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Synthesis of Alkylene-linked Dimers of (-)-Huperzine A

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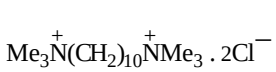
Abstract: Six alkylene-linked dimers of (-)-huperzineA (**6**) were prepared. All these dimers are less potent than (-)-Hup A in inhibition of AChE.

Keywords: Alkylene-linked dimers, (-)-huperzineA, Acetylcholinesterase.

In literature there are a number of dimer drugs or bio-active compounds which are derived structurally from the same pharmacophoric moiety *via* connection of two monomers by a tether chain. Decamethonium chloride **1** is the first depolarizing neuromuscular blocking agent. The SAR study on bis-quaternary ammonium salts with varying numbers of methylene unites demonstrated that maximal neuromuscular blockade occurred with 10 to 12 methylene unites. Another example is succinylcholine chloride **2**, which represents two molecules of acetylcholine connected at the carbons of acetyl groups[1].



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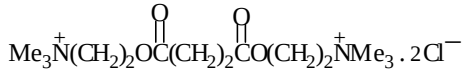

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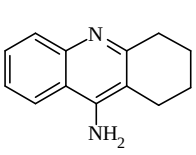

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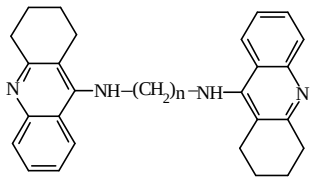

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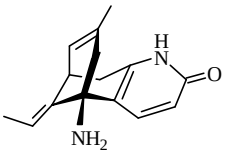

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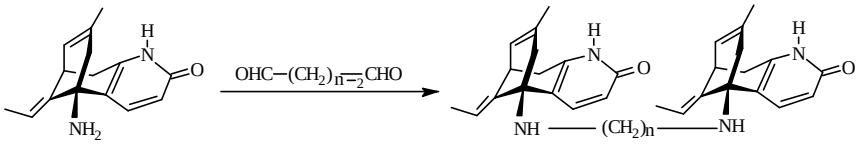
In 1996, Pang and his co-workers reported the synthesis and AChE inhibition of the alkylene linked bis-tacrine analogs **4**. Among them, the heptylene-linked bis-tacrine (**4,** n=7) showed the optimal inhibitory potency and selectivity of AChE. It was found to be 149 times more potent and 250 times more selective in inhibiting rat brain AChE than tacrine **3**[2,3,4] as a result of simutaneous binding of two tacrine unites to both central and peripheral active sites of *Torpedo* AChE. (-)-Huperzine A (**5**, HupA), an alkaloid isolated from Chinese herb *Huperzia serrata*, is a potent, selective and reversible AChE inhibitor that shows promise for the palliative treatment of Alzheimer's disease. It is 4-fold more potent *in vitro* for AChE inhibition than tacrine[5].

The X-ray crystallographic studies of HupA-*Torpedo* AChE complex by Sussman *et al*. demonstrated that **5** was bound in the central catalytic site of the active gorge of AChE[6]. However, the docking studies by Pang *et al*. showed the affinity of **3** for both central and peripheral sites of AChE[7].

Based on the hypothesis with respect to two binding sites in the active gorge of AChE and the good example of bis-tacrine, we undertook the synthetic and docking studies of bis-HupA with various length of the alkylene tethers.

For the preparation of bis-HupA analogs **6**, a few methods were tested including the reaction of HupA **5** with α, ω-dihaloalkane in the presence of silver carbonate, and α, ω-diacylalkane dihalides as well, but no desired products were obtained. When reductive-amination of α, ω-alkylenedial with **5** was conducted by sodium cyanoborohydride, the corresponding analogs **6** were furnished in reasonable yields. Six dimers of (-)-HupA were thus prepared (**Scheme 1**).

Scheme 1



5 **6**

Reagents and conditions: $NaBH_3CN$, HOAc, EtOH, rt., 24 h, 25-45%.

The reaction was carried out in medium at pH 5-6, and the dials, due to the instability, should be used immediately after preparation by PCC oxidation of the corresponding diols. The yields of dimers decreased with shortening of tethers. No dimer was obtained when 1,6-hexandial was used for this reaction. In consideration of the sterically hindered amino group and the bulky bridged monomer, it is resonable that yields of all dimers are less than 50%. The results are shown in **Table 1**. All these dimers are less active than HupA in inhibition of AChE.

General procedure for preparation of dimers of (-)-Hup A 6

To a stirred solution of HupA (49.4 mg, 0.2 mmol) and dial (0.1mmol,freshly prepared) in anhydrous ethanol (1.0 mL) was added $NaBH_3CN$ (40.0 mg, 0.6 mmol), then the pH of the solution was adjusted to 5-6 with acetic acid. The mixture was continuously stirred at room temperature for 24 h. The solvent was removed *in vacuo*, water (5 mL) was then added to. The pH of the mixture was adjusted to 9 with 10% NaOH, and the mixture was extracted with methylene chloride (3×5 mL), and the combined organic layer was dried over anhydrous Na_2SO_4.

Table 1 Physical properties and AChE inhibition of dimers of HupA (**6**)

n	$[\alpha]_D^{25}$ (c=0.1,$CHCl_3$)	mp°C, (dec)	Yield (%)	IC_{50} (μM)*
7	-74.9	113-115	25	0.455
8	-95.3	139-141	38	>2
9	-78.8	153-155	35	1.19
10	-67.5	143-145	41	>2
11	-73.6	163-166	33	>2
12	-91.2	185-187	45	>2
(-)-HupA				0.08

* AChE inhibitory activity of the dimers was measured *in vitro* according to the method of Ellman *et al.*[8] using rat brain cortex crude homogenates. The assay samples were mixed with PVP (**6**:PVP = 1:99).

After evaporation of the solvent *in vacuo*, the residue was purified by preparative thin layer chromatography (silica gel, CH_2Cl_2 : EtOH = 10:1) to give the corresponding dimer[9].

In summary, six (-)-HupA dimers with 7-12 methylene unites as tethers were prepared. It was found that these dimers were less potent in inhibition of AChE than (-)-HupA. Further studies on dimeric (-)-HupA with varying tethers are in progress and will be reported in due course.

Acknowledgment

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References and Notes

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9. Analytical data: **6** (n=7): ^{1}H NMR (400 MHz, $CDCl_3$, ppm): 13.10 (br.s., 2H), 7.51 (d, 2H, J=9.3 Hz), 6.40 (d, 2H, J=9.3Hz), 5.38 (m, 4H), 3.58 (m, 2H), 2.85 (dd, 2H, J=4.1, 12.0Hz), 2.64 (d, 2H, J=16.8Hz), 2.39 (dd, 2H, J=7.2, 10.5Hz), 2.11 (m, 8H), 1.60 (d, 6H, J=4.3Hz), 1.37 (s, 6H), 1.40 (d, 4H, J=3.2Hz), 1.19 (t, 6H, J=3.8Hz); ^{13}C NMR (100MHz, $CDCl_3$, ppm): 12.4, 22.6, 27.2, 29.4, 30.7, 33.1, 34.3, 42.6, 50.4, 58.9, 112.0, 117.0, 119.8, 124.7, 134.2, 137.7, 140.7, 143.9, 165.2; IR (KBr): 3388, 2929, 1656,1612, 1554, 1452, 1122, 838, 661 cm^{-1}. HRMS (*m/z*) calcd. for $C_{37}H_{48}O_2N_4$: 580.3739, found 580.3758.

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