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Synthesis and in vitro evaluation of N-substituted aza-trozamicol analogs as vesicular acetylcholine transporter ligands

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Abstract—As dysfunction of cerebral cholinergic neurotransmission is one of the main features in patients with Alzheimer's disease, in vivo imaging of the vesicular acetylcholine transporter (VAChT) can be of great value for the early diagnosis of this disease. Two series of positional isomers of *m*-iodobenzyltrozamicol (MIBT): 3-hydroxy-4-(*N*-phenylpiperazinyl)piperidine and 4-hydroxy-3-(*N*-phenylpiperazinyl)piperidine substituted by benzyl, aryl, alkyl or vinyl groups at the nitrogen have been synthesized. These compounds have been evaluated in vitro by competition studies and five compounds (*N*-benzyl derivatives) showed high affinity for the VAChT ($11\ nM < IC_{50} < 66\ nM$). These compounds will be soon radiolabeled with [^{125}I] for further biological evaluation using single photon emission computed tomography (SPECT).

Cholinergic neurotransmission plays an important role in the central nervous system which requires the synthesis of acetylcholine (ACh) in the cytoplasm, accumulation in presynaptic vesicles, and release from the terminal neuron. The vesicular acetylcholine transporter (VAChT) which is responsible for ACh accumulation into presynaptic vesicles plays a crucial role in regulating cholinergic neurotransmission as it concentrates and makes ACh available. Moreover, as the VAChT is located exclusively on cholinergic neurons, it has emerged as a useful target to study cholinergic function.[1–3] Alzheimer's disease (AD) is a progressive and neurodegenerative disorder which affects the cholinergic system and leads to cognitive deficits. Moreover, as this disease is progressive, the initial stages are not well defined compared to normal age 'decline' and it is classified as mild cognitive impairment. Exploration of cholinergic neurotransmission and especially the detection and quantification of the VAChT in vivo should provide important and useful information for the early diagnosis of this disease. As vesamicol (Fig. 1) binds to the VAChT but also to sigma receptors, structure–activity relationship studies (SARs) have been employed[4] to aid the development of radiopharmaceuticals suitable for use in single photon emission computed tomography (SPECT) and positron emission tomography (PET).[5] Among those radioligands, (−)-(2*R*,3*R*)-2-hydroxy-3-(4-phenylpiperidino)-5-[^{123}I]iodotetralin [(−)-[^{123}I]-5-IBVM] (Fig. 1) is the most promising SPECT

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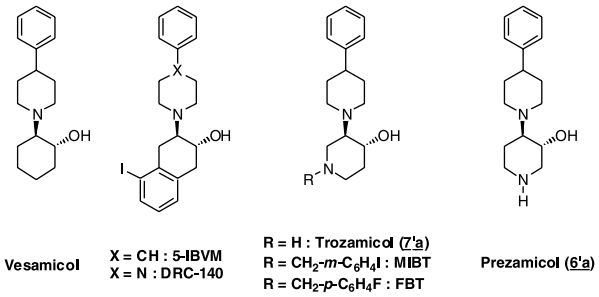


Figure 1. Vesamicol analogs.

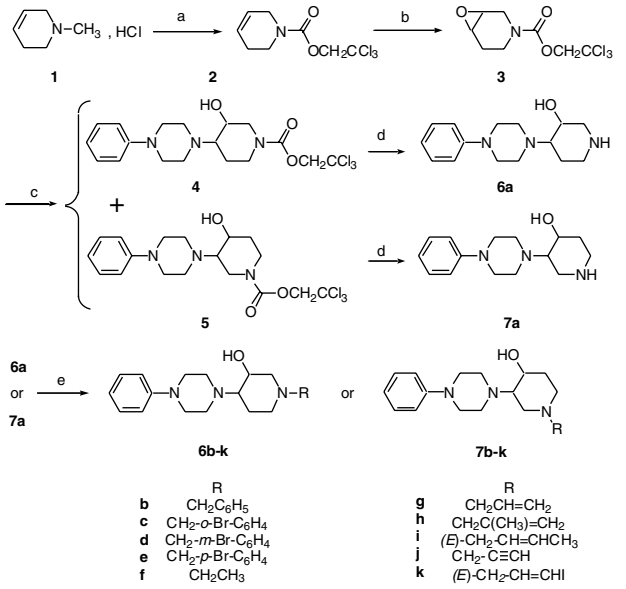
compound to be used in human studies. This radioiodinated derivative was found to be a reliable marker of cholinergic integrity at 22 h postinjection, as its binding correlates with choline acetyltransferase (ChAT) activity.[6] In addition, a 3.7% reduction per decade in cortical binding has been reported with [^{123}I]IBVM in normal aging and an inverse relationship between cortical [^{123}I]IBVM binding and the severity of AD has been reported.[7] Furthermore, although its gastrointestinal toxicity is not an issue, its slow pharmacokinetics are far from ideal.[7,8]

To improve these parameters, SARs have been performed on vesamicol structures which revealed that the piperidine group of vesamicol is important to obtain compounds with high affinity for the VAChT.[9] More recently, Bando et al.[10] have reported that the replacement of the piperidine ring of IBVM by a piperazine ring resulting in the formation of DRC140 derivative (Fig. 1) afforded a high affinity compound ($K_{i\text{DRC140}}$ = 1.21 nM) with high selectivity for VAChT over σ receptors.

Alkylation of the trozamicol scaffold (Fig. 1) at nitrogen by a *m*-iodobenzyl group afforded a highly potent derivative for the VAChT ($K_{i(+)\text{-MIBT}}$ = 4.8 ± 1.1 nM).[11] Similarly, [(−)-[^{18}F]-FBT] (Fig. 1) has proved to be a suitable radioligand in non-human primate studies using PET,[12] but has yet to be assessed in human studies.

From these results, we hypothesized that a fused skeleton between DRC-140 and MIBT structures (Scheme 1, target compounds) should result in compounds with a high affinity for the VAChT. Here, we report the synthesis of new aza-trozamicol derivatives and their in vitro evaluation on a human VAChT cell line.

Two series of compounds (**6a**–**k** and **7a**–**k**) were prepared as presented in Scheme 1. The synthesis started with 1-methyl-1,2,3,6-tetrahydropyridine hydrochloride **1**, which was converted into its corresponding carbamate **2** by treatment with 2,2,2-trichloroethylchloroformate under reflux. The crude carbamate was then treated with *m*-chloroperbenzoic acid which generated the required epoxide[9] **3**. The addition of phenylpiperazine to the epoxide **3** afforded two regioisomers **4** and **5** which were separated by flash chromatography (silica gel, EtOH/Et_3N, 10/1). The amine function was then deprotected (**6a** and **7a**) by reduction with zinc in acetic acid[13] in quantitative yield.



Scheme 1. Reagents and conditions: (a) $Cl_3CCH_2O_2CCl$, reflux, 3 h; (b) *m*-CPBA, Et_2O, rt, 5 h; (c) 1-phenylpiperazine, EtOH, reflux, 20 h; (d) Zn/AcOH, rt, 48 h; (e) RBr (2.5 equiv), EtOH (10 mL/mmol), Et_3N (140 μL/mmol), KI, reflux, 16 h.

The exact regioisomer (3-OH or 4-OH) of the piperidine ring was confirmed by X-ray diffraction measurements carried out on compound **7a**.[14] Both piperidine and piperazine rings were found to exist in the chair conformation with the piperidine and piperazine rings nearly perpendicular: 80.9° for the (*S*,*S*) derivative and −83.5° for the (*R*,*R*) derivative. These angles conformed to those described for (−)-vesamicol[9] and a trozamicol derivative.[11]

N-Alkylation of **6a** or **7a** was performed with the appropriate alkyl bromide reagent in ethanol which gave the corresponding N-substituted-4-hydroxy-3-(*N*-phenylpiperazinyl)piperidine **6b**–**j** or N-substituted-3-hydroxy-4-(*N*-phenylpiperazinyl)piperidine **7b**–**j** in 50–71% yield. Compounds **6k** or **7k** were obtained in two steps. Stannyl analogs of **6k** or **7k** were prepared by N-alkylation of **6a** or **7a** with (*E*)-3-chloro-1-tributylstannylprop-2-ene in ethanol[15] in 56% and 59% yield, respectively. Treatment with iodine of these stannyl derivatives in $CHCl_3$ resulted in compounds **6k** or **7k** in 66% and 71% yield, respectively. All target compounds were characterized by ^{1}H NMR and mass spectrometry and gave results consistent with the assigned structure.

Human VAChT stably expressed in the PC12 cell line,[16] which is a common model system for neurosecretory phenomena, was used to screen target compounds. Because the cell line expresses a relatively large amount of human VAChT (>2 pmol/mg postnuclear supernatant), displacement of bound [^{3}H]-vesamicol by competing ligands is monophasic and simple. A low concentration of [^{3}H]-vesamicol was used so that the IC_{50} value for displacement is essentially equal to the K_d value. Cells were grown in quantity, harvested, and homogenized to prepare a low-speed, postnuclear super-

Table 1. IC_{50} values (nM) at the human VAChT compared to the literature data

Compound	R	Series **6**[a]	Series **6′**[b]	Series **7**[a]	Series **7′**[b]
a	H	~100	5000 (±700)	>100	2900 (±400)
b	$CH_2C_6H_5$	57 (±26)	83 (±7)	>100	30 (±7)
c	CH_2–*o*-Br–C_6H_4	11 (±4)	55 (±8)	47 (±18)	22 (±6)
d	CH_2–*m*-Br–C_6H_4	66 (±24)	—	46 (±20)	21 (±8)
e	CH_2–*p*-Br–C_6H_4	>100	310 (±130)	>100	25 (±10)
f	CH_2CH_3	>1000	—	>1000	—
g	$CH_2CH{=}CH_2$	>100	—	>100	—
h	$CH_2C(CH_3){=}CH_2$	>100	—	>100	—
i	(*E*)-CH_2–$CH{=}CHCH_3$	~100	—	>100	—
j	$CH_2C{\equiv}CH$	>1000	—	>100	—
k	(*E*)-CH_2–$CH{=}CHI$	>100	—	>100	—

[a] Values are means of three experiments, standard deviation is given in parentheses. In the same conditions, (−)-vesamicol showed an IC_{50} = 20 (±2.3) nM.

[b] Literature data obtained on VAChT isolated from the electric organ of *Torpedo californica*,[17] (±)-vesamicol showed a K_d = 34 (±6) nM.

natant rich in synaptic-like microvesicles containing human VAChT. Competition was allowed to proceed for 24 h to ensure equilibration of low concentrations of target compounds. It could not be carried out at 37 °C due to the length of incubation. However, binding of vesamicol is not strongly temperature dependent.

Because IBVM and MIBT are compounds with high affinities for the VAChT; IC_{50} values have only been determined for potent compounds with IC_{50} lower than 100 nM. Affinities of synthesized compounds are presented in Table 1.

The only structural difference between prezamicol analogs (**6′a–e**),[17] and compounds **6a–e**, trozamicol analogs (**7′a–e**)[17] and compounds **7a–e** is the replacement of the 4-phenylpiperidine group for a *N*-phenylpiperazine group. Comparison of their in vitro affinities would provide information on the impact of an additional nitrogen at this part of the molecule. Even compounds **6a** ($IC_{50} \sim 100$ nM) and **7a** ($IC_{50} > 100$ nM) displayed higher affinities for the VAChT compared to prezamicol (**6′a**; IC_{50} = 5000 nM) and trozamicol (**7′a**; IC_{50} = 2900 nM),[17] compounds **6b**,**6c**, and **6e** (IC_{50} = 57, 11, and >100 nM, respectively) exhibited similar affinities for the VAChT compared to their prezamicol analogs **6′b**,**6′c**, and **6′e** (IC_{50} = 83, 55, and 310 nM, respectively).[17] Moreover, even compounds **7c** and **7d** (IC_{50} = 47 and 46 nM, respectively) showed VAChT affinities in the same range of magnitude as their trozamicol analogs **7′c** and **7′d** (IC_{50} = 22 and 21 nM, respectively), while compounds **7b** and **7e** (IC_{50} = >100 nM) are less potent compared to compounds **7′b** and **7′e** (IC_{50} = 30 and 25 nM, respectively). Because the substitution of the 4-phenylpiperidine group for *N*-phenylpiperazine generated compounds with or without improved affinities for the VAChT, an additional nitrogen at this part of the molecule cannot be considered as essential for the binding to the VAChT.

Except for the benzyl group for which the VAChT affinity could be considered significantly different for compounds **6b** and **7b**, the VAChT affinity in series 6 and 7 is in the same range for each R substituent. In these series of compounds, the orientation of the nitrogen in the piperidine ring relative to the hydroxyl group (in position 3 or 4) seems not to influence the binding affinity. These results contrast with those obtained previously for trozamicol and prezamicol congeners.[17,18] For these compounds, the 1,4 orientation of the nitrogen relative to the hydroxyl group (trozamicol series) was found to be preferred.

Regarding the nature of the R group, substitution of compounds **6a** and **7a** with alkyl, alkenyl, and alkynyl groups was based on results obtained for hydroxylated decahydroquinoline vesamicol derivatives.[18] In this study, a (*E*)-iodoprop-2-enyl group was successfully introduced to obtain a compound with nanomolar VAChT affinity. Contrary to these results, the N-alkylation of compounds **6a** or **7a** failed to obtain compounds with VAChT potency. Only some of the benzyl derivatives displayed IC_{50} less than 100 nM (**6b–d** and **7c–d**). Moreover, the position of the bromide atom on the benzyl group induces variability in the VAChT affinity as the *para*-substituted derivatives (**6e** and **7e**) showed low VAChT affinities. Here also, these results contrast with those obtained for benzyl-substituted prezamicol or trozamicol derivatives as the biological activity was not influenced by the position of the substituent on the ring.[17]

Although it is well known that interaction at the VAChT binding site is stereoselective,[9] only racemic mixtures have been screened. We can expect that enantiomeric separation of compounds **6b–d** and **7c–d** would provide compounds with improved VAChT affinity. In parallel, as compound **6c** is the most potent of this series, its radiolabeling with bromide-76 but also with iodine-125/123 is underway to further evaluate its in vivo pharmacological profile.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.02.033.

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