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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,

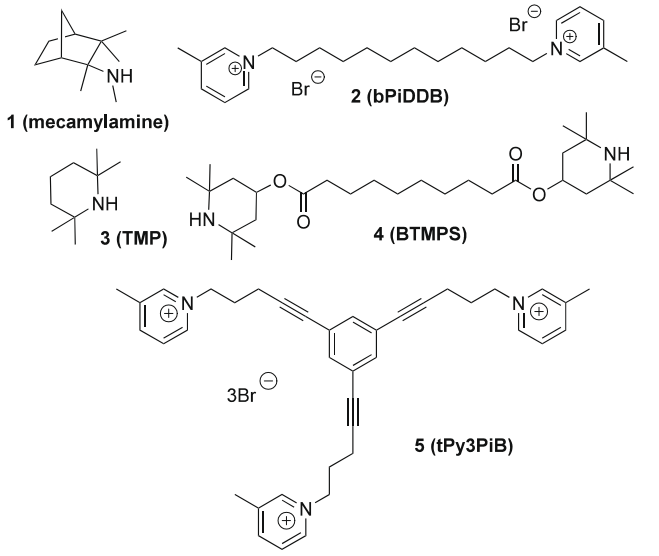

Figure 1. Structures of mecamylamine (**1**), bPiDDB (**2**), TMP (**3**), BTMPS (**4**), and tPy3PiB (**5**).

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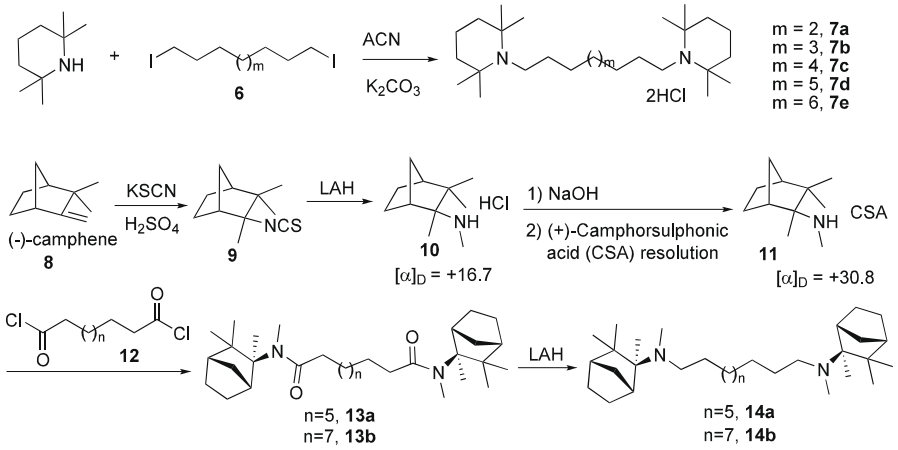
our lead candidate, *N*,*N*′-dodecyl-1,12-diyl-bis-3-picolinium dibromide (bPiDDB, **2**, Fig. 1),[10] a brain-bioavailable azaaromatic quaternary ammonium analog,[11] was demonstrated to be more selective than mecamylamine for nicotinic receptors inhibiting NIC-evoked DA release, and was the first example 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.

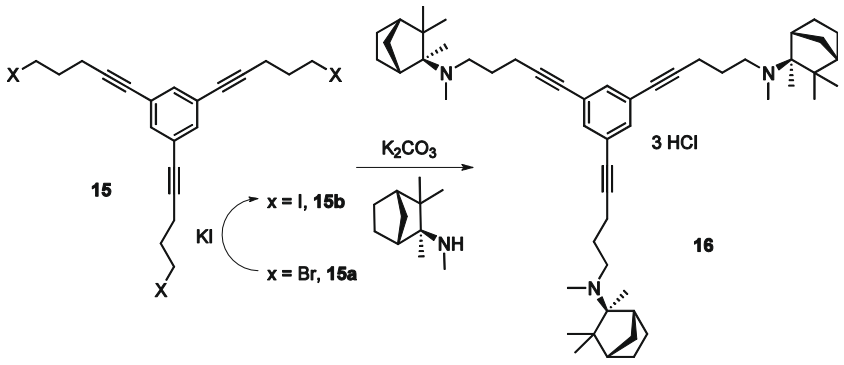
Mecamylamine (**1**, Fig. 1) is an example of such an azacyclic amino compound that is a non-selective nAChR channel blocker and non-competitive inhibitor of NIC-evoked DA release. Other examples of azacyclic compounds that are nAChR channel blockers, include the azacyclic tertiary amine, 1,2,2,6,6-pentamethylpiperidine (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**.



Scheme 2. Synthesis of tris-mecamylamine analog **16**.

pure *S*-(+)-enantiomer, which was then utilized in the N-alkylation reaction with the appropriate di- or tri-iodo intermediate.

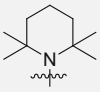
The same procedure as utilized in the synthesis of analogs **7a**–**7e** could be adopted to afford the bis-mecamylamine analogs. However, with *S*-(+)-mecamylamine, the employment of a large excess of starting mecamylamine is not practical, due to the amount of effort involved in the preparation of this compound. Thus, another procedure was devised, and the *S*-(+)-mecamylamine was initially N-acylated with either 1,10-decandioic chloride or 1,12-dodecandioic chloride to afford the bis-amides **13a** and **13b**, respectively, which were each cleanly reduced by LAH to afford **14a** and **14b**, respectively. Since compound **5** (tPy3PiB, Fig. 1) was considered to be a valuable lead compound in our systematic structural elaboration of the original quaternary ammonium series, a tris-analog of **5** was synthesized in which the 3-picolinium head groups had been replaced with *S*-(+)-mecamylamine moieties (i.e., analog **16**). The preparation of the starting tri-bromide **15a** is reported in our previous study.[8] Tri-bromide **15a** was transformed into the corresponding tri-iodide **15b** and this intermediate was then utilized in the synthesis of **16**.

Utilizing an initial probe concentration of 100 nM, compounds **7a**–**7e**, **14a**, **14b**, and **16** were evaluated for their ability to inhibit NIC-evoked [^{3}H]DA release from superfused rat striatal slices. The most active compounds (>50% inhibition in the probe assay) were then evaluated across a full concentration range, to determine IC_{50} and I_{max} values for inhibition of NIC-evoked [^{3}H]DA release (Table 1).

[^{3}H]DA release assays were performed according to a previously published method.[17] Initially, analog-induced inhibition of nicotine-evoked [^{3}H]DA release was determined using 10 μM NIC and 100 nM analog concentrations. Amount of inhibition is presented as a percentage of the response to NIC under control conditions (in the absence of analog) and the results are provided in Table 1 and Fig. 2. Full concentration response (1 nM to 10 μM) was performed for the most promising analogs (Table 1 and Fig. 3), and IC_{50} and I_{max} values were determined using an iterative non-linear least squares curve-fitting program, PRISM version 4.0 (GraphPAD Software, Inc., San Diego, CA).

The current study introduces for the first time a novel series of nAChR antagonists produced by incorporating TMP or mecamylamine molecules into a bis- or tris-structural scaffold. In our search for novel nAChR antagonists with potential as smoking cessation agents, the NIC-evoked [^{3}H]DA release assay was used as an initial screen to identify lead compounds, since the ability of NIC to release DA is believed to be associated with the rewarding properties of NIC. Also, the neuronal tissue utilized in this assay, striatum, is important for identifying and anticipating reward and organizing goal-directed behavior.[18] Compounds **7e**, **14b** and **16** were identified as hits in the single 100 nM concentration screen because they

Table 1
Inhibition of nicotine-evoked [^{3}H]DA release from superfused rat striatal slices[a]

Compound			DA release	
No.	Head group	Linker	% Inhibition (100 nM)[a]	IC_{50} nM (CI) and I_{max}[b]
7a		1,8-Octane	18 ± 9%	ND[c]
7b		1,9-Nonanel	5 ± 5%	ND
7c		1,10-Decane	22 ± 8%	ND
7d		1,11-Undecane	36 ± 10%	ND
7e		1,12-Dodecane	56 ± 14%	2.2 (0.9–5.2) I_{max} = 87%
14a		1,10-Decane	35 ± 16%	319(143–712) I_{max} = 90%
14b		1,12-Dodecane	52 ± 7%	46 (8–259) I_{max} = 83%
16		Tris-linker	73 ± 2%	107 (25–443) I_{max} = 62%
2		1,12-Dodecane (bPiDDB)	ND	2.0 ± 0.1 I_{max} = 78%
5		Tris-linker (tPy3PiB)	40 ± 12%	0.2 ± 0.07 I_{max} = 67%

[a] Percentage of inhibition at 100 nM are presented unless otherwise specified. Each value represents data from at least three independent experiments, each performed in duplicate.
[b] IC_{50} and I_{max} from full concentration response assays; data from 4 to 6 independent experiments.
[c] Not determined.

produced greater than 50% inhibition of NIC-evoked [^{3}H]DA release (Fig. 2, Table 1). From previous studies, we have found that the length of the linker unit plays an important role in the effect of these bis- and tris-compounds in inhibiting NIC-evoked striatal [^{3}H]DA release. The linker length SAR was evaluated over a C_8- to C_{12}-methylene linker range with TMP as the head group moiety (analogs **7a**–**7e**). From the results obtained in the single concentration screen, with the exception of C_8, all analogs inhibited NIC-evoked [^{3}H]DA release, with **7e**, the C_{12}-linker derivative, being the most efficacious compound in the series at the probe concentration of 100 nM. The other TMP derivatives were less efficacious, and there was a noticeable dependence of inhibitory potency on linker length. Except for the C_8-linker derivative **7a**, the other four TMP analogs exhibited systematically higher inhibition, as linker length was increased. In the full concentration–response analysis, **7e** was observed to inhibit NIC-evoked [^{3}H]DA release from superfused rat striatal slices with an IC_{50} value of 2.2 nM and an I_{max} of 87%. This compares very favorably with bPiDDB (IC_{50} = 2 nM; I_{max} = 78%). Thus, replacing the two 3-picolinium head groups of bPiDDB with TMP moieties resulted in a bis-tertiary amino analog with similar inhibitory potency. Analog **7e** was the most potent compound in the series, and can therefore be considered as a lead compound, and a more 'drugable' analog of bPiDDB.

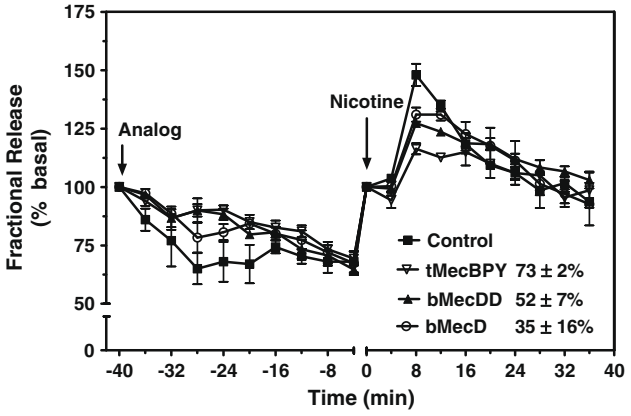


Figure 2. *S*-(−)-Nicotine-evoked fractional [^{3}H]DA release from rat striatal slices superfused with 100 nM **14a** (bMecD), **14b** (bMecDD) and **16** (tMecBPY). Data are expressed as mean ± SEM fractional release as a percent of basal fractional release, that is, percent of samples prior to the addition of analog or nicotine. Control represents the amount of fractional release evoked by *S*-(−)-nicotine in the absence of analog; n = 3 rats/analog.

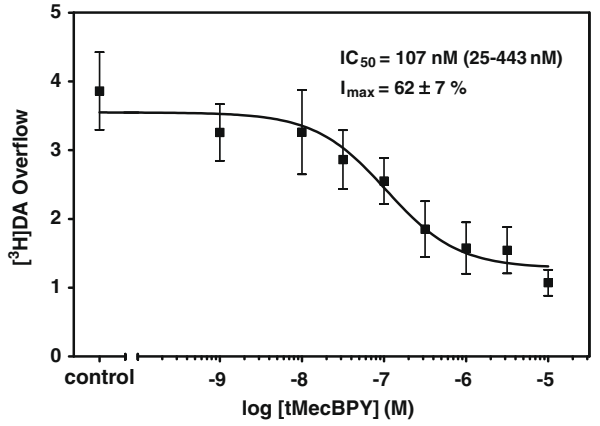


Figure 3. Analog **16** (tMecBPY) inhibited *S*-(−)-nicotine-evoked [^{3}H]DA overflow from rat striatal slices in a concentration-dependent manner. Control represents [^{3}H]DA overflow in response to 10 μM nicotine in the absence of analog and is expressed as a percent of tissue [^{3}H] content, mean ± S.E.M, n = 4 rats.

With these encouraging results, we next investigated the bis-analogs containing *S*-(+)-mecamylamine as the replacement head-group for the 3-picolinium moiety in bPiDDB. Analogs with C_{10}- and C_{12}-methylene linkers were evaluated. Similar to **7e**, the C_{12} mecamylamine derivative, **14b** (bMecDD), was designated as a hit (52% inhibition) in the NIC-evoked [^{3}H]DA release probe assay (Fig. 2, Table 1). Analog **14b** afforded an IC_{50} of 46 nM with an I_{max} of 83% in the full concentration response assay. The greatest percentile inhibition in the probe assay was obtained with the tris-mecamylamine analog **16** (tMecBPY; 73% inhibition, Fig. 2). Compound **16** was 100-fold more potent than mecamylamine in inhibiting NIC-evoked DA release, with a IC_{50} value of 107 nM and an I_{max} of 62% (Fig. 3, Table 1). These results clearly demonstrate that both quaternary ammonium and tertiary amino head groups can be utilized in the design of bis- and tris-analogs as potent inhibitors of nAChRs which mediate NIC-evoked DA release.

It should be noted that unlike the prototypical CNS-active nAChR inhibitor mecamylamine, **14b** and **16** did not inhibit NIC-evoked DA release from striatal slices completely (I_{max} = 83% and 62%, respectively). These results are in agreement with previous literature indicating that multiple nAChRs mediate NIC-evoked DA release, and that **14b** and **16** are likely acting as antagonists at only a subset of these nAChR subtypes. This observation is consistent with previous results showing that TMPH blocked some, but not all, of the CNS effects of NIC, indicating that this compound has a unique selectivity for specific nAChR receptor subtypes in the brain.[15]

In conclusion, linking TMP and mecamylamine head groups with lipophilic *n*-alkane linkers of variable lengths, or with a conformationally restrained tris-linker moiety, affords a novel series of compounds with tertiary amine head groups replacing the quaternary ammonium head groups present in the nAChR antagonists, bPiDDB and tPy3PiB. These molecules demonstrated high potency in inhibiting NIC-evoked DA release from striatal tissue, and can be considered lead compounds in the development of therapeutic agents to treat nicotine addiction. Since such tertiary amino derivatives will have improved membrane permeation capabilities due to their physicochemical properties, and have the potential to be orally effective with good brain bioavailability.

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