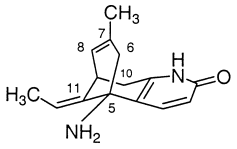
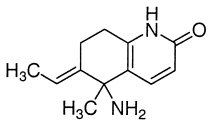
Dimerization of an Inactive Fragment of Huperzine A Produces a Drug with Twice the Potency of the Natural Product**

Paul R. Carlier,* Da-Ming Du, Yi-Fan Han,* Jing Liu, Emanuele Perola, Ian D. Williams, and Yuan-Ping Pang

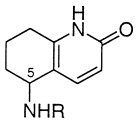
Bivalency is an effective strategy for improving drug potency and selectivity.[1] When multiple recognition sites for the same substrate exist, homodimers can exhibit dramatic performance enhancements relative to the corresponding monomers.[1a, 2] In this vein, homodimers of structurally complex natural products have recently been investigated.[3] Herein we demonstrate a new strategy in bivalent drug design by dimerizing an easily synthesized but pharmacologically inactive fragment of Huperzine A (−)-**1**: the optimum dimers (*S*,*S*)-(−)-**4h**,**i** are more than twice as potent as the natural product.



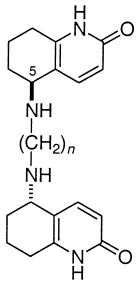
(−)-**1**



(±)-**2**



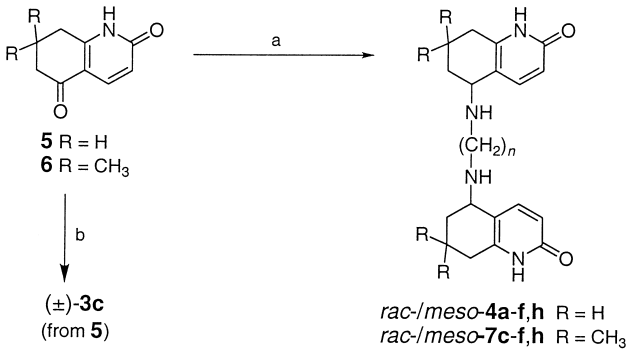
(±)-**3a** R = CH_2Ph
(±)-**3b** R = CH_2CH_2Ph
(±)-**3c** R = *n*Bu
(±)-**3d** R = Me



(*S*,*S*)-(−)-**4h** *n* = 12
(*S*,*S*)-(−)-**4i** *n* = 13

Huperzine A (−)-**1**, isolated from the clubmoss *Huperzia serrata*, is a selective and potent reversible acetylcholinesterase (AChE) inhibitor that shows promise for the palliative treatment of Alzheimer's disease.[4] Asymmetric syntheses of (−)-**1** as short as 12 steps have been reported,[5] and considerable effort has been directed towards the development of more easily synthesized analogues.[6] To date 5-amino-2(1*H*)-quinolinones which lack the C6–C8 bridge of **1** have not significantly inhibited AChE (e.g. (±)-**2**, (±)-**3a**, (±)-**3b**, $IC_{50} > 100\,000$ nM).[6] Yet, based on the X-ray crystal structure analysis of the *Torpedo* AChE·(−)-**1** complex,[7] analogue **3** appears to possess much of the requisite functionality. In particular **3** retains the pyridone oxygen atom and NH group of **1**, which form hydrogen bonds to Tyr-130 and to Gly-117/Glu-199 (through a water molecule), respectively, and the 5-amino group of **1**, which undergoes a cation–π-interaction with Trp-84/Phe-330. We conjectured that high affinity of analogues like **3** would be possible, if the loss of hydrophobic contact in the active site could be offset by additional interactions at the AChE peripheral site (12 Å distant from the active site)[8]. Our studies of alkylene-linked tacrine homodimers[2a, 9] and heterodimers[10] have demonstrated that this site binds a variety of protonated amine ligands, including moieties related to **3**.

Thus **5** and **6** (each available in two steps)[11] were condensed with α,ω-diamines and reduced, affording dimers **4a**–**f**,**h** and **7c**–**f**,**h** as mixtures of the *rac*- and *meso*-diastereomers (Scheme 1). These compounds were tested



5 R = H
6 R = CH_3

(±)-**3c**
(from **5**)

rac-/*meso*-**4a**-**f**,**h** R = H
rac-/*meso*-**7c**-**f**,**h** R = CH_3

Scheme 1. Synthesis of dimeric inhibitors. a) 0.5 equiv $NH_2(CH_2)_nNH_2$, cat. CH_3CO_2H, benzene reflux, 24 h; $NaBH_4$, MeOH, room temperature, 12 h, *rac*-/*meso*-**4a**–**f**,**h**: 38–58%, *rac*-/*meso*-**7c**–**f**,**h**: 22–35%; b) *n*$BuNH_2$, cat. CH_3CO_2H, benzene reflux, 24 h; $NaBH_4$, MeOH, room temperature, 12 h, 51%. Lettering scheme (see also Scheme 2): **a**, *n* = 5; **b**, *n* = 6; **c**, *n* = 7; **d**, *n* = 8; **e**, *n* = 9; **f**, *n* = 10; **g**, *n* = 11; **h**, *n* = 12; **i**, *n* = 13; **j**, *n* = 14.

for AChE and butyrylcholinesterase (BChE) inhibition.[12] As expected, monomer (±)-**3c** proved an extremely weak AChE inhibitor ($IC_{50} \sim 500\,000$ nM, Table 1, entry 1). However corresponding dimers *rac*-/*meso*-**4a**–**f**, **h** showed dramatically enhanced potency, with the highest potency observed at a tether length of 12 methylene groups (*rac*-/*meso*-**4h**, 159 nM, Table 1, entry 2). Dimers *rac*-/*meso*-**7c**–**f**, **h** were similarly optimized at *n* = 12, but were less potent than *rac*-/*meso*-**4h** (cf. Table 1, entries 2, 3; data for **4a**–**f**, **7c**–**f** not shown).

As a test of the design strategy, 2-methoxypyridine analogue *rac*-/*meso*-**8h** was prepared from **5** (Scheme 2). The 31-

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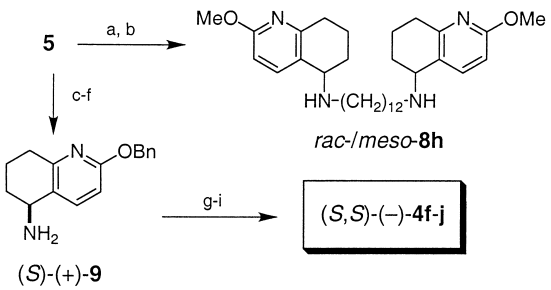
Table 1. Cholinesterase inhibition by huperzine A fragment dimers and controls.

Entry	Drug[a]	n[b]	AChE IC_{50} [nM][c]	BChE IC_{50} [nM][d]	Selectivity for AChE[e]
1	(±)-**3c**	na	500 000[f]	500 000[f]	1
2	*rac*-/*meso*-**4h**	12	159 ± 26	24 400 ± 910	153
3	*rac*-/*meso*-**7h**	12	3620 ± 110	60 000 ± 3230	16.6
4	*rac*-/*meso*-**8h**	12	5000 ± 190	78 900 ± 14 500	15.8
5	(*S*,*S*)-(−)-**4f**	10	151 ± 36	1820 ± 70	12.1
6	(*S*,*S*)-(−)-**4g**	11	84 ± 5	1160 ± 80	13.8
7	(*S*,*S*)-(−)-**4h**	12	**52 ± 8**	9600 ± 300	185
8	(*S*,*S*)-(−)-**4i**	13	**52 ± 9**	16 700 ± 650	321
9	(*S*,*S*)-(−)-**4j**	14	240 ± 50	59 500 ± 10 100	248
10	(*R*,*R*)-(+)-**4h**	12	3130 ± 790	297 000 ± 78 400	94.9
11	(−)-**1**[g]	na	115 ± 1	135 000 ± 6000	1200
12	tacrine	na	231 ± 16	77.2 ± 5.8	0.3

[a] Drugs **3c**, **4** were assayed as the hydrochloride salts; drugs *rac*-/*meso*-**7** and *rac*-/*meso*-**8** were assayed as the fumaric acid salts. Elemental analyses matched the proposed salt formulations (C, H, N, Cl ± 0.4%). [b] na = not applicable. [c] Assay performed using rat cortex homogenate, in the presence of ethopropazine as a specific BChE inhibitor. [d] Assay performed using rat serum, in the presence of BW284c51 as a specific AChE inhibitor. [e] Selectivity for AChE is defined as IC_{50}(BChE)/IC_{50}(AChE). [f] Estimated values based on ~50% inhibition of AChE and BChE at 0.5 mM, the highest drug concentration tested. [g] $[\alpha]_D^{20} = -149^\circ$ ($c = 0.17$, $CHCl_3$).



Scheme 2. Synthesis of enantiomerically pure dimers. a) MeI, Ag_2CO_3, $CHCl_3$, room temperature, 48 h, 81%; b) 0.5 equiv $NH_2(CH_2)_{12}NH_2$, cat. CH_3CO_2H, benzene, reflux, 24 h; $NaBH_4$, MeOH, room temperature, 12 h, 57%; c) BnBr, Ag_2CO_3, toluene, 48 h, 80%; d) $NH_2OBn \cdot HCl$, pyridine, room temperature, 16 h, 95%; e) $BH_3 \cdot THF$, room temperature, 12 h, reflux 2 h; 20% KOH, reflux 1.5 h, 66%; f) (*R*)-(−)-mandelic acid, MeOH, two recrystallizations; CH_2Cl_2/aq. NaOH, 29%; g) 0.5 equiv $ClC(O)-(CH_2)_{n-2}C(O)Cl$, Et_3N, benzene, reflux 4 h, 80–91%; h) 6 equiv $BH_3 \cdot THF$, room temperature 12 h, reflux 2 h; 20% KOH, reflux 1.5 h, 87–92%; i) H_2, 10% Pd/C, EtOH, 24 h, 86–91%. Lettering scheme (see Scheme 1).

fold lower potency relative to *rac*-/*meso*-**4h** (Table 1, entries 2, 4) suggests that, as hoped, hydrogen bonding of the pyridone with the active site contributes to affinity. The synthesis of pure enantiomers of **4f–j** was then undertaken (Scheme 2). Pyridone **5** was converted to (±)-**9** in 50% yield over three steps, and resolved with (*R*)-(−)-mandelic acid; an X-ray crystal structure analysis of the salt established the *S* configuration for (+)-**9**.[13] The enantiomeric free bases were transformed to (*S*,*S*)-(−)-**4f–j** and (*R*,*R*)-(+)-**4h** in 62–74% yield over three steps. The 60-fold greater potency of (*S*,*S*)-(−)-**4h** relative to its enantiomer (Table 1, entries 7 and 10) is suggestive of a multipoint interaction with the AChE active site. The optimization of AChE IC_{50} in the *S*,*S* series at a tether length of 12–13 methylene groups is consistent with simultaneous binding of these drugs to the catalytic and peripheral sites of AChE.[9, 10] Remarkably the optimum drugs (*S*,*S*)-(−)-**4h–i** are nearly 10 000-fold more potent than the related monomer (±)-**3c**, and are twice as potent as (−)-**1**, the natural product which inspired their synthesis. Enzyme kinetics confirmed the superior potency of (*S*,*S*)-(−)-**4h** ($K_I = 19.6$ nM) relative to (−)-**1** ($K_I = 47.1$ nM). Although (*S*,*S*)-(−)-**4h**,**i** are not as potent as 10-axial-methyl-huperzine[4] or tacrine/huperzine hybrids,[10b, 14] they are easily synthesized and are superior to the latter in terms of selectivity for AChE.

As a prelude to computational and experimental evaluations of the binding of these dimers to AChE, studies of the docking[15] of monomers (*R*)- and (*S*)-**3d** (protonated at N5) into the active site of AChE were carried out. Both enantiomers were found to bind in a similar way to (−)-**1**, and binding of (*R*)-**3d** was 3 kcal mol^{-1} more exothermic than binding of (*S*)-**3d**. However, examination of all the computed binding orientations of (*R*)-**3d** reveals that the 5-amino group does not easily accommodate a C_{12}-alkylene tether leading upwards towards the peripheral site (Figure 1, magenta); in contrast (*S*)-**3d** encounters no such problem (Figure 1, cyan). We therefore attribute the observed 60-fold enantio-preference for (*S*,*S*)-(−)-**4h** to the disposition of the tether leading to the peripheral site.

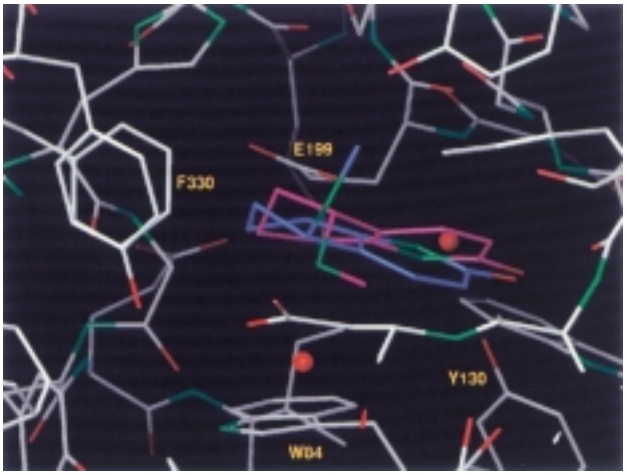


Figure 1. Overlay of the energetically favorable binding orientations of (*R*)-**3d**·H^+ (magenta) and (*S*)-**3d**·H^+ (cyan) at the catalytic site of *Torpedo* AChE (white); in each the nitrogen and oxygen atoms are green and red, respectively. F = Phe; E = Glu; Y = Tyr; W = Trp. The perspective shows a cross-section of the catalytic site.

We have demonstrated that dimerization of an easily synthesized but inactive fragment of Huperzine A can produce a drug with greater potency than the natural product itself. Given the importance of structurally complex natural products in drug discovery, we anticipate that the simple approach described here will be useful in developing new leads for other biological targets.

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[13] Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-137010. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
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[15] The docking study was performed with the SYSDOC program by employing the AMBER 95 force field and the RESP charge model, as described in the Supporting Information.

Naked-Eye Detection of Anions in Dichloromethane: Colorimetric Anion Sensors Based on Calix[4]pyrrole**

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The development of chemosensors for specific chemical species is emerging as a research area of considerable importance within the generalized field of supramolecular chemistry.[1] One of the more appealing approaches in this context involves the construction of optical sensors.[2–6] Such systems generally contain some combination of substrate-recognition functionality (receptor) and optical-signaling capacity (chromophore), either directly linked[2, 3, 5] or appropriately associated in a noncovalent manner,[4] and are designed to permit the detection of substrates by binding-induced changes in absorption or emission properties (termed colorimetric and fluorescent sensors, respectively). While the utility of these approaches are becoming increasingly appreciated in terms of both qualitative and quantitative analysis,[5] the number of colorimetric sensors available at present for anionic substrates remains quite limited.[6] Indeed, only a few systems are known that undergo color changes of sufficient magnitude that they can be used for the direct "naked-eye" sensing of anions.[4b, 6b, c] Here we report the synthesis of a new class of covalently linked calix[4]pyrrole – anthraquinone conjugates and show that they act as powerful naked-eye sensors for selected anions (namely, F^-, Cl^-, $H_2PO_4^-$) in dichloromethane.

The calix[4]pyrroles,[7] colorless macrocycles rich in pyrrole NH hydrogen bond donor functionality, are an easy-to-make class of uncharged anion receptors that show considerable promise in the area of anion sensing. In previous work we demonstrated that anion sensors could be generated from calix[4]pyrroles either by attaching a fluorescent reporter group to the basic tetrapyrrolic skeleton[7d] or through the use of a displacement process involving competition with *para*-nitrophenolate anions.[4b] Unfortunately, we were unable to produce inherently colored calix[4]pyrrole derivatives that functioned as naked-eye anion sensors. Recently, however, we discovered that calix[4]pyrroles functionalized at the β-pyrrolic positions[8] may be made using a modified Sonogashira coupling procedure.[9] By taking advantage of this approach as well as the current availability of the ethynyl-substituted calix[4]pyrrole **1**,[8] we have now succeeded in preparing (in 73% yield) an anthraquinone-functionalized system (**2**) that bears an appended chromophore directly

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