Studies on a new series of THA analogues: Effects of the aromatic residues that line the gorge of AChE

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Abstract A series of *N*-monoalkylsubstituted 1,2,3,4-tetrahydro-9-aminoacridines have been prepared after modelling simulation of the AChE–inhibitor complex. Molecular modelling has predicted a number of hydrophobic residues to be involved in the catalytic mechanism of this interaction between the binding sites of AChE and this series of aminoacridines. In these compounds the acridine moiety becomes sandwiched between the rings of PHE330 and TRP84. In particular, the alkyl chain shows the important role of aromatic groups as binding sites. Their in vitro inhibitory properties (enzyme from *Electrophorus electricus*) confirm the aromatic groups as a general and significant characteristic of the mechanism of AChE inhibition.

Key words: Acetylcholinesterase catalysis; Acetylcholinesterase mechanism; Tacrine analogues; Acetylcholinesterase inhibitors

1. Introduction

The main biological role of acetylcholinesterase (AChE) is the termination of neurotransmission at cholinergic synapses by rapid hydrolysis of acetylcholine. AChE inhibitors are very important for the symptomatic treatment of diseases associated with acetylcholine depletion [1]. Particularly, two AChE inhibitors, physostigmine and 1,2,3,4-tetrahydro-9-aminoacridine (THA) are employed in the therapeutic approach to senile dementia of Alzheimer type.

Since 1986 our group [2–4] reported the inhibitory effects of a series of physostigmine derivatives; these compounds carbamoylate the esterasic site of AChE with a mechanism typical of irreversible inhibitors. The values of their bimolecular rate constants have suggested the existence of a hydrophobic interaction in an area adjacent to the esterasic site of the enzyme. Also the reversible competitive inhibitor THA [5] interacts with the active site of AChE; in the inhibitor–enzyme complex THA is sandwiched between the rings of PHE330 and TRP84; the last ring has been identified as part of the putative anionic (choline) binding site [6].

The hypothesis of the present study was to verify if an equivalent hydrophobic interaction is also operating for reversible THA analogues. In order to answer this question, we have studied a number of *N*-monoalkylsubstituted 1,2,3,4-tetrahydro-9-aminoacridines as probes in the docking with AChE from *Torpedo californica*. According to the results of the docking simulation we have selected and prepared a series of these inhibitors whose anticholinesterase activity in vitro has been investigated.

2. Materials and methods

2.1. Monoalkylsubstituted 1,2,3,4-tetrahydro-9-aminoacridines

Compounds were synthesized by heating a mixture of 9-chloro-1,2,3,4-tetrahydroacridine (10.9 g, 0.05 mol) and *n*-alkylamine (0.12 mol) in phenol (50 g) at 125–130°C for 5 h, [7]. Their identities were confirmed by spectroscopic methods. For nuclear magnetic resonance (NMR) spectra each compound was dissolved in $CDCl_3$. Trimethylsilane was included as an internal standard. The ^{1}H- (300 MHz) and ^{13}C-NMR (75.5 MHz) were obtained on a Varian Gemini spectrometer (Fig. 1A,B).

2.2. Enzyme inhibition in vitro

All reagents were purchased from Sigma Chemical Co. (St. Louis, MO). AChE activity from *Electrophorus electricus* was determined by the colorimetric method of [8] using acetylthiocholine as substrate. The assay medium contained 0.5 mM acetylthiocholine iodide, 0.25 mM dithio-bis-dinitrobenzoic acid, 0.1 M sodium phosphate, pH 8.0, and the reaction was monitored at 410 nm by means of graphics software (Varian Cary 3E) at 25° using thermostated (Haake) cells. The Michaelis constant (K_m) at 25°C had been determined previously (average ± S.D., 0.14 ± 0.0007 mM). Each experiment was repeated 3 times. Samples were assayed in the absence of inhibitor and served as control. The inhibitor competitive constant (K_i) for each THA derivative was determined from the relationship between the K_i and the concentration of inhibitor which causes 50% inhibition (IC_{50}) of the competitive enzymatic reaction, according to the formula: $IC_{50} = K_i(1+[S]/K_m)$ [9].

2.3. Molecular modelling

The X-ray crystallographic coordinates (at 2.8 Å resolution, entry 1ACJ) for AChE from *Torpedo californica* [10] were obtained from the Brookhaven Protein Data Bank [11]. Optimization was carried out with the special method used by ANNEAL (hot region, 10 Å; interesting region, 16 Å). The POWELL minimizer, which belongs to the conjugate gradient family of minimization methods, was selected. The TRYPOS force field consisted of only non-bonded energies: hydrogen-bonding, van der Waals, torsional and bending energy terms; the electrostatic term was ignored. The cut-off criterion was 8 Å radius for all interactions. The energy convergence criterion was ±0.05 kcal·mol^{-1}.

The structure of THA analogues was built by using SYBYL. The geometry of the enzyme–inhibitor complex was adjusted according to the crystallographic structures previously reported [5,10].

3. Results and discussion

In this investigation we used for our fit model the AChE from *Torpedo californica*. [5,10]. This enzyme has a simpler

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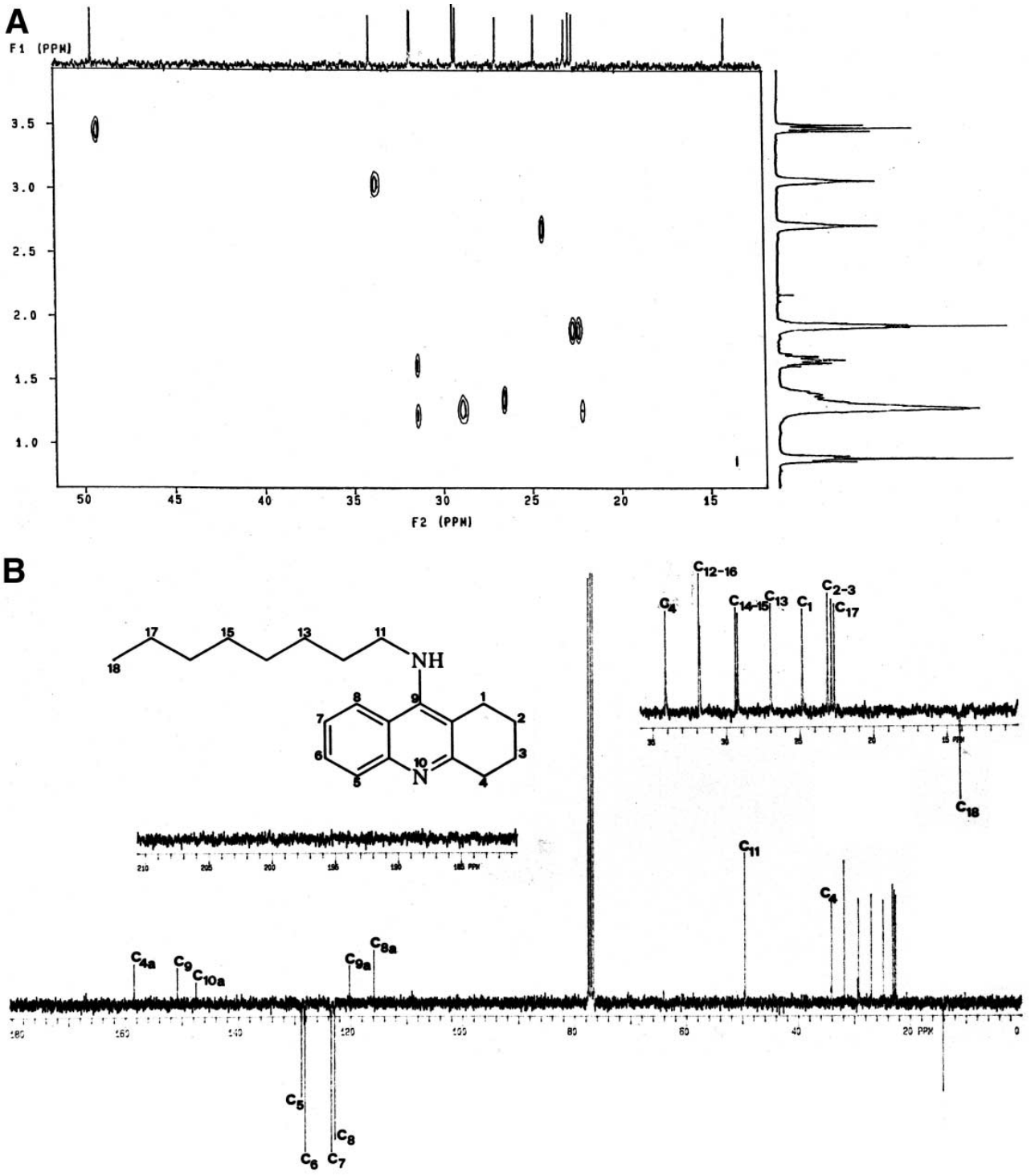
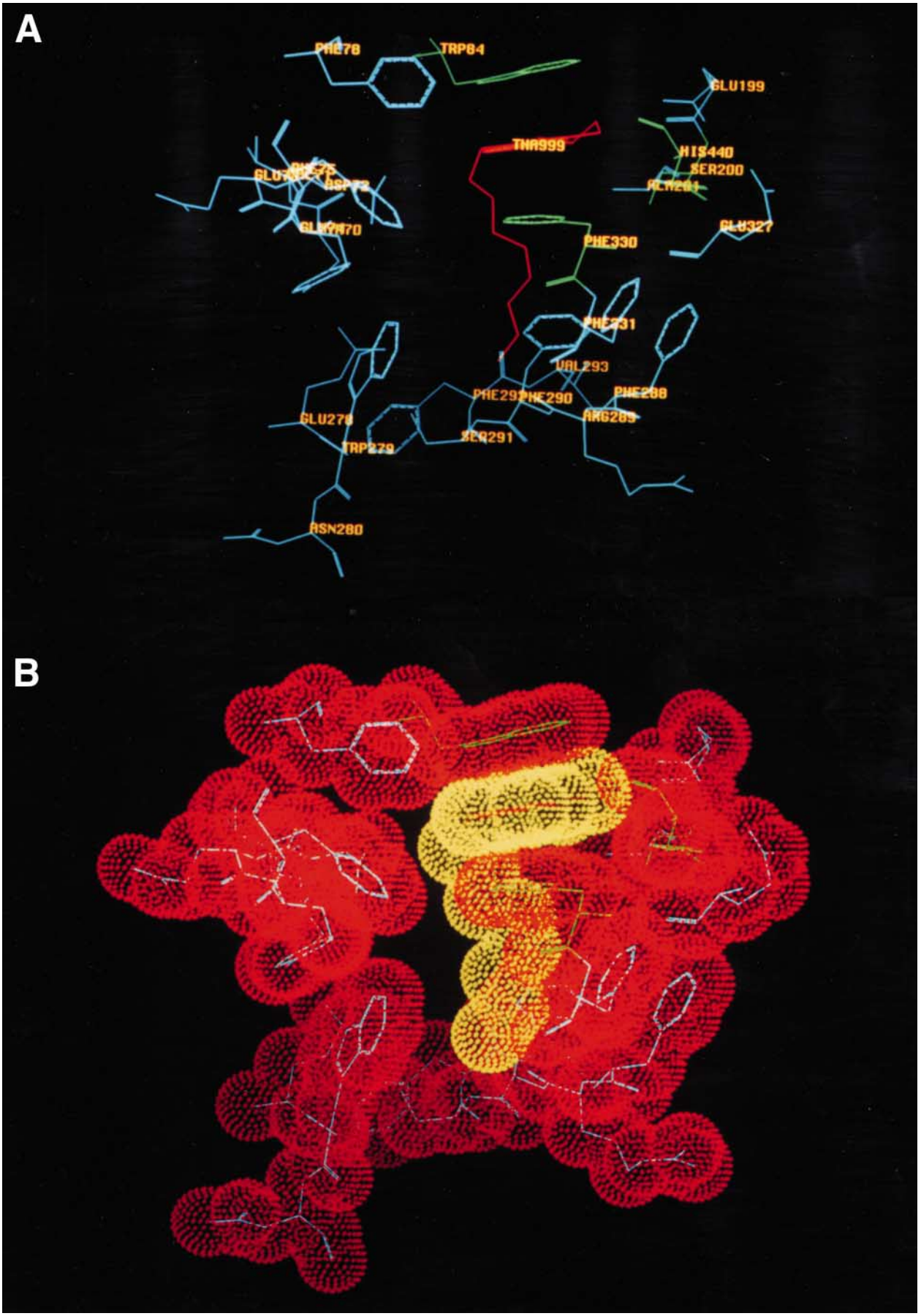


Fig. 1. Nuclear magnetic resonance spectrum of octyl-THA. A: The ^{1}H- and ^{13}C-NMR were obtained on a Varian Gemini spectrometer operating at 300 MHz. The assignment of each NMR signal to the pertinent proton and carbon was achieved by HETCOR measurements. B: 75.5-MHz ^{13}C NMR spectrum for the same sample; APT assignments.

quaternary structure (homodimer) than AChE from *Electrophorus*, for which the three-dimensional structure has not yet been determined, although a preliminary characterisation has been reported [12]. Fig. 2A shows the stereoview of conserved aromatics in the active site gorge, the catalytic triad (Ser^{200}–His^{440}–Glu^{327}) and the inhibitor molecule. The most remarkable feature of this figure is the long and narrow alkyl chain which penetrates halfway into the enzyme. The molecular mechanics simulation of *Torpedo* AChE–inhibitor complex shows, in addition, that the planar tacrine moiety is placed between the aromatic rings of PHE330 and TRP84 (Fig. 2B,C). On the other hand, the residues that line the 'aromatic gorge' also play an important role [5] in the formation of this complex, by an additional binding hydrophobic effect that, for THA analogues, is greater for heptyl and octyl chains.

Following this simulation, THA analogues produce a mod-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PHE78
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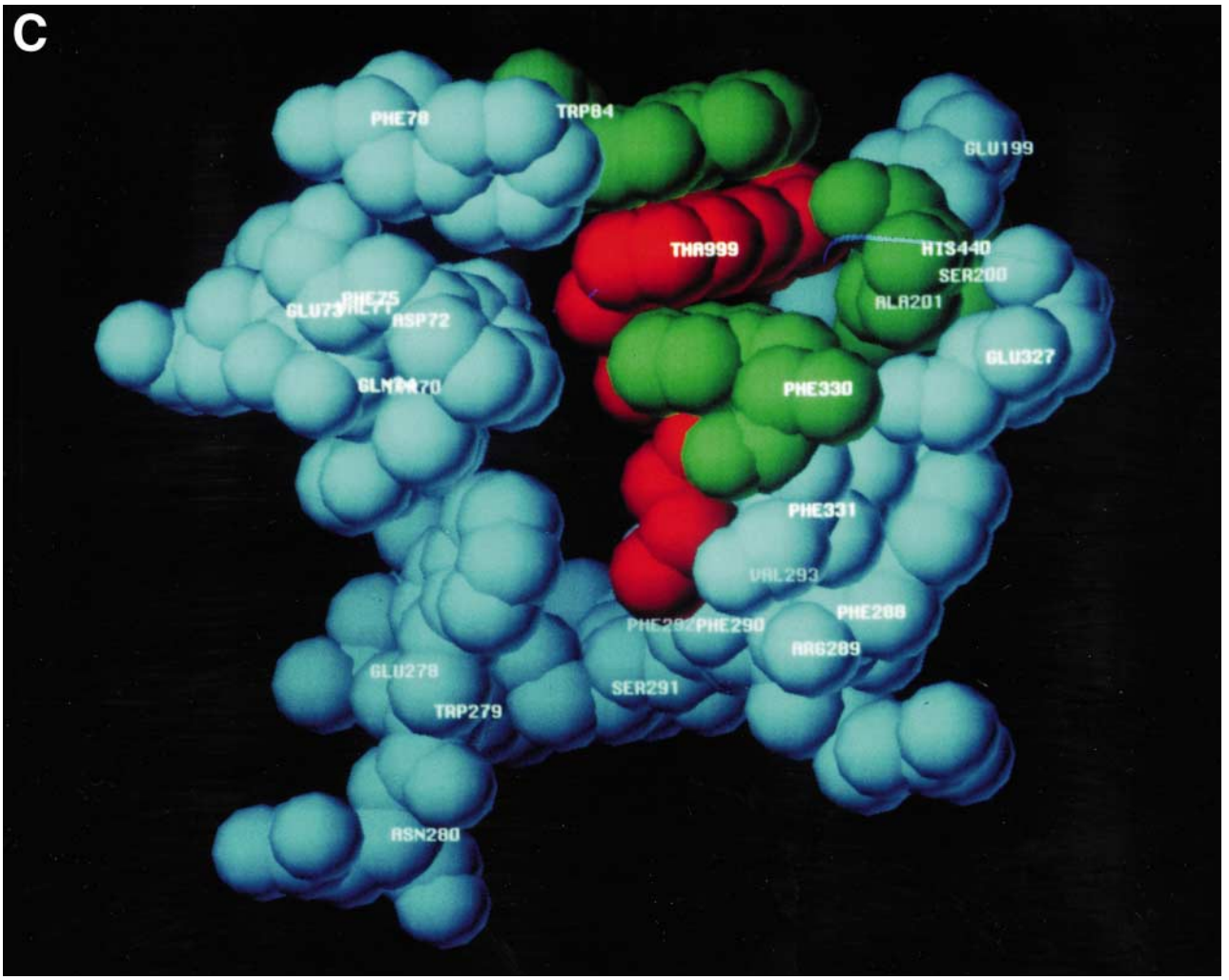

Fig. 2. The crystal structure of AChE, complexed with tacrine [5,10] was used as the basis for modelling procedures. Octyl-THA was modelled and minimized using Sybyl program. A: Stereo drawing that includes the residues contributing to the active site gorge interaction (Brookhaven Protein Data Base, entry 1ACJ). The AChE inhibitor, octyl-THA, is red-coloured. B: Stereo drawing of a thin slice through the AChE (monomer). The red-dot van der Waals surface shows the narrow gorge and the active site; the yellow dot shows the van der Waals surface of octyl-THA. C: Space-filling stereo view of the complex between AChE and octyl-THA.

ulation of the anti-AChE activity, as already reported for irreversible inhibitors [12,13,4]. Furthermore, it is interesting to note that the effect of binding interaction between the alkyl chain and the aromatic residues modulates the interaction between the acridine ring of THA analogues with the PHE330 aromatic ring (Fig. 3).

These results have been confirmed also by the enzyme inhibition in vitro (Fig. 4) which is stronger for heptyl- and octyl-THA. The non-linear characteristic relationship between the number of carbon atoms and K_i values suggests that the conformational distortion of AChE is small. In fact, the modulation of the inhibitory effect can be justified by the presence of aromatic residues which fill a substantial portion of the surface of the gorge. It has been also noted by the present modelling studies that the binding interaction of the chain, due to an enhancement of the steric effect, decreases in the case of the decyl analogue. Moreover, these studies in vitro show that also for reversible inhibitors the activity is modulated by the length of the *n*-alkyl chain and suggest that future inhibition and reactivation data may be reasonably anticipated.

From a biochemical point of view, this study shows that the K_i of THA analogues is principally affected by three factors: (a) the interactions between enzyme molecule and the planar tacrine ring; (b) the high aromatic content of the walls of the active site gorge, which may help explain previous biochemical studies of homologous series of organophosphorus [13] and carbamic [4] inhibitors; (c) the steric effects which, for longer residues, as in the example of decyl, reduce the conformational fitting of the alkyl chain which projects inside the aromatic gorge of the enzyme. In fact, if only the steric effects were acting for all the analogues, the THA derivatives should have a constant decrease, proportional to the length of the alkyl chain, of the inhibitory activity. On the contrary, the high affinity of octyl- and heptyl-THA for the enzyme suggests that some hydrophobic interactions successfully antagonize the steric effect introduced in the THA molecule by the alkyl radical.

Finally, it would appear from preliminary results that octyl-THA is less toxic than THA whose toxicity has been reported in the literature [15] to be >20 mg/kg; in fact, in experiments

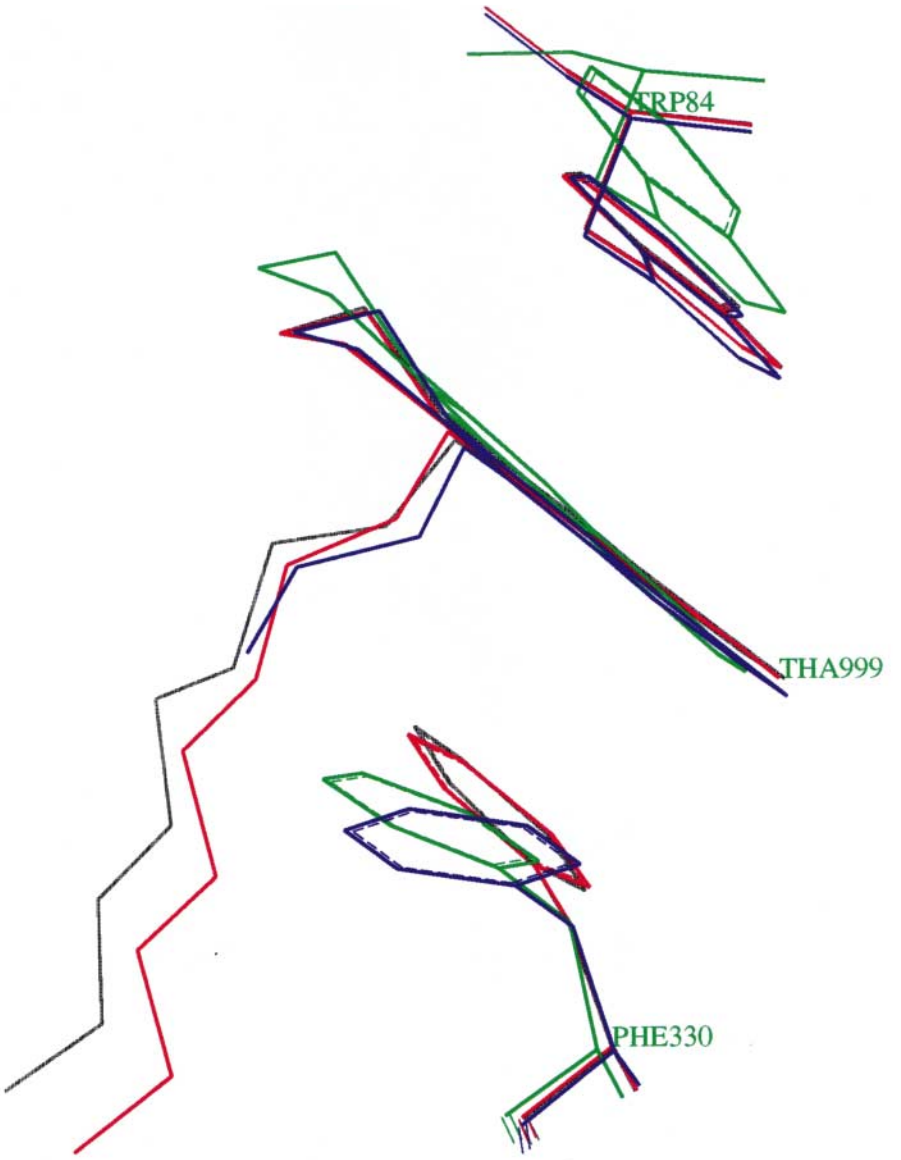

Fig. 3. Interaction between inhibitor acridine rings and PHE330 aromatic ring. Decyl-THA, black; octyl-THA, red; propyl-THA, blue; and THA, green colour. For octyl-THA the PHE330 aromatic ring rotates (29°) with respect to THA complex; for propyl-THA the rotation (−9°) causes a decrease in binding.

in which the acute toxicity was measured in CD-1 mice strain the LD_{50} of octyl-THA was >31 mg/kg.

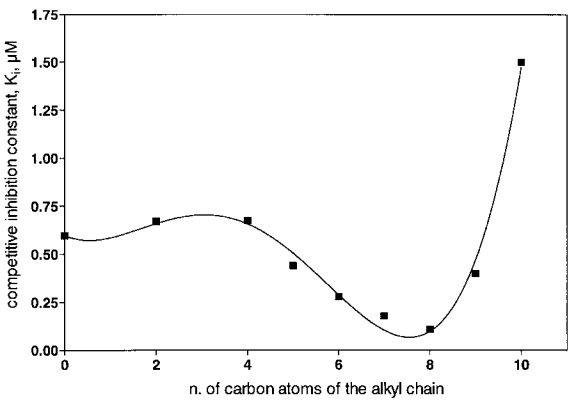

Fig. 4. Effect of alkyl-chain on competitive inhibition constant (K_i).

In conclusion, in view of the growing interest in new agents for the therapeutic treatment of Alzheimer's disease [14] octyl- or heptyl-THA could provide an attractive approach in the search for additional cholinergic compounds.

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References

[1] Taylor, P. (1990) in: The pharmacological basis of therapeutics (Gilman, A.G., Rall, T.W., Nies, A. and Taylor, P., Eds.) Pergamon, New York.
[2] M. Brufani, M. Marta, M. Pomponi, Eur. J. Biochem. 157 (1986) 115–120.
[3] M. Marta, M. Pomponi, Biochem. Biom. Acta 47 (1988) 285–288.
[4] M. Marta, F. Gatta, M. Pomponi, Biochim. Biophys. Acta 1120 (1992) 262–266.

[5] M. Harel, I. Schalk, L. Ehret-Sabatier, F. Bouet, M. Goeldner, C. Hirth, P.H. Axelsen, I.N.L. Silman, J.L. Sussman, P.N.A.S. 90 (1993) 9031–9035.
[6] C. Weise, H.-J. Kreienkamp, R. Raba, A. Pedak, A. Aaviksaar, F. Hucho, EMBO J. 9 (1990) 3885–3888.
[7] W.P. Brian, B.L. Souther, J. Med. Chem. 8 (1965) 143–144.
[8] G.L. Ellman, K.D. Courtney, V. Andres Jr., M. Featherstone, Biochem. Pharmacol. 7 (1961) 88–95.
[9] Y.-C. Cheng, W.H. Prusoff, Biochem. Pharmacol. 22 (1973) 3099–3108.
[10] J.L. Sussman, M. Harel, F. Frolow, C. Oefner, A. Goldman, L. Toker, I. Silman, Science 253 (1991) 872–879.
[11] F.C. Bernstein, T.F. Koetzle, G.J.B. Williams, E.F. Meyer, M.D. Brice, J.R. Rodgers, O. Kennard, T. Shimanouchi, M. Tasumi, J. Mol. Biol. 112 (1977) 535–542.
[12] J.D. Schrag, M.F. Schmid, D.J. Morgan, G.N. Phillips Jr., W. Chiu, L. Tang, J. Biol. Chem. 263 (1988) 9795–9800.
[13] M.I. Kabachnik, A.P. Brestkin, N.N. Godovikov, M.J. Michelson son, E.V. Rosengart, V.I. Rozengart, Pharmacol. Rev. 22 (1970) 355–388.
[14] Enz, A., Meier, D. and Spiegel, R. (1994) in: Alzheimer Disease: Therapeutic Strategies (Giacobini, E. and Becker R., Eds.) Birkhäuser, Boston.
[15] A.J. Hunter, T.K. Murray, J.A. Jones, A.J. Cross, A.R. Green, Br. J. Pharmacol. 98 (1989) 79–86.