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Novel bis-2,2,6,6-tetramethylpiperidine (bis-TMP) and bis-mecamylamine antagonists at neuronal nicotinic receptors mediating nicotine-evoked dopamine release

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ABSTRACT

By linking two or three mecamylamine or 2,2,6,6-tetramethylpiperidine (TMP) molecules together via a linear lipophilic bis-methylene linker or a specially designed conformationally restricted tris-linker, a series of bis- and tris-tertiary amine analogs has been synthesized and evaluated as potent antagonists at nAChRs mediating nicotine-evoked [^{3}H]dopamine release from rat striatal slices. Compounds **7e**, **14b** and **16** demonstrated high potency in decreasing nicotine-evoked [^{3}H]dopamine release (IC_{50} = 2.2, 46, and 107 nM, respectively). The preliminary structure–activity data obtained with these new analogs suggest the importance of the length of the methylene linker in the bis-analog series. Such bis-tertiary amino analogs may provide a new strategy for the design of drugable ligands that have high inhibitory potency against nAChRs mediating nicotine-evoked dopamine release in striatum, which have been suggested to be target receptors of interest in the development of potential smoking cessation therapies.

Tobacco smoking is the number one health problem accounting for more illnesses and deaths in the US than any other single factor.[1] Despite some success of currently available pharmacotherapies, relapse rates continue to be high, indicating that novel medications are still needed.[2] Based on the observation that the non-selective nicotinic acetylcholine receptor (nAChR) antagonist, mecamylamine (1, Fig. 1) has some efficacy as a tobacco use cessation agent, but is limited by its peripherally-mediated side-effects, which range from constipation to hypotension,[3] we hypothesized that subtype-selective nAChR antagonists will have both efficacy and therapeutic advantages (i.e., limited side-effect profile) as tobacco use cessation agents. We concluded that antagonist molecules that selectively inhibit central nAChRs mediating nicotine (NIC)-evoked dopamine (DA) release will decrease NIC self-administration and/or cue-induced reinstatement of NIC seeking, and thus have potential as effective and safe pharmacotherapeutics for the treatment of NIC addiction.[4]

The classical discovery that the bis-trialkylammonium nAChR channel blockers, hexamethonium and decamethonium, exhibit subtype selectivity between ganglionic nAChRs and muscle type nAChRs,[5] led us to adopt a similar molecular approach in the discovery of antagonists of nAChRs mediating NIC-evoked DA release. This resulted in the identification of a series of novel structural scaffolds incorporating both flexible and conformationally restrained bis-,[6,7] tris-[8] and tetrakis-[9] frameworks to which were appended a variety of quaternary ammonium head groups. Initially,

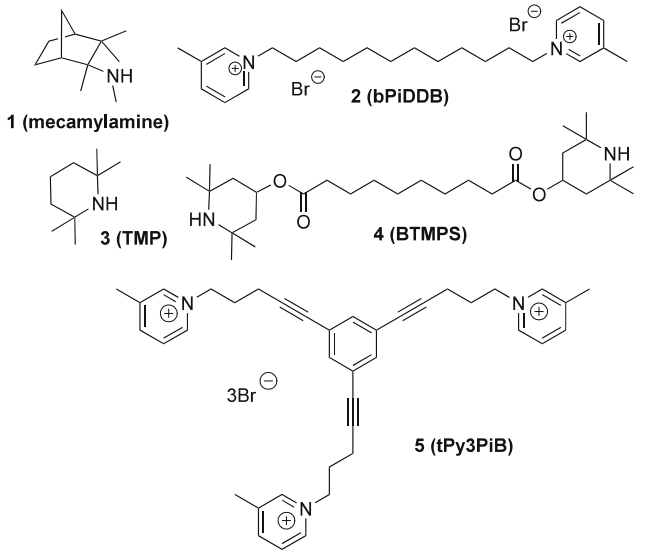

Figure 1. Structures of mecamylamine (**1**), bPiDDB (**2**), TMP (**3**), BTMPS (**4**), and tPy3PiB (**5**).

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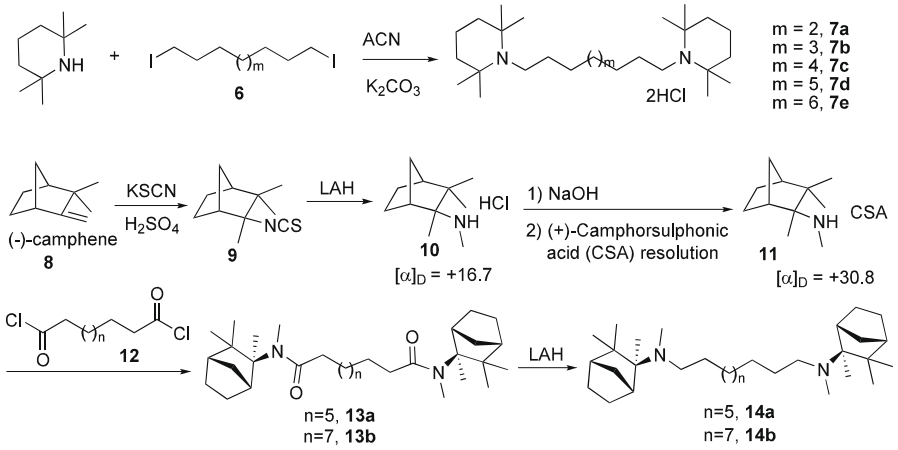
our lead candidate, *N*,*N*′-dodecyl-1,12-diyl-bis-3-picolinium dibromide (bPiDDB, **2**, Fig. 1),[10] a brain-bioavailable azaaromatic quaternary ammonium analog,[11] was demonstrated to be more selective than mecamylamine for nicotinic receptors inhibiting NIC-evoked DA release, and was the first example of a small molecule version of the α6-selective neuropeptide, α-conotoxin MII.[12] Structural iterations on bPiDDB included the development of novel scaffolds to which three or four cationic head groups were appended (i.e., tris- and tetrakis-azaaromatic quaternary ammonium sub-libraries), which afforded a selection of unique, high potency analogs that inhibited NIC-evoked DA release.[8,9] Results from pharmacokinetic studies utilizing radiolabeled ^{14}C-bPiDDB indicated that despite their cationic charge and polarity, the azaaromatic bis-quaternary ammonium analogs were brain-bioavailable after subcutaneous delivery, due to their facilitated transport via the blood-brain barrier choline transporter,[11,13] although bPiDDB has limited bioavailability when given by the oral route (Albayati et al., unpublished data). Since oral delivery is the preferred clinical route for development of a pharmaceutical product, we sought to optimize our synthetic strategies to focus on the design of analogs with improved oral bioavailability while maintaining inhibitory potency at α6-containing nAChRs.

The current study was initiated to determine if quaternary ammonium head groups in the structures of the first generation bis- and tris-analogs could be replaced with a variety of azacyclic tertiary amino moieties that have pK_a values in the range 7–9. This would allow the molecules to be protonated under physiological conditions (i.e., they will be cationic), with the ability to partition through cell membranes. In the selection of the tertiary amino head group, we chose to utilize small molecule tertiary amines that were previously shown to be non-competitive antagonists at nAChRs,[14,15] since our bis- and tris-quaternary amino analogs do not appear to interact with the acetylcholine binding site.

Mecamylamine (**1**, Fig. 1) is an example of such an azacyclic amino compound that is a non-selective nAChR channel blocker and non-competitive inhibitor of NIC-evoked DA release. Other examples of azacyclic compounds that are nAChR channel blockers, include the azacyclic tertiary amine, 1,2,2,6,6-pentamethylpiperidine (pempidine) and its N-demethylated analog, TMP (**3**, Fig. 1).[14] In this regard, it has already been reported that bis-(2,2,6,6-tetramethyl-4-piperidinyl) sebacate (BTMPS; **4**, Fig. 1) is a non-competitive, use-dependent antagonist at nAChRs.[15] Thus, TMP and mecamylamine were incorporated as the tertiary amine replacement head groups in the current study because, like the azaaromatic bis-quaternary ammonium analogs, they would be predicted to not interact with the acetylcholine binding site on nAChRs.

TMP analogs were synthesized by linking two TMP molecules through the same lipophilic 1,12-dodecanyl linker as in bPiDDB. The linker length was also varied from 8 to 12 methylene units, in order to determine the effect of linker length on potency for inhibition of NIC-evoked [^{3}H]DA release. N-Alkylation of TMP with the appropriate diiodoalkane (**6**, Scheme 1) was employed for the linking chemistry. Initial attempts at linking two molecules of TMP with 1,12-dibromododecane in the presence of potassium carbonate in refluxing acetonitrile produced a mixture containing the desired product accompanied by many other components, including the mono-alkylation product, elimination products of 1,12-dibromododecane, that is, bromododecenes, and monoalkylated elimination products. Steric hindrance caused by the four α-methyl groups of TMP also impeded efficient N-alkylation. Thus, the more reactive 1,12-diiododododecane was employed in the presence of an excess of TMP, and the reaction was carried out in a sealed tube. These conditions afforded the desired product, **7e**, in good yield (60–70%). Compounds **7a**–**7d** were prepared in a similar fashion from the appropriate diiodoalkane.

Since there is a chiral center in the mecamylamine molecule, the incorporation of two or more (±)-mecamylamine moieties into one bis- or tris-molecule becomes complicated, due to the generation of multiple diastereomeric products. It has been shown that there is little difference in the IC_{50} values of *S*-(+)- and *R*-(−)-mecamylamine at a given nAChR receptor subtype.[15] However, in oocyte expression systems, there appears to be significant differences in the off-rates of the two mecamylamine enantiomers from nAChR receptors. Specifically, *S*-(+)-mecamylamine appears to dissociate more slowly from α4β2 and α3β4 receptors than does *R*-(−)-mecamylamine.[16] The more active *S*-(+)-mecamylamine enantiomer was employed in the synthesis of the bis- and tris-mecamylamine analogs, in order to simplify the synthetic process and to better understand the SAR. The preparation of *S*-(+)-mecamylamine and the bis- and tris-mecamylamine analogs **14a**, **14b**, and **16** are summarized in Scheme 2. Commercially available (−)-camphene (**6**, Scheme 2, and 80% chemical purity) was treated with sulfuric acid and potassium thiocyanate to afford isothiocyanate **9** and a small amount of the corresponding thiocyanate. Reduction of this intermediate with LAH afforded crude *S*-(+)-mecamylamine that was contaminated with the corresponding thioalcohol generated from the thiocyanate. After acid extraction to remove the thioalcohol, the resulting *S*-(+)-mecamylamine was resolved further with camphorsulfonic acid to afford the chirally



Scheme 1. Synthesis of bis-TMP analogs **7a**–**7e**, and bis-*S*-(+)-mecamylamine analogs **14a** and **14b**.