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tris-Azaaromatic quaternary ammonium salts: Novel templates as antagonists at nicotinic receptors mediating nicotine-evoked dopamine release

Guangrong Zheng, Sangeetha P. Sumithran, Agripina G. Deaciuc, Linda P. Dwoskin and Peter A. Crooks*

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

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Abstract—A series of tris-azaaromatic quaternary ammonium salts has been synthesized and evaluated for their ability to inhibit neuronal nicotinic acetylcholine receptors (nAChRs) mediating nicotine-evoked [^{3}H]dopamine release from superfused rat striatal slices and for inhibition of [^{3}H]nicotine and [^{3}H]methyllycaconitine binding to whole rat brain membranes. The 3-picolinium compound 1,3,5-tri-{5-[1-(3-picolinium)]-pent-1-ynyl}benzene tribromide (tPy3PiB), **3b**, exhibited high potency and selectivity for nAChR subtypes mediating nicotine-evoked [^{3}H]dopamine release with an IC_{50} of 0.2 nM and I_{max} of 67%.

Neuronal nicotinic acetylcholine receptors (nAChRs) are pentameric ligand-gated ion channels composed of five (α and/or β) subunits, each spanning the membrane four times and aligning around the central ion channel.[1–3] To date, 10 α (α1–α10) and four β (β1–β4) subunits have been identified.[4] Combinations of different subunits establish the various nAChR subtypes, and have distinct pharmacological profiles.[5,6] Activation of specific subtypes of nAChRs contributes to the reinforcing effects of nicotine by stimulating release of the neurotransmitter dopamine (DA).[7–13] Nicotine-evoked DA release is calcium-dependent and is blocked completely by the classical nAChR inhibitors, mecamylamine and DHβE, demonstrating that this effect is a direct action at nAChRs.[10]

Subtype-selective nAChR antagonists, which inhibit nicotine-evoked DA release, have been proposed as potential therapeutic agents for nicotine addiction.[14–17] To date, the nAChR subtype(s) mediating nicotine-evoked DA release and the mechanism of inhibition of the inhibitors at this receptor subtype have not been elucidated conclusively. Also, in contrast to extensive research on subtype-selective nAChR agonists, less attention has focused on the development of subtype-selective nAChR antagonists.[16,17] The availability of subtype-selective nAChR antagonists would augment the identification of the antagonist pharmacophore(s) for the nAChR subtype(s) mediating nicotine-evoked DA release. Moreover, such subtype-selective antagonists may have potential as treatments for nicotine dependence.

Previous studies from our laboratories have identified *N*,*N*′-dodecane-1,12-diyl-bis-3-picolinium dibromide (bPiDDB; **1**; Fig. 1) as a potent inhibitor (IC_{50} = 5 nM) of nAChRs mediating nicotine-evoked [^{3}H]DA release from superfused rat striatal slices.[18,19] In vivo microdialysis studies also demonstrated that pretreatment with bPiDDB dose-dependently reduced nicotine-evoked extracellular DA release in rat nucleus accumbens.[20] Moreover, behavioral studies in rats showed that bPiDDB dose-dependently decreased intravenous nicotine self-administration, but not sucrose-maintained responding, suggesting a specific inhibition of nicotine reward.[21] bPiDDB was shown to have no affinity at α4β2* and α7* nAChR, ligand binding sites.[18,22] bPiDDB inhibited nicotine-evoked [^{3}H]DA overflow by a maximum of 68% in striatum,[18] suggesting the involvement of at least two nAChR subtypes in this response to nicotine. Similarly, the *Conus* neurotoxin, α-conotoxin-MII (α-CTX MII), was found to inhibit

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* Corresponding author. Tel.: +1 859 257 1718; fax: +1 859 257 7585; e-mail: pcrooks@email.uky.edu

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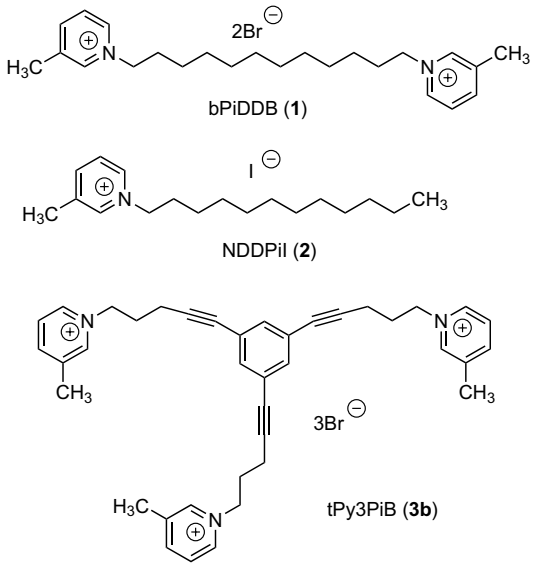


Figure 1. Structures of bPiDDB (**1**), NDDPiI (**2**), and tPy3PiB (**3b**).

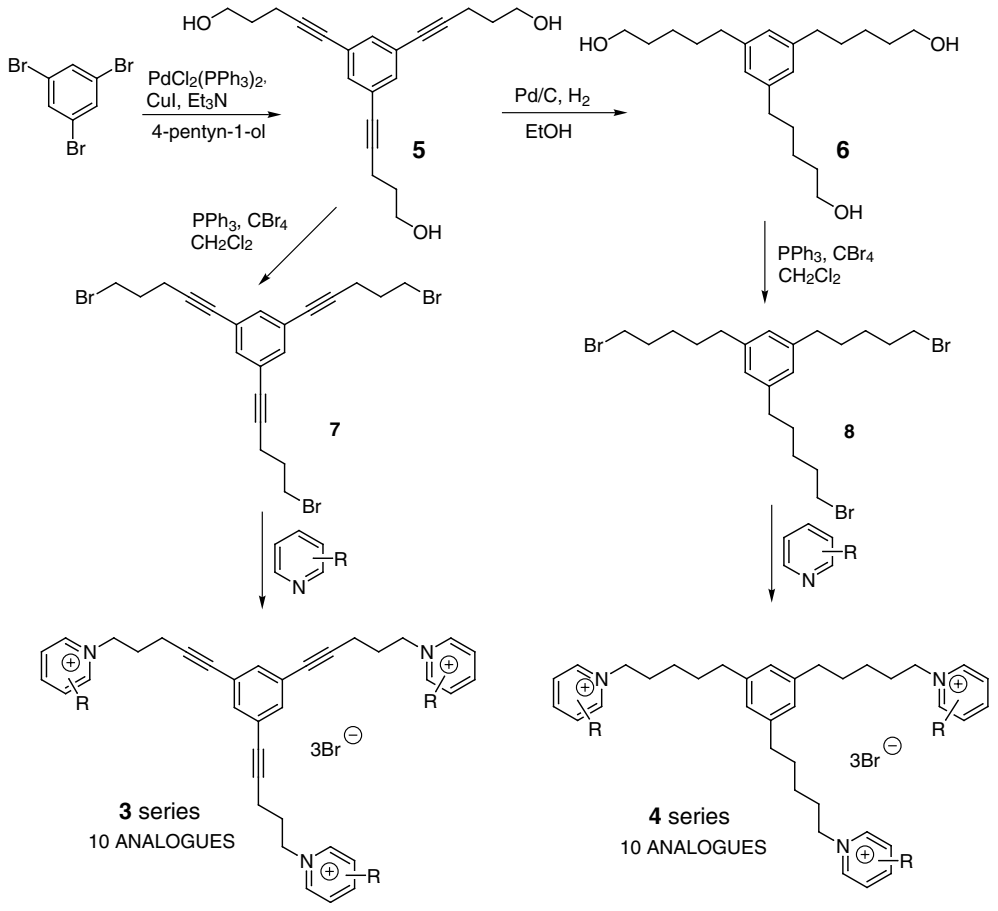
50% of nicotine-evoked [^{3}H]DA overflow from striatal preparations,[23–29] consistent with the contention that more than one nAChR subtype mediates nicotine-evoked DA release.

bPiDDB is a member of the bis-azaaromatic quaternary ammonium series[18,22] and is characterized by the presence in the molecule of two cationic head groups connected by a N–N lipophilic linker. Based on the structural characteristics of this series of compounds, one may speculate that these molecules bind at complementary anionic binding sites at the nAChR protein. In initial studies, a series of mono-azaaromatic quaternary ammonium compounds was synthesized and assessed for activity in the nicotine-evoked [^{3}H]DA release assay.[18,30] From these studies, *N*-*n*-dodecyl-3-picolinium iodide (NDDPiI; **2**; Fig. 1), a compound containing only one cationic head group rather than the two cationic head groups in the bPiDDB molecule, inhibited nicotine-evoked [^{3}H]DA release with an IC_{50} of 30 nM and an I_{max} of 62%.[18] Taken together, these results suggest that the second positively charged picolinium head group increased the affinity, but did not appear to change the selectivity for specific nAChRs mediating nicotine-evoked DA release. The focus of the current study was to assess the effect of introducing a third picolinium head group into the bPiDDB molecule on inhibition of nicotine-evoked DA release. Thus, analogues with three cationic head groups were synthesized and evaluated herein. An increase in inhibitory potency in the progression from mono to bis to tris cationic head groups in these molecules would indicate at least three polar interactions between the cationic groupings in the molecule and the anionic sites on the receptor protein.

Two series of tris-azaaromatic quaternary ammonium compounds, that is, 1,3,5-tri-(pent-1-ynyl-5-azaaromatic quaternary ammonium)-benzene salts (**3** series, Scheme 1) and 1,3,5-tri-(*n*-pentyl-5-azaaromatic quaternary ammonium)-benzene salts (**4** series, Scheme 1), were prepared for evaluation. The tris-quaternary ammonium compounds were constructed using a scaffold incorporating a central phenyl ring from which was appended three identical quaternary ammonium head groups in a 1,3,5 orientation, and tethered to the phenyl ring via linker units to afford *N*-*N*′ inter-atomic distances approximating that in bPiDDB (**1**). The linker units were either saturated or unsaturated; the unsaturated linker incorporating a triple bond conjugated to the central phenyl ring. These compounds were prepared from corresponding azaaromatic free bases, including 2-, 3-, and 4-picolines, 3,4- and 3,5-lutidines, quinoline, isoquinoline, 3-phenyl- and 3-*n*-butylpyridines, and nicotine (Table 1), by reacting each free base with tribromide 1,3,5-tri-(5-bromopent-1-ynyl)-benzene (**7**) or 1,3,5-tri-(5-bromopentyl)-benzene (**8**). Tribromide **7** was synthesized by initial Sonogashira coupling[31] of 1,3,5-tribromobenzene with 4-pentyn-1-ol, to afford the tri-hydroxyl compound **5**, followed by bromination using PPh_3/CBr_4.[32] Tribromide **8** was prepared by catalytic hydrogenation of the Sonogashira coupling product **5**, to afford compound **6**, followed by PPh_3/CBr_4 bromination (Scheme 1). The resulting tris-azaaromatic quaternary ammonium compounds were evaluated for inhibition of nAChRs mediating nicotine-evoked [^{3}H]DA release from superfused rat striatal slices. Also, interaction of these compounds with $\alpha4\beta2^*$ and $\alpha7^*$ nAChR subtypes was assessed in (*S*)-[^{3}H]nicotine (NIC) and [^{3}H]methyllycaconitine (MLA) binding assays using rat brain membranes, respectively. The effect of each of these novel analogues was determined at 100 nM using rapid-throughput screening assays. Promising compounds were then evaluated across a full concentration range to determine the IC_{50} and I_{max} values of inhibition of nicotine-evoked DA release.

[^{3}H]NIC and [^{3}H]MLA binding assays were performed according to previous methods,[33] using 3 nM and 2.5 nM concentrations of radioligand, respectively, and 10 μM cytisine and 10 μM nicotine to assess nonspecific binding to whole brain membranes. Analogues were evaluated at a probe concentration of 100 nM. Amount of inhibition is presented as a percentage of radioligand binding in the absence of analogue (control, Table 1). [^{3}H]DA release assays were performed according to a previously published method.[33] Analogue-induced inhibition of nicotine-evoked [^{3}H]DA release was determined using 10 μM NIC and 100 nM analogue concentrations. Amount of inhibition is presented as a percentage of the response to nicotine under control conditions (in the absence of analogue) and the values are provided in Table 1. IC_{50} and I_{max} values of inhibition of nicotine-evoked [^{3}H]DA release were determined using 10 μM NIC and a full concentration range (1 nM to 10 μM) of analogue, and then calculated using an iterative nonlinear least squares curve-fitting program, PRISM version 4.0 (GraphPAD Software, Inc., San Diego, CA).[33]

All the analogues in the **3** and **4** series with the exception of the tris-nicotinium compounds exhibited low or no



Scheme 1. The synthesis of 1,3,5-tri-(pent-1-ynyl-5-azaaromatic quaternary ammonium)-benzene salts, **3**, and 1,3,5-tri-(*n*-pentyl-5-azaaromatic quaternary ammonium)-benzene salts, **4**.

affinity at $\alpha4\beta2^*$ nAChRs probed by [^{3}H]NIC binding. tris-Nicotinium compounds in both the **3** and **4** series (**3f** and **4f**, respectively) displayed significant potency at $\alpha4\beta2^*$, affording 44% and 88% inhibition, respectively. These results with the nicotinium analogues are consistent with our previous results with mono-nicotinium and bis-nicotinium analogues.[19,30,34] Several analogues, that is, **3g**, **3i**, **3j**, and **4i**, displayed some potency at $\alpha7^*$ nAChRs. Notably, all of these four analogues bear bulky, more hydrophobic cationic head groups. The absence or presence of unsaturation in the linker units also appears to contribute to the inhibitory potency at the [^{3}H]MLA binding site, that is, analogues **3g**, **3i**, and **3j** in the **3** series were more potent when compared to their more flexible counterparts, **4g**, **4i**, **4j**, in the **4** series, indicating that rigidity is important for binding of these compounds at $\alpha7^*$ nAChRs.

In general, a large number of compounds in the tris-series of quaternary ammonium compounds exhibited inhibitory potency in the nicotine-evoked [^{3}H]DA release assay, that is, 47% of the compounds exhibited inhibition of >40%. Furthermore, the absence or presence of unsaturation in the linker units had less impact on inhibition of nicotine-evoked [^{3}H]DA release. From the data in Table 1, it is apparent that introducing identical head group changes in general structures **3** and **4** does not result in a parallelism in the inhibition of nicotine-evoked DA release. Thus, no clear structure-activity relationship (SAR) is obvious within these series of compounds. Theoretically, parallel structural changes within two series of analogues can result in parallel shifts in potency if the two series of compounds are binding in a similar manner to the *same* binding site. However, based on the available data, it is premature to conclude that molecules in the **3** and **4** series are binding to different sites on the receptor protein.

Evaluation of the full concentration response relationship for inhibition of nicotine-evoked [^{3}H]DA release for several selected lead compounds, that is, **3a**, **3b**, and **4b**,[35] revealed IC_{50} value of 4.0 nM, 0.2 nM, and 2.0 nM, respectively (Table 1). Notably, the tris-compound **3b** (1,3,5-tri-{5-[1-(3-picolinium)]-pent-1-ynyl} benzene tribromide (tPy3PiB)), which has the same 3-picolinium head groups as in bPiDDB molecule, is 25-fold more potent than bPiDDB in inhibiting nicotine-evoked DA release. I_{max} values for **3a**, **3b**, and **4b** were in the range 50–67%, which is similar to the I_{max} values for NDDPiI and bPiDDB, indicating that the mono-, bis-, and tris-series of quaternary ammonium compounds that contain 3-picolinium head groups likely exhibit similar selectivity for inhibition of nicotine-evoked DA release. To our knowledge, tPy3PiB is the most potent non-peptide small molecule inhibitor of nicotine-evoked DA release.

Table 1. Inhibition of [^{3}H]NIC binding (probing α4β2* nAChRs) and [^{3}H]MLA binding (probing α7* nAChRs) to rat brain membranes, and nicotine-evoked [^{3}H]DA release from superfused rat striatal slices

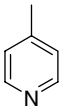
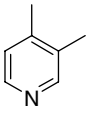
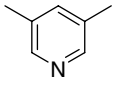
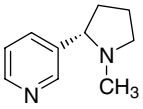
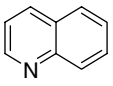
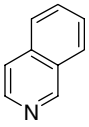
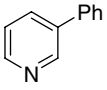
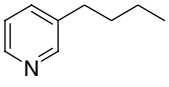
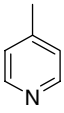
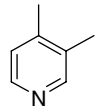
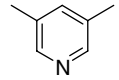
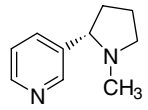
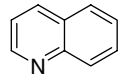
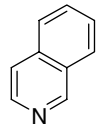
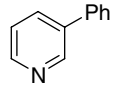
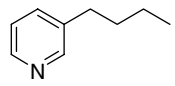
	Compounds		[^{3}H]NIC binding (% inhibition at 100 nM)[a]	[^{3}H]MLA binding (% inhibition at 100 nM)[a]	Inhibition of nicotine-evoked [^{3}H]DA release (% inhibition at 100 nM)[a]
					
3 Series	**3a**		4.5 ± 2.0%	0 ± 0%	51 ± 15% IC_{50} = 4 nM[b]
	3b		10 ± 1.9%	0 ± 0%	40 ± 12% IC_{50} = 0.2 nM[b]
	3c		21 ± 4.5%	0 ± 0%	32 ± 8%
	3d		4.0 ± 1.4%	1.8 ± 1.8%	18 ± 18%
	3e		8.9 ± 2.9%	0 ± 0%	27 ± 12%
	3f		44 ± 3.1%	4.2 ± 2.2%	59 ± 17%
	3g		7.0 ± 3.8%	21 ± 6.0%	29 ± 18%
	3h		9.2 ± 5.0%	4.5 ± 2.6%	44 ± 11%
	3i		15 ± 0.9%	39 ± 2.2%	29 ± 12%
	3j		6.80 ± 2.0%	30 ± 2.0%	28 ± 15%
4 Series	**4a**		8.6 ± 7.2%	0 ± 0%	27 ± 10%
	4b		14 ± 6.1%	1.2 ± 1.2%	58 ± 20% IC_{50} = 2 nM[b]
	4c		6.0 ± 3.4%	0 ± 0%	37 ± 17%

Table 1 (*continued*)

Compounds		[^{3}H]nicotine binding (% inhibition at 100 nM)[a]	[^{3}H]MLA binding (% inhibition at 100 nM)[a]	Inhibition of nicotine-evoked [^{3}H]DA release (% inhibition at 100 nM)[a]
				
4d		9.3 ± 4.1%	0.2 ± 0.2%	50 ± 21%
4e		11 ± 2.9%	6.5 ± 3.6%	39 ± 18%
4f		81 ± 2.3%	7.9 ± 7.1%	26 ± 17%
4g		5.7 ± 5.7%	6.8 ± 0.9%	8.0 ± 6.4%
4h		8.4 ± 7.6%	2.5 ± 1.5%	28 ± 11%
4i		14 ± 5.8%	19 ± 1.7%	6.0 ± 5.0%
4j		6.2 ± 4.0%	4.4 ± 3.3%	68 ± 14%

[a] Each value represents data from at least three independent experiments, each performed in duplicate.
[b] IC_{50} of full concentration response assay, data from at least five independent experiments, each performed in duplicate.

The IC_{50} values obtained for the mono-, bis-, and tris-picolinium analogues, NDDPiI, bPiDDB, and tPy3PiB were 30 nM, 2.0 nM, and 0.2 nM, respectively, with I_{max} values of 62%, 68%, and 67%, respectively. These results clearly indicate that introduction of additional 3-picolinium head groups into the NDDPiI structure greatly augments inhibitory potency and may be attributed to an increase in the number of ionic interactions with putative negatively charged binding sites on nAChR proteins mediating nicotine-evoked DA release. However, one must also consider the potential contributions of the central planar aromatic moiety and triple bond in the linker units with respect to the improved potency of tPy3PiB compared with bPiDDB and NDDPiI. Compound **3b** has also been evaluated in the functional assay by carrying out surmountability experiments to determine orthosteric or allosteric inhibition of *S*-(−)-nicotine, and preliminary results (Smith et al., unpublished results) indicate that the inhibitory effect of compound **3b** can be overcome by increasing concentrations of *S*-(−)-nicotine, consistent with an orthosteric mechanism of inhibition.

In summary, the novel tris-quaternary ammonium compounds described in the current study represent new leads in our search for subtype-selective nAChR antagonists as treatments for nicotine addiction.

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35. Spectral data of compounds **3a**, **3b**, and **4b**: for compound **3a**, ^{1}H NMR (300 MHz, CD_3OD) δ 9.01 (dd, J = 6.3, 0.9 Hz, 3H), 8.44 (dt, J = 7.8, 1.5 Hz, 3H), 7.92–8.07 (m, 6H), 7.41 (s, 3H), 4.81 (t, J = 6.0 Hz, 6H), 2.98 (s, 9H), 2.70 (t, J = 7.2 Hz, 6H), 2.29 (m, 6H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 157.1, 146.8, 146.6, 134.9, 131.6, 127.1, 125.4, 90.1, 81.6, 58.4, 29.9, 20.7, 17.3 ppm; ESI-MS: *m/z* 710.0/711.9/713.9 $[M]^{1+}$ (calcd for $[C_{39}H_{42}N_3Br_2]^{1+}$ 710.18/712.18/714.18), 632.1 $[M]^{2+}$ (calcd for $[C_{39}H_{42}N_3Br]^{2+}$ 631.26/633.26); **3b**, ^{1}H NMR (300 MHz, CD_3OD) δ 9.02 (s, 3H), 8.92 (d, J = 6.0 Hz, 3H), 8.40 (d, J = 8.4 Hz, 3H), 8.01 (dd, J = 8.1, 6.0 Hz, 3H), 7.35 (s, 3H), 4.81 (t, J = 7.2 Hz, 6H), 2.64 (t, J = 6.9 Hz, 6H) 2.58 (s, 9H), 2.35 (m, 6H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 147.5, 145.8, 143.4, 141.3, 134.9, 128.8, 125.3, 89.9, 81.5, 62.2, 31.0, 18.7, 17.2 ppm; ESI-MS: *m/z* 711.7 $[M]^{1+}$ (calcd for $[C_{39}H_{42}N_3Br_2]^{1+}$ 710.18/712.18/714.18), 632.0 $[M]^{2+}$ (calcd for $[C_{39}H_{42}N_3Br]^{2+}$ 631.26/633.26); **4b**, ^{1}H NMR (300 MHz, CD_3OD) δ 8.94 (s, 3H), 8.84 (d, J = 6.0 Hz, 3H), 8.42 (d, J = 8.4 Hz, 3H), 7.98 (dd, J = 8.1, 6.0 Hz, 3H), 6.86 (s, 3H), 4.62 (t, J = 7.8 Hz, 6H), 2.59 (s, 9H), 2.57 (t, J = 7.5 Hz, 6H) 2.06 (m, 6H), 1.69 (m, 6H), 1.43 (m, 6H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 147.2, 145.5, 143.4, 143.0, 141.2, 128.7, 127.2, 62.9, 36.7, 32.5, 32.2, 27.0, 18.7 ppm; ESI-MS: *m/z* 722.1/724.1/726.1 $[M]^{1+}$ (calcd for $[C_{39}H_{42}N_3Br_2]^{1+}$ 722.27/724.27/726.27).