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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 $\sigma_1$ and $\sigma_2$ 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 $\sigma_1$ and $\sigma_2$ 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 $\sigma_1$ 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 $\sigma_1$ and $\sigma_2$, 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 $\sigma_1$ and $\sigma_2$ 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 $\sigma_1/\sigma_2$ 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 $\sigma_1$ and $\sigma_2$ 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 $\sigma_1/\sigma_2$ 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′-

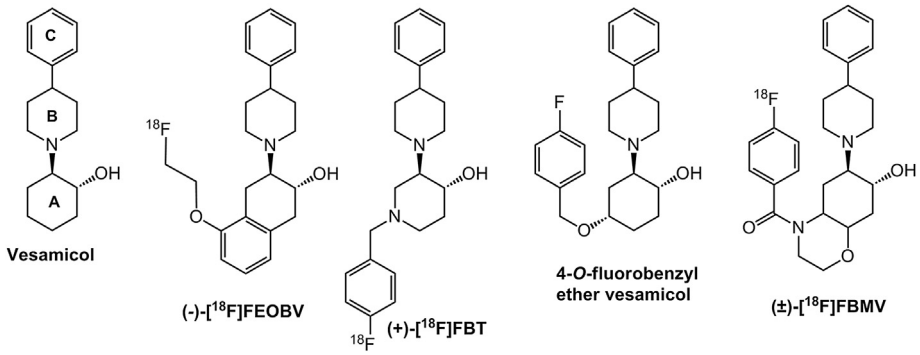


**Fig. 1.** Vesamicol and selected VAChT ligands.