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Potent, Easily Synthesized Huperzine A-Tacrine Hybrid Acetylcholinesterase Inhibitors

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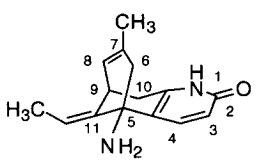
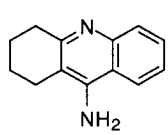
Abstract: Hybrid acetylcholinesterase inhibitors composed of a key fragment of huperzine A and an intact tacrine unit were prepared. The syntheses are quite direct, proceeding in a maximum of 4 linear steps from commercially available starting materials. The optimum hybrid inhibitor (±)-**9g** is 13-fold more potent than (-)-huperzine A, and 25-fold more potent than tacrine.

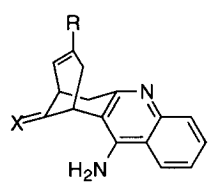
Huperzine A **1**, a potent reversible acetylcholinesterase (AChE) inhibitor isolated from the clubmoss *Huperzia serrata*, shows considerable promise for the palliative treatment of Alzheimer's disease (AD).[2] Prompted by the scarcity of the natural product, total syntheses of **1**[3,4] and numerous analogs[5-8] have been undertaken. Recently a novel class of huperzine A analogs have been developed which feature fusion of **1** with tacrine **2**, which was the first AChE inhibitor approved for treatment of AD.



1
(-)-huperzine A



2
tacrine



	X	R
(±)-**3a**	CH-CH_3 (*E*-)	CH_3
(±)-**3b**	H_2	CH_3
(±)-**3c**	H_2	CH_2CH_3

Compound (±)-**3a**, the drug which most resembles a hybrid of the huperzine A and tacrine structures, is a less potent AChE inhibitor than tacrine **2**.[9] However, removal of the C-11 ethylidene resulted in compounds (±)-**3b-c**, which were 2- and 3.4-fold more potent than **2**, respectively. In this communication we report a new class of huperzine A-tacrine hybrids; the optimum drug is 25-fold more potent than tacrine **2**, and 13-fold more potent than (-)-huperzine A **1.**

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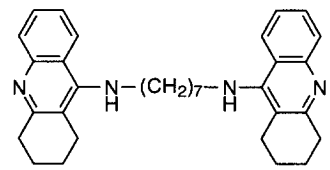
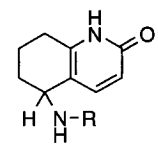
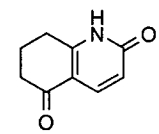
We have previously reported that heptylene-linked bis-tacrine **4** is 150-fold more potent and 250-fold more selective for AChE inhibition than monomeric tacrine **2**, as a consequence of dual-site binding to the enzyme.[10,11] The synthesis of **4** and its homologs was undertaken on the basis of docking studies which indicated affinity of **2** for both the catalytic and peripheral sites of *Torpedo* AChE.[12] Since these studies also indicated affinity of **1** to both sites,[12] we undertook syntheses of heterodimeric inhibitors comprised of tacrine and huperzine A-like monomers.



4



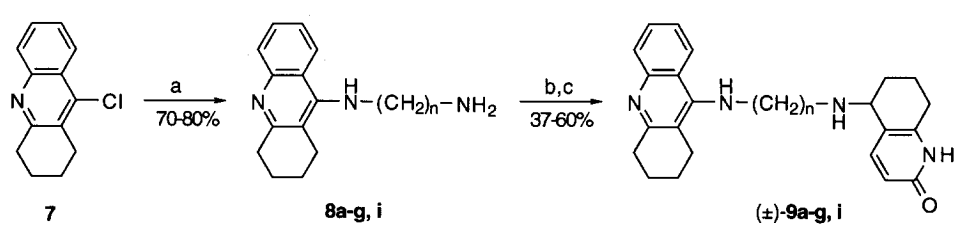
(±)-**5a**: R = $PhCH_2CH_2$
(±)-**5b**: R = *n*-Bu
(±)-**5c**: R = H



6

In view of the expense of **1** itself, and for synthetic expediency, we decided to simplify the huperzine A monomer by removing both the three-carbon bridge (C_6-C_8) and the C_{11}-ethylidene. Although simple 5-amino-5,6,7,8-tetrahydro-2(1H)-quinolinones **5** are known to be extremely weak AChE inhibitors [(±)-**5a**: IC_{50} > 100,000 nM],[6] previous studies in our laboratories have demonstrated that monomers which have no discernable affinity for AChE can still serve as effective peripheral site ligands, when incorporated in a bivalent drug.[13] Thus ketone **6** (available in 40% yield over two steps from methyl propiolate and 1,3-cyclohexanedione[14]) was selected as a starting material.

The synthesis of the desired huperzine A-tacrine hybrids (±)-**9** proved quite straightforward. 9-chloro-1,2,3,4-tetrahydroacridine **7** can be prepared in one step from commercially available 1,2,3,4-tetrahydro-9-acridanone, or from anthranilic acid and cyclohexanone in 89% yield over two steps.[11] Treatment of **7** with 3 equivalents of α,ω-diamines (n = 4-10, 12) in refluxing 1-pentanol provided functionalized tacrines **8** in good yield (70-80%). Condensation with **6** (benzene reflux, Dean-Stark trap) provided imines, which were reduced with sodium borohydride to give (±)-**9** in 37-60% yield over two steps (Table 1).



7 **8a-g, i** (±)-**9a-g, i**

a)3 equiv. H_2N-$(CH_2)_n$-NH_2, 1-pentanol, reflux, 18 hours. b)1 equiv. **6**, cat. CH_3CO_2H, benzene reflux, Dean-Stark trap, 24 hours. c)$NaBH_4$, MeOH, 4 hours.

To serve as an additional control, 5-*n*-butylamino-5,6,7,8-tetrahydro-2(1H)-quinolinone (±)-**5b** was prepared analogously from **6** and *n*-butylamine. The free bases were converted to the hydrochloride or oxalic acid salts

to impart water solubility and improved handling, as indicated in Table 1. Elemental analysis (CHN) matched the formulae shown in Table 1 within 0.4% in each case.

Table 1. Physical properties of huperzine A-tacrine hybrids (±)-**9a-g,i** and (±)-**5b**

drug	n	yield (free base)	formula (salt)[a]	mp (°C)
(±)-**9a**	4	51	$C_{26}H_{32}N_4O$•2HCl•3.5H_2O	197-198 (dec)
(±)-**9b**	5	52	$C_{27}H_{34}N_4O$•2HCl•0.5H_2O	216-217.5 (dec)
(±)-**9c**	6	54	$C_{28}H_{36}N_4O$•2.5HCl•2.5H_2O[b]	169-171 (dec)
(±)-**9d**	7	58	$C_{29}H_{38}N_4O$•2.5HCl•1.5H_2O[b]	129-131
(±)-**9e**	8	59	$C_{30}H_{40}N_4O$•2($C_2H_2O_4$)•0.5H_2O	160-162
(±)-**9f**	9	56	$C_{31}H_{42}N_4O$•2($C_2H_2O_4$)•1.5H_2O	180-182
(±)-**9g**	10	60	$C_{32}H_{44}N_4O$•2($C_2H_2O_4$)•0.8H_2O	152-154
(±)-**9i**	12	37	$C_{34}H_{48}N_4O$•2($C_2H_2O_4$)•1H_2O	154-155
(±)-**5b**	na	51	$C_{13}H_{20}N_2O$•2HCl•0.8H_2O	124-126

[a]Elemental analyses (CHN) match the formulae within ±0.4%.
[b]Formula further supported by Cl analysis.

Table 2. Cholinesterase inhibition by hybrids (±)-**9a-g, i** and controls.

drug	AChE IC_{50} (nM)[a]	BChE IC_{50} (nM)[b]	Selectivity for AChE[c]
(±)-**9a**	42.6±6.5	228±27	5.4
(±)-**9b**	122±11	220±38	1.8
(±)-**9c**	37.1±4.4	223±44	6.0
(±)-**9d**	53.1±13.5	233±17	4.4
(±)-**9e**	26.2±14.1	166±4	6.3
(±)-**9f**	36.3±4.7	150±11	4.1
(±)-**9g**	**8.8±1.0**	81.5±8.2	**9.3**
(±)-**9i**	112±9	434±102	3.9
(±)-**5b**	≈500,000[d]	≈500,000[d]	≈1
tacrine (**2**)	223±11	92±2	0.4
(-)-huperzine A (**1**)	114±1	135,000±6,000	1170
4	1.5±0.3	149±23	99.4

[a]Assay performed using rat cortex homogenate, in the presence of ethopropazine as a specific BChE inhibitor.
[b]Assay performed using rat serum, in the presence of BW284c51 as a specific AChE inhibitor.
[c]Selectivity for AChE is defined as IC_{50}(BChE)/IC_{50}(AChE)
[d]Estimated values based on ~50% inhibition of AChE and BChE at 0.5 mM, the highest drug concentration tested.

Assays for rat AChE and BChE inhibition potency were carried out by the Ellman method[15] with some minor modifications, as described previously;[11] the data is given in Table 2. Cholinesterase inhibition constants for controls **2** and **4** were within error of their previous determinations.[11]

As can be seen, huperzine A-tacrine hybids (±)-**9** are all more potent for AChE inhibition than tacrine **2**. The optimum drug (±)-**9g** is easily synthesized, has nanomolar affinity for AChE (IC_{50} = 8.8 nM), and is 13-fold more potent than (-)-huperzine A **1**. Although (±)-**9g** does not possess the exquisite selectivity of (-)-huperzine A **1**, it is considerably more selective than tacrine **2**. As expected, simplified huperzine A monomer (±)-**5b** is an extremely weak inhibitor ($IC_{50} \approx$ 500,000 nM). Nevertheless, attachment of the parent monomer **5c** to tacrine **2** via an alkylene tether results in significant enhancement in AChE inhibition potency and selectivity. We therefore propose that (±)-**9g**, like **4**, derives its potency and selectivity from simultaneous binding to the peripheral and catalytic sites of AChE. The present work offers insights into crafting more potent and selective AChE inhibitors, and provides further incentive for use of the dual-site binding approach in structure-based drug design. Further studies on alkylene-linked huperzine A heterodimers and homodimers are in progress and will be reported in due course.

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