (s), 66.4 (s, CH–N cyclohexane), 66.0 (s, CH–OH), 63.1 (s, CH–N pip), 52.9 (s, CH_2 pip), 50.5 (s, 2xCH_2-N pip), 44.3 (s, CH_2 pip), 38.1 (s, CH_2 cyclohexane), 29.2 (s, CH_2 pip), 28.8 (s, CH_2 pip), 26.6 (s, 2xCH_2 pip), 26.3 (s, CH_2 cyclohexane), 25.0 (s, CH_2 pip), HRMS (ESI): m/z = 315.24323 ($M + H^+$) calc. 315.24309 m/z = 337.22531 ($M + Na^+$) calc. 337.22503.

(±)-**9m** (±)-3-(4-(pyridin-4-yl)piperidin-1-yl)-1,2,3,4-tetrahydronaphthalen-2-ol: The product crystallized in the reaction mixture, was isolated and recrystallized from EtOH at 4 °C to give 67 mg (16%) of colorless crystals. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 8.53 (d, $^2J_{HH}$ = 6 Hz, 2H), 7.20–7.05 (m, 6H), 4.33 (bs, 1H, CH–OH), 3.96 – 3.89 (m, 1H, CH–OH), 3.36 – 3.30 (m, 1H, CH_2 cyclohexane), 3.07–2.77 (m, 7H, CH–N, 2xCH_2 cyclohexane, CH_2 pip), 2.64–2.38 (m, 2H, CH_2 pip), 2.01–1.64 (m, 4H, 2xCH_2 pip); ^{13}C NMR (101 MHz, $CDCl_3$) δ in ppm: 154.8 (s), 150.1 (s), 134.9 (s), 134.2 (s), 129.5 (s), 129.3 (s), 126.4 (s), 126.3 (s), 122.5 (s), 66.8 (s, CH–OH), 65.9 (s, CH–N), 53.4 and 45.1 (s, 2xCH_2 pip), 42.4 (s, CH-Ph), 38.1 (s, CH_2 cyclohexane), 33.6 and 33.2 (s, 2xCH_2 pip), 26.4 (s, CH_2 cyclohexane); HRMS (ESI): m/z = 309.19632 ($M + H^+$) calc. 309.19614.

*4.1.2.4. General procedure for aminobenzovesamicol derivatives (±)-**11a**-(±)-**12h**.* To synthesize the epoxide precursor, 1-naphthylamine was selectively reduced under Birch conditions in liquid ammonia using sodium and *tert*-butanol resulting in 5,8-dihydro naphthylamine which was protected with *p*-fluorobenzoyl chloride using standard conditions to obtain the *p*-fluorobenzamide. Epoxidation with *m*-chloroperbenzoic acid resulted in the epoxide (±)-**10**. The aminobenzovesamicol derivatives were synthesized nonregioselectively, stirring the amine (1.4 eq) with epoxide in EtOH under reflux for several days. After evaporation of the solvent, the crude product was purified by column chromatography and the regioisomers were separated by semi-preparative HPLC as described in detail for each derivative.

(±)-**11a** (±)-(4-fluoro-N-(-6-hydroxy-7-(4-phenylpiperidin-1-yl)-5,6,7,8-tetrahydronaphthalen-1-yl)benzamide): 4-Phenylpiperidine (**a**) was added dropwise to the epoxide in EtOH and the mixture was stirred under reflux for six days. After evaporation of the solvent, the crude product was purified by column chromatography (silica 60, $CHCl_3$/MeOH/NH_3 10:1:0.1) and the regioisomers were separated by semi-preparative HPLC (Reprosil Basic C-18 HD, MeOH/40 mM NH_4OAc 65:35 v/v, flow rate 5 ml/min). **11a** was yielded as the less polar isomer (37 mg, 4%). ^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ in ppm: 8.16–8.06 (m, 2H), 7.30–7.11 (m, 6H), 7.06–6.97 (m, 2H), 6.78 (dd, $^1J_{HH}$ = 7.5 Hz, 1H), 6.34 (d, $^1J_{HH}$ = 7.5 Hz, 1H), 3.76 (td, $^1J_{HH}$ = 9.9, $^1J_{HH}$ = 5.4 Hz, 1H, CH–OH), 3.11–2.93 (m, 2H, CH_2 pip), 2.92–2.78 (m, 2H, CH_2 pip), 2.71–2.54 (m, 3H, CH–N, CH_2 pip, cyclohexene), 2.44–2.36 (m, 3H, CH-Ph, CH_2 pip, cyclohexene), 1.80–1.56 (m, 4H, 2xCH_2 pip); ^{13}C NMR (75 MHz, $(CD_3)_2SO$) δ in ppm: 163.6 (C=O), 162.7 (d, $^1J_{CF}$ = 242.1 Hz), 154.1 (s), 147.3 (s), 141.5 (d, $^4J_{CF}$ = 2.6 Hz), 133.5 (s), 130.6 (d, $^3J_{CF}$ = 8.0 Hz), 129.1 (s), 128.9 (s), 127.4 (s), 126.6 (s), 125.3 (s), 121.3 (s), 118.5 (s), 113.7 (d, $^2J_{CF}$ = 20.5 Hz), 67.5 (s, CH–N), 66.9 (s, CH–OH), 52.1 (s) and 47.5 (s, CH_2 pip), 43.1 (s, CH-Ph), 39.8 (s, CH_2CH–OH), 34.6 and 34.4 (s, CH_2 pip), 24.1 (s, CH_2CH–N); HRMS (ESI): m/z = 445.22823 ($M + H^+$) calc. 445.22858, m/z = 467.21023 ($M + Na^+$) calc. 467.21053.

(±)-**12a** (±)-(4-fluoro-N-(-7-hydroxy-6-(4-phenylpiperidin-1-yl)-5,6,7,8-tetrahydronaphthalen-1-yl)benzamide): See description above, **12a** was yielded as the polar isomer (54 mg, 6%). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ in ppm: 9.86 (bs, 1H, NH), 8.15–7.97 (m, 2H), 7.41–7.33 (m, 2H), 7.32–7.23 (m, 4H), 7.21–7.12 (m, 3H), 7.09–7.02 (m, 1H), 4.46 (s, 1H, OH), 3.93–3.80 (m, 1H, CH–OH), 3.08 (dd, J = 16.6, 5.6 Hz, 1H, CH_2CH–OH), 3.00–2.78 (m, 4H, CH2 pip, CH_2CH–N), 2.74–2.61 (m, 2H, CH–N, CH2 pip), 2.61–2.41 (m, 3H, CH2 pip, CH-Ph), 1.83–1.54 (m, 4H, 2xCH2 pip). ^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ in ppm: 164.2 (s, C=O), 164.0 (d, $^1J_{CF}$ = 248.8 Hz), 146.5 (s), 136.6 (s), 135.8 (s), 131.0 (s), 130.9 (d, $^4J_{CF}$ = 2.8 Hz), 130.3 (d, $^3J_{CF}$ = 9.0 Hz), 128.3 (s), 126.7 (s), 125.9 (s), 125.7 (s), 124.4 (s), 115.3 (d, $^2J_{CF}$ = 21.8 Hz), 66.0 (s, CH–OH), 65.2 (s, CH–N), 51.1 and 47.4 (s, CH2 pip), 42.4 (s, CH-Ph), 33.9 and 33.8 (s, CH_2 pip), 33.6 (s, CH_2CH–OH), 27.7 (s, CH_2CH–N); HRMS (ESI): m/z = 445.22869 ($M + H^+$) calc. 445.22858.

(±)-**11h** (±)-(N-(7-(4-benzylpiperidin-1-yl)-6-hydroxy-5,6,7,8-tetrahydronaphthalen-1-yl)-4-fluorobenzamide): Amine **h** (2.45 mmol) was added dropwise to the epoxide (1.78 mmol) in EtOH and the mixture was stirred under reflux for seven days. After evaporation of the solvent, the crude dark oil was extracted with 20% citric acid solution (5 × 3 ml). The combined aqueous phase was treated with 6 M NaOH solution and extracted with $CHCl_3$ (5 × 4 ml). After evaporation of the solvent, the resulting orange oil was purified by column chromatography (silica 60, $CHCl_3$/MeOH/NH_3 10:1:0.1) and the regioisomers were separated by semi-preparative HPLC (ReprosilBasic C-18 HD, MeOH/40 mM NH_4OAc_{aq} 70:30 v/v, flow rate 5 ml/min). **11h** was yielded as the less polar isomer (21 mg, 3%). ^{1}H NMR (300 MHz, $CDCl_3$) δ in ppm: 7.96–7.82 (m, 2H), 7.50 (d, $^1J_{HH}$ = 7.8 Hz, 1H), 7.32–7.08 (m, 8H), 7.02 (d, $^1J_{HH}$ = 7.5 Hz, 1H), 3.94–3.80 (m, 1H, CH–OH), 3.31 (dd, $^1J_{HH}$ = 16.2, 5.4 Hz, 1H, CH_2CH–OH), 2.95–2.58 (m, 7H, 2xCH_2 pip, CH–N, CH_2CH–OH, CH_2CH–N), 2.55 (d, $^1J_{HH}$ = 6.8 Hz, 2H, CH_2Ph), 1.82–1.16 (m, 5H, 2xCH_2 pip, CHCH$_2$Ph). ^{13}C NMR (75 MHz, $CDCl_3$) δ in ppm: 165.2 (d, $^1J_{CF}$ = 253.1 Hz), 165.1 (s, C=O), 140.5 (s), 135.5 (d, $^4J_{CF}$ = 3.3 Hz), 129.8 (d, $^3J_{CF}$ = 8.9 Hz), 129.3 (s), 128.4 (s), 127.5 (s), 127.1 (s), 126.1 (s), 122.7 (s), 116.2 (d, $^2J_{CF}$ = 22.0 Hz), 66.5 (s, CH–N), 65.3 (s, CH–OH), 53.0 and 45.0 (s, CH_2 pip), 43.3 (s, CH_2Ph), 38.4 (s, CHCH$_2$Ph), 38.2 (s, CH_2CH–OH) 33.1 and 32.6 (s, CH_2 pip), 21.9 (s, CH_2CH–N); HRMS (ESI): m/z = 459.24391 ($M + H^+$) calc. 459.24423, m/z = 481.22580 ($M + Na^+$) calc. 481.22618.

(±)-**12h** (±)-(N-(6-(4-benzylpiperidin-1-yl)-7-hydroxy-5,6,7,8-tetrahydronaphthalen-1-yl)-4-fluorobenzamide): See description above, **12h** was yielded as the polar isomer (22 mg, 3%). ^{1}H NMR (300 MHz, $CDCl_3$) δ in ppm: 7.92–7.85 (m, 2H), 7.79 (d, $^1J_{HH}$ = 7.8 Hz, 1H), 7.64 (s, 1H, NH), 7.31–7.14 (m, 8H), 6.95 (d, $^1J_{HH}$ = 7.5 Hz, 1H), 3.89 (d, $^1J_{HH}$ = 6.2 Hz, 1H, CH–OH), 3.32–3.20 (m, 1H, CH_2CH–OH), 2.98–2.59 (m, 7H, CH_2CH–OH, CH_2CH–N), 2.58 (d, $^1J_{HH}$ = 6.8 Hz, 2H, CH_2Ph), 2.33–2.19 (m, 1H, CH_2 pip), 1.81–1.30 (m, 5H, 2xCH_2 pip, CHCH$_2$Ph). ^{13}C NMR (75 MHz, $CDCl_3$) δ in ppm: 165.2 (d, $^1J_{CF}$ = 252.9 Hz), 164.9 (s, C=O), 140.5 (s), 135.7 (s), 131.1 (d, $^4J_{CF}$ = 3.1 Hz), 129.7 (d, $^3J_{CF}$ = 9.0 Hz), 129.3 (s), 128.5 (s), 127.1 (s), 126.6 (s), 126.2 (s), 121.6 (s), 116.1 (d, $^2J_{CF}$ = 22.0 Hz), 66.1 (s, CH–N), 66.0 (s, CH–OH), 53.1 and 45.4 (s, CH_2 pip), 43.23 (s, CH_2Ph), 38.33

(s, CHCH$_2$Ph), 33.84 (s, CH$_2$CH–OH), 32.5 and 32.4 (s, CH$_2$ pip), 26.8 (s, CH$_2$CH–N); HRMS (ESI): m/z = 459.24422 (M + H$^+$) calc. 459.24423, m/z = 481.22650 (M + Na$^+$) calc. 481.22618, m/z = 939.46324 (2M + Na$^+$) calc. 939.46313.

DeHVes [56] (±)-6-(4-phenylpiperidin-1-yl)cyclohex-3-enol: The synthesis of DeHVes was accomplished according to the reference, however the authors do not provide NMR data in this report: ^{1}H NMR (300 MHz, CDCl$_3$) δ in ppm: 7.34–7.29 (om, 2H), 7.25–7.18 (om, 3H), 5.64 (m, 1H, ethylene), 5.56 (m, 1H, ethylene), 4.34 (bs, OH), 3.75 (m, 1H, CH–OH), 2.93 (m, 1H, CH$_2$ pip), 2.86–2.47 (om, 5H, 2 × CH$_2$ pip, 1 × CH$_2$ cyclohexane, CH–N, CH pip), 2.31 (m, 1H, CH$_2$ pip), 2.19–2.04 (om, 3H, 2 × CH$_2$ pip, CH$_2$ cyclohexane), 1.92–1.65 (om, 4H, 2 × CH$_2$ pip, 2 × CH$_2$ cyclohexane); ^{13}C NMR (75.4 MHz, CDCl$_3$) δ in ppm: 146.2 (s), 128.5 (s), 126.9 (s), 126.3 (s), 125.3 and 124.6 (s, ethylene), 66.3 (s, CH–OH), 65.5 (s, CH–N), 53.7 (s, CH$_2$ pip), 45.1 (s, CH pip), 43.0 (s, CH$_2$ pip), 34.4 (s, CH$_2$ cyclohexane), 34.3 and 33.9 (s, CH$_2$ pip), 22.2 (s, CH$_2$ cyclohexane); HRMS (ESI): m/z = 258.18507 (M + H$^+$) calc. 258.18524.

5-ABV (5-Aminobenzovesamicol) [16] ((±)-5-amino-3-(4-phenylpiperidin-1-yl)-1,2,3,4-tetrahydronaphthalen-2-ol), **8-ABV** (8-Aminobenzovesamicol) [16] ((±)-8-amino-3-(4-phenylpiperidin-1-yl)-1,2,3,4-tetrahydronaphthalen-2-ol), **BV** (Benzovesamicol) [16] ((±)-3-(4-phenylpiperidin-1-yl)-1,2,3,4-tetrahydronaphthalen-2-ol), **FBT** (Fluorobenzyltrozamicol) [57] ((±)-1′-(4-fluorobenzyl)-4-phenyl-[1,3′-bipiperidin]-4′-ol), and **FBBV** (*p*-Fluorobenzoylbenzovesamicol) [37] ((±)-4-fluorophenyl) (1-(3-hydroxy-1,2,3,4-tetrahydronaphthalen-2-yl)piperidin-4-yl)methanone) were synthesized according to references and verified with NMR and HRMS.

4.2. Competitive binding assays

4.2.1. Preparation of rVAChT-PC12 cells

rVAChT-PC12 cells were grown in Dulbecco's modified Eagle Medium with 4.5 g/L D-glucose and supplemented with 5% cosmic calf serum, 10% equine serum, 100 U/mL penicillin-streptomycin and the neomycin analog G-418 (0.125 g/L) to confluence at incubator conditions (37 °C, 5% CO$_2$). Cells were harvested by scraping, pelleted and washed with PBS. After resuspension in assay buffer (50 mM Tris–HCl pH 7.4 with 120 mM NaCl, 50 mM KCl, 1 mM MgCl$_2$, 2 mM CaCl$_2$) they were counted and stored at −30 °C. For the assay aliquots were thawed and suspended with test buffer to get cells resp. cell debris in a concentration of 1 × 10^7 VAChT-PC12 per mL. After intensive homogenization they were ready for use.

4.2.2. Preparation of rat brain membranes

Female Sprague Dawley rats (150–200 g) were anaesthetized with ether and decapitated. Their brains were rapidly removed from the skull. The brain tissue without cerebellum was homogenized in 10 volumes (w/v) of ice-cold 250 mM sucrose solution using a Teflon-glass homogenizer (50 strokes). Aliquots were stored at −30 °C until use. After thawing homogenate was diluted to 50 mL water and intensively homogenized. Cell debris were removed by rotation (1000 g, 5 min). Crude cell membranes for drug competition assays were obtained by centrifugation of the supernatant at 15 000 g for 15 min at 4 °C, washed with 250 mM sucrose solution and distilled water and re-suspended in assay buffer (50 mM Tris pH 7.4 containing 120 mM NaCl, 5 mM KCl, 2 mM CaCl$_2$, 1 mM MgCl$_2$).

4.2.3. Preparation of rat liver

Female Sprague Dawley rats (150–200 g) were anaesthetized and decapitated. Liver was isolated and homogenized in 10 volumes (w/v) of 50 mM TRIS-HCl, pH 7.4 at 4 °C, using a Teflon-glass homogenizer. Homogenates were centrifuged at 15.000 rpm for 10 min at 4 °C, and the sediments were re-suspended and washed as described before, and aliquots stored at −30 °C until use. After thawing, homogenates were washed twice as described before and finally re-suspended in assay buffer (50 mM TRIS-HCl, pH 7.4 at 21 °C, containing 120 mM NaCl, 5 mM KCl, 2 mM CaCl$_2$, 1 mM MgCl$_2$).

4.2.4. VAChT binding

For the VAChT standard equilibrium assay 1.5 × 10^6 VAChT-PC12 cells (250 ± 30 μg protein) together with 1.5 pmol (−)-[^{3}H]vesamicol ([^{3}H]2-(4-phenylpiperidinyl cyclohexanol, specific radioactivity 1258 GBq/mmol, PerkinElmer Life Sciences, Boston, MA, USA) and the relevant compound (concentrations between 10^{-11} and 10^{-5} mol/L) were incubated in a volume of 1 mL in duplicate for 2 h at 25 °C under agitation (200 min^{-1}). Nonspecific binding was determined in the presence of 10 μM (±)-vesamicol.

After incubation, bound and free radioligand were separated by a rapid vacuum filtration system (cell harvester Brandel Gaithersburg, MD, USA) through Whatman GF/B glass-fiber filters presoaked in 0.5% polyethylenimine for 3 h. The filters were washed 3 times with 4 mL ice-cold Tris buffer (0.05 mol/L, pH 7.4). The dried filters were slightly shaken in 4 mL Rotiscint eco plus cocktail (Carl Roth GmbH, Karlsruhe, Germany) for about 30 min, maintained in the dark for at least 8 h and the radioactivity was measured in a Tri-Carb2900TR Liquid Scintillation Counter (PerkinElmer Life Sciences Boston, MA, USA) with a counting efficiency of 70%.

4.2.5. σ_1 receptor and σ_2 receptor binding

Affinity toward σ_1 receptors was estimated using homogenates of rat cortical membranes and (−)-[^{3}H]pentazocine (specific activity: 1070 GBq/mmol, PerkinElmer Life Sciences, Boston, USA). Nonspecific binding was determined in the presence of 10 μM haloperidol.

Affinity toward σ_2 receptors was estimated using homogenates of rat liver membranes and [^{3}H]DTG (specific activity: 1110 GBq/mmol, PerkinElmer Life Sciences, Boston, USA) in the presence of 1 μM dextrallorphan (Roche) to block the binding of [^{3}H]DTG toward σ_1 receptors. Nonspecific binding was determined in the presence of 10 μM haloperidol. All procedures were the same as those described for VAChT binding assay above.

IC_{50} values were calculated from competitive binding curves by a nonlinear curve fitting, using Graphpad Prism, version 3 (GraphPad Software, Inc., San Diego, USA). Specific binding of the radioligand was defined as total binding minus nonspecific binding. The apparent inhibition constant (K_i) was derived from IC_{50} values according to the Cheng–Prusoff equation: $K_i = IC_{50}/(1 + C/K_D)$ where C is the concentration of the radioligand and K_D is the dissociation constant of the radioligand (K_D ((−)-[^{3}H]vesamicol) = 25.6 nM to VAChT on rVAChT-PC12 cells, K_D ((−)-[^{3}H]pentazocine) = 6.9 nM to σ_1 receptors on rat cortical membranes, K_D ([^{3}H]DTG) = 29 nM for σ_2 receptors on rat liver membranes with blocking of σ_1 receptors using dextrallorphan (unpublished data).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.05.033.

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