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PII: S0968-0896(17)30146-3
DOI: http://dx.doi.org/10.1016/j.bmc.2017.01.041
Reference: BMC 13520

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 18 November 2016
Revised Date: 19 January 2017
Accepted Date: 23 January 2017

Please cite this article as: Roslin, S., Rosa, M.D., Deuther-Conrad, W., Eriksson, J., Odell, L.R., Antoni, G., Brust, P., Larhed, M., Synthesis and *in vitro* evaluation of 5-substituted benzovesamicol analogs containing *N*-substituted amides as potential positron emission tomography tracers for the Vesicular Acetylcholine Transporter, *Bioorganic & Medicinal Chemistry* (2017), doi: http://dx.doi.org/10.1016/j.bmc.2017.01.041

Synthesis and *in vitro* evaluation of 5-substituted benzovesamicol analogs containing *N*-substituted amides as potential positron emission tomography tracers for the Vesicular Acetylcholine Transporter[†]

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Abstract

Herein, new ligands for the vesicular acetylcholine transporter (VAChT), based on a benzovesamicol scaffold, are presented. VAChT is acknowledged as a marker for cholinergic neurons and a positron emission tomography tracer for VAChT could serve as a tool for quantitative analysis of cholinergic neuronal density. With an easily accessible triflate precursor, aminocarbonylations were utilized to evaluate the chemical space around the C5 position on the tetrahydronaphthol ring. Synthesized ligands were evaluated for their affinity and selectivity for VAChT. Small, preferably aromatic, *N*-substituents proved to be more potent than larger substituents. Of the fifteen compounds synthesized, benzyl derivatives **(±)-7i** and **(±)-7l** had the highest affinities for VAChT. Compound **(±)-7i** was chosen to investigate the importance of stereochemistry for binding to VAChT and selectivity towards the σ_1 and σ_2 receptor. Enantiomeric resolution gave **(+)-7i** and **(-)-7i**, and the eutomer showed seven times better affinity. Although racemate **(±)-7i** was initially promising, the affinity of **(-)-7i** for VAChT was not better than 56.7 nM which precludes further preclinical evaluation. However, the nanomolar binding together with the ready synthesis of **[^{11}C]-(±)-7i** shows that **(-)-7i** can serve as a scaffold for future optimizations to provide improved ^{11}C-labelled VAChT PET tracers.

Keywords

Vesicular acetylcholine transporter, VAChT, PET tracer, aminocarbonylation, ^{11}C-labelling

1. Introduction

In the cholinergic nerve terminal, acetylcholine (ACh) is accumulated in synaptic vesicles for storage. This accumulation is accomplished by the vesicular acetylcholine transporter (VAChT).[1,2] The expression of VAChT has been shown to correlate well with the brain cholinergic innervation, which can be found in areas such as the striatum, cortex, hippocampus and amygdala.[3–6] VAChT has been acknowledged as a biological marker for the integrity of cholinergic neurons due to the presynaptic location.

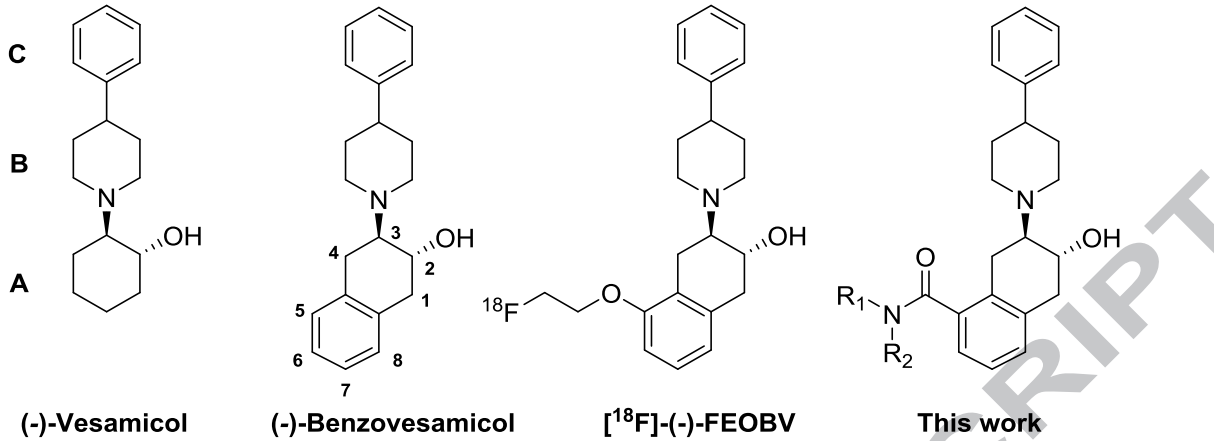
Abbreviations

Acetylcholine, ACh; vesicular acetylcholine transporter, VAChT; choline acetyltransferase ChAT; positron emission tomography, PET; (±)-*trans*-2-(4-phenylpiperidino)cyclohexanol, vesamicol; 3-(4-phenyl-piperidin-1-yl)-1,2,3,4-tetrahydronaphthalen-2-ol, benzovesamicol; structure-activity relationship, SAR; [^{3}H]-1,3-di-*o*-tolylguanidine, [^{3}H]DTG; *trans*-bisacetato)-bis[*o*-(di-*o*-tolyl-phosphino)benzyl]di-palladium(II), Herrmann's palladacycle; 2-dicyclo-hexylphosphino-2',4',6'-triisopropylbiphenyl, XPhos; (dimethylamino)pyridine (DMAP); Palladium(dibenzylidene acetone), Pd(dba)$_2$; SFC, Supercritical fluid chromatography.

The cholinergic system is involved in neurodegenerative diseases associated with cognitive deficits, as the loss of cholinergic neurons has been correlated to the impairment of cognitive abilities and memory.[7,8] In the case of Alzheimer's disease (AD), this has been known as the cholinergic hypothesis and it has stimulated significant research over the years although it has been demonstrated that not only cholinergic neurons are degenerated in AD.[9] The related research has shown a loss of cortical cholinergic neurons in AD patients and a correlation of cortical synapse loss with cognitive impairment.[7,10,11] More recent studies have shown a reduced expression of VAChT in brains of AD patients.[5] Another neurodegenerative disease is Parkinson's disease (PD), where the motor symptoms are the most pronounced symptoms but non-motor co-morbidities are also likely to affect PD patients. Dementia is such a co-morbidity and demented patients with PD have been shown to have a loss of cholinergic neurons in the basal forebrain similar to that of AD patients.[12] The study of the cholinergic system via the study of VAChT would therefore offer insight into the disease pathology and could be a complement in the diagnosis of neurodegenerative diseases where loss of cholinergic neurons is part of the etiology.[13–15]

VAChT has been studied with imaging techniques such as positron emission tomography (PET) and single-photon emission computed tomography (SPECT).[16] PET is a molecular imaging technique where a tracer is used to investigate biochemical or physiological processes *in vivo*. The tracer is a molecule that has been radiolabeled with a positron-emitting radionuclide, such as ^{11}C and ^{18}F. Several attempts at synthesizing a PET tracer for VAChT have been made but only a limited number have progressed to *in vivo* preclinical evaluations.[17–24] All VAChT ligands synthesized thus far are structurally related to (±)-*trans*-2-(4-phenylpiperidino)-cyclohexanol (vesamicol, Figure 1). This single lead compound has been invaluable in the discovery and subsequent characterization of VAChT.[25] Vesamicol acts as an allosteric inhibitor at a stereoselective binding site with (-)-vesamicol being the preferred enantiomer.[2,26,27] Vesamicol itself is unsuitable as a PET tracer for VAChT, because of the lack of a suitable position for labeling along with a high affinity to other receptors.[28] Specifically, the competitive binding to the σ_1 and σ_2 receptor causes a selectivity problem and for a successful PET tracer, selectivity for the target is essential. This selectivity issue is, together with a low affinity for VAChT, the main reason for failure of new VAChT ligands.[17,18] The affinity criteria is set by the binding potential (BP, $BP = B_{max}/K_D$) and for neuronal targets, the affinity should be in the low nanomolar to subnanomolar range.[29–32]

A structurally related analog (3-(4-phenylpiperidin-1-yl)-1,2,3,4-tetrahydronaphthalen-2-ol (benzovesamicol), Figure 1) of vesamicol, first synthesized by Rogers *et al*. [33], has also been used as a scaffold for different VAChT ligands.[33,34] The benzovesamicol scaffold has shown a better selectivity over the σ receptors, compared to vesamicol derivatives, and the tetrahydronaphthol ring allows for introduction of different substituents and for different possibilities of labeling.[30,35,36] (-)-5-[^{18}F]-fluoroethoxybenzovesamicol is the most promising PET tracer candidate reported to date and has been evaluated in healthy human volunteers ((-)-[^{18}F]FEOBV, Figure 1).[37] The tracer candidate distributed to brain regions known to contain cholinergic innervation but long scan times were needed to reach equilibrium in the binding to VAChT. The selectivity over the σ_1 receptor is however a concern and has not been verified in *in vivo* studies. *In vitro* binding studies has measured the $K_{i(VAChT)}$ to 19.6 nM and the $K_{i(\sigma\text{-}1)}$ to 210 nM for (-)-FEOBV.[38]



(-)-Vesamicol **(-)-Benzovesamicol** **[^{18}F]-(-)-FEOBV** **This work**

Figure 1. Vesamicol and selected VAChT ligands.

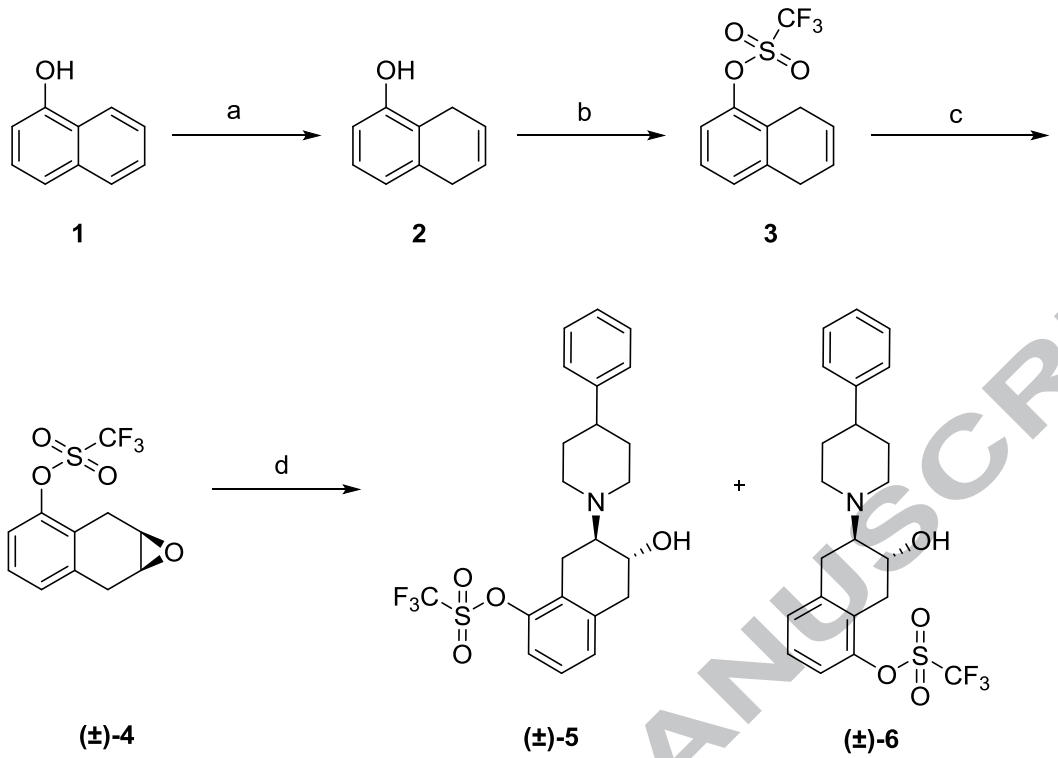
In a previous paper, modifications of rings B and C were explored (see vesamicol, Figure 1) by exchanging the piperidine ring with a piperazine ring to allow for [^{11}C]-aminocarbonylations for introduction of a carbonyl function between the B and C ring.[39] The tolerance for these modifications was however low, probably due to the conformation of the ligands imparted by the amide group. In the continued effort to find a PET tracer for VAChT, we wanted to explore the chemical space around the C5 position of a benzovesamicol scaffold and to evaluate the affinity and selectivity of potential PET tracers for VAChT (Figure 1). VAChT ligands with substituents on the C5 position have previously been shown to have a better binding to VAChT than their C8 regioisomers.[30,33,35,40] With PET and radiochemistry in mind, an amide was placed in the C5 position to be able to label all potentially interesting ligands. Inclusion of the amide functional group allowed the use of different amines to explore a structure-activity relationship (SAR) around the above mentioned position. Furthermore, the positron emitting radionuclide ^{11}C can be readily introduced in the amide moiety through incorporation of [^{11}C]CO via an aminocarbonylation reaction.

2. Results and discussion

The implication of cholinergic dysfunction in neurodegenerative diseases makes a PET tracer for VAChT a desirable tool in order to better understand the pathology of the neurodegenerative diseases. The work presented herein reflects the struggle of finding such a PET tracer candidate, one with high affinity and selectivity. The synthesis of fifteen ligands from a benzovesamicol scaffold via a novel and facile synthetic route was followed by *in vitro* determination of the affinity for VAChT and the σ_1 and σ_2 receptors, as well as ^{11}C-labeling of one ligand by aminocarbonylation.

2.1 Chemistry

The traditional route to 5-substitued benzovesamicol derivatives has started from 1-aminonaphthalene and has led to 5-aminobenzovesamicol derivatives.[33,35,40] To synthesize derivatives, the amino group has been transformed via diazotization reactions or protected with 4-fluorobenzoyl chloride.[30,40,41] The ambition in this project was to explore the SAR around the 5 position by using aliphatic and aromatic substituents. We therefore sought a precursor that could undergo an aminocarbonylation reaction, with both CO and [^{11}C]CO. To avoid the use of highly regulated and carcinogenic 1-aminonaphthalene, α-naphthol was identified as a viable alternative from which a triflate precursor could be synthesized in four steps. Thus a shortened route and a precursor for aminocarbonylation both with CO and [^{11}C]CO was developed.

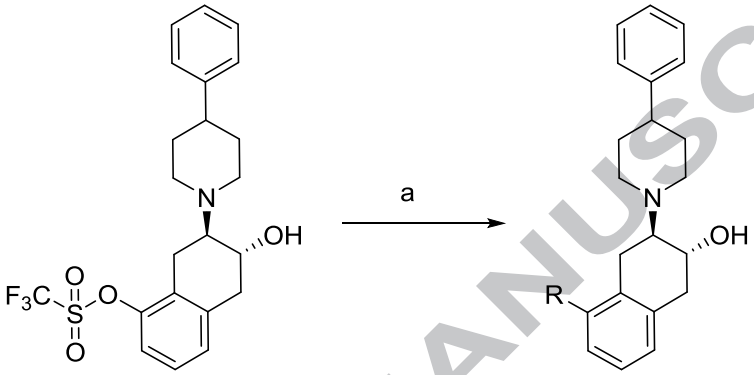


Scheme 1. a) Liquid ammonia, Li, absolute EtOH, 1 h, -78 °C, 73%; b) *N*-Phenyl-bis(trifluoromethane-sulfonimide), K_2CO_3, THF, MW, 120 °C, 6 min, 62%; c) *m*-CPBA, DCM, 0 °C to rt, 16 h, 77%; d) $LiClO_4$, 4-phenylpiperidine, MeCN, reflux, 16 h, 33% yield (**(±)-5**) and 49% yield (**(±)-6**).

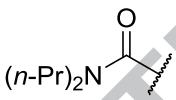
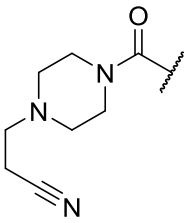
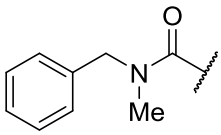
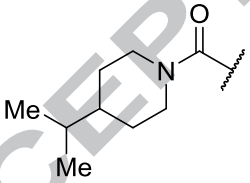
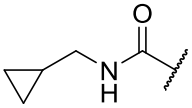
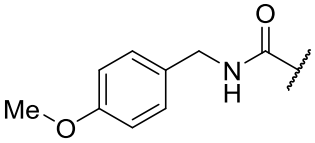
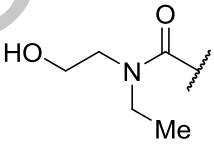
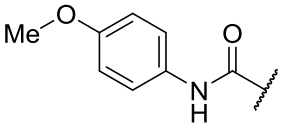
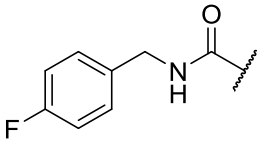
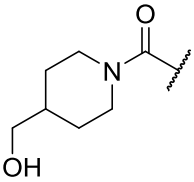
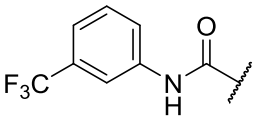
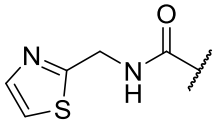
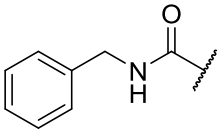
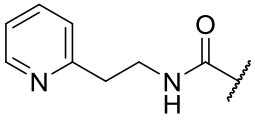
Synthesis of **(±)-5** started with a Birch reduction of commercially available α-naphthol (**1**) to **2** (Scheme 1).[42] The selectivity for reduction of the unsubstituted ring was >99%. Compound **2** was then converted by a microwave-assisted triflation, using *N*-phenyl-*bis*(trifluoromethanesulfonimide) as triflate source, to **3** in only 6 minutes.[43] The isolated double bond in **3** was epoxidized, giving the racemate **(±)-4** after an overnight reaction.[44] 4-Phenylpiperidine and **(±)-4** were refluxed overnight in acetonitrile (MeCN), affording **(±)-5** and **(±)-6**.[45] The C8 regioisomer **(±)-6** was obtained in higher yield than the desired C5 regioisomer **(±)-5** (49% and 33%, respectively), possibly due to a steric clash between the 4-phenylpiperidine on C3 and the triflate group on C5. Compound **(±)-5** was next used as starting material for the microwave-assisted aminocarbonylation to obtain **(±)-7a-n** (Scheme 2). For the aminocarbonylation, molybdenum hexacarbonyl ($Mo(CO)_6$) was used as a solid source of carbon monoxide together with the appropriate amine and *trans*-bis(acetato)bis[*o*-(di-*o*-tolylphosphino)-benzyl]-dipalladium(II) (Herrmann's palladacycle) as the catalyst.[46,47] There are only limited literature examples describing the use of aryl triflates as coupling partners in aminocarbonylations, mainly because they are not as reactive as the corresponding iodides and are generally more expensive than the corresponding bromide.[46,48] In our case, the triflate **(±)-5** was selected as the key intermediate due to its ease of synthesis as compared to the corresponding iodide. The overall transformation was accomplished in only four reaction steps and provides a straightforward route to the ligand precursor **(±)-5**. The aminocarbonylations were run at 160 °C for 3-8 h with the ligand 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (XPhos), which recently has been used in carbonylation reactions for synthesis of 1,4-diketones and ureas.[49,50] The long reaction times are needed for full consumption of triflate **(±)-5**. The yields for compounds **(±)-7a-n** were in the range of 5%-37%, after flash column chromatography followed by semi-preparative HPLC and isolation as the TFA salts (see experimental section for ^{19}F NMR of **(±)-7g** and **(±)-7j**). Part of the

starting material was reduced to the corresponding arene or formed the corresponding acid in the aminocarbonylation.

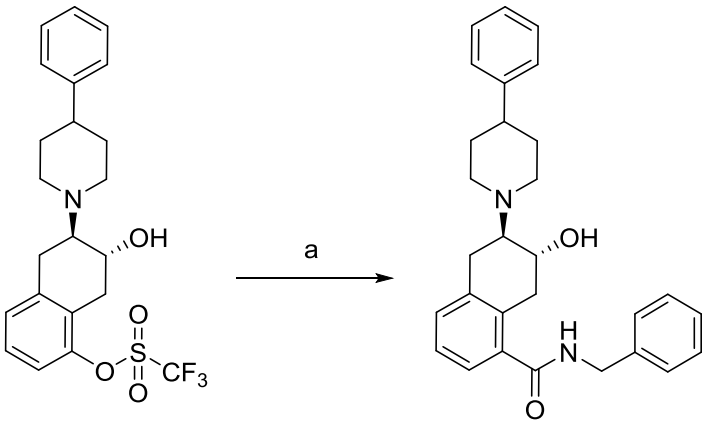
The amines were chosen in order to explore the SAR around the C5 position. Both primary and secondary amines with aliphatic and aromatic side chains were used. Previous studies has indicated that a certain amount of bulk can be tolerated in this region, thus bulky aliphatic amines were investigated.[33,51,52] With the possibility of finding a hydrogen bond interaction, amines with hydrogen bond acceptors and donators were also used. As for the amines with aromatic rings in the side chain, variation of the electron density in the ring was explored by different ring substituents and by using heteroaromatic rings.



(±)-5 **(±)-7a-n**

Compd.	R =	Compd.	R =	Compd.	R =
(±)-7a		**(±)-7e**		**(±)-7j**	
(±)-7b		**(±)-7f**		**(±)-7k**	
(±)-7c		**(±)-7g**		**(±)-7l**	
(±)-7d		**(±)-7h**		**(±)-7m**	
		(±)-7i		**(±)-7n**	

Scheme 2. a) Amine, Cs_2CO_3, $Mo(CO)_6$, XPhos, Herrmann's palladacycle, DMAP, 1,4-dioxane or MeCN, 160 °C, MW, 3-8 h, 5%-37% yield (isolated as TFA-salts, more information can be found in part 4. Experimental section).



(±)-6 **(±)-8**

Scheme 3. a) Amine, Cs_2CO_3, $Mo(CO)_6$, XPhos, Herrmann's palladacycle, DMAP, 1,4-dioxane, 160 °C, MW, 5 h, 22% yield (isolated as a TFA-salt).

Resolution of racemate **(±)-7i** into enantiomers **(+)-7i** and **(-)-7i** was performed by SFC on an YMC Chiral Cellulose-SB column (10 mm x 250 mm) with 30% methanol and 0.2% diethylamine in CO_2 as eluent. Compound **(+)-7i** eluted after 2.2 min and compound **(-)-7i** eluted after 2.95 min, both isolated in 99% enantiomeric excess.
In order to prove the difference in affinity for the C5 and C8 regioisomer, the C8 regioisomer **(±)-8** was synthesized from **(±)-6** (Scheme 3).

2.2 *In vitro* binding studies

Compounds **(±)-7a-n**, **(+)-7i**, and **(-)-7i** were evaluated for their affinity toward VAChT in an *in vitro* binding study (Table 1). Compound **(±)-8**, the regioisomer of **(±)-7i**, was also investigated to confirm the expected lower affinity for ligands with substituents in the C8 position. The binding study for VAChT was performed on PC12 cells stably transfected with a human VAChT gene, using [^{3}H]vesamicol as a high affinity VAChT radioligand (K_D = 2.39 nM).[53] As affinity for σ_1 and σ_2 receptors is a known problem for VAChT ligands, the compounds were also evaluated for their affinity to the σ_1 and σ_2 receptors and hence their selectivity for VAChT was determined (expressed as the selectivity factor, Table 1). The binding studies for the σ receptors were performed on membrane homogenates from rat cortex or rat liver using (+)-[^{3}H]pentazocine (K_D = 6.9 nM) and [^{3}H]DTG (K_D = 29 nM) as high affinity radioligands, for σ_1 and σ_2 respectively.[54] The ability of **(±)-7a-n**, **(+)-7i**, and **(-)-7i** to compete with [^{3}H]vesamicol for binding to VAChT is expressed by a K_i value, which was determined according to the Cheng-Prusoff equation.[55]
For ligands **(±)-7a-n**, the affinity for VAChT seems to be more dependent on steric effects rather than on electronic effects (see below), which is in line with previously reported data.[56] Structures **(±)-7a-f**, with aliphatic side chains on the nitrogen, generally showed a low affinity and low selectivity toward VAChT. Here, **(±)-7c** and **(±)-7f** were exceptions with moderate affinities ($K_{i(VAChT)}$ of 295 and 205 nM, respectively, see Table 1). Ligand **(±)-7c** also showed a very good selectivity relative to the σ_1 and σ_2 receptors. Both **(±)-7c** and **(±)-7f** have small side chains, as compared to the other, larger aliphatic derivatives with affinities close to or in the micromolar range. Most notable is nitrile **(±)-7e** with the

largest side chain and the lowest affinity towards VAChT. Another noteworthy point is the fact that compounds **(±)-7a**, **(±)-7b** and **(±)-7d** have higher affinity for σ receptors than for VAChT. Again, a common denominator is their large amide side chains.

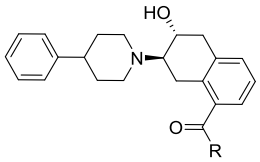
Compounds **(±)-7g-n**, with an aromatic or heteroaromatic ring on the amide nitrogen, generally showed a moderate to good affinity and selectivity towards VAChT with the benzyl derivatives **(±)-7i-l** having the highest affinities for VAChT. As for the selectivity for VAChT, most of the compounds showed a higher selectivity regarding the σ_1 than the σ_2 receptor. Benzyl substituted ligands **(±)-7i** and **(±)-7l** had a $K_{i(VAChT)}$ of 67.0 nM and 63.5 nM, respectively, making them the best compounds in the series. In addition, the selectivity of both compounds toward the σ_1 and σ_2 receptors is comparable. Methylated analog **(±)-7j** had an almost three times lower affinity than **(±)-7i**, an indication that the N-H fragment might be engaged in a hydrogen bond to an amino acid residue in VAChT. However, the loss in affinity for VAChT is not followed by the same loss in affinity for either σ receptor. The selectivity factors for **(±)-7i** are 20 and 6 for the σ_1 and σ_2 receptors, respectively, whereas the selectivity factors are 10 and 3 for **(±)-7j**. This indicates that the affinities for VAChT and the σ_1 and σ_2 receptors is not necessarily governed by the same factors. The impact of electron density in the aromatic ring on affinity was investigated through compounds **(±)-7g-h** and **(±)-7k-l** and found not to have any particular impact. This has previously been shown by Tu *et al*. [55], who also modified the A-ring.[56]

Comparing the effect of the position of the side chain on the fused benzo-cyclohexanol ring, the C5 position was more favorable than the C8 position. Compound **(±)-7i** had an affinity of 67 nM as compared to the C8 regioisomer **(±)-8** with an affinity of 795 nM. This regioisomeric difference is in accordance with previously acquired results.[30,33,35] Interesting to note is that **(±)-8** showed higher affinity for the σ_1 receptor than for VAChT.

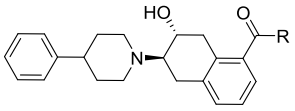
As for ligands **(±)-7a-n** selectivity for VAChT over the σ receptors, the majority of the compounds had a higher affinity for VAChT than for the σ receptors (Table 1). Racemic vesamicol had a higher affinity for the σ_1 receptor than for VAChT (Table 1). Although VAChT ligands with a benzovesamicol-based scaffold often show a higher selectivity for VAChT than for σ receptors compared to vesamicol-based ligands, the selectivity is still an issue and points to a close structural resemblance of VAChT ligands and σ receptor ligands. A sterical effect seems to be the most important determinant for the selectivity, and compounds **(±)-7a, (±)-7b** and **(±)-7d** with their large side chains even showed higher affinities for either the σ_1 or the σ_2 receptor than for VAChT. In general, most compounds, whether they had an aliphatic or aromatic side chain, showed a higher selectivity for VAChT over the σ_1 receptor than the σ_2 receptor. This is in contrast to previously reported results for benzovesamicol-based VAChT ligands, with modifications in the C-ring, which have shown higher selectivity for VAChT over the σ_2 receptor.[19,20,30,35] The results presented here indicate that modifications in the A-ring could have a negative effect on the affinity for the σ_2 receptor, a notion that can be of use when designing future VAChT ligands.

Because of the aforementioned stereoselective binding properties of VAChT, resolution of racemate **(±)-7i** to enantiomers **(+)-7i** and **(-)-7i** was performed. In Table 1, the binding affinities are shown. The (-)-enantiomer ($K_{i(VAChT)}$ = 56.7 nM) showed a seven times higher affinity than the (+)-enantiomer ($K_{i(VAChT)}$ = 393 nM), a result in line with the enantioselective characteristics of VAChT reported in the literature.[19,20,36] In addition, the eutomer **(-)-7i** shows higher selectivities over the σ receptors in comparison to the racemate **(±)-7i** and the distomer **(+)-7i**.

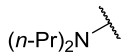
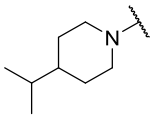
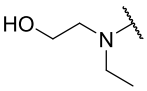
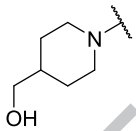
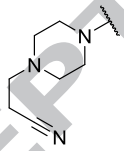
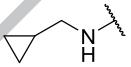
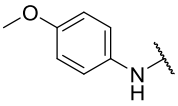
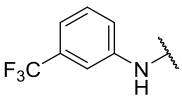
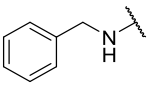
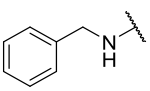
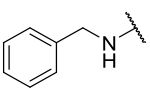
Table 1. Inhibition constants (K_i) of (±)-vesamicol and compounds **(±)-7a-n**, **(+)-7i**, **(-)-7i** and **(±)-8** for their binding to VAChT and the σ_1 and σ_2 receptors.

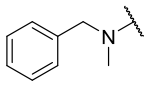
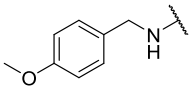
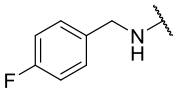
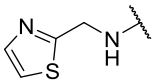
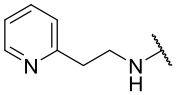
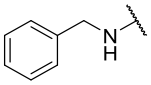


(±)-7a-n



(±)-8

Compound		K_i (nM)[a]			Selectivity factor[b]	
	R	VAChT[c]	σ_1[d]	σ_2[e]	K_i (σ_1/VAChT)	K_i (σ2/VAChT)
(±)-vesamicol[f]		47.7	20.4	112	0.43	2.3
(±)-7a		1 547; 2 666	4 687; 3 874	753; 863	2.0	0.4
(±)-7b		953; 947	669; 1 078	359; 377	0.9	0.4
(±)-7c		328; 262	13 000; 9 900	15 000; 4 900	> 34	34
(±)-7d		3 500; 2 300	1 700; 2 100	> 10 000	0.66	> 3.4
(±)-7e		> 10 000	> 100 000	> 30 000	-	-
(±)-7f		197; 212	665; 1 785	436; 513	6.0	2.3
(±)-7g		193; 219	1 844; 1 961	1 970; 1 245	9.2	7.8
(±)-7h		256; 241	614; 835	2 246; 1 190	2.9	6.9
(±)-7i[g]		67.0 ± 25.4	1 326 ± 142	384 ± 36	20	5.7
(+)-7i[g]		393 ± 69.6	6 214 ± 2 144	915 ± 81	18	2.7
(-)-7i[g]		56.7 ± 20.8	2 974 ± 1 242	612 ± 270	60	12

(±)-7j		177; 214	1 909; 2 027	430; 566	10	2.5
(±)-7k		148; 145	707; 519	250;306	4.2	1.9
(±)-7l		70.1; 53.5	1 495; 1 827	418; 518	26	7.4
(±)-7m		694; 469	8 358; 5 515	7 932; 6 447	12	12
(±)-7n		317; 364	> 10 000	1 302; 1 415	> 29	4.0
(±)-8		834; 755	267; 215	2 397; 2 377	0.30	3.0

[a] K_i-values were determined in competitive binding studies using radiolabeled ligands ([^{3}H]vesamicol for VAChT, (+)-[^{3}H]pentazocine for the σ_1 receptor and [^{3}H]DTG for the σ_2 receptor) in the presence of the compounds in concentrations ranging from 100 µM – 0.1 nM. Individual K_i-values from the two experiments performed are shown.

[b] Calculated from the mean of the K_i-values.

[c] Binding to membrane homogenates from PC12 cells stably transfected with human VAChT gene. The equilibrium dissociation constant of [^{3}H]vesamicol has been determined with K_D = 2.39 nM in this assay. Nonspecific binding of [^{3}H]vesamicol was determined in the presence of 10 µM vesamicol.

[d] Binding to membrane homogenates from rat cortex. The equilibrium dissociation constant of (+)-[^{3}H]pentazocine has been determined with K_D = 6.9 nM in this assay. Nonspecific binding of (+)-[^{3}H]pentazocine was determined in the presence of 100 µM haloperidol. σ_1 receptors were masked with 1 µM dextrallorphan.

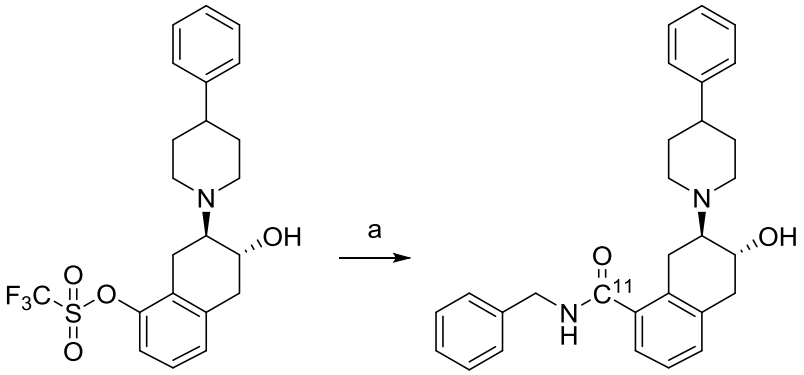
[e] Binding to membrane homogenates obtained rat liver. The equilibrium dissociation constant of [^{3}H]DTG has been determined with K_D = 29 nM in this assay. Nonspecific binding of [^{3}H]DTG was determined in the presence of 100 µM haloperidol.

[f] Reported results were obtained with (-)-[^{3}H]-vesamicol and vesicles from the electric organ of *Torpedo californica*.

[g] The K_i-values are the mean ± SD (nM) of 5-6 experiments.

2.3 Radiochemistry

In order to investigate whether this C5 substituted benzovesamicol-based scaffold could be evaluated using PET, radiolabelling of **(±)-7i** was pursued. Again using **(±)-5** as a precursor together with benzylamine and [^{11}C]CO, **[^{11}C]-(±)-7i** was labelled through an aminocarbonylation (Scheme 4). The reaction was carried out in a disposable glass vial where [^{11}C]CO was efficiently added in xenon gas resulting in ambient pressure conditions.[57,58] Without much optimization of the method, **[^{11}C]-(±)-7i** could be isolated in 9 ± 0.3% decay-corrected radiochemical yield, >99% radiochemical purity and with a molar activity of 55-78 GBq/µmol at the end of synthesis (n = 2). As an example, when starting from 16.4 GBq of [^{11}C]CO, the product was isolated with 411 MBq activity and a molar activity of 55 GBq/µmol. This amount of activity is sufficient to perform preclinical experiments. These results show the usefulness of **(±)-5** as a precursor for labeling of C5 substituted benzovesamicol-based VAChT ligands in addition to its potential use as a precursor for synthesis of new ligands.



(±)-5 **[^{11}C]-(±)-7i**

Scheme 4. a) Benzylamine, $Pd(dba)_2$, XPhos, triethylamine, tetrabutylammonium iodide, [^{11}C]CO, THF, 150 °C, 5 min, RCY = 9 ± 0.3% (decay corrected, n = 2).

3. Conclusion

In summary, fifteen VAChT ligands with different amide *N*-substituents in the C5 position on the tetrahydronaphthol ring were synthesized. With a novel synthetic route for VAChT ligands, a benzovesamicol scaffold was synthesized in a facile manner for further modification by employing aminocarbonylation reactions. When exploring both aliphatic and aromatic side chains on the amide nitrogen, ligands with affinities extending from the micromolar to the nanomolar range were obtained.

Benzyl substituted ligands had the highest affinities, with **(±)-7i** and **(±)-7l** showing the strongest binding to VAChT and good selectivities towards both σ receptors. In order to prove the stereoselectivity of VAChT's binding site, the enantiomers of **(±)-7i** were evaluated for their affinity. Eutomer **(-)-7i** showed the best affinity for VAChT. Although racemate **(±)-7i** initially looked promising, the affinity of **(-)-7i** does not warrant further preclinical experiments but the nanomolar affinity for VAChT and the high selectivity over the σ_1 receptor make **(-)-7i** an interesting lead structure for future development. Compound **(±)-5** could be used as a precursor for both synthesis of ligands and as a precursor for ^{11}C-labeling. As **[^{11}C]-(±)-7i** could be isolated with radioactivity high enough for preclinical PET experiments, the interest in **(-)-7i** as a scaffold for development of new ^{11}C-labelled VAChT PET tracers is further supported.

4. Experimental Section

4.1 General chemistry information

Reagents and solvents were obtained from Sigma-Aldrich (St. Louis, MO, USA) and Fischer (Pittsburgh, PA, USA) and used without further purification. Thin layer chromatography (TLC) was performed on aluminum sheets precoated with silica gel 60 F_{254} (0.2 mm, Merck KGaA, Darmstadt, Germany). Column chromatography was performed using silica gel 60 (40–63 µm, Merck KGaA, Darmstadt, Germany). Carbon-11 was prepared by the ^{14}N(p,α)^{11}C nuclear reaction using 17 MeV protons produced by a Scanditronix MC-17 Cyclotron at PET Centre, Uppsala University Hospital, and obtained as [^{11}C]carbon dioxide. The target gas used was nitrogen (AGA Nitrogen 6.0) containing 0.05% oxygen (AGA Oxygen 4.8). The [^{11}C]CO_2 was transferred to the low-pressure xenon-system in a stream of helium gas. [^{11}C]CO_2 and [^{11}C]CO were concentrated on anhydrous silica traps at -196 °C and released from the same traps by removal of liquid nitrogen dewars and heating the traps with a

coiled heater. [^{11}C]CO_2 was reduced to [^{11}C]CO over zinc heated to 400 °C and Ascarite was used to remove residual [^{11}C]CO_2. Before transferring [^{11}C]CO into the reaction vial through a transfer needle placed in the capped reaction vial, the carrier gas was changed from helium to xenon (>99.9%, 1.5 mL/min). For a schematic view of the process, see Eriksson *et al.* [57] and Chow *et al.* [58]. Microwave reactions were carried out in a Smith Synthesizer™ or in an Initiator™ single-mode microwave cavity producing controlled irradiation at 2450 MHz. Analytical GC-MS were performed on an Agilent Technologies system with a CP-SIL 8 CB low bleed (30 m × 0.25 mm) capillary column using a 70−300 °C temperature gradient and an EI ionization at 70 eV. Analytical reversed phase HPLC-MS was performed on a Dionex Ultimate 3000 system using MeCN/H_2O (0.05% HCOOH) as the mobile phase with MS detection, equipped with a C18 (Phenomenex Kinetex SB-C18 (4.8 × 50 mm)) column using UV (214 or 254 nm) detection or on a Dionex Ultimate 3000 system using MeCN/0.05% HCOOH in H_2O as the mobile phase with MS detection, equipped with a C18 (Phenomenex Kinetex SB-C18 (4.8 × 50 mm)) column using a UV diode array detector. Semi-preparative reversed phase HPLC was performed on a Gilson-Finnigan ThermoQuest AQA system equipped with a C8 (Zorbax SB-C8 (5 µm, 150 × 21.2 mm)) column using MeCN/H_2O (0.1% TFA) as the mobile phase with UV or on a Gilson GX-271 system equipped with a C18 (Macherey-Nagel Nucleodur HTec (5 µm, 125 × 21 mm)). Purity determinations were done on the Dionex Ultimate 3000 system with MeCN/H_2O (0.05% HCOOH) as the mobile phase and the C18 (Phenomenex Kinetex SB-C18 (4.8 × 50 mm)) column with UV detection at 254 nm. Resolution of enantiomers was performed with supercritical fluid chromatography (SFC). Analytical measurements were performed on a SFC Waters Investigator system connected to a Waters 2998 PDA detector. The column was an YMC Chiral Cellulose-SB (5 µm, 4.6 mm × 150 mm). The column temperature was set to 45 °C. An isocratic condition of 30% methanol and 0.1% diethylamine in CO_2 was applied at a flow rate of 5 mL/min. Preparative runs were performed on a SFC Waters Investigator system connected to a Waters 2998 PDA detector. The column was an YMC Chiral Cellulose-SB (5 µm, 10 mm × 250 mm). The column temperature was set to 45 °C. An isocratic condition of 30% methanol and 0.2% diethylamine in CO_2 was applied at a flow rate of 15 mL/min. Preparative purification of ^{11}C-labeled **(±)-7i** was done on a VWR La Prep Sigma system with a LP1200 pump, 40D UV detector and a Bioscan flowcount radiodetector. The mobile phase was MeCN/ammonium formate buffer (pH 3.5) and the column used was Phenomenex Kinetex C18 (5 µm, 150x10.0 mm). The identities, concentration and radiochemical purities of the purified labeled compound **[^{11}C]-(±)-7i** were determined either with VWR Hitachi Elite LaChrom system, consisting of L-2130 pump, L-2200 autosampler, L-2300 column oven, L-2450 diode array detector in series with a Bioscan β^+-flowcount radiodetector or with Elite LaChrom VWR international, equipped with a LaPrep P206 pump, an Elite LaChrom L-2400 UV detector in series with a Bioscan β+-flowcount detector, and using **(±)-7i** as reference. Mobile phase consisted of MeCN/ammonium carbonate (8.1 mM) and column used was Reprosil-Pur basic C18 (5 µm, 100 x 4.6 mm) from Dr Maisch GmbH or MeCN/ammonium formate buffer (50 mM) on a Merck Chromolith Performance RP-18 column (4.6 × 100 mm). Optical rotation was measured on a Rudolph research analytical autopol II automatic polarimeter at 23°C and wavelength 589 nm using a 100 mm cell. NMR spectra were recorded on a Varian Mercury plus spectrometer (^{1}H at 399.8 MHz, ^{13}C at 100.5 MHz and ^{19}F at 376.5 MHz) at ambient temperature. Chemical shifts (δ) are reported in ppm, indirectly referenced to tetrametylsilane (TMS) via the residual solvent signal (^{1}H: $CHCl_3$ δ 7.26, CD_2HOD δ 3.31, CD_2HCN δ 1.94, $(CHD_2)(CD_3)CO$; ^{13}C: $CDCl_3$ δ 77.16, CD_3OD δ 49.00, CD_3CN δ 1.32, $(CD_3)_2CO$ δ 29.84, 206.26.

4.2 Synthetic procedures and characterization of compounds 2 - (±)-8 and [^{11}C]-(±)-7i

4.2.1 5,8-Dihydronaphthalen-1-ol[42] (2)

A two-necked flask equipped with a cold finger was charged with *α*-naphthol (**1**, 2.0 g, 0.014 mol). Whilst vigorously stirring, liquid ammonia (30 mL) was added. After the *α*-naphthol had gone into solution (~30 min), lithium metal (0.41 g, 0.059 mol) was added in small pieces over 30 min. When the addition of the metal was completed, the solution (blue in color) was stirred for additional 1 h before absolute ethanol (4 mL) was added dropwise. The cold finger was removed, stirring was continued and the excess ammonia was evaporated in a stream of air introduced through an inlet tube. The residue was dissolved in water (20 mL) and washed with diethyl ether (5 mL). The aqueous phase was carefully acidified (pH < 1) using conc. HCl. An off-white solid precipitated which was extracted to diethyl ether (3 x 5 mL). The collected organic layers were washed with brine (2 x 5 mL), dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure, affording the crude product which was purified by silica gel flash column chromatography, using ethyl acetate/petroleum ether 1:19, affording 1.5 g of **2** as white solid (73% isolated yield), re-crystallized from petroleum ether. ^{1}H NMR (399.8 MHz, Chloroform-d_1): δ 7.06-7.02 (m, 1H), 6.73-6.71 (m, 1H), 6.63-6.61 (m, 1H), 5.91-5.90 (m, 2H), 4.62 (s, 1H, *OH*), 3.42-3.39 (m, 2H), 3.29-3.26 (m, 2H). ^{13}C NMR (100.5 MHz, Chloroform-d_1): δ 153.1, 135.9, 126.7, 124.5, 123.8, 121.2, 121.1, 112.2, 29.6, 24.0. MS (EI): *m/z* $[M]^+$: 146.1 (100).

4.2.2 5,8-Dihydronaphthalen-1-yl trifluoromethanesulfonate (3)

A 2-5 ml processor vial was charged with **2** (256 mg, 1.75 mmol), *N*-phenyl-*bis*(trifluoromethane sulfonamide) (614 mg, 1.74 mmol), K_2CO_3 (734 mg, 5.31 mmol) and THF (3 mL). The vial was sealed and irradiated in the reactor cavity at 120 °C for 6 min. After cooling to room temperature, the reaction mixture was filtered through a plug of celite which was rinsed with ethyl acetate. The filtrate was concentrated under reduced pressure affording the crude product, which was purified by silica gel flash column chromatography using ethyl acetate/petroleum ether 1:19 as eluent, to get 383 mg of **3** as yellow oil, in 79% isolated yield. ^{1}H NMR (399.8 MHz, Chloroform-d_1): δ 7.23 (t, J = 7.9 Hz, 1H), 7.16-7.11 (m, 2H), 5.92-5.91 (m, 2H), 3.46-3.39 (m, 4H). ^{13}C NMR (100.5 MHz, Chloroform-d_1): δ 147.9, 137.6, 128.4, 127.6, 127.1, 124.0, 123.1, 118.6 (d, J = 320 Hz, CF_3), 118.5, 29.4, 24.4. MS (EI): *m/z* $[M]^+$: 278.0 (100).

4.2.3 1a,2,7,7a-Tetrahydronaphtho[2,3-b]oxiren-3-yl trifluoromethanesulfonate [(±)-4]

A solution of **3** (200 mg, 0.72 mmol) in dichloromethane (10 mL) was cooled to 0 °C and *m*-chloroperbenzoic acid (186 mg, 1.08 mmol) was added. The reaction mixture was gradually warmed to room temperature and stirring was continued over night (16 h). An aqueous solution of saturated $NaHCO_3$ (5 mL) was added to quench the excess of acid and the mixture was stirred for 30 min. Phases were separated and the organic layer was washed with brine (2 x 5 mL), dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure affording the crude product, which was purified by silica gel flash column chromatography using 15% ethyl acetate in petroleum ether, to get 159 mg of **(±)-4** as white solid, in 77% isolated yield. ^{1}H NMR (399.8 MHz, Chloroform-d_1): δ 7.25-7.21 (m, 1H), 7.13-7.09 (m, 2H), 3.57-3.49 (m, 3H), 3.42-3.37 (m, 1H), 3.26-3.22 (m, 1H), 3.09-3.04 (m, 1H). ^{13}C NMR (100.5 MHz, Chloroform-d_1): δ 148.3, 135.1, 129.5, 127.8, 125.3, 119.5, 118.7 (d, J = 318 Hz, CF_3), 51.3, 50.7, 29.8, 24.1. HRMS for $C_{11}H_9F_3O_4S$ $[M+H]^+$: 295.0252, Found: 295.0251.

4.2.4 6-hydroxy-7-(4-phenylpiperidin-1-yl)-5,6,7,8-tetrahydro-naphthalen-1-yl trifluoromethane-sulfonate [(±)-5] and 7-Hydroxy-6-(4-phenylpiperidin-1-yl)-5,6,7,8-tetrahydro-naphthalen-1-yl trifluoromethanesulfonate [(±)-6]

A solution of **(±)-4** (60.0 mg, 0.20 mmol) in MeCN (5 mL) was treated with lithium perchlorate (32.0 mg, 0.30 mmol) under nitrogen flow and stirred at room temperature until complete dissolution of the salt. Then 4-phenylpiperidine (138 mg, 0.86 mmol), was added and the reaction mixture was heated and refluxed for 16 h. After cooling to room temperature, MeCN was concentrated under reduced pressure affording the crude product, which was a mixture of the two regioisomers **(±)-5** and **(±)-6**. The crude product was purified by silica gel flash column chromatography using 30% ethyl acetate in petroleum ether, affording 30 mg of compound **(±)-5** as white solid and 45 mg of compound **(±)-6** as off-white solid, in 33% and 49% isolated yields, respectively. **(±)-5** was re-crystallized from petroleum ether. ^{1}H NMR (399.8 MHz, Chloroform-d_1): δ 7.35-7.31 (m, 2H), 7.26-7.22 (m, 4H), 7.14 (dd, J = 7.5, 3.5 Hz, 2H), 4.35 (s, 1H, *OH*), 3.88 (td, J = 10.0, 6.0 Hz, 1H), 3.50 (dd, J = 16.8, 6.0 Hz, 1H), 3.04-2.75 (m, 6H), 2.70 (dd, J = 16.8, 10.0 Hz, 1H), 2.57 (tt, J = 11.6, 3.9 Hz, 1H), 2.39 (td, J = 11.6, 1.8 Hz, 1H), 1.96-1.72 (m, 4H). ^{13}C NMR (100.5 MHz, Chloroform-d_1): δ 148.2, 146.0, 138.7, 129.3, 128.6, 127.7, 127.5, 126.9, 126.5, 119.6 (d, J = 318 Hz, CF_3), 119.1, 65.8, 65.3, 53.7, 45.3, 43.0, 34.3, 33.9, 32.4, 26.5. HRMS for $C_{22}H_{24}F_3NO_4S$ $[M+H]^+$: 456.1456, Found: 456.1451. HPLC purity > 99%. **(±)-6** was re-crystallized from petroleum ether. ^{1}H NMR (399.8 MHz, Chloroform-d_1): δ 7.65-7.61 (m, 2H), 7.57-7.50 (m, 4H), 7.47-7.41 (m, 2H), 4.67 (s, 1H, *OH*), 4.21 (td, J = 10.2, 5.7 Hz, 1H), 3.68 (dd, J = 16.4, 5.7 Hz, 1H), 3.42 (dd, J = 15.9, 4.5 Hz, 1H), 3.31 (dt, J = 10.5, 3.4 Hz, 1H), 3.23-3.04 (m, 5H), 2.92 (tt, J = 11.9, 4.1 Hz, 1H), 2.75 (td, J = 11.4, 2.4 Hz, 1H), 2.29-2.07 (m, 4H). ^{13}C NMR (100.5 MHz, Chloroform-d_1): δ 148.4, 146.0, 137.8, 129.4, 128.6, 128.4, 127.8, 127.0, 126.5, 119.0, 118.8 (d, J = 318 Hz, CF_3), 66.0, 65.1, 53.7, 45.2, 42.8, 38.0, 34.2, 33.8, 20.8. HRMS for $C_{22}H_{24}F_3NO_4S$ $[M+H]^+$: 456.1456, Found: 456.1458. HPLC purity > 99%.

2D NMR experiments (COSY, HMBC) were also performed and confirmed the assignment of both regioisomers, according ref. [33].

4.2.4 General procedure for synthesis of compounds (±)-7a-n and (±)-8

To a 2-5 mL processor vial, **(±)-5** or **(±)-6** (1 equiv.), amine (3 equiv.), Cs_2CO_3 (3 equiv.), $Mo(CO)_6$ (1-1.5 equiv.), 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (Xphos, 7.5 mol%), *trans*-di(μ-acetato)bis[*o*-(di-*o*-tolyl-phosphino)benzyl]dipalladium(II) (Herrmann's palladacycle, 2.5 mol%), 4-(dimethylamino)pyridine (DMAP, 2 equiv.) and either 1,4-dioxane or MeCN was added. The reaction was sealed and irradiated for 3-8 h at 160 °C. The reaction mixture was allowed to cool to room temperature before filtration. The filtrate was washed with ethyl acetate and concentrated in vacuo. The crude product was purified by silica gel flash column chromatography using either ethyl acetate in *i*-hexane or methanol and triethylamine (NEt_3) in dichloromethane, followed by a second purification on semi-preparative reversed phase HPLC to isolate the compounds **(±)-7a-n** and **(±)-8** as their TFA-salts.

4.2.4.1 6-hydroxy-7-(4-phenylpiperidin-1-yl)-*N,N*-dipropyl-5,6,7,8-tetrahydro-naphthalene-1-carboxamide [(±)-7a]

Synthesized according to the general procedure using **(±)-5** (55.8 mg, 0.12 mmol), di-*N*-propylamine (62.0 mg, 0.61 mmol), Cs_2CO_3 (122 mg, 0.37 mmol), $Mo(CO)_6$ (32.6 mg, 0.12 mmol), Xphos (4.3 mg, 9 μmol), Herrmann's palladacycle (2.9 mg, 3 μmol) and 1,4-dioxane (1.5 mL) and irradiated for 8 h.

Crude product was purified first by silica gel flash column chromatography with 1:4 to 1:2 ethyl acetate in *i*-hexane, followed by purification on semi-preparative HPLC (MeCN/H_2O (0.1% TFA)). 10.5 mg of compound **(±)-7a** was isolated as the TFA-salt (16% yield). ^{1}H NMR (399.8 MHz, Methanol-d_4) δ 7.36 – 7.23 (m, 7H), 7.13 (d, *J* = 7.2 Hz, 1H), 4.24 (s, 1H), 3.75 – 3.36 (m, 7H), 3.23 – 2.87 (m, 6H), 2.25 – 1.98 (m, 4H), 1.67 (d, *J* = 83.6 Hz, 4H), 1.04 (t, *J* = 7.4 Hz, 3H), 0.92 – 0.85 (m, 1H), 0.76 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100.5 MHz, , Methanol-d_4) δ 172.8, 144.9, 137.5, 136.1, 130.8, 129.8, 128.27, 128.0, 127.7, 125.5, 87.1, 85.5, 70.0, 68.2, 66.2, 52.1, 47.9, 41.1, 39.6, 32.0, 31.6, 22.8, 21.9, 11.9, 11.4 (One aromatic carbon missing). HRMS for $C_{30}H_{39}F_3N_2O_4$ $[M+H]^+$: 435.3012, Found: 435.3008. HPLC purity > 90%.

4.2.4.2 (6-hydroxy-7-(4-phenylpiperidin-1-yl)-5,6,7,8-tetrahydronaphthalen-1-yl)(4-isopropyl-piperidin-1-yl)methanone [(±)-7b]

Synthesized according to the general procedure using compound **(±)-5** (50.0 mg, 0.11 mmol), 4-isopropylpiperidine (67.0 mg, 0.55 mmol), Cs_2CO_3 (107 mg, 0.33 mmol), $Mo(CO)_6$ (30.0 mg, 0.11 mmol), Xphos (3.9 mg, 8 µmol), Herrmanns palladacycle (2.6 mg, 3 µmol), DMAP (26.8 mg, 0.22 mol) and 1,4-dioxane (1.5 mL) and irradiated for 8 h. Crude product was purified first by silica gel flash column chromatography with 1:1 ethyl acetate in *i*-hexane, followed by purification on semi-preparative HPLC (MeCN/H_2O (0.1% TFA)). 10.0 mg of compound **(±)-7b** was isolated as the TFA-salt (16% yield). ^{1}H NMR (399.8 MHz, Methanol-d_4) δ 7.37 – 7.21 (m, 7H), 7.17 – 7.08 (m, 1H), 4.79 – 4.70 (m, 1H), 4.33 – 4.18 (m, 1H), 3.80 – 3.39 (m, 6H), 3.28 – 2.76 (m, 7H), 2.11 (d, *J* = 32.6 Hz, 4H), 1.93 – 1.85 (m, 1H), 1.76 – 1.61 (m, 1H), 1.55 – 1.11 (m, 4H), 0.97 – 0.89 (m, 6H). ^{13}C NMR (100.5 MHz, Chloroform-d_1) δ 143.1, 129.9, 129.8, 129.0, 127.4, 127.4, 127.3, 126.9, 124.9, 124.5, 52.5, 52.32, 48.4, 48.0, 46.9, 46.4, 42.9, 42.6, 40.7, 38.4, 32.5, 30.7, 30.4, 29.8, 29.4, 19.8, 19.7 (The carbonyl carbon was not detected). HRMS for $C_{32}H_{41}F_3N_2O_4$ $[M+H]^+$: 461.3168, Found: 461.3166. HPLC purity > 93%.

4.2.4.3 *N*-ethyl-6-hydroxy-*N*-(2-hydroxyethyl)-7-(4-phenylpiperidin-1-yl)-5,6,7,8-tetrahydro-naphthalene-1-carboxamide [(±)-7c]

Synthesized according to the general procedure using compound **(±)-5** (39.2 mg, 0.09 mmol), 2-ethylaminoethanol (22.9 mg, 0.26 mmol), Cs_2CO_3 (89.3 mg, 0.27 mmol), $Mo(CO)_6$ (34.2 mg, 0.13 mmol), Xphos (4.0 mg, 8 µmol), Herrmanns palladacycle (2.3 mg, 2 µmol), DMAP (21.3 mg, 0.17 mol) and MeCN (2.5 mL) and irradiated for 3 h. Crude product was purified first by silica gel flash column chromatography with 1:19 methanol in dichloromethane with 0.1% NEt_3, followed by two purifications on semi-preparative HPLC (MeCN/H_2O (0.1% TFA)). 8.1 mg of compound **(±)-7c** was isolated as the TFA-salt (18%) yield. ^{1}H NMR (399.8 MHz, Acetonitrile-d_3, a few drops of D_2O) δ 7.37 – 7.17 (m, 7H), 7.13 – 7.04 (m, 1H), 4.25 – 4.15 (m, 1H), 3.82 – 3.75 (m, 1H), 3.68 – 3.20 (m, 10H), 2.95 – 2.83 (m, 3H), 2.17 – 2.00 (m, 4H), 1.23 (t, *J* = 7.1 Hz, 1H), 1.01 (t, *J* = 7.1 Hz, 2H). ^{13}C NMR (100.5 MHz, Acetonitrile-d_3, a few drops of D_2O) δ 172.2, 145.0, 130.3, 130.2, 129.7, 128.0, 127.8, 127.8, 127.7, 127.7, 124.7, 67.6, 65.8, 60.1, 59.1, 47.1, 45.0, 40.3, 40.2, 39.0, 31.4, 31.0, 14.0, 13.0. HRMS for $C_{28}H_{35}F_3N_2O_5$ $[M+H]^+$: 423.2648, Found: 423.2639. HPLC purity > 99%.

4.2.4.4 (6-hydroxy-7-(4-phenylpiperidin-1-yl)-5,6,7,8-tetrahydronaphthalen-1-yl)(4-(hydroxyl-methyl)piperidin-1-yl)methanone [(±)-7d]

Synthesized according to the general procedure using compound **(±)-5** (63.6 mg, 0.14 mmol), 4-piperidinemethanol (49.5 mg, 0.43 mmol), Cs_2CO_3 (150 mg, 0.46 mmol), $Mo(CO)_6$ (39.4 mg, 0.15

mmol), Xphos (5.0 mg, 10 µmol), Herrmanns palladacycle (3.6 mg, 4 µmol), DMAP (36.6 mg, 0.30 mol) and 1,4-dioxane (3 mL) and MeCN (0.5 mL) and irradiated for 3 h. Crude product was purified first by silica gel flash column chromatography with 1:19 methanol in dichloromethane with 1% NEt_3, followed by two purifications on semi-preparative HPLC (MeCN/H_2O (0.1% TFA)). 13.6 mg of compound **(±)-7d** was isolated as the TFA-salt (17% yield). ^{1}H NMR (399.8 MHz, Acetonitrile-d_3, a few drops of D_2O) δ 7.37 – 7.32 (m, 2H), 7.30 – 7.18 (m, 5H), 7.06 (dd, *J* = 22.5, 7.3 Hz, 1H), 4.60 (t, *J* = 13.1 Hz, 1H), 4.28 – 4.14 (m, 1H), 3.67 – 3.33 (m, 8H), 3.30 – 3.09 (m, 2H), 2.98 – 2.73 (m, 5H), 2.17 – 1.98 (m, 5H), 1.87 – 1.56 (m, 3H), 1.24 – 1.18 (m, 1H). ^{13}C NMR (100.5 MHz, Acetonitrile-d_3, a few drops of D_2O) δ 170.2, 145.0, 135.8, 130.4, 129.7, 129.4, 128.1, 128.0, 127.9, 127.7, 124.9, 85.4, 67.7, 67.0, 66.7, 65.7, 51.5, 47.6, 42.5, 40.3, 39.5, 39.4, 39.0, 31.4, 31.1, 9.1. HRMS for $C_{30}H_{37}F_3N_2O_5$ $[M+H]^+$: 449.2804, Found: 449.2822. HPLC purity > 92%.

4.2.4.5 3-(4-(6-hydroxy-7-(4-phenylpiperidin-1-yl)-5,6,7,8-tetrahydro-naphthalene-1-carbonyl)-piperazin-1-yl)propanenitrile [(±)-7e]

Synthesized according to the general procedure using compound **(±)-5** (52.3 mg, 0.12 mmol), 3-(1-piperazinyl)-propionitrile (33.6 mg, 0.24 mmol), Cs_2CO_3 (119 mg, 0.37 mmol), $Mo(CO)_6$ (38.7 mg, 0.15 mmol), Xphos (4.7 mg, 10 µmol), Herrmanns palladacycle (3.2 mg, 3 µmol), DMAP (30.2 mg, 0.25 mol) and 1,4-dioxane (2 mL) and irradiated for 8 h. Crude product was purified first by silica gel flash column chromatography with 1:4 methanol in dichloromethane with 1% NEt_3, followed by purification on semi-preparative HPLC (MeCN/H_2O (0.1% TFA)). 15.8 mg of compound **(±)-7e** was isolated as the TFA-salt (22% yield). ^{1}H NMR (399.8 MHz, Acetonitrile-d_3) δ 7.69 (br. s, 1H), 7.39 – 7.34 (m, 2H), 7.32 – 7.23 (m, 5H), 7.16 – 7.13 (m, 1H), 4.24 (td, *J* = 10.3, 5.6 Hz, 2H), 3.68 – 3.40 (m, 11H), 3.30 (dd, *J* = 16.2, 5.6 Hz, 2H), 3.25 – 3.03 (m, 3H), 3.01 – 2.84 (m, 5H), 2.26 – 2.02 (m, 4H).^{13}C NMR (100.5 MHz, Acetonitrile-d_3) δ 169.6, 144.9, 135.8, 135.0, 131.0, 129.7, 128.1, 127.9, 127.8, 127.6, 125.4, 117.8, 67.9, 67.3, 65.7, 52.9, 52.7, 52.5, 51.5, 44.6, 40.2, 39.3, 39.0, 31.2, 14.2. HRMS for $C_{31}H_{37}F_3N_4O_4$ $[M+H]^+$: 473.2917, Found: 473.2904. HPLC purity > 99%.

4.2.4.6 *N*-(cyclopropylmethyl)-6-hydroxy-7-(4-phenylpiperidin-1-yl)-5,6,7,8-tetrahydronaphthalene-1-carboxamide [(±)-7f]

Synthesized according to the general procedure using compound **(±)-5** (62.0 mg, 0.14 mmol), aminomethylcyclopropane (29.1 mg, 0.41 mmol), Cs_2CO_3 (133 mg, 0.41 mmol), $Mo(CO)_6$ (57.3 mg, 0.22 mmol), Xphos (4.8 mg, 10 µmol), Herrmanns palladacycle (3.1 mg, 3 µmol), DMAP (33.5 mg, 0.27 mol) and MeCN (2 mL) and irradiated for 5 h. Crude product was purified first by silica gel flash column chromatography with 1:19 methanol in dichloromethane with 0.5% NEt_3, followed by purification on semi-preparative HPLC (MeCN/H_2O (0.1% TFA)). 15.1 mg of compound **(±)-7f** was isolated as the TFA-salt (21% yield). ^{1}H NMR (399.8 MHz, Acetonitrile-d_3, a few drops D_2O) δ 7.37 – 7.32 (m, 2H), 7.30 – 7.22 (m, 6H), 4.19 (td, *J* = 10.2, 5.6 Hz, 1H), 3.61 – 3.47 (m, 3H), 3.44 – 3.33 (m, 3H), 3.28 – 3.14 (m, 4H), 2.96 – 2.85 (m, 2H), 2.22 – 1.99 (m, 4H), 1.12 – 1.01 (m, 1H), 0.53 – 0.47 (m, 2H), 0.28 – 0.23 (m, 2H). ^{13}C NMR (100.5 MHz, Acetonitrile-d_3, a few drops D_2O) δ 170.0, 144.9, 137.8, 135.6, 131.4, 130.7, 129.7, 127.9, 127.9, 127.6, 126.4, 68.5, 65.9, 52.4, 49.3, 44.8, 40.3, 39.1, 31.4, 31.1, 26.3, 11.6, 3.9, 3.8. HRMS for $C_{28}H_{33}F_3N_2O_4$ $[M+H]^+$: 405.2542, Found: 405.2550. HPLC purity > 99%.

4.2.4.7 6-hydroxy-*N*-(4-methoxyphenyl)-7-(4-phenylpiperidin-1-yl)-5,6,7,8-tetrahydronaphthalene-1-carboxamide [(±)-7g]

Synthesized according to the general procedure using compound **(±)-5** (50.0 mg, 0.11 mmol), 4-methoxyaniline (29.1 mg, 0.54 mmol), Cs_2CO_3 (108 mg, 0.33 mmol), $Mo(CO)_6$ (29.0 mg, 0.22 mmol), Xphos (3.9 mg, 8 µmol), Herrmanns palladacycle (2.7 mg, 3 µmol), DMAP (28.8 mg, 0.22 mol) and 1,4-dioxane (2 mL) and irradiated for 8 h. Crude product was purified first by silica gel flash column chromatography with 1:1 ethyl acetate in *i*-hexane, followed by purification on semi-preparative HPLC (MeCN/H_2O (0.1% TFA)). 11.0 mg of compound **(±)-7g** was isolated as the TFA-salt (18% yield). ^{1}H NMR (399.8 MHz, Acetonitrile-d_3) δ 8.57 (s, 1H), 7.61 – 7.56 (m, 2H), 7.44 (dd, *J* = 6.7, 2.3 Hz, 1H), 7.37 – 7.21 (m, 7H), 6.97 – 6.92 (m, 2H), 4.20 (td, *J* = 9.9, 5.5 Hz, 1H), 3.79 (s, 3H), 3.64 – 3.55 (m, 2H), 3.52 – 3.44 (m, 2H), 3.38 – 3.21 (m, 4H), 2.97 – 2.85 (m, 2H), 2.32 – 2.19 (m, 1H), 2.11 – 2.02 (m, 3H). ^{13}C NMR (100.5 MHz, Chloroform-d_1) δ 167.9, 157.4, 142.9, 135.9, 135.2, 131.8, 130.6, 130.3, 129.0, 127.7, 127.4, 126.8, 125.6, 122.7, 114.6, 77.4, 68.0, 66.3, 55.7, 53.0, 46.8, 40.5, 38.4, 30.5, 25.4. ^{19}F NMR (376.5 MHz, Acetonitrile-d_3) δ -75.6. HRMS for $C_{31}H_{33}F_3N_2O_5$ $[M+H]^+$: 457.2491, Found: 457.2512. HPLC purity > 99%.

4.2.4.8 6-hydroxy-7-(4-phenylpiperidin-1-yl)-*N*-(3-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydro-naphthalene-1-carboxamide [(±)-7h]

Synthesized according to the general procedure using compound **(±)-5** (51.0 mg, 0.11 mmol), 3-(trifluoromethyl)aniline (53.7 mg, 0.33 mmol), Cs_2CO_3 (110 mg, 0.34 mmol), $Mo(CO)_6$ (31.8 mg, 0.12 mmol), Xphos (5.2 mg, 11 µmol), Herrmanns palladacycle (2.6 mg, 3 µmol), DMAP (27.7 mg, 0.23 mol) and 1,4-dioxane (3 mL) and MeCN (0.5 mL) and irradiated for 8 h. Crude product was purified first by silica gel flash column chromatography with 1:19 methanol in dichloromethane with 1% NEt_3, followed by purification on semi-preparative HPLC (MeCN/H_2O (0.1% TFA)). 6.8 mg of compound **(±)-7h** was isolated as the TFA-salt (10% yield). ^{1}H NMR (399.8 MHz, Acetone-d_6) δ 9.87 (s, NH), 8.34 (s, 1H), 8.02 (d, *J* = 8.3 Hz, 1H), 7.62 (t, *J* = 8.0 Hz, 1H), 7.53 – 7.46 (m, 2H), 7.36 – 7.30 (m, 6H), 7.25 – 7.20 (m, 1H), 4.26 (td, *J* = 9.8, 5.4 Hz, 1H), 3.80 – 3.56 (m, 4H), 3.54 – 3.34 (m, 3H), 3.30 (dd, *J* = 16.0, 5.4 Hz, 1H), 3.03 – 2.93 (m, 4H), 2.50 – 2.38 (m, 2H). ^{13}C NMR (100.5 MHz, Acetonitrile-d_3) δ 168.8, 145.1, 140.6, 136.8, 136.5, 136.4, 132.1, 131.6, 131.3, 131.2, 130.8, 129.7, 127.8, 127.8, 127.6, 126.9, 126.8, 124.5, 121.5, 68.3, 66.4, 52.6, 48.6, 40.3, 39.3, 31.3, 31.1, 26.3. HRMS for $C_{29}H_{29}F_3N_2O_2$ $[M+H]^+$: 495.2233, Found: 495.2259. HPLC purity > 99%.

4.2.4.9 *N*-benzyl-6-hydroxy-7-(4-phenylpiperidin-1-yl)-5,6,7,8-tetrahydro-naphthalene-1-carboxamide [(±)-7i]

Synthesized according to the general procedure using compound **(±)-5** (54.8 mg, 0.12 mmol), benzylamine (36.7 mg, 0.36 mmol), Cs_2CO_3 (139 mg, 0.43 mmol), $Mo(CO)_6$ (63.4 mg, 0.24 mmol), Xphos (8.7 mg, 18 µmol), Herrmanns palladacycle (5.6 mg, 6 µmol), DMAP (29.0 mg, 0.24 mol) and 1,4-dioxane (1.5 mL) and irradiated for 8 h. Crude product was purified first by silica gel flash column chromatography with ethyl acetate with 1% NEt_3, followed by purification on semi-preparative HPLC (MeCN/H_2O (0.1% TFA)). 19.8 mg of compound **(±)-7i** was isolated as the TFA-salt (30% yield). ^{1}H NMR (399.8 MHz, Acetonitrile-d_3) δ 9.15 (br. s, 1H), 7.44 – 7.22 (m, 13H), 5.23 (br. s, 1H), 4.54 (d, *J* = 6.2 Hz, 2H), 4.17 (td, *J* = 10.1, 5.6 Hz, 1H), 3.61 – 3.50 (m, 2H), 3.47 – 3.39 (m, 1H), 3.29 (ddd, *J* = 28.3, 16.1, 5.5 Hz, 2H), 3.22 – 3.09 (m, 3H), 2.94 – 2.81 (m, 2H), 2.31 – 2.16 (m, 1H), 2.11 – 2.01 (m, 3H).^{13}C NMR (100.5 MHz, Acetonitrile-d_3) δ 170.0, 145.0, 140.4, 137.5, 136.0, 131.4, 131.0, 129.7, 129.6, 128.6, 128.1, 127.9, 127.8, 127.7, 126.3, 68.3, 66.3, 52.6, 48.6, 43.9, 40.4, 39.2, 31.3, 31.1, 26.2. HRMS for $C_{31}H_{33}F_3N_2O_4$ $[M+H]^+$: 441.2542, Found: 441.2527. HPLC purity > 99%.

Resolution of compound (±)-7i

Chromatographic resolution of compound **(±)-7i** was accomplished by SFC (30% MeOH and 0.2% diethylamine in CO_2) to give 33.0 mg of **(+)-7i** (retention time, 2.2 min) and 33.3 mg of **(-)-7i** (retention time, 2.95 min). The specific rotation, $[\alpha]_{589}$, of **(+)-7i** was 34.1° and the optical rotation was 0.058° (c 0.17 g/100 mL in acetone, 23 °C). The specific rotation,$[\alpha]_{589}$, of **(-)-7i** was -26.4° and the optical rotation was -0.058° (c 0.22 g/100 mL in acetone, 23 °C). The enantiomeric excess of **(+)-7i** was 99% and the enantiomeric excess of **(-)-7i** was 99%.

4.2.4.10 *N*-benzyl-6-hydroxy-*N*-methyl-7-(4-phenylpiperidin-1-yl)-5,6,7,8-tetrahydronaphthalene-1-carboxamide [(±)-7j]

Synthesized according to the general procedure using compound **(±)-5** (56.2 mg, 0.12 mmol), N-methylbenzylamine (45.1 mg, 0.37 mmol), Cs_2CO_3 (133 mg, 0.41 mmol), $Mo(CO)_6$ (50.5 mg, 0.19 mmol), Xphos (5.8 mg, 12 µmol), Herrmanns palladacycle (3.4 mg, 4 µmol), DMAP (33.8 mg, 0.28 mol) and 1,4-dioxane (2 mL) and irradiated for 8 h. Crude product was purified first by silica gel flash column chromatography with 1:99 methanol in dichloromethane with 0.5% NEt_3, followed by purification on semi-preparative HPLC (MeCN/H_2O (0.1% TFA)). 24.7 mg of compound **(±)-7j** was isolated as the TFA-salt (34% yield). ^{1}H NMR (399.8 MHz, Acetonitrile -d_3, a few drops of D_2O) δ 7.46 – 7.40 (m, 3H), 7.39 – 7.05 (m, 16H), 4.37 (s, 1H), 4.17 (td, *J* = 10.3, 5.6 Hz, 1H), 3.61 – 3.48 (m, 3H), 3.26 – 3.16 (m, 2H), 3.08 (s, 2H), 2.94 – 2.83 (m, 3H), 2.74 (s, 2H), 2.20 – 1.97 (m, 6H). ^{13}C NMR (100.5 MHz, Acetonitrile-d_3, a few drops of D_2O) δ 172.5, 171.8, 145.0, 144.8, 138.3, 137.6, 135.7, 130.6, 130.5, 129.8, 129.8, 129.7, 129.4, 128.7, 128.6, 128.2, 128.2, 128.0, 127.9, 127.9, 127.7, 127.7, 125.1, 125.0, 67.3, 65.6, 51.9, 51.0, 40.4, 40.3, 38.9, 38.8, 37.0, 34.1, 31.3, 31.2, 31.0, 31.0. Because **(±)-7j** exists as two conformers, the integrals in the ^{1}H NMR and the number of carbons in the ^{13}C NMR does not add up. After running the NMR at 50°C, the integrals in the ^{1}H NMR adds up. There are still too many signals in the ^{13}C NMR but fewer than when the sample was run at room temperature. ^{1}H NMR (399.8 MHz, Acetonitrile-d_3, a few drops of D_2O, 60°C) δ 7.48 – 7.40 (m, 2H), 7.40 – 7.18 (m, 9H), 7.18 – 7.06 (m, 2H), 4.81 – 4.66 (m, 1H), 4.38 (s, 1H), 4.15 (s, 1H), 3.64 – 3.41 (m, 2H), 3.39 – 2.99 (m, 5H), 2.98 – 2.83 (m, 3H), 2.76 (s, 2H), 2.29 – 2.15 (m, 1H), 2.15 – 2.02 (m, 4H). ^{13}C NMR (100.5 MHz, Acetonitrile-d_3, a few drops of D_2O, 60°C) δ 171.8, 136.2, 130.7, 130.6, 129.9, 129.6, 129.4, 128.8, 128.8, 128.5, 128.4, 128.2, 128.1, 128.1, 127.9, 125.5, 125.3, 68.3, 66.4, 55.6, 51.3, 49.8, 49.2, 40.7, 39.2, 37.1, 34.1, 31.5, 31.3, 26.2. ^{19}F NMR (376.5 MHz, Acetonitrile-d_3) δ -75.6. HRMS for $C_{32}H_{35}F_3N_2O_4$ $[M+H]^+$: 455.2699, Found: 455.2705. HPLC purity > 99%.

4.2.4.11 6-hydroxy-*N*-(4-methoxybenzyl)-7-(4-phenylpiperidin-1-yl)-5,6,7,8-tetrahydronaphthalene-1-carboxamide [(±)-7k]

Synthesized according to the general procedure using compound **(±)-5** (57.8 mg, 0.13 mmol), 4-methoxybenzylamine (52.5 mg, 0.38 mmol), Cs_2CO_3 (125 mg, 0.39 mmol), $Mo(CO)_6$ (53.3 mg, 0.20 mmol), Xphos (5.2 mg, 11 µmol), Herrmanns palladacycle (3.3 mg, 4 µmol), DMAP (30.5 mg, 0.25 mol) and 1,4-dioxane (2 mL) and irradiated for 5 h. Crude product was purified first by silica gel flash column chromatography with 1:99 methanol in dichloromethane with 0.5% NEt_3, followed by purification on semi-preparative HPLC (MeCN/H_2O (0.1% TFA)). 19.6 mg of compound **(±)-7k** was isolated as the TFA-salt (26% yield). ^{1}H NMR (399.8 MHz, Acetonitrile-d_3, a few drops of D_2O) δ 7.38 – 7.22 (m, 10H), 6.95 – 6.90 (m, 2H), 4.51 – 4.37 (m, 2H), 3.73 (s, 3H), 3.58 – 3.47 (m, 2H), 3.46 – 3.39 (m, 1H), 3.29 – 3.05 (m, 5H), 2.93 – 2.81 (m, 2H), 2.21 – 1.97 (m, 4H). ^{13}C NMR (100.5 MHz, Acetone-

d_6, a few drops of D_2O) δ 170.1, 159.6, 145.0, 137.1, 135.7, 132.2, 131.1, 130.9, 130.0, 129.4, 127.5, 127.5, 127.5, 126.2, 114.6, 67.9, 65.7, 55.5, 51.7, 48.9, 43.1, 40.4, 39.0, 31.3, 31.0, 26.4. HRMS for $C_{32}H_{35}F_3N_2O_5$ $[M+H]^+$: 471.2648, Found: 471.2639. HPLC purity > 99%.

4.2.4.12 *N*-(4-fluorobenzyl)-6-hydroxy-7-(4-phenylpiperidin-1-yl)-5,6,7,8-tetrahydronaphthalene-1-carboxamide [(±)-7l]

Synthesized according to the general procedure using compound **(±)-5** (56.2 mg, 0.12 mmol), 4-fluorobenzylamine (46.0 mg, 0.37 mmol), Cs_2CO_3 (119 mg, 0.37 mmol), $Mo(CO)_6$ (52.1 mg, 0.20 mmol), Xphos (4.6 mg, 10 µmol), Herrmanns palladacycle (2.8 mg, 3 µmol), DMAP (29.5 mg, 0.24 mol) and 1,4-dioxane (2 mL) and irradiated for 5 h. Crude product was purified first by silica gel flash column chromatography with 1:99 methanol in dichloromethane with 0.5% NEt_3, followed by purification on semi-preparative HPLC (MeCN/H_2O (0.1% TFA)). 26.5 mg of compound **(±)-7l** was isolated as the TFA-salt (37% yield). ^{1}H NMR (399.8 MHz, Acetonitrile-d_3) δ 7.46 – 7.40 (m, 2H), 7.38 – 7.32 (m, 2H), 7.31 – 7.22 (m, 6H), 7.15 – 7.08 (m, 2H), 4.50 (s, 2H), 4.15 (td, *J* = 10.2, 5.6 Hz, 1H), 3.60 – 3.50 (m, 2H), 3.47 – 3.39 (m, 1H), 3.32 – 3.08 (m, 5H), 2.95 – 2.80 (m, 2H), 2.28 – 2.13 (m, 1H), 2.10 – 2.00 (m, 3H). ^{13}C NMR (100.5 MHz, Acetonitrile-d_3) δ 170.3, 162.9 (d, *J* = 242.8 Hz), 145.0, 137.2, 136.4 (d, *J* = 3.2 Hz), 136.0, 131.5, 130.9, 130.6 (d, *J* = 8.4 Hz), 129.7, 127.9, 127.8, 127.7, 126.3, 116.2 (d, *J* = 21.5 Hz), 68.0, 66.0, 52.2, 48.7, 43.1, 40.4, 39.1, 31.2, 31.0, 26.3 .HRMS for $C_{31}H_{32}F_4N_2O_4$ $[M+H]^+$: 459.2448, Found: 459.2433. HPLC purity > 99%.

4.2.4.13 6-hydroxy-7-(4-phenylpiperidin-1-yl)-*N*-(thiazol-2-ylmethyl)-5,6,7,8-tetrahydro-naphthalene-1-carboxamide [(±)-7m]

Synthesized according to the general procedure using compound **(±)-5** (56.2 mg, 0.12 mmol), 2-aminomethylthiazole (60.4 mg, 0.53 mmol), Cs_2CO_3 (130 mg, 0.40 mmol), $Mo(CO)_6$ (54.6 mg, 0.21 mmol), Xphos (4.4 mg, 9 µmol), Herrmanns palladacycle (2.9 mg, 3 µmol), DMAP (31.5 mg, 0.26 mol) and 1,4-dioxane (2.5 mL) and irradiated for 8 h. Crude product was purified first by silica gel flash column chromatography with 1:99 methanol in dichloromethane with 0.5% NEt_3, followed by two purifications on semi-preparative HPLC (MeCN/H_2O (0.1% TFA)). 3.1 mg of compound **(±)-7m** was isolated as the TFA-salt (5% yield). ^{1}H NMR (399.8 MHz, Acetonitrile-d_3, a few drops of D_2O) δ 7.73 (d, *J* = 3.3 Hz, 1H), 7.49 (d, *J* = 3.3 Hz, 1H), 7.38 – 7.33 (m, 3H), 7.31 – 7.26 (m, 4H), 7.26 – 7.23 (m, 1H), 4.81 (s, 2H), 4.19 (td, *J* = 10.2, 5.6 Hz, 1H), 3.61 – 3.46 (m, 3H), 3.44 – 3.27 (m, 3H), 3.26 – 3.16 (m, 1H), 2.95 – 2.86 (m, 2H), 2.24 – 1.97 (m, 5H). ^{13}C NMR (100.5 MHz, Acetonitrile-d_3, a few drops of D_2O) δ 170.5, 145.0, 143.3, 136.5, 135.9, 131.9, 131.0, 129.7, 127.9, 127.87, 127.7, 127.6, 126.5, 68.0, 65.7, 52.1, 49.0, 41.7, 40.3, 39.0, 31.3, 31.0, 26.4. HRMS for $C_{28}H_{30}F_3N_3O_4S$ $[M+H]^+$: 448.2059, Found: 448.2047. HPLC purity > 99%.

4.2.4.14 6-hydroxy-7-(4-phenylpiperidin-1-yl)-*N*-(2-(pyridin-2-yl)ethyl)-5,6,7,8-tetrahydro-naphthalene-1-carboxamide [(±)-7n]

Synthesized according to the general procedure using compound **(±)-5** (61.7 mg, 0.14 mmol), 2-(2-aminoethyl)pyridine (50.0 mg, 0.41 mmol), Cs_2CO_3 (137 mg, 0.42 mmol), $Mo(CO)_6$ (56.9 mg, 0.22 mmol), Xphos (5.2 mg, 11 µmol), Herrmanns palladacycle (3.2 mg, 3 µmol), DMAP (34.5 mg, 0.28 mol) and 1,4-dioxane (2 mL) and irradiated for 5 h. Crude product was purified first by silica gel flash column chromatography with 1:19 methanol in dichloromethane with 0.5% NEt_3, followed by two purifications on semi-preparative HPLC (MeCN/H_2O (0.1% TFA)). 4.8 mg of compound **(±)-7n** was isolated as the TFA-salt (6% yield). ^{1}H NMR (399.8 MHz, Acetonitrile-d_3, a few drops of D_2O) δ 8.70 –

8.66 (m, 1H), 8.36 (td, *J* = 7.9, 1.6 Hz, 1H), 7.88 (d, *J* = 8.1 Hz, 1H), 7.80 – 7.75 (m, 1H), 7.37 – 7.32 (m, 2H), 7.30 – 7.24 (m, 3H), 7.23 – 7.17 (m, 3H), 4.17 (td, *J* = 10.1, 5.6 Hz, 1H), 3.81 – 3.74 (m, 2H), 3.59 – 3.46 (m, 3H), 3.44 – 3.28 (m, 5H), 3.23 (dd, *J* = 16.0, 5.6 Hz, 1H), 3.18 – 3.09 (m, 1H), 2.96 – 2.83 (m, 2H), 2.07 (s, 4H). ^{13}C NMR (100.5 MHz, Acetonitrile-d_3, a few drops of D_2O) δ 170.7, 156.6, 146.3, 145.1, 143.1, 136.5, 136.0, 131.7, 131.2, 129.7, 128.4, 127.8, 127.7, 127.9, 126.6, 125.7, 68.1, 65.9, 51.5, 49.9, 40.4, 39.4, 39.1, 34.7, 31.4, 31.1, 26.7. HRMS for $C_{31}H_{34}F_3N_3O_4$ $[M+H]^+$: 456.2651, Found: 456.2654. HPLC purity > 99%.

4.2.4.15 *N*-benzyl-7-hydroxy-6-(4-phenylpiperidin-1-yl)-5,6,7,8-tetrahydro-naphthalene-1-carboxamide [(±)-8]

Synthesized according to the general procedure using compound **(±)-6** (67.0 mg, 0.15 mmol), benzylamine (47.1 mg, 0.44 mmol), Cs_2CO_3 (160 mg, 0.49 mmol), $Mo(CO)_6$ (59.7 mg, 0.23 mmol), Xphos (5.1 mg, 11 µmol), Herrmanns palladacycle (3.9 mg, 4 µmol), DMAP (36.4 mg, 0.30 mol) and 1,4-dioxane (2 mL) and irradiated for 5 h. Crude product was purified first by silica gel flash column chromatography with 1:99 methanol in dichloromethane with 0.5% NEt_3, followed by purification on semi-preparative HPLC (MeCN/H_2O (0.1% TFA)). 18.2 mg of compound **(±)-8** was isolated as the TFA-salt (22% yield). ^{1}H NMR (399.8 MHz, Acetonitrile-d_3, a few drops of D_2O) δ 7.39 – 7.32 (m, 6H), 7.32 – 7.22 (m, 7H), 4.56 – 4.45 (m, 2H), 4.14 (td, *J* = 10.2, 5.6 Hz, 1H), 3.64 – 3.45 (m, 3H), 3.44 – 3.26 (m, 4H), 3.20 – 3.10 (m, 1H), 2.97 – 2.88 (m, 2H), 2.26 – 1.99 (m, 4H).^{13}C NMR (100.5 MHz, Acetonitrile-d_3, a few drops of D_2O) δ 170.1, 145.0, 140.1, 137.3, 134.2, 132.3, 131.6, 129.7, 129.5, 128.4, 128.1, 127.9, 127.7, 127.6, 126.7, 67.4, 66.2, 52.0, 48.9, 43.8, 40.4, 36.7, 31.3, 31.1, 28.8. HRMS for $C_{31}H_{33}F_3N_2O_4$ $[M+H]^+$: 441.2542, Found: 441.2553. HPLC purity > 99%.

4.3 *In vitro* binding studies

The VAChT assay was performed with the radioligand [^{3}H]vesamicol (1539 GBq/µmol; Perkin Elmer) using membrane homogenates obtained from PC-12 cells stably transfected with human VAChT gene (obtained from Ali Roghani, Texas Tech University, Lubbock, TX, USA). The thawed membrane preparation (~100 µg protein) was incubated with seven concentrations of test compounds (100 µM – 0.1 nM), 2 nM [^{3}H]vesamicol, and buffer (50 mM TRIS, pH 7.4, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$) in a total volume of 1 mL for 60 min at room temperature. The incubation was terminated by rapid filtration through GF-B glass fibre filters presoaked in PEI (0.3%, 60 min, room temperature). The receptor-bound [^{3}H]vesamicol trapped on the filters was measured by liquid scintillation counting. Nonspecific binding of [^{3}H]vesamicol was determined with 10 µM vesamicol. The equilibrium dissociation constant of [^{3}H]vesamicol has been determined with K_D = 2.39 nM in this assay.

The σ_1 assay was performed with the radioligand (+)-[^{3}H]pentazocine (1288 GBq/µmol; Perkin Elmer) using membrane homogenates obtained from rat cortex (SPRD, female, 10 – 12 weeks). The thawed membrane preparation (~100 µg protein) was incubated with seven concentrations of test compounds (100 µM – 0.1 nM), 2 nM (+)-[^{3}H]pentazocine, and buffer (50 mM TRIS, pH 7.4, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$) in a total volume of 1 mL for 60 min at room temperature. The incubation was terminated by rapid filtration through GF-B glass fibre filters presoaked in polyethyleneimine (PEI) (0.3%, 60 min, room temperature). The receptor-bound (+)-[^{3}H]pentazocine trapped on the filters was measured by liquid scintillation counting. Nonspecific binding of (+)-[^{3}H]pentazocine was determined with 100 µM haloperidol. The equilibrium dissociation constant of (+)-[^{3}H]pentazocine has been determined with K_D = 6.9 nM in this assay.

The σ_2 assay was performed with the radioligand [^{3}H]-1,3-di-*o*-tolylguanidine ([^{3}H]DTG, 1761 GBq/µmol; Perkin Elmer) using membrane homogenates obtained from rat liver (SPRD, female, 10 – 12 weeks). The thawed membrane preparation (~100 µg protein) was incubated with seven concentrations of test compounds (100 µM – 0.1 nM), 2 nM [^{3}H]DTG, 1 µM dextrallorphan (Roche) and buffer (50 mM TRIS, pH 7.4, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$) in a total volume of 1 mL for 120 min at room temperature. The incubation was terminated by rapid filtration through GF-B glass fibre filters presoaked in PEI (0.3%, 60 min, room temperature). The receptor-bound [^{3}H]DTG trapped on the filters was measured by liquid scintillation counting. Nonspecific binding of [^{3}H]DTG was determined with 100 µM haloperidol. The equilibrium dissociation constant of [^{3}H]DTG has been determined with K_D = 29 nM in this assay.

4.4 Radiochemistry

A small ligand screen was performed in order to find conditions for labeling of 5-substituted benzovesamicol ligands from triflate **(±)-5** (see Table S1, Supporting information). Compound **(±)-7i** was labeled by adding 1.10 mg of **(±)-5**, 0.75 mg $Pd(dba)_2$, 2.45 mg XPhos, 0.92 mg tetrabutylammonium iodide, 5 µl benzylamine and 2.5 µl triethylamine to a 900 µl pear-shaped reaction vial. 200 µl of freshly distilled THF was added and the vial was capped and flushed with nitrogen gas. After collection of [^{11}C]CO in the vial, the activity was measured before 5 min heating at 150 °C commenced. After the reaction was finished, the activity was measured again whereby the vial was vented and flushed with nitrogen gas to remove unreacted [^{11}C]CO or volatile compounds. The reaction mixture was diluted with 200 µl of MeCN to improve chromatography. Preparative purification was performed with 30% ammonium formate in MeCN as mobile phase (flow 5 mL/min) and **[^{11}C]-(±)-7i** eluted after 12.7 min. Analytical HPLC showed >99% radiochemical purity measured using two methods with different columns and mobile phases (see Supporting information for chromatograms). An aliquot of **(±)-7i** was added to the analytical sample of **[^{11}C]-(±)-7i** and retention times were compared to confirm the identity. The synthesis was repeated twice with decay-corrected yields 8.9% and 9.3% (decay-corrected to collection of [^{11}C]CO) and 61%-85% of the carbon monoxide had been converted to non-volatile products at the end of the reaction (calculated by dividing the decay-corrected activity after the reaction with the activity after the nitrogen purge). For experiment 1, starting with 16.4 GBq of [^{11}C]CO, the product was isolated with 411 MBq activity after 45 min from end of bombardment. The concentration of the product was 1.2 µg/mL (volume 2.7 mL, concentration derived from HPLC analysis) and the molar activity was 55 GBq/µmol at the end of synthesis (calculated as activity/amount of substance). For experiment 2, starting with 10.1 GBq of [^{11}C]CO, the product was isolated with 235 MBq activity after 47 min from end of bombardment. The concentration of the product was 1.3 µg/mL (volume 2.0 mL) and the molar activity was 78 GBq/µmol at the end of synthesis.

Supporting information

Supplementary data associated with this article can be found in the online version.

Acknowledgments

We thank Hooman Hamdi for help with the chemistry and Dr Patrik Nordeman for help with the radiochemistry. We gratefully acknowledge the financial support from the Disciplinary domain of Medicine and Pharmacy, Uppsala University, and the Swedish Society for Medical Research (SSMF).

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