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Bis-azaaromatic quaternary ammonium salts as ligands for the blood–brain barrier choline transporter

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ABSTRACT

A series of *bis*-azaaromatic quaternary ammonium compounds containing flexible polymethylenic linkers as well as conformationally restricted linkers were evaluated for their affinity for the blood–brain barrier choline transporter (BBB-ChT). The preliminary structure–activity relationships obtained from this study suggest that incorporating a linear, conformationally restricted linker into the molecule improves affinity for the BBB-ChT.

The blood–brain barrier (BBB) choline transporter (ChT) facilitates the transport of the highly polar, positively charged choline molecule across the BBB from periphery to the central nerves system.[1] Choline is a biochemical precursor of the neurotransmitter acetylcholine, and of other essential components of cell membrane phospholipids such as phosphatidylcholine.[2] In vivo and in vitro experiments have demonstrated that carrier-mediated transport is the primary mechanism of total brain uptake of choline.[3,4] The BBB-ChT has an anionic binding area that accommodates positively charged quaternary ammonium moieties or simple cations, such as tetramethylammonium ion.[4] Thus, the BBB-ChT has been suggested as a possible portal for the delivery of pharmacologically relevant concentrations of positively charged drugs, which normally exhibit restricted permeation by passive diffusion across the BBB. In this respect, recent studies have shown that this transport system can be utilized as a brain delivery vector for a series of newly developed azaaromatic quaternary ammonium compounds, which are antagonists at neuronal nicotinic acetylcholine receptors (nAChRs), and that have potential use as smoking cessation agents.[5–9] For example, *N*-n-octylnicotinium iodide (NONI; **1**; Fig. 1), which binds to the BBB-ChT with an affinity comparable (K_i ~49 μM) to that of choline (K_i ~45 μM), was demonstrated to be transported by the BBB-ChT into the brain from the periphery.[5] In addition, *N*,*N*′-dodecyl-*bis*-picolinium bromide (bPiDDB; **2**; Fig. 1), a *bis*-quaternary ammonium compound which is currently being investigated as a lead compound for smoking cessation,[10–18] has also been shown to have good affinity (K_i ~36 μM) for the BBB-ChT, and enters the brain at least in part via this transporter.[8,9,19] These results are very promising, given the fact that NONI and bPiDDB are impermeable to the BBB through passive transport because of their high polarity and hydrophilicity as quaternary ammonium compounds.

Prompted by these results, we set out to design analogs of these molecules that would further enhance the affinity of these agents for the BBB-ChT in order to increase their accessibility to the brain.

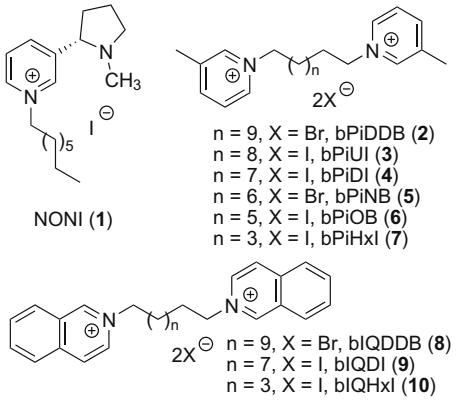

Figure 1. Structures of NONI (**1**), bPiDDB (**2**), and bPiDDB analogs **3**–**10**.

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Accordingly, we initially focused on generating structure–affinity relationships (SAR) of bPiDDB and its analogs, with the goal of identifying the structural features that would allow such compounds to pass the BBB through carrier-mediated transport.

In the absence of the crystal structure of the BBB-ChT, design of potential ligands from lead structures such as bPiDDB (**2**) becomes a valuable method in exploring the SAR and probing the topology of the transporter binding site. bPiDDB contains two 3-picolinium head groups and a linear 12-methylene linker moiety (Fig. 1). Comparative molecular field analysis (COMFA) models have been constructed to explain the binding of bPiDDB at BBB-ChT, and it has been speculated that the tethered second cationic group is required for high affinity interaction or binding at the BBB-ChT. The incorporation of two cationic moieties into the molecule may compensate for the lack of a hydroxyl group thought to be necessary for hydrogen bonding at the choline binding site. One of the cationic moieties may initially bind to the anionic site where choline binds on the BBB-ChT, anchoring the molecule to the transporter. Subsequently, the second cationic moiety may then bind to a second anionic site on the transporter, which is different from the hydrogen bonding site that normally accommodates the hydroxy group of choline.[8,20,21] Because of the high degree of flexibility of the polymethylenic linker in bPiDDB, the molecule may twist or bend, when too long, to allow the two cationic head groups to access the two complementary anionic binding sites on the transporter. If this is the case, an optimal length of polymethylenic linker should be identifiable when exploring the SAR in an homologous series. Thus, we first evaluated a series of bPiDDB homologs with shorter *N,N′*-alkyl linker units (Fig. 1) for their binding affinity at the BBB-ChT. Unlike the results from the dopamine (DA) release assay, which showed that analogs with shorter linker units have decreased potency in inhibition of nicotine-evoked DA release in rat striatum when compared with bPiDDB,[16] shortening the *N,N′*-alkyl linker units from 12 methylene units to 11, 10, 9, 8, or even 6 methylene units (corresponding to compounds **3**–**7**, respectively, Fig. 1) caused little or no change in affinity for the BBB-ChT (K_i in the range 22–36 μM, Table 1).

Apart from the bPiDDB homologs, previous studies have also revealed that the affinities of the *bis*-isoquinolinium homologs (Fig. 1), **8** (12-methylene linker), **9** (10-methylene linker), and **10** (6-methylene linker) for the BBB-ChT were also similar to each other (K_i in the range of 9.5–29 μM, Table 1).[8]

One possible explanation for the above phenomenon is that multiple secondary cationic interaction sites at various distances from the primary cationic interaction site (i.e., where the cationic head group of choline binds) may exist in the binding pocket, which can accommodate a range of *bis*-quaternary ammonium compounds that incorporate a variety of linker lengths. Another possible explanation is that the two cationic interaction sites in the BBB-ChT binding pocket are rather close to each other, allowing the flexible *bis*-quaternary ammonium compounds to bind to the transporter in a folded conformation. In the former binding mode, analogs bind in somewhat similar low energy conformations, whereas in the latter mode, they bind in similar manner but in a comparatively higher energy conformation.

To further understand the SAR of the binding of these *bis*-quaternary ammonium compounds, we next investigated the possible binding conformation of the bPiDDB molecule at the BBB-ChT. As mentioned above, bPiDDB is a highly flexible molecule, which can adopt a large number of conformations, from low energy, fully extended conformations to high energy folded hairpin-like conformations. A better understanding of the potential binding conformation of bPiDDB at the BBB-ChT may help elucidate the binding mode, and may also provide some insight into the nature of the pharmacophore requirements for optimal binding at this transporter. Rigid analogs that conformationally restrict molecules into folded, extended or other unique structures can help determine the active conformation of the flexible *bis*-azaaromatic aromatic quaternary ammonium compounds. A series of previously reported conformationally restricted bPiDDB analogs, **11**–**13** (Fig. 2), which incorporate a benzene ring and two triple bonds into the middle of the *N,N′* linker unit, thereby constraining these molecules into an extended or angular geometry, served as model compounds for the purpose of this study.[22]bPiDDB (**2**) and its homologs **3–7** were prepared according to a previous report by Ayers et al.[23] The synthesis of the conformationally restricted analogs **11–14** has also been described previously.[22] Analogs **15–17** were prepared in the same manner as analogs **13** and **14** utilizing the appropriate azaaromatic head group precursors.

The above *bis*-azaaromatic quaternary ammonium analogs were evaluated for their ability to inhibit [^{3}H]-choline uptake into brain, providing an indication of the ability of these analogs to interact with the choline transporter. These assays were conducted using the in situ rat brain perfusion method of Takasato et al.,[24] as modified by Allen and Smith.[1,25] Inhibition coefficients (K_i, concentration of analog inhibiting 50% of [^{3}H]-choline uptake into brain) were determined (n = 4–6 animals) using a single inhibitor concentration as previously described.[26] K_i values were compared by ANOVA followed by Bonferoni's multiple comparisons test to determine analog inhibition of [^{3}H]-choline uptake.

Among these conformationally restricted bPiDDB analogs, the 1,2-isomer, compound **11** (K_i = 319 μM), and the 1,3-isomer, compound **12** (K_i = 140 μM), exhibited significantly lower binding affinity at BBB-ChT than the 1,4-isomer, compound **13**

Table 1
Affinity of choline, NONI, *bis*-3-picolinium, and *bis*-isoquinolinium compounds for the BBB-ChT

Compound	K_i (μM)
Choline	41
NONI (**1**)	49
bPiDDB (**2**)	36
3	36
4	22
5	30
6	24
7	26
8	22
9	10
10	29

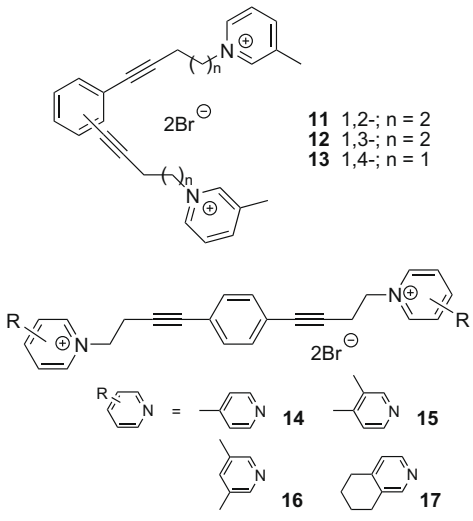

Figure 2. Structures of conformationally restricted analogs **11**–**17**.

Table 2
Affinity of compounds **11**–**17** for the BBB-ChT

Compound	K_i (μM)
11	319
12	140
13	15
14	8.5
15	8.4
16	7.4
17	5.4

(K_i = 14.8 μM) (Table 2). In addition, between the two angular compounds, the BBB-ChT binding affinity of the 1,2-isomer **11** is slightly lower than the less folded 1,3-isomer **12**. These results may indicate that the active conformation of bPiDDB for binding at BBB-ChT is in an extended conformation, rather than in a more angular or folded conformation, as is reflected in the structures of the 1,2- and 1,3-isomers.

Investigations into the SAR of the cationic head-groups of the bPiDDB molecule were also carried out. As shown in Table 1, changing the head groups from 3-picolinium to isoquinolinium was well tolerated by the BBB-ChT, as indicated from the similarity of the binding affinities between these two homologous series. Similarly, modification of the cationic head groups of the conformationally restricted 1,4-isomer **13** by replacing the 3-picolinium head groups with 4-picolinium (compound **14**), 3,4-lutidinium (compound **15**), 3,5-lutidinium (compound **16**), or 5,6,7,8-tetrahydroisoquinolinium (compound **17**) (Fig. 2) head groups resulted in little change in affinity for the BBB-ChT (Table 2). These results indicate that the cationic interaction sites on the BBB-ChT are generally insensitive to the changes in the azaaromatic quaternary ammonium head groups, which provides an opportunity to further improve the nAChR potency and selectivity of bPiDDB analogs through head groups modification without altering affinity for BBB-ChT.

Generally, the conformationally restricted 'extended' analogs **13–17** were more potent ligands than the flexible bPiDDB analogs, which further indicates that bPiDDB binds to BBB-ChT in a linear conformation, and also precludes the possible folded binding mode. The *N,N′* distance in compound **13** is 16 Å (energy-preferred measurement), which is in-between the *N,N′* distance of a 12-carbon methylene linker (16.7 Å) and an 11-carbon methylene linker (15.3 Å) in bPiDDB series. In addition, the *N,N′* distances in compound **11** (9.9 Å) and compound **12** (15.4 Å) are close to a 6-carbon (9.1 Å) and an 11-carbon (15.3 Å) methylene linker, respectively. Since bPiDDB analogs with shorter linker units had similar binding affinity at the BBB-ChT compared to bPiDDB, one would assume that shortening the linker length in the conformationally restricted analogs should not significantly change the affinity for this transporter. A possible explanation for the significantly decreased affinity of the 'angular' compounds is that the choline binding pocket of the BBB-ChT is likely narrow or restricted; thus, the linkers in compound **11** and **12** are too bulky and rigid for effective binding.

In summary, bPiDDB analogs with flexible linkers, as well as analogs with conformationally restricted linkers were evaluated for their affinity for the BBB-ChT. Improvements in affinity for the BBB-ChT have been achieved with these conformationally restricted bPiDDB analogs. Thus, incorporating a linear, conformationally restricted linker into the bPiDDB structure improves affinity for the BBB-ChT. The results suggest that an 'extended' conformation is preferred by the BBB-ChT binding site. In addition, modifications of the head groups appear to be well tolerated. This SAR information can be incorporated into the design of a new generation of subtype-selective nAChR inhibitors with high affinity for the BBB-ChT, and which will be effective in vivo inhibitors of neuronal nAChRs involved in nicotine-evoked DA release.

Acknowledgment

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