Synthesis and Acetylcholinesterase Inhibitory Activity of Several Pyrimidone Analogues of Huperzine A

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Syntheses of four new pyrimidone analogues of the acetylcholinesterase (AChE) inhibitor huperzine A are reported together with the inhibitory potencies of these compounds for foetal bovine calf serum AChE; β-lactone formation followed by a thermal cycloreversion reaction serves as the key step for introduction of the ethylidene appendage of **12** in the stereochemically correct form.

Huperzine A is a potent inhibitor of AChE, the key brain enzyme involved in the processing of the neurotransmitter acetylcholine.[1] Because numerous studies have pointed to the existence of a cholinergic deficit in patients suffering from neurodegenerative diseases of the Alzheimer's type, considerable credence has been given to the use of acetylcholinesterase inhibitors in the amelioration of the Alzheimer's symptomatology.[2] In this regard, the considerable duration of action of huperzine A combined with its potency and lack of side effects strongly recommend its further study both clinically as well as in the laboratory.[3]

In the present efforts we chose to explore the effect of introducing an additional heteroatom into the huperzine A structure. Specifically, we envisaged that replacement of its pyridone ring by a pyrimidone ring might possibly lead to an analogue showing improved AChE inhibitory potency due to its ability to engage in additional hydrogen-bonding (Fig. 1).

Thus, starting from cyclohexane-1,4-dione monoethylene ketal **1**, reaction with methyl formate in the presence of sodium methoxide[4] gave the hydroxymethylene derivative **2**, which was benzoylated[5] and reacted with *O*-methylisourea hydrogensulfate[6] to afford quinazoline **4**. Next, the quinazoline was deketalized, and a methoxycarbonylation reaction carried out using Mander's reagent.[7] Following steps worked out in our original synthesis of huperzine A,[8] **6** was now subjected to the tandem Michael–aldol reaction employing methacrolein as the electrophile to afford the expected ketol intermediate in 85% yield, which was immediately subjected to mesylation and elimination. Unfortunately, the desired elimination product was obtained in only 8% yield. Accordingly, we turned our attention to an alternative palladium assisted bicycloannulation strategy.[9] As shown in Scheme 1, reaction of **6** with 2-methylene-1,3-propanediol diacetate in the presence of palladium acetate, triphenylphosphine and DBU[10] delivered the exocyclic olefin **7** in 76% yield. Since the exocyclic double bond of **7** failed to undergo isomerization upon treatment with triflic acid or $RhCl_3$,[9,11] we decided to install the remaining ethylidene appendage at this juncture, and to reserve the isomerization step until the final stage of the synthesis.

Initial attempts to carry out the olefination reaction proved to be problematic. Application of the standard Wittig reaction protocol to **7** using ethylidenetriphenylphosphorane led to the product of the reverse Dieckmann reaction in 70% yield. Also, use of the Takai olefination procedure failed.[12,13] Success was ultimately achieved through a two-step protocol which involved the initial construction of a β-lactone.[14] Thus, the phenylthiol ester of propionic acid was converted to its enolate and reacted with **7** to provide **8** as a single stereoisomer. The stereochemistry of **8** is surmised to be as drawn based both upon extensive COSY and NOESY experiments with the model β-lactone containing a methoxypyridine ring in place of the pyrimidine ring of **8** as well as its subsequent conversion to **9**. [2 + 2] Cycloreversion of this β-lactone with evolution of carbon dioxide was then induced by heating with silica gel in dry toluene. The reaction was stereospecific, and only the *E*-olefin **9** was isolated in 67% yield. Next, the ester was saponified, the resulting acid was treated with diphenyl azidophosphate,[15] and the intermediate isocyanate was reacted with methanol to provide urethane **10**. Deprotection of **10** with trimethylsilyl iodide proceeded with some isomerization of the exocyclic double bond. Further reaction with triflic acid drove the isomerization to completion, yielding the desired pyrimidone analogue **12** in 87% yield. To preclude rearrangement of the exocyclic double bond during the deprotection stage, **10** was reacted with lithium *n*-propylthiolate in HMPA[16] to provide solely **11**. Further reaction of **11** with triflic acid led to **12** in 84% yield.†

As an aide to the present study, we were also interested in gaining access to the huperzine A analogue in which the pyrimidone ring is annealed to the bicyclo[3.3.1]nonane at the C–C bond site more remote from the amino group. To acquire this analogue, we started from ketone **13**, an intermediate readily accessible from the monoketal **1** as described previously.[17] This ketone was hydroxymethylenated, and the resulting product benzoylated. A 9:1 mixture of the *cis* and *trans* stereoisomers resulted, which was reacted in turn with *O*-methylisourea hydrogensulfate to provide methoxypyrimidine **17**. Me_3SiI promoted deprotection of **17** led to a mixture of the pyrimidones **18** and **19**. Further reaction of this mixture with triflic acid drove the isomerization reaction to completion, affording the thermodynamically favoured product **18**. Pure **19** was available from **17** through the simple expedient of using lithium *n*-propylthiolate to bring about the deprotection step. It is of some interest to note that just as in the huperzine series

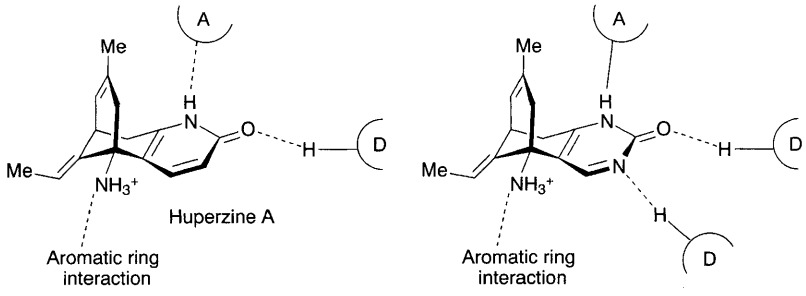
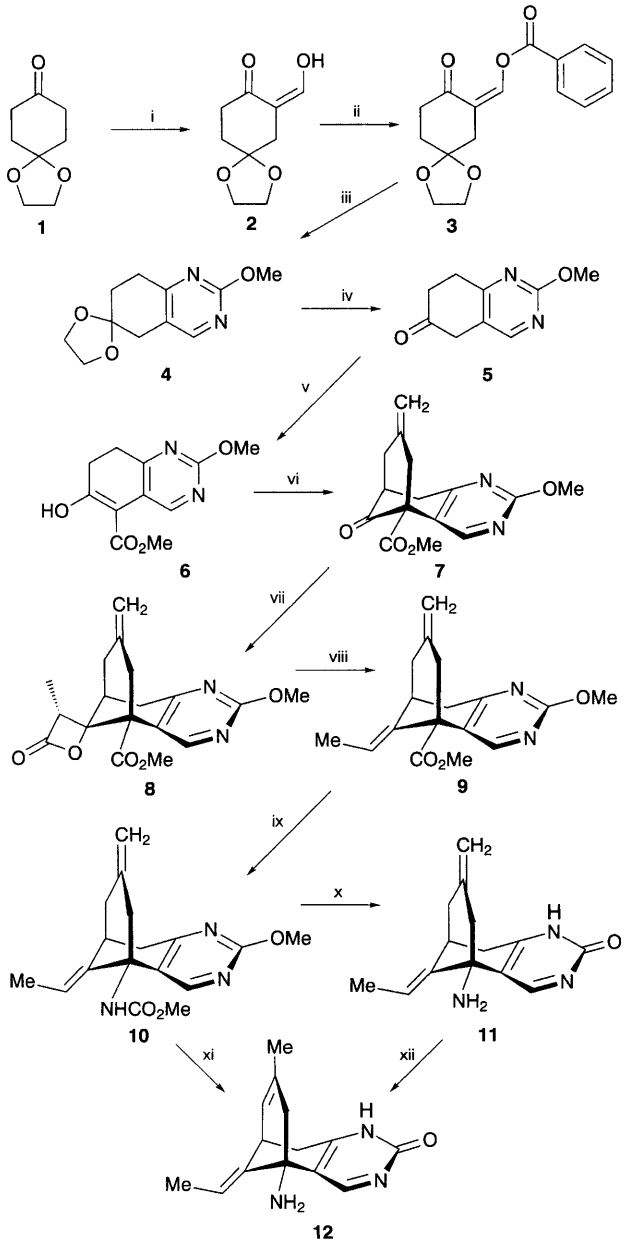


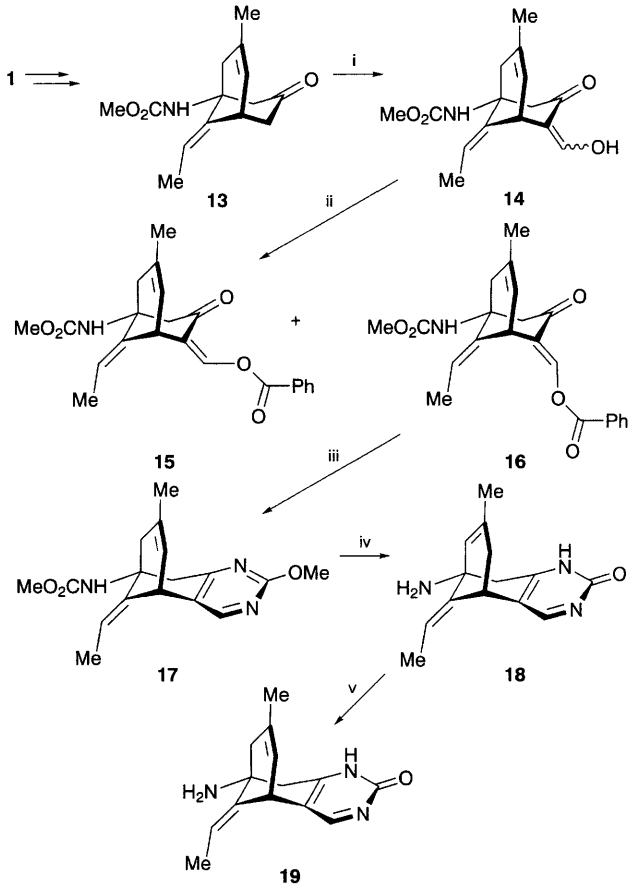
Fig. 1 Illustration of the possible binding interactions of huperzine A and its pyrimidone analogue (A = acceptor; D = donor)

itself, the thermodynamically preferred position of the double bond is in the direction of the methylene group attached to the heterocyclic ring.

The AChE inhibitory data for the four new pyrimidone analogues are provided in Table 1 along with the K_I for racemic huperzine A.[18,19] In view of the relatively more dramatic structural alterations embodied by the alternately fused pyrimidone analogues **18** and **19**, it is not surprising to find that these compounds are less active than huperzine A itself. The former compound is about 800-fold less active, while the latter is only about 36-fold less active. Somewhat less expected, however, is the finding that compounds **11** and **12** are even less active than **18** and **19** (>15 000-fold and 1540-fold, respectively), in spite of the fact that the former structures are more closely related to huperzine A. Apparently, the extra nitrogen atom present in these structures must confer an undesirable electrostatic interaction with the enzyme in addition to the unfavourable change in the energetics of desolvation.‡ To date, the only structural modification to huperzine A that leads to a modest improvement in its activity is one that does not alter the electrostatic field of the molecule. The present work serves to underscore the importance of huperzine A's electronic field to its interaction with AChE. An in depth understanding of the ability of the alternatively fused pyrimidone **19** to serve as a modestly active AChE inhibitor will require the docking of this molecule to the X-ray structure available for *T. californica*, a study now underway.[20]



Scheme 1 Synthesis of the pyrimidone analogue of huperzine A. *Reagents and Conditions* i, HCO_2Me, NaOMe; ii, PhCOCl, Et_3N; iii, $H_2NC(OMe)NH{\cdot}0.5\,H_2SO_4$, Et_3N, EtOH; iv, Pr^iOH, 5% HCl; v, LDA, $NCCO_2Me$; vi, 2-methylene-propane-1,3-diol diacetate, $Pd(OAc)_2$, Ph_3P, DBU; vii LDA, PhSCOEt; viii, heat, SiO_2, toluene; ix, 1. NaOH; 2. $(PhO)_2P(O)N_3$, Et_3N; 3. MeOH; x, Pr^nSLi, HMPA; xi, 1. Me_3SiI; 2. MeOH; 3. CF_3SO_2OH; xii, CF_3SO_2OH.



Scheme 2 Synthesis of the alternatively fused pyrimidone analogues **18** and **19**. *Reagents*: i, HCO_2Me, sodium *tert*-pentoxide; ii, PhCOCl, Et_3N; iii, $H_2NC(OMe)NH{\cdot}0.5H_2SO_4$, Et_3N, DMF; iv, 1. Me_3SiI, $CHCl_3$; 2. MeOH; 3. CF_3SO_2OH; v, Pr^nSLi, HMPA.

Table 1 K_I Values (average of three experiments) for the inhibition of FBS AChE by the pyrimidone analogues

Compound	K_I/μmol dm^{-3} [a]
(±)-Huperzine A	0.02 ± 0.005
11	> 300
12	30.8 ± 10.0
18	16.6 ± 4.0
19	0.73 ± 0.1

[a] K_I values are the mean ± standard deviation.

We are indebted to the National Institute on Aging and MURST-Roma (Italy) for support of our research program. We thank Professor Alessandro Sega for his help with the NMR experiments.

Received, 15th September 1994; Com. 4/05622A

Footnotes

† *Spectral and physical data*: **11** colourless prisms; mp 240 °C (decomp.) (from ethyl acetate); R_f = 0.41 [methanol–chloroform (18 : 72)]; IR (KBr) ν/cm^{-1} 3400–3300, 1660, 1390, 1215, 740; ^{1}H NMR (D_2O), δ 8.31 (s, 1 H), 5.74 (q, 1 H, *J* 6.8 Hz), 4.88 (m, 1 H), 4.60 (m, 1 H), 3.57 (m, 1 H), 3.04 (dd, 1 H, *J* 19.6, 6.9 Hz), 2.76 (d, 1 H, *J* 19.4 Hz), 2.41 (m, 4 H), 1.78 (d, 3 H, *J* 6.8 Hz); ^{13}C NMR (CD_3OD), δ 12.7 (CH_3), 31.4 (CH), 38.4 (CH_2), 42.7 (CH_2), 53.2 (CH_2), 56.0 (C), 114.8 (CH_2), 115.9 (CH), 123.9 (C), 140.7 (C), 143.7 (C), 151.2 (br, CH), 158.7 (C), 171.5 (br, C); MS, *m/z* 243 (80%, M$^+$), 228 (100%), 214, 202, 188, 162. Satisfactory elemental analysis was obtained. **12**: white solid; mp 203 °C (decomp.) (ethyl acetate); IR (KBr) 3420–3340, 1662, 737 cm^{-1}; ^{1}H NMR (D_2O), δ 8.49 (s, 1 H), 5.62 (q, 1 H, *J* 6.8 Hz), 5.55 (m, 1 H), 3.75 (m, 1 H), 2.95 (dd, 1 H, *J* 18.4, 4.9 Hz), 2.79 (dd, 1 H, *J* 17.8, 1.4 Hz), 2.41 (ABq, 2 H, *J* 17.3 Hz), 1.70 (d, 3 H, *J* 6.7 Hz), 1.55 (s, 3 H); ^{13}C NMR (CD_3OD) δ 12.6 (CH_3), 22.6 (CH_3), 33.9 (CH), 39.6 (CH_2), 51.1 (CH_2), 54.7 (C), 114.0 (CH), 124.4 (C), 125.7 (CH), 135.0 (C), 141.6 (C), 154.9 (br, CH), 158.9 (C), 169.0 (br, C); MS, *m/z* 243 (100%, M$^+$), 228, 214, 200, 188, 174. Satisfactory elemental analysis was obtained.

‡ We note here that while the pyrimidones are displayed as discrete structures, they will be in equilibrium with their tautomeric forms. The definition of the preferred tautomer is, therefore, a moot point, for these molecules will automatically adjust to the receptor as needed.

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