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Novel bis-, tris-, and tetrakis-tertiary amino analogs as antagonists at neuronal nicotinic receptors that mediate nicotine-evoked dopamine release

Zhenfa Zhang, Guangrong Zheng, Marharyta Pivavarchyk, A. Gabriela Deaciuc, Linda P. Dwoskin, Peter A. Crooks *

College of Pharmacy, Department of Pharmaceutical Sciences, University of Kentucky, Lexington, KY 40536-0082, USA

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ABSTRACT

A series of tertiary amine analogs derived from lead azaaromatic quaternary ammonium salts has been designed and synthesized. The preliminary structure–activity relationships of these new analogs suggest that such tertiary amine analogs, which potently inhibit nicotine-evoked dopamine release from rat striatum, represent drug-like inhibitors of α6-containing nicotinic acetylcholine receptors. The bis-tertiary amine analog **7** exhibited an IC_{50} of 0.95 nM, while the tris-tertiary amine analog **19** had an IC_{50} of 0.35 nM at nAChRs mediating nicotine-evoked dopamine release.

Nicotine, the principal alkaloidal component in tobacco, is believed to be primarily responsible for producing the rewarding effects of smoking. Nicotine produces this effect via its interaction with neuronal nicotinic acetylcholine receptors (nAChRs) in the brain to stimulate dopamine (DA) release from nucleus accumbens and striatum.[1,2] Stimulation of the brain DA pathways is believed also to be involved in the mechanism of action of many other drugs of abuse.[3] Thus, nAChR antagonists that inhibit nicotine-evoked DA release have been suggested to have potential as efficacious therapies for the treatment of tobacco dependence.[4,5]

As part of a continuing drug discovery effort in our laboratories to identify novel nAChR antagonists that may have potential as smoking cessation agents,[6–14] we found that *N*,*N*′-dodecane-1, 12-diyl-bis-3-picolinium dibromide (bPiDDB; **1**; Fig. 1) potently inhibited α6-containing nAChR subtype(s) mediating nicotine-evoked [^{3}H]DA release from superfused rat striatal slices (IC_{50} = 2 nM).[15] In vivo microdialysis studies showed that bPiDDB dose-dependently reduced the increase in extracellular DA in rat nucleus accumbens following acute or repeated nicotine administration.[16] Also, behavioral studies in rats showed that bPiDDB dose-dependently decreased nicotine self-administration, but not sucrose-maintained responding, suggesting a specific inhibition of nicotine reward.[17] The ability of bPiDDB to inhibit the effect of nicotine in nucleus accumbens and decrease nicotine self-administration in rats provides support for considering bPiDDB as a potential lead compound in the development of novel pharmacotherapies for nicotine dependence.

Further structural elaboration of the bPiDDB molecule led to the discovery of 1,2-bis-(5-isoquinolinium-pent-1-ynyl) benzene dibromide (bPyiQB; **2**; Fig. 1), a conformationally constrained bis-quaternary ammonium salt;[12] 1,3,5-tri-{5-[1-(3-picolinium)]-pent-1-ynyl}ben-zene tribromide (tPy3PiB; **3**, Fig. 1), a tris-quaternary ammonium compound;[13] and 1,2,4,5-tetrakis-{5-[(3-(3-hydroxypropyl)-pyridinium)]-pentanyl}-benzene tetrabromide (tkP3HPPB; **4**; Fig. 1), a tetrakis-quaternary ammonium

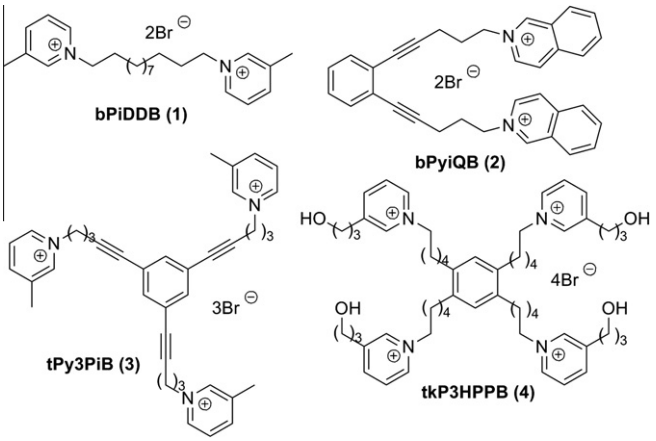

Figure 1. Structures of quaternary ammonium lead compounds.

* Corresponding author. Tel.: +1 859 257 1718; fax: +1 859 257 7585.
E-mail address: pcrooks@email.uky.edu (P.A. Crooks).

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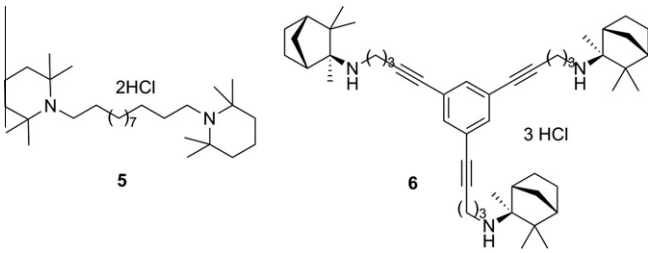
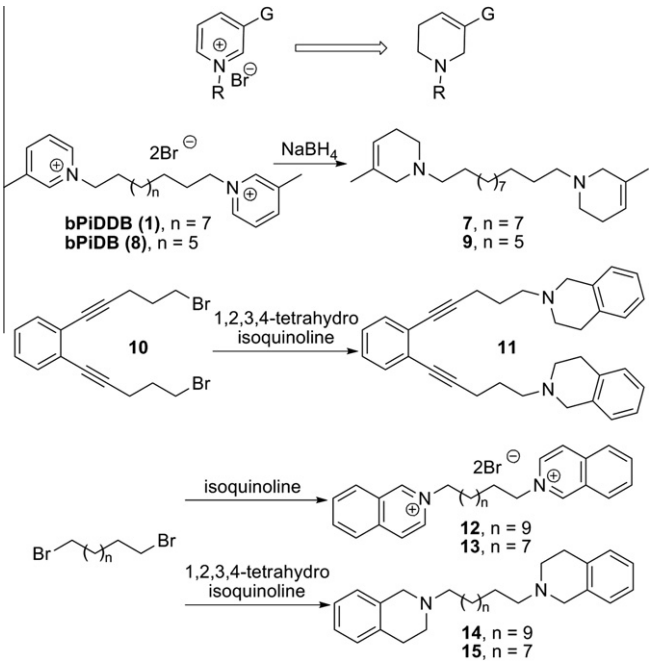


Figure 2. Structures of the TMP and mecamylamine containing compounds.

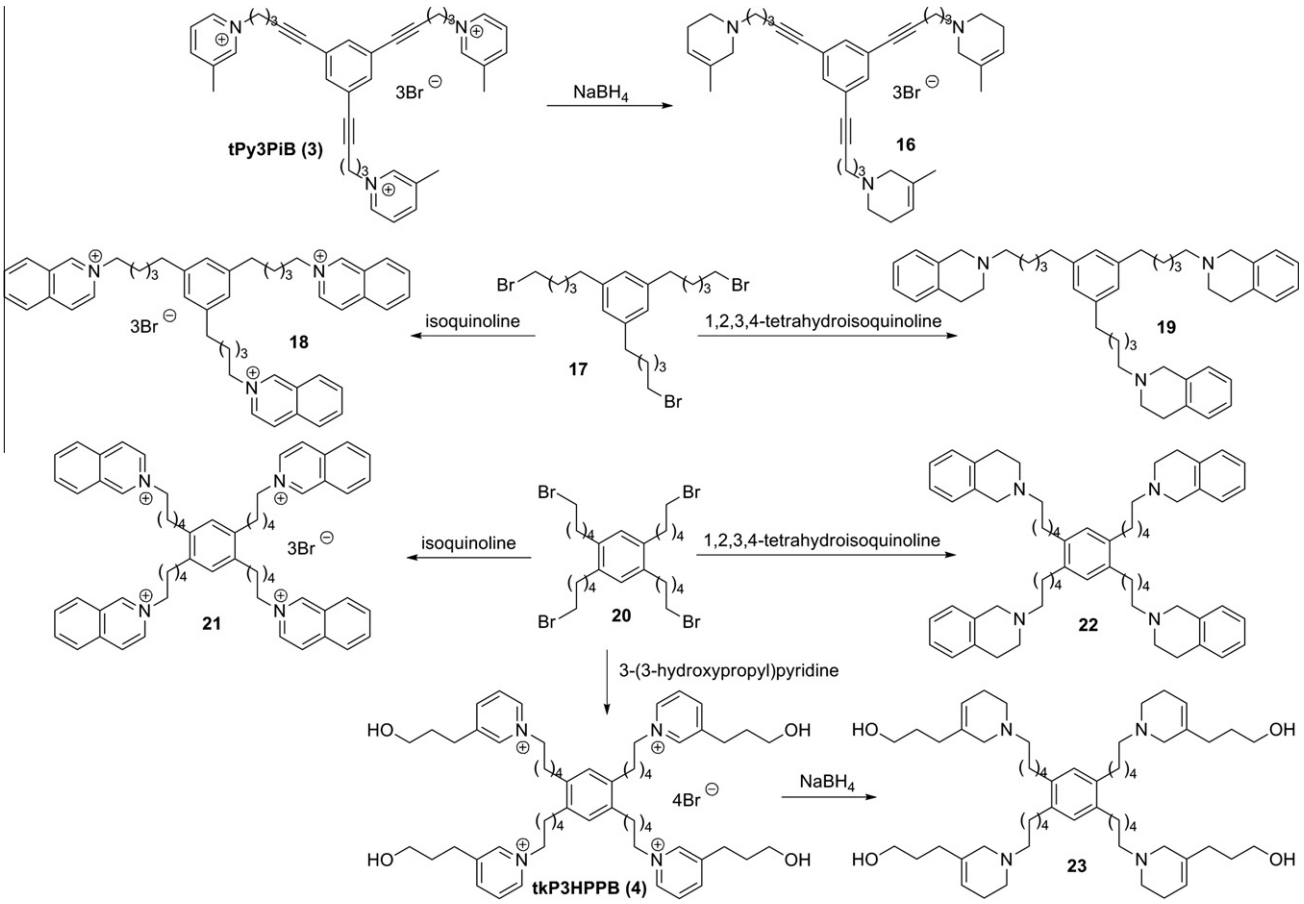
compound;[14] all of these compounds exhibited high potency and selectivity for nAChR subtype(s) mediating nicotine-evoked [^{3}H]DA release with IC_{50} values ranging from 0.2 to 3.0 nM.[12–14]

The blood–brain barrier (BBB) is comprised of juxtaposed brain capillary endothelial cells producing tight cell membrane junctions which are essentially impermeable to hydrophilic compounds.[18] Because of the cationic nature, high polarity and water-solubility of the bPiDDB molecule, access to brain by passive transport is very limited. However, results from pharmacokinetic studies utilizing radiolabeled ^{14}C-bPiDDB showed that bPiDDB was brain-bioavailable after subcutaneous delivery, due to its facilitated transport via the BBB choline transporter.[19,20] Nevertheless, quaternary ammonium compounds are generally not suitable for oral delivery, and bPiDDB has been shown to have limited bioavailability when given by the oral route (Albayati et al., unpublished data). Since oral delivery is the preferred clinical route for development of pharmaceutical products, 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.



Scheme 1. Synthesis of compounds **7**, **9**, **11**, **14** and **15**.

A quaternized pyridinium moiety is the common characteristic feature in bPiDDB, bPyiQB, tPy3PiB and tkP3HPPB molecules. Conceivably, ionic interactions of such cationic pyridinium moieties with the nAChR binding site(s) may be an important factor in



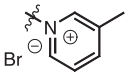
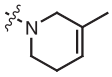
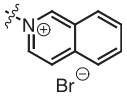
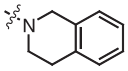
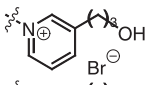
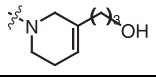
Scheme 2. Synthesis of compounds **16**, **18**, **19**, and **21**–**23**.

understanding mechanism of inhibition. In this respect, we considered that the ionic interaction of a protonated tertiary amine with binding sites on nAChRs may involve similar binding characteristics as a quaternized pyridinium moiety when the protonated tertiary amine moieties are appended to a common structural scaffold. Based on this premise, we hypothesized that analogs derived from the above quaternized ammonium lead compounds, in which the quaternary pyridinium moieties had been replaced with tertiary amine moieties (capable of being protonated at physiological pH) may retain their inhibitory interactions with nAChRs mediating nicotine-evoked DA release from striatum.

In our previous report,[21] we have shown that replacing the quaternary ammonium head groups in compound **1** and **3** with classical nAChR antagonists, mecamylamine or TMP (e.g., compounds **5** and **6**, respectively; Fig. 2) resulted in a retention of inhibitory potency. Since bPiDDB, bPyiQB, tPy3PiB, and tkP3HPPB were identified as the most important leads in the search for inhibitors of nicotine-evoked DA release, we designed tertiary amino analogs of these closely related compounds, viz.: **7** (Scheme 1), **11** (Scheme 1), **16** (Scheme 2), and **23** (Scheme 2), in which the 3-picolinium, isoquinolinium, or 3-(3-hydroxypropyl)-pyridinium headgroups in these lead compounds have been reductively transformed into their corresponding tertiary amine headgroups: 3-methyl-1,2,5,6-tetrahydropyridine, 1,2,3,4-tetrahydroisoquinoline, and 3-(3-hydroxypropyl)-1,2,5,6-tetrahydropyridine, respectively. In these structural modifications, the central structural scaffold is retained, while the head groups are de-aromatized. Initial designs in these tertiary amino analogs included retention of one double bound in the resulting piperidine ring, in order to eliminate the introduction of a chiral center into the azaheterocyclic ring, which would have led to multiple enantiomeric and diastereomeric products. The design also maintains to some degree the planar characteristics of the pyridinium moiety in the lead molecules. Additionally, compounds **9**, **14**, **15**, **19**, and **22** were synthesized; these compounds were generated from reduction of the 3-picolinium and isoquinolinium head groups in compounds **8**, **12**, **13**, **18**, and **21**, affording the corresponding analogs containing 3-methyl-1,2,5,6-tetrahydropyridine and/or 1,2,3,4-tetra-hydroisoquinoline head groups (Schemes 1 and 2).

The synthesis of the non-quaternary analog **7** was achieved through $NaBH_4$ reduction of bPiDDB (Scheme 1). A similar reductive procedure was used in the synthesis of analogs **9**, **16**, and **23**

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

	Compound		Nicotine-evoked DA Release	
	Head group	Linker	Inhibition (100 nM)[a]	IC_{50} (nM)and I_{max}[b]
bPiDDB **1**		bis-1,12-dodecane	ND[c]	2.0 ± 1.0[d] 63%
bPiDB **8**		bis-1,10-decane	ND	180 ± 110 63%
tPy3PiB **3**		tris-linker (unsaturated)	40 ± 12%	0.2 ± 0.07[e] 67%
7		bis-1,12-dodecane	50 ± 21%	0.95 ± 0.30 60%
9		bis-1,10-decane	36 ± 12%	37.4 ± 18.7 65%
16		tris-linker (unsaturated)	83 ± 2%	3.22 ± 1.36 67%
bPyiQB **2**		rigid bis-linker	ND	63 ± 39[f] 59%
12		bis-1,12-dodecane	ND	40 ± 30 53%
13		bis-1,10-decane	ND	70 ± 50 95%
18		tris-linker (saturated)	28 ± 11%	ND
21		tetrakis-linker	ND	56 ± 45 52%
11		rigid bis-linker	18 ± 18%	ND
14		bis-1,12-dodecane	ND	8.59 ± 3.27 76%
15		bis-1,10-decane	ND	9.91 ± 9.23 74%
19		tris-linker (saturated)	58 ± 9%	0.35 ± 0.09 58%
22		tetrakis-linker	84 ± 4%	205 ± 132 64%
tkP3HPPB **4**		tetrakis-linker	41 ± 15%	3.0 ± 3.0[g] 63%
23		tetrakis-linker	40 ± 23%	30 ± 16 64%

[a] Percentage inhibition at 100 nM is 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 four to six independent experiments.
[c] Not determined.
[d] Taken from results published in Ref. 15.
[e] Taken from results published in Ref. 13.
[f] Taken from results published in Ref. 12.
[g] Taken from results published in Ref. 14.

from the corresponding quaternary ammonium analogs, bPiDB (**8**), tPy3PiB (**3**) and tkP3HPPB (**4**) (Scheme 1 and 2, Table 1). The corresponding tertiary amine analog of bPyiQB (**2**), that is, compound **11**, was prepared from dibromide **10** through direct substitution with 1,2,3,4-tetrahydroisoquinoline (Scheme 1). A similar method to that utilized in the synthesis of compound **11** was applied to the synthesis of analogs **14**, **15**, **19**, and **22** (Scheme 1 and 2, Table 1). The bromide precursors **10**, **17**, and **20**, were prepared according to previously reported procedures.[12–14]

The resulting analogs[22] were initially evaluated for inhibition of nAChRs mediating nicotine-evoked [^{3}H]DA release from superfused rat striatal slices using a probe concentration of 100 nM. [^{3}H]DA release assays were performed according to a previously published method.[7] Analog-induced inhibition of nicotine-evoked [^{3}H]DA release was determined using 10 μM nicotine and 100 nM analog concentrations. The amount of inhibition is presented as a percentage of the response to nicotine under control conditions (i.e., in the absence of analog) and the values are provided in Table 1. The most active compounds (>40% inhibition) were then evaluated across a full concentration range, to determine IC_{50} and I_{max} values for inhibition of nicotine-evoked [^{3}H]DA release (Table 1).

With the exception of compound **11**, all of the tertiary amine analogs that were directly derived from the corresponding quaternary ammonium lead compounds demonstrated high potency in inhibiting nicotine-evoked DA release from rat striatal slices. These results support the validity of the hypothesis that both the quaternary ammonium lead compounds and their reduced tertiary amine analogs possibly interact at a common site on α6-containing nAChRs, and that the tertiary amine analogs likely interact at these sites in their protonated forms via ionic interactions. It is important to note that the IC_{50} values of these tertiary amine derivatives are generally within an order of magnitude of the IC_{50} values of their corresponding parent quaternary ammonium molecules. The bis-analog **7** and the tris-analog **19** were the most potent inhibitors in the tertiary amine series of compounds, with IC_{50} values of 0.91 nM and 0.35 nM, respectively.

All of the tertiary amino compounds evaluated exhibited incomplete inhibition of nicotine-evoked DA release, as indicated by their I_{max} values, which ranged from 58% to 76%. These results are consistent with previous literature, which indicates that multiple nAChRs mediate nicotine-evoked DA release. Thus, these analogs are likely acting as antagonists at only a subset of nAChR subtypes mediating nicotine-evoked DA release, and may have a unique selectivity for specific nAChR subtypes in brain.[23,24]

Due to high polarity, the quaternary ammonium lead compounds are not able to pass through the BBB through passive diffusion. However, bPiDDB has been demonstrated to be transported from the periphery into the brain by facilitated transport via the BBB choline transporter.[19,25] On the other hand, the tertiary amine analogs are expected to readily pass the BBB via passive diffusion due to improved drug-like properties such as enhanced membrane permeation properties and improved log *P* values, resulting in better brain distribution, as well improved oral bioavailability. Thus, the results from this study reveal a new direction for the discovery of more drug-like derivatives of bPiDDB and its analogs in the search for potent agents that inhibit nicotine-evoked DA release.

In conclusion, in the search for potent agents that inhibit nicotine-evoked DA release from striatum, a series of tertiary amine analogs derived from lead azaaromatic quaternary ammonium salts has been identified as having potential as tobacco cessation agents. The preliminary results suggest that these novel tertiary amine analogs, which are protonated at physiological pH, may interact with the same target nAChRs as their parent quaternary ammonium compounds. However, in contrast to their parent compounds, the tertiary amine analogs are expected to pass the BBB easily through passive diffusion, and may exhibit improved plasma and brain bioavailability via the oral route of administration.

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22. *Spectra data of selective compounds*: **7**, ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.43 (ddd, J = 6.9, 3.3, 1.8 Hz, 2H), 2.82 (dd, J = 1.8, 0.9 Hz, 4H), 2.48 (t, J = 5.7 Hz, 4H), 2.38 (dd, J = 7.8 Hz, 4H), 2.14 (m, 4H), 1.64 (d, J = 1.5 Hz, 6H), 1.53 (m, 4H), 1.20–1.38 (m, 16H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 132.29, 119.50, 58.95, 57.31, 50.14, 29.87, 27.96, 27.43, 26.32, 21.34 ppm; **16**, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.23 (s, 3H), 5.42–5.44 (m, 3H), 2.85 (br, 3H), 2.49–2.57 (m, 12H), 2.43 (t, J = 7.2 Hz, 6H), 2.10–2.16 (m, 6H), 1.77–1.87 (m, 6H), 1.63 (br, 9H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 133.71, 132.22, 124.39, 119.63, 90.90, 79.89, 57.67, 57.32, 50.17, 26.51, 26.28, 21.34, 17.85 ppm; **19**, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.00–7.16 (m, 12 H), 6.84 (s, 3H), 3.64 (s, 6H), 2.86 (t, J = 6 Hz, 6H), 2.68 (t, J = 6.0 Hz, 6H), 2.62 (t, J = 6.0 Hz, 6H), 2.48 (t, J = 6.0 Hz, 6H), 1.62–1.68 (m, 12 H), 1.41–1.46 (m, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 142.75, 135.11, 134.56, 128.84, 126.82, 126.26, 126.12, 125.76, 58.87, 56.64, 51.40, 36.29, 31.96, 29.54, 27.83, 27.56 ppm; **22**, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.08–17.10 (m, 16 H), 6.92 (s, 2H), 3.63 (s, 8H), 2.90 (t, J = 6 Hz, 8H), 2.73 (t, J = 6 Hz, 8H), 2.56 (t, J = 6 Hz, 8 H), 1.59–1.70 (m, 16 H), 1.41–1.49 (m, 8H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.78, 135.03, 134.52, 130.05, 128.81, 126.77, 126.24, 125.72, 58.87, 56.59, 51.40, 32.72, 31.79, 29.48, 28.24, 27.57 ppm; **23**, ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.88 (s, 2H), 5.46 (br, 4H), 3.59 (t, J = 6 Hz, 8 Hz), 2.85 (s, 8H), 2.43–2.60 (m, 16H), 2.37–2.49 (m, 8H), 2.15 (m, 8H), 1.98–2.03 (m, 8H), 1.58–1.69 (m, 24H), 1.36–1.43 (m, 8H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.69, 135.69, 130.03, 119.44, 62.59, 58.96, 56.02, 50.49, 32.61, 31.91, 31.67, 31.06, 28.19, 27.24, 26.17 ppm.
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