Docking Analysis of a Series of Benzylamino Acetylcholinesterase Inhibitors with a Phthalimide, Benzoyl, or Indanone Moiety

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The enzyme-binding mode of a series of acetylcholinesterase inhibitors has been analyzed on the basis of the crystal structure of the *Torpedo* enzyme using docking programs DOCK and directed-DOCK. The inhibitors have a benzyl group connected to tertiary ammonium nitrogen at one end and a phthalimide, benzoyl, or indanone moiety at the other. Our modeling results have indicated that the benzyl group interacts with Trp 84, which is located near the bottom of the binding pocket and is postulated to be the quaternary ammonium binding site for acetylcholine. The other aromatic ring has been found to interact with Trp 279 at the peripheral hydrophobic site. In addition, the hydrogen-bonding interaction between a carbonyl group of the inhibitor and Tyr 121 OH seems to play an important role. Our active-orientation model is, at least qualitatively, consistent with structure–activity data for more than 50 compounds and should be useful for the design of more potent inhibitors.

Introduction

Acetylcholinesterase (AChE) plays a crucial role in terminating transmission at cholinergic synapses by rapid hydrolysis of acetylcholine (ACh). Controlled inhibition of this enzyme can be of therapeutic importance for diseases associated with ACh depletion, especially senile dementia of the Alzheimer type (SDAT). Actually, tetrahydroaminoacridine (THA), an AChE inhibitor, has been reported to be effective for the treatment of SDAT,[1] and much attention has been focused on AChE inhibitors. Recently, one of us (Y.I.) and his co-workers have synthesized a series of benzylamino AChE inhibitors with a phthalimide, benzoyl, or indanone moiety with the aim of creating effective drugs for the treatment of SDAT.[2] In designing these compounds, they assumed, without the knowledge of the enzyme structure, that there are two hydrophobic binding sites (HBS-1 and HBS-2) in AChE: HBS-1 is located near the esteratic subsite and HBS-2 some distance away from HBS-1. Although their assumption is consistent with the structure–activity data for this series of compounds, no study has been carried out to define the residues involved in HBS-1 and HBS-2.

More recently, Sussman *et al.* have determined the crystal structure of AChE from *Torpedo californica* and have shown that the catalytic site is located at the bottom of a deep and narrow gorge surrounded by 14 aromatic amino acids.[3] By docking studies, Trp 84 located near the bottom of the gorge has been identified as the primary site of interaction with the quaternary group of ACh. They have also reported the crystal structures of some AChE–inhibitor complexes, indicating that Trp 84 is the binding site for the quaternary ammonium group of decamethonium and edrophonium and for the aromatic ring of THA.[4] In addition, Trp 279 at the peripheral site has been shown to be involved in the binding of the second quaternary group in decamethonium. Therefore, these Trp residues could correspond to HBS-1 and HBS-2 mentioned above.

As the X-ray analysis of the AChE–inhibitor complex cannot be easily done, we chose a theoretical approach, "docking". Docking a ligand in the receptor has been one of the main subjects of computer-assisted drug design,[5] and some programs for this purpose have been reported.[6,7] Among them, DOCK developed by Kuntz *et al.*[6] is one of the most successful. It represents the negative image of the binding site as a cluster of spheres, and docks the ligand molecule by a distance-matching algorithm. A modified version of DOCK (directed-DOCK) combined with a systematic conformational search is also available for docking flexible ligands.[8] In this paper, we have applied DOCK and directed-DOCK to the compounds mentioned above in order to elucidate their binding mode to AChE and to explain the known structure–activity data. This study should be the first step toward the structure-based design of more potent AChE inhibitors.

Methods

(1) Enzyme Structure. The crystal structure of the *Torpedo* AChE determined by Sussman *et al.*[3] (1ACE in the Protein Data Bank[9]) was used for this study with a slight modification, described below. Although no three-dimensional structure is available for the human AChE, its amino acid sequence has been determined[10] and shown to have high homology with the *Torpedo* AChE. Especially, the residues constituting the surface of the ligand-binding pocket are identical except for Phe 330, which is replaced with Tyr in the human AChE. Therefore, we replaced Phe 330 by Tyr with the identical conformation, and used it as a model for the human AChE without energy minimization. Three of the bound water molecules (4, 43, and 58) were also included in the model; H_2O 43 and 58 are inside the enzyme and not exposed to the solvent while H_2O 4 is at the bottom of the pocket, binding tightly (B-factor = 4.0) with Tyr

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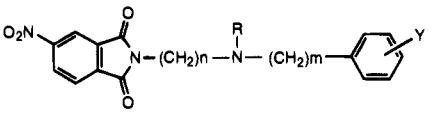
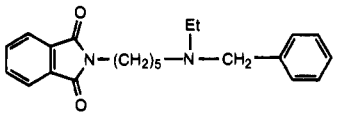
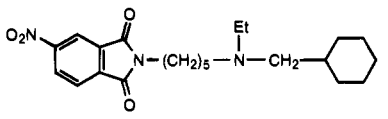
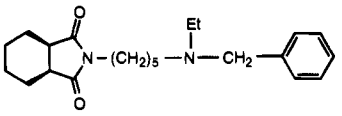
Table 1. Structure–Activity Data for Compounds Studied

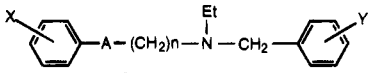
Series 1



compd	*n*	*m*	R	Y	IC_{50} (nM)	compd	*n*	*m*	R	Y	IC_{50} (nM)
1	5	1	Et	3-OCONHMe	0.38	**8**	6	1	Et	H	607
2	5	1	Et	H	151	**9**	5	2	Et	H	770
3	5	1	Et	2-OMe	24	**10**	5	3	Et	H	161
4	5	1	Et	3-OMe	45	11	4	1	Me	H	8230
5	5	1	Et	2,3-$(OMe)_2$	274	**12**	5	1	Me	H	1360
6	5	1	Et	4-OMe	269	**13**	6	1	Me	H	474
7	4	1	Et	H	252	**14**	5	1	Pr	H	2650

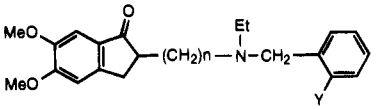
compd	structure	IC_{50} (nM)	compd	structure	IC_{50} (nM)
15		3370	**17**		1090
16		18500			

Series 2



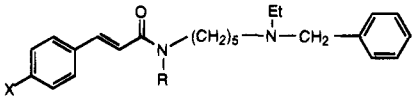
compd	*n*	A	X	Y	IC_{50} (nM)	compd	*n*	A	X	Y	IC_{50} (nM)
18	6	C=O	3,4-$(OMe)_2$	2-OMe	10	**21**	5	C=O	4-pyrrolidino	H	305
19	5	C=O	3,4-$(OMe)_2$	2-OMe	24	**22**	6	O	3,4-$(OMe)_2$	2-OMe	920
20	5	C=O	4-pyrrolidino	2-OMe	50	**23**	6	CH_2	3,4-$(OMe)_2$	2-OMe	1070

Series 3



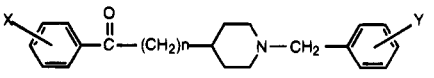
compd	*n*	Y	IC_{50} (nM)	compd	*n*	Y	IC_{50} (nM)
24	4	OMe	20	**26**	6	OMe	100
25	5	OMe	6	**27**	4	H	76

Series 4



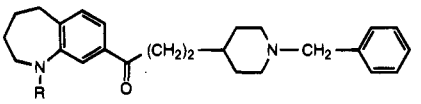
compd	R	X	IC_{50} (nM)	compd	R	X	IC_{50} (nM)
28	H	H	11800	**30**	H	NO_2	3000
29	Ac	H	2300	**31**	Ac	NO_2	539

Series 5

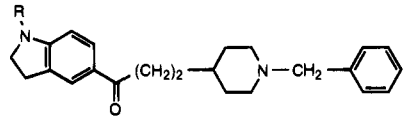


compd	*n*	X	Y	IC_{50} (nM)	compd	*n*	X	Y	IC_{50} (nM)
32	1	4-pyrrolidino	H	19000	**36**	2	4-pyrrolidino	3-OMe	5320
33	2	4-pyrrolidino	H	28	**37**	2	4-pyrrolidino	4-OMe	30200
34	3	4-pyrrolidino	H	220	**38**	2	3,4-$(OMe)_2$	H	52
35	2	4-pyrrolidino	2-OMe	1900					

Table 1 (Continued)

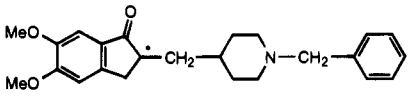


compd	R	IC_{50} (nM)	compd	R	IC_{50} (nM)
39	H	98	**42**	Pr	1330
40	Me	81	**43**	CH_2Ph	3140
41	Et	376			

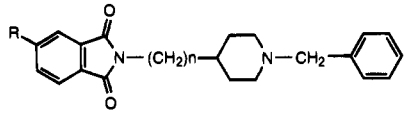


compd	R	IC_{50} (nM)	compd	R	IC_{50} (nM)
44	H	20	**47**	Pr	22
45	Me	10	**48**	CH_2Ph	68
46	Et	11			

Series 6



compd	IC_{50} (nM)	compd	IC_{50} (nM)
49 (*R*)	5.3	**50** (*S*)	7.7



compd	*n*	R	IC_{50} (nM)	compd	*n*	R	IC_{50} (nM)
51	2	H	30	**53**	1	NO_2	3000
52	0	NO_2	27000	**54**	2	NO_2	13

130 and Glu 199 through hydrogen bonding. The numbering of AChE residues in this paper is based on the *Torpedo* enzyme.

(2) Compounds. The compounds studied in this paper are listed in Table 1 with their AChE inhibitory activities.[18] These compounds have a phthalimide, benzoyl, or indanone moiety at one end of the molecule (except for series 4) and a benzyl moiety connected to a tertiary ammonium group at the other. In addition to the compounds synthesized by Ishihara *et al.*,[2] similar compounds reported by another group[11,12] (series 6 in Table 1) were also taken into account. The activities of the studied compounds range from 0.38 nM to 30 μM.

(3) DOCK. Version 3.0 of DOCK[6c] was used for compound **33** with standard parameters. Details are described in the original paper and are not repeated here. The ligand–receptor interaction was estimated according to the grid-based force field scores, and a distance-dependent dielectric constant ($\epsilon = 4r$) was used in the calculation of electrostatic energy.

(4) Directed-DOCK with Conformational Search.[8] This was used for docking compounds **1** and **3**. Briefly, the ligand molecule was decomposed into anchor and flexible fragments, and the anchor fragment was docked into the receptor with directed-DOCK, which considers hydrogen-bonding interactions explicitly. Then, the flexible fragment was attached to the anchor, and a systematic conformational search was performed in the binding site of the receptor. Sterically allowed orientations thus obtained were clustered and energy minimized as described below.

(5) Energy Minimization. After docking [either by (directed-)DOCK or manually], the AChE–ligand complex was energy minimized using Discover (Biosym Technologies) with the enzyme atoms fixed. Acidic and basic residues except for histidines were assumed to be charged and a distance-dependent dielectric constant ($\epsilon = 4r$) was adopted. A cutoff of 11 Å was used, and no solvent molecule was included in the calculation except for those mentioned above. Energy minimization was carried out for 500 steps with the steepest descent minimizer and subsequently for 1000 steps with the conjugate gradient minimizer with the CVFF force field in Discover.

Results and Discussion

(1) Compound 1. Compound **1** has a carbamoyl ester moiety, which will be attacked by the catalytic Ser 200 to form a tetrahedral intermediate. Therefore it is relatively easy to locate the benzyl moiety of the inhibitor in the binding pocket of the enzyme. We first studied a model shown in Figure 1 as an anchor

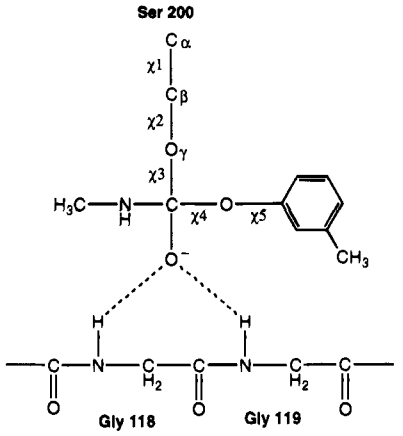


Figure 1. Anchor fragment of **1** connected to Ser 200 Oγ of AChE.

fragment of 1. Due to the steric constraints, the allowed values for χ^1, χ^2, and χ^3 were limited only near 180°, 180° and −60°, respectively. χ^4 and χ^5 were tentatively assumed to be 180° and −90° (where there were no bad contacts), and energy minimization was carried out with the enzyme atoms fixed. The minimized structure was used for the following analysis. In this structure, the oxyanion of the inhibitor formed hydrogen bonds with amide hydrogens of Gly 118 and Gly 119 that constitute the "oxyanion hole". With χ^1–χ^3 fixed to the minimized values, a systematic conformational search was done for χ^4 and χ^5 by rotating the bonds in 10° increments in the presence of the enzyme atoms. The global minimum was found at $\chi^4 = 170°$ and $\chi^5 = -90°$, and conformers with $\chi^4 = 160°-170°$ and $\chi^5 = -100°$ to −80°, 20°, or 60°−100° were within 10 kcal/mol of the global minimum. Eleven representative conformers within this range were chosen and used as anchor fragments. The flexible fragment of **1** was attached to each anchor and a systematic search was performed with Leach's program.[8] Both stereoisomers of the tertiary ammonium moiety were taken into account. A total of 4008 orientations were obtained, of which 696 had at least one hydrogen bond with the enzyme and were within 10 kcal/mol of the best orientation according to the grid-based force field score. These 696 orientations were clustered into 50 groups as described,[8] and the top 20 representative orientations were energy minimized with Discover. The result is shown in Table 2, in which each orientation is ordered by the total energy (E_{tot}).

According to the structure–activity data of the related compounds (e.g. **18** vs **22** or **23**), at least one carbonyl group of the phthalimide is essential for high activity and is supposed to form a hydrogen bond with the enzyme. Of the 20 energy-minimized structures, only orientations 3 and 7 satisfy this requirement. In 3, one carbonyl oxygen formed a hydrogen bond with Tyr 121 OH (O—O distance = 2.87 Å), and in 7, a hydrogen bond with Tyr 70 OH (O—O distance = 3.30 Å) was formed. As 3 has the best interaction energy with the enzyme both before (E_{grid}) and after minimization (E_{bnd}), 3 was assumed to be the active orientation of **1** (Figure 2a).[13]

In this orientation, the benzyl group was located in the hydrophobic pocket near Trp 84, which is deep in the binding pocket and is presumed to be the binding site for quaternary ligands. As shown in Figure 2b, the benzyl moiety of **1** partly overlapped with the quaternary ammonium group of ACh.[3] The benzene ring of

Table 2. Summary of Energy Calculation for Compound 1–AChE Complex

orientation	E_{tot}[a]	E_{lig}[b]	E_{bnd}[c]	E_{grid}[d]
1	88.0	150.9	−62.9	1.8
2	91.2	150.8	−59.6	1.2
3	92.4	155.9	−63.5	0.0
4	94.0	154.1	−60.1	1.1
5	94.0	154.3	−60.3	1.2
6	94.1	153.1	−59.0	0.2
7	94.6	153.3	−58.7	2.0
8	95.4	156.2	−60.8	3.7
9	95.5	153.6	−58.1	1.4
10	95.6	150.4	−54.8	3.8
11	96.3	155.7	−59.4	2.1
12	96.6	152.0	−55.4	1.0
13	97.9	154.8	−56.9	3.5
14	98.1	152.5	−54.4	1.7
15	99.0	152.3	−53.3	3.9
16	100.5	157.3	−56.8	3.2
17	102.2	156.1	−53.9	3.8
18	102.5	158.2	−55.7	0.5
19	103.0	157.5	−54.5	2.8
20	103.0	156.2	−53.2	3.3

[a] Total energy after minimization by Discover (kcal/mol). [b] Intramolecular energy of the ligand after minimization (kcal/mol). [c] Interaction energy between the enzyme and ligand after minimization (kcal/mol). [d] Grid-based energy before minimization (relative to orientation 3) (kcal/mol).

the phthalimide moiety was in the "peripheral" hydrophobic pocket, interacting orthogonally with Trp 279. This pocket is surrounded by a cluster of aromatic residues such as Phe 290 and Phe 331, and seems suitable for the binding of an aromatic group. In addition, Trp 279 is the site for a quaternary group of decamethonium as shown by X-ray analysis[4] and is also suggested by mutational experiments[14] to be the binding site for propidium, an inhibitor with aromatic rings. Our modeling result is compatible with these experimental data and indicates that HBS-1, as postulated previously, corresponds to Trp 84 and HBS-2 to Trp 279, as expected.

The methylene chain was in the crevice surrounded by Tyr 330 and Tyr 334, and all CH_2—CH_2 bonds but one adopted a trans conformation. The terminal NMe group was in a small hydrophobic pocket consisting of Phe 288, Phe 290, Phe 331, and Trp 233. This site is close to the postulated binding site for the acetyl group of ACh (Figure 2b). The proton of the tertiary ammonium group did not interact directly with the enzyme; the positive charge is supposed to be stabilized by the negative charge of Asp 72 and the aromatic rings of Trp 84 and Tyr 330 through the aromatic–ammonium interaction.[15] One of the two C=O's of the phthalimide group did not form a hydrogen bond with the enzyme, and is probably not essential for the binding. This is consistent with the experimental data that some compounds with one C=O have high activity (e.g. **18** and **25**).

(2) Compound 3. Next we studied compound **3**, which has the highest activity in series 1 except for compound **1**. The carbamoyl ester moiety of **1** was removed from the model and a 2-OMe group was introduced. Two possibilities (models 1 and 2) were considered for the position of 2-OMe, and each was subjected to energy minimization (Figure 3a). As compound **3** has a pseudosymmetric structure with an aromatic ring at both terminals, it is possible that this molecule binds to the enzyme with a "reversed" orientation compared with **1**. Therefore a systematic analysis was also done using Leach's program. The phthalimide

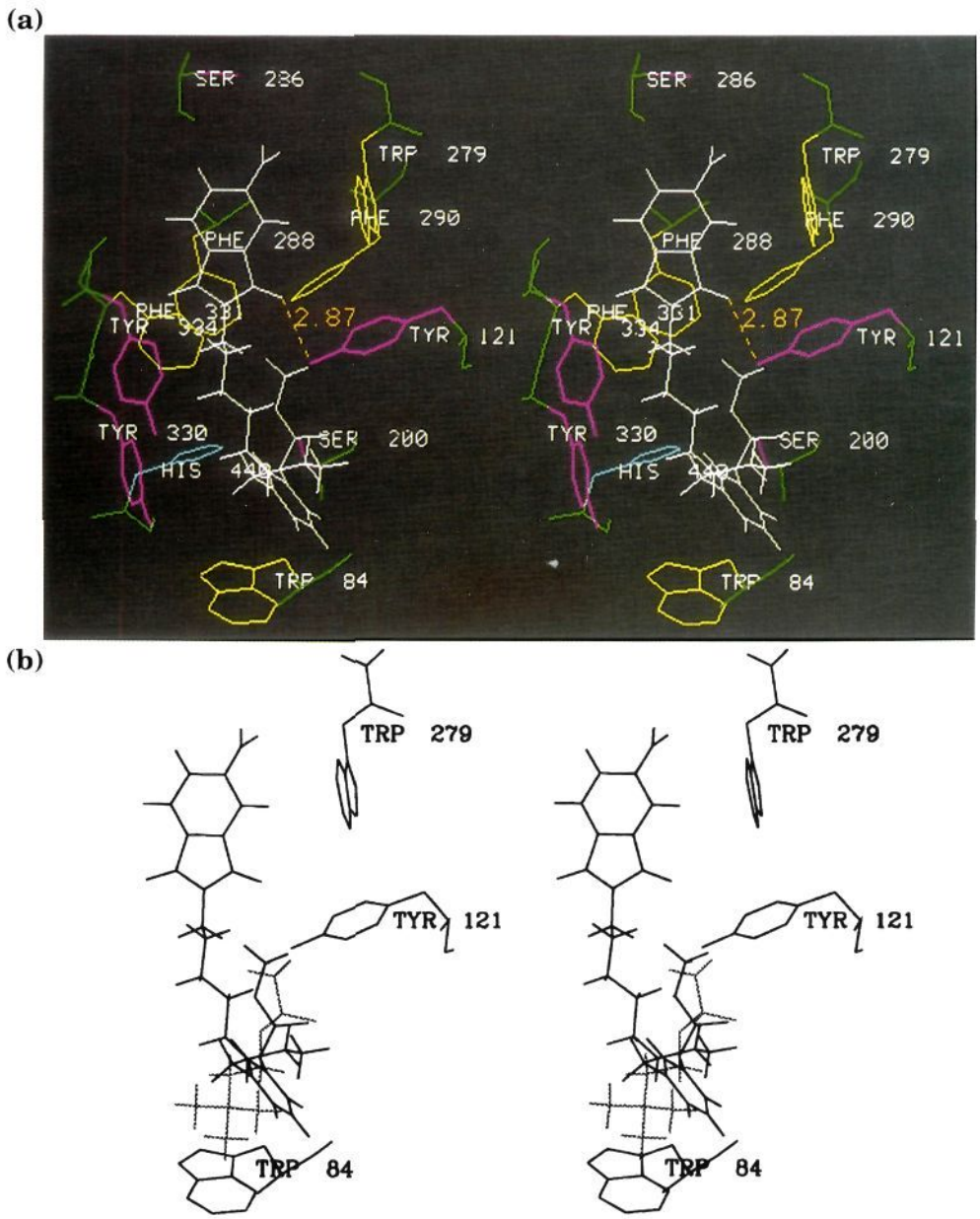


Figure 2. (a) Binding mode of **1** to AChE. Aromatic residues in the binding pocket as well as Ser 200 and Ser 286 are shown. Color codes: white, compound **1** (orientation 3); green, main chains; yellow, Phe and Trp side chains; magenta, Ser and Tyr side chains; cyan, His side chain. Hydrogen atoms are shown only for the ligand. (b) Comparison of the binding mode of **1** (orientation 3, solid lines) and ACh (dotted lines). The orientation of ACh is from Sussman *et al.*[3]

moiety was chosen as an anchor fragment, and two local minimum conformers were taken into account for the NO_2 group. Of the 2245 anchor orientations obtained by directed-DOCK, 596 had at least one hydrogen bond with the enzyme and were within 10 kcal/mol of the best orientation. These were classified into 100 clusters and the flexible fragment was attached to the best orientation of each cluster. About 80% of the anchor orientations were located near the bottom of the binding pocket, not in the peripheral pocket like **1**. After the systematic search by Leach's program, 58 273 orientations were obtained and pruned to 1416 with the same criteria as above. They were classified into 200 clusters, and the top 20 (15 with reversed and 5 with nonreversed orientations) were subjected to energy minimization with the enzyme atoms fixed (Table 3). The top two had reversed orientations (Figure 3b for 1), and the best one with a nonreversed orientation (3) was 2.9 kcal/mol less stable than 1. However, the models built from **1** (model 1 and 2) had better E_{tot} than 3, and model 2 was only 0.8 kcal/mol less stable than 1.[16] Furthermore, the interaction energy (E_{bnd}) of model 2 was the best among these orientations. From these results alone, it was difficult to tell which orientation (reversed or not) is more reasonable. We therefore compared these orientations with structure–activity data.[2] The data indicate that the substituent in the phthalimide ring is more permissible than that in the benzyl group (not shown in Table 1). In the compound **1**-like orientation, the phthalimide ring is at the peripheral site, and there is sufficient room for the substituent. On the other hand, the benzyl moiety is deep in the pocket, and not much space is available around the benzene ring. This is compatible with the structure–activity data. In the

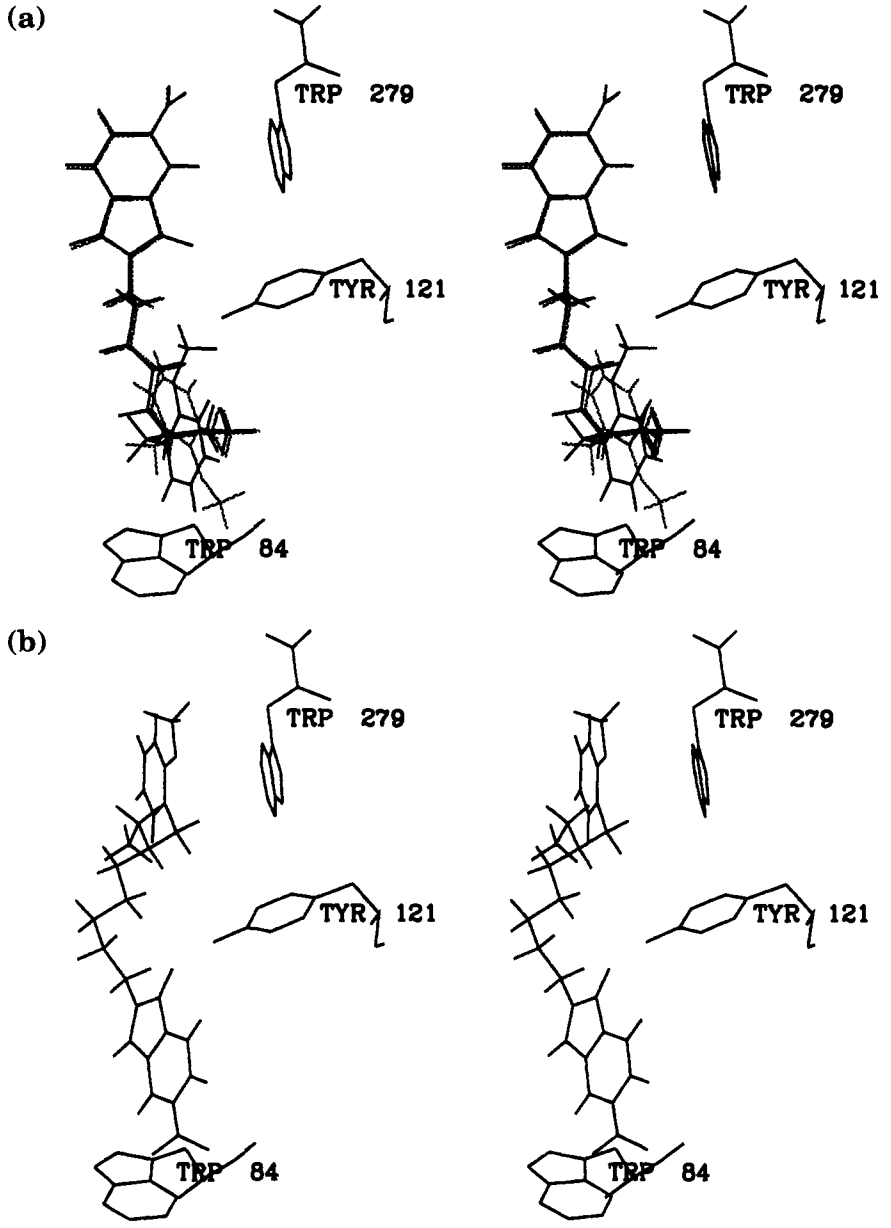


Figure 3. Binding models for **3**: (a) manual model **1** (dotted lines) and model 2 (solid lines), (b) best score orientation. Trp 84, Tyr 121, and Trp 279 are also shown. Hydrogen atoms are shown only for the ligand.

Table 3. Summary of Energy Calculation for Compound **3**–AChE Complex[c]

orientation	E_{tot}	E_{lig}	E_{bnd}	E_{grid}
1	66.8	141.5	−74.7	−47.8
2	69.6	143.9	−74.3	−49.6
3[a]	69.7	143.4	−73.7	−41.2
4[a]	71.0	146.3	−75.3	−45.1
5[a]	72.6	144.6	−72.0	−43.4
6	72.8	143.4	−70.6	−47.6
7	73.1	145.2	−72.1	−48.2
8	73.8	150.8	−76.2	−47.5
9	76.3	145.9	−69.6	−48.0
10	76.8	144.8	−68.0	−47.2
11	76.9	143.1	−66.2	−49.4
12[a]	77.9	148.1	−70.2	−41.0
13	78.6	144.5	−65.9	−47.2
14	80.9	144.8	−63.9	−48.3
15[a]	81.3	153.6	−72.3	−45.0
	Manual Modeling[b]			
model 1	68.0	144.4	−76.4	
model 2	67.6	145.3	−77.7	

[a] Compound 1-like orientations. [b] Modeling based on orientation 3 of compound 1. [c] See Table 2 for details. Absolute values are shown for E_{grid}. Orientations 16–20 are omitted.

case of the reversed orientation, however, there is not much space around the phthalimide moiety, inconsistent with the experimental results. Accordingly, it is reasonable to assume that compound **3** also adopts a compound **1**-like orientation. Judging from the energy values, we chose model 2 as the binding mode for **3**.[13] This conformation is nearly identical to that of **1** except for the orientation of the benzene ring (Figure 4); the benzene ring of **3** is orthogonal to Trp 84. The methyl group of 2-OMe interacts with Phe 290 and Phe 331, and is close to the position of NMe in **1**.

(3) Compound 3-Related Compounds. Based on this model, the structure–activity relations of compound **3**-related compounds (series 1 in Table 1) were examined. The unsubstituted molecule (**2**) is 6–7 times less active than **3**. This can be attributed to lack of the OMe–enzyme interaction described above. The 3-OMe derivative (**4**) also has high activity. As shown in Figure 5, 3-OMe can occupy nearly the same position as 2-OMe. The oxygen atom of 3-OMe can form a hydrogen bond

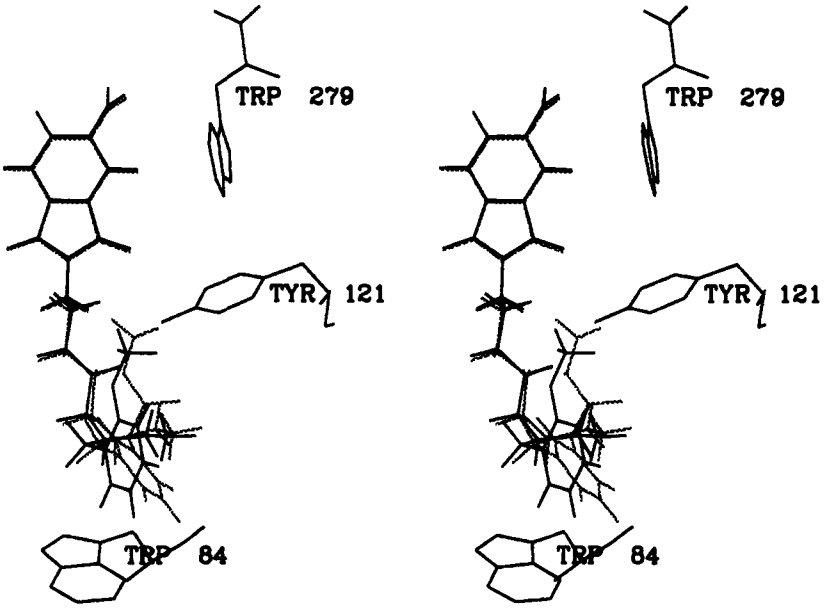


Figure 4. Comparison of the binding mode of **1** (dotted lines) and **3** (solid lines).

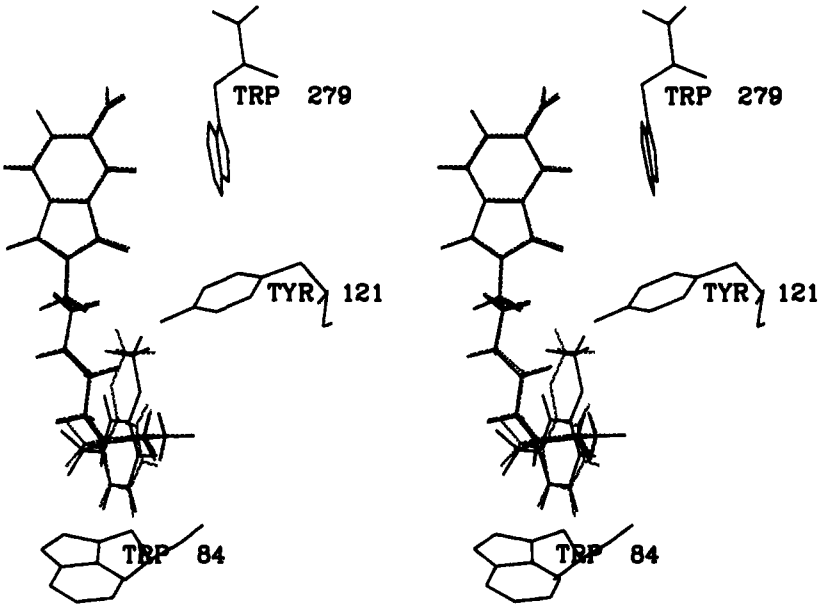


Figure 5. Comparison of the binding mode of **3** (2-OMe, dotted lines) and **4** (3-OMe, solid lines).

with Ser 200, but this interaction might not be very strong, as this Ser already interacts with His 440. There is not much space around position 4 of the benzene ring, and introduction of 4-OMe in the model caused bad contacts. This is consistent with the data that the 4-OMe derivative (**6**) is less active than the unsubstituted compound. The 2,3-$(OMe)_2$ derivative (**5**) is also weakly active. As can be seen from Figure 5, at least one OMe needs to change its conformation compared with the 2-OMe or 3-OMe derivative to avoid a bad contact between the two OMe groups; however, this will cause steric hindrance with the enzyme.

Regarding the substituent at the ammonium group, ethyl is optimal. If this is replaced with propyl (**14**) in the model, some bad contacts are caused with the enzyme. If it is replaced with methyl (**12**), a cavity is formed between the enzyme and inhibitor, which is unfavorable for the interaction. In either case, our modeling results are in agreement with the experimental data.

As for the substituent at position 5 of phthalimide, hydrophilic and electron-withdrawing groups are favorable[2a] (data not shown in Table 1). As this substituent is located at the peripheral site and fairly exposed to the solvent, it is reasonable that hydrophilic groups are preferable. The electron-withdrawing capacity of the substituent might alter the hydrogen-bonding capability with Ser 286. Besides, the electron-density change caused by the substituent would affect the interaction with Trp 279.

The length of the methylene chain is also an important factor. When R = Et, the activity is highest for **2** ($n = 5$), but it is not greatly decreased for **7** ($n = 4$). We found that compound **7** can adopt an orientation similar to that of **2** by assuming an all-trans conformation in the methylene chain (Figure 6). In the case of **8** ($n = 6$), the activity is decreased more. This can be attributed to the inability of this compound to form a hydrogen bond with Tyr 121, at least with a stable conformation. When R = Me, compound **13** ($n = 6$) has

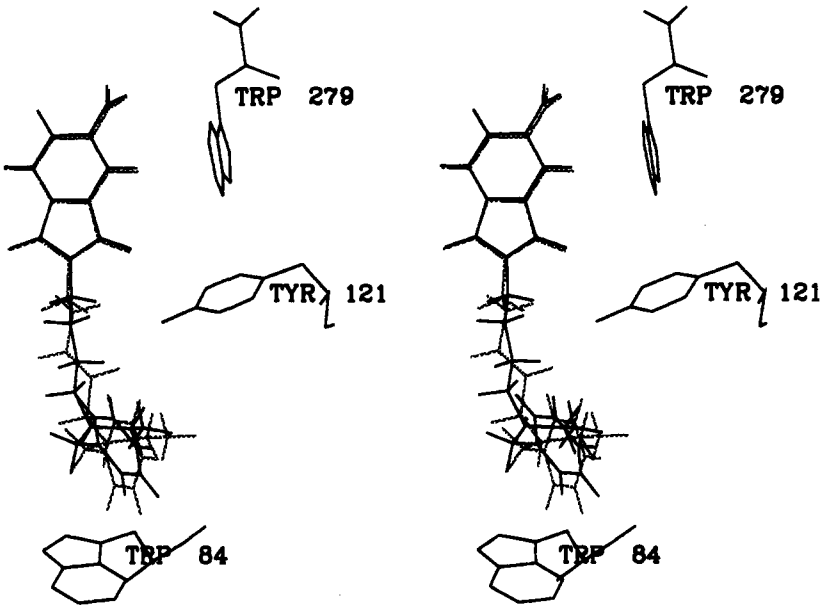


Figure 6. Comparison of the binding mode of **2** ($n = 5$, dotted lines) and **7** ($n = 4$, solid lines).

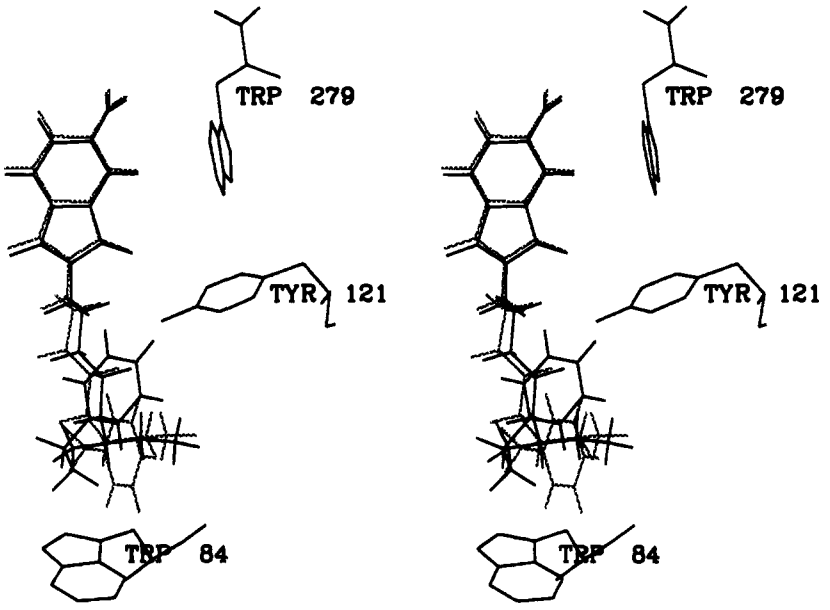


Figure 7. Comparison of the binding mode of **2** ($m = 1$, dotted lines) and **10** ($m = 3$, solid lines).

higher activity than **12** ($n = 5$) and **11** ($n = 4$). Although we have not constructed a model for these compounds, a reasonable explanation would be that they can bind more deeply in the pocket due to the smaller size of the methyl group and as a result a longer methylene chain is more favorable.

When the length m between the ammonium group and the benzene ring is made longer, the activity is decreased for **9** ($m = 2$) but not for **10** ($m = 3$). In the case of **9**, it was difficult to avoid bad contacts between the enzyme and the benzene ring without a significant change in the orientation and/or conformation (data not shown). On the other hand, a reasonable orientation was obtained for **10** (Figure 7), in which the benzene ring was located in the pocket surrounded by Phe 290 and Phe 331, close to the site of 2-OMe in **3**. Although we have not performed a systematic search for **10** due to its high flexibility, the above orientation would be at least one of the reasonable orientations.

Replacement of an aromatic ring with an aliphatic ring decreases the activity (**16** and **17**). The modeling results indicated that, in the case of **16**, the cyclohexane ring moved away from the enzyme and was more exposed to the solvent compared with the corresponding phthalimide derivative (**15**). The cyclohexane ring of **17** was found to cause steric hindrance with the enzyme due to its bulkier shape than a benzene ring.

(4) Modification of the Phthalimide Moiety (Series 2 and 3). Compounds with a benzoyl or indanone moiety instead of a phthalimide also have high activity (series 2 and 3). Binding models for **18** and **25** were constructed based on the model of **3** (Figure 8). In these models, the hydrogen bond between C=O and Tyr 121 OH was conserved, and the benzoyl or indanone ring interacted with Trp 279 like the phthalimide ring in **3**. The location of the benzyl group was nearly identical to that for **3**. This is in agreement with a common structure–activity relationship that 2-OMe increases the activity (**3** vs **2**, **20** vs **21**, **24** vs **27**).

Regarding the number of atoms between C=O and the ammonium nitrogen, the optimum was six, but the activity was also high when it was five. This is

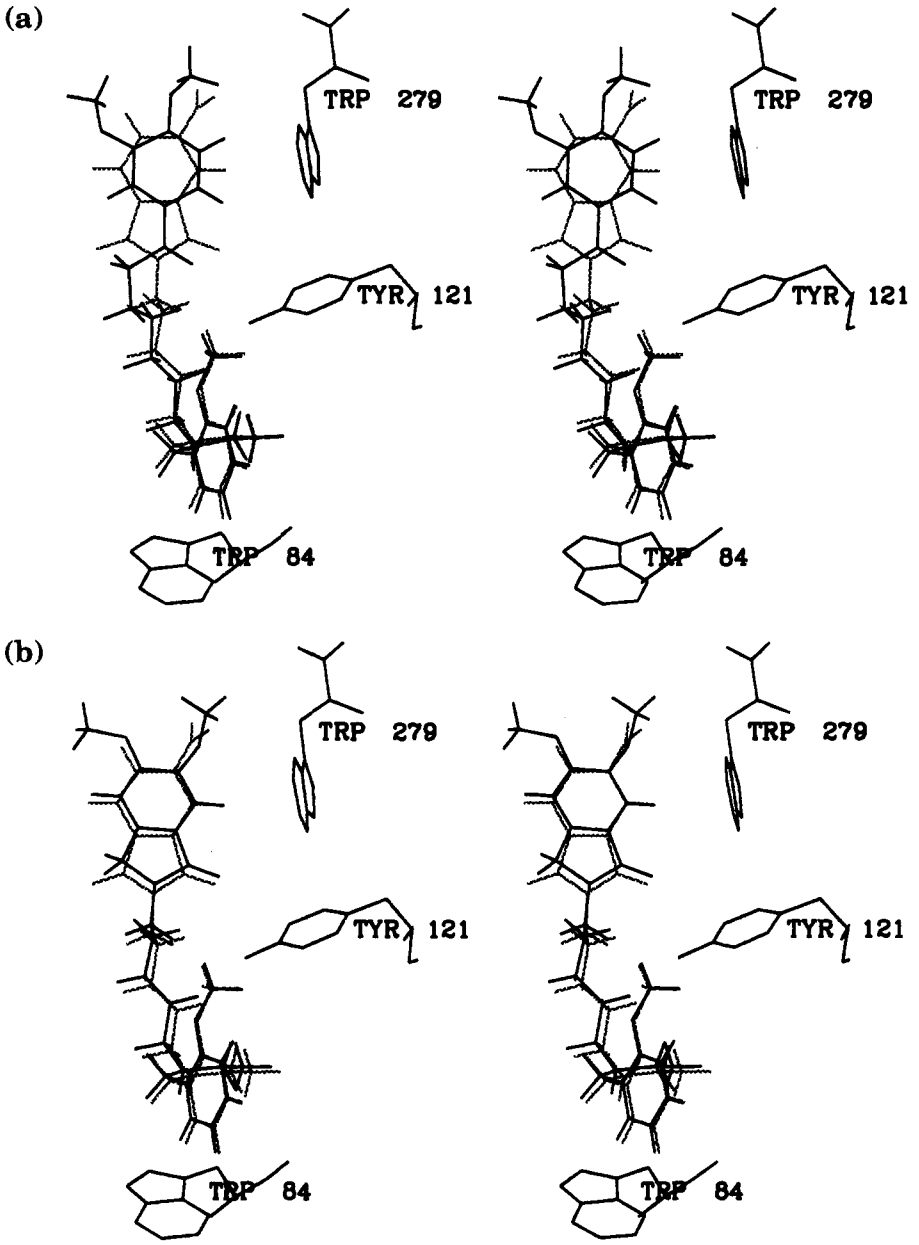


Figure 8. Comparison of the binding mode of (a) **3** (dotted lines) and **18** (solid lines) and (b) **3** (dotted lines) and **25** (solid lines).

consistent with the data for series 1 and can be explained by essentially the same model as described above.

(5) Series 4. Compounds in this series are generally weakly active. We built a model for this series of compounds and found that the benzene ring of the styrene moiety did not interact with Trp 279, but the vinyl moiety did (data not shown). An acetyl group is better than hydrogen for R, but in the model, the C=O of the acetyl group was not involved in hydrogen-bonding interactions, and the van der Waals interactions of the methyl group seem more important for the binding. The NO_2 moiety formed a hydrogen bond with Ser 286.

(6) Piperidine Derivatives (Series 5). In series 5, a part of the methylene chain was replaced with a piperidine ring. The model for one of the most potent compounds of this series (**33**) was first built manually on the basis of the model for series 1-3. A reasonable model was obtained in which the benzyl group interacted with Trp 84 and the benzoyl group with Trp 279, and C=O formed a hydrogen bond with Tyr 121 OH (Figure 9). We also carried out a systematic search for **33**. As this compound has fewer degrees of freedom than those mentioned above, possible local minimum conformers were generated before docking by use of Search–Compare (Biosym Technologies). Conformers were built with 120° intervals for sp3–sp3 bonds and 45° intervals for the others and subjected to energy minimization. Forty-two conformers within 5 kcal/mol of the global minimum were docked into the enzyme using DOCK, and the top orientation for each conformer was chosen. The top 20 of these orientations were energy minimized by Discover with the enzyme atoms fixed (Table 4).

The top six had orientations similar to the manual model with the benzoyl moiety at the peripheral site. Among high-score orientations, only number 4 formed a hydrogen bond between C=O and the enzyme. In 4, the locations of the benzyl group and piperidine ring were similar to those in the manual model. Although the manual model has good E_{tot} and E_{bnd} values, this

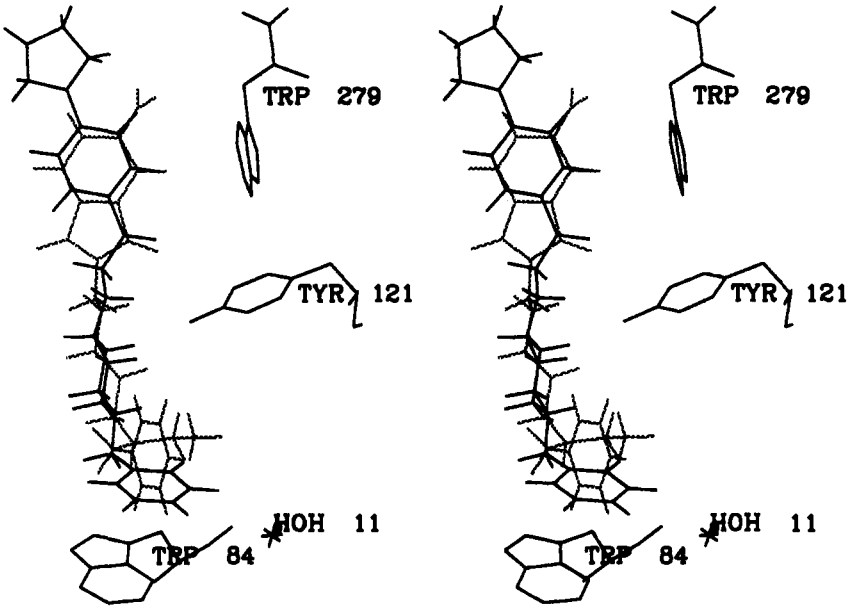


Figure 9. Comparison of the binding mode of **2** (dotted lines) and **33** (solid lines). Bound water molecule **11** is also shown.

Table 4. Summary of Energy Calculation for Compound **33**–AChE Complex[c]

orientation	E_{tot}	E_{lig}	E_{bnd}	E_{grid}
1[a]	61.1	126.1	−65.0	−32.4
2[a]	61.2	124.0	−62.8	−37.9
3[a]	62.7	125.0	−62.3	−35.8
4[a]	62.7	123.2	−60.5	−34.8
5[a]	63.1	124.6	−61.5	−35.5
6[a]	64.4	125.9	−61.5	−34.0
7	64.9	124.8	−59.9	−32.6
8[a]	65.1	123.3	−58.2	−32.5
9[a]	65.2	125.9	−60.7	−35.3
10	65.3	127.8	−62.5	−35.9
11[a]	66.6	124.7	−58.1	−32.9
12	66.8	123.9	−57.1	−32.9
13[a]	67.6	125.6	−58.0	−37.1
14[a]	67.9	126.6	−58.7	−34.1
15	68.0	123.9	−55.9	−31.8
16	69.8	126.7	−56.9	−32.6
17	70.1	125.7	−55.6	−32.3
18[a]	70.6	126.1	−55.5	−34.4
19	71.5	125.1	−53.6	−31.7
20	73.8	125.2	−51.4	−32.2
	Manual Modeling[b]			
	62.3	127.3	−64.9	

[a] Compound 1-like orientations. [b] Modeling based on the orientation of compound 1. [c] See Table 2 for details. Absolute values are shown for E_{grid}.

orientation was not obtained by the above search. This is probably due to the fact that the conformation of the manual model is a little shifted from the local minimum. Actually, when the manual model conformer was docked into the enzyme using DOCK, the best orientation was similar to the model orientation. Considering the importance of the hydrogen bonding of C=O, either orientation 4 or the manual model should be adopted as the active orientation of **33**. In 4, however, the benzoyl moiety (especially its substituent) was more exposed to the solvent than in the manual model, and the interaction energy with the enzyme was worse. As the substituent at the benzoyl group has some effects on the activity (**33** vs **38**), the manual model seems more reasonable. Therefore we chose this as the active orientation for **33**.[13]

In this model, the benzene ring of the benzyl group had a stacking interaction with Trp 84 (Figure 9), which is different from the orthogonal interaction observed in **3** and related compounds. This difference in the orientation of the benzene ring might be related to the difference in the substituent effect on the benzene ring. In series 1, 2-OMe and 3-OMe increase the activity while 4-OMe decreases the activity. In series 5, on the other hand, the activity is significantly decreased in every case in the order 2-OMe > 3-OMe > 4-OMe. When 4-OMe was introduced into the model (**37**), steric hindrance with the enzyme was unavoidable with any conformation of the OMe group. In the case of 3-OMe (**36**), a bad contact with a bound water (H_2O 11) was inevitable, and it was necessary to remove this water for this compound to bind to the enzyme (this water was not included in the above calculations). As the water molecule forms hydrogen bonds with Tyr 130 OH and Gly 117 C=O, its replacement would be energetically unfavorable. 2-OMe could be permitted with some limited conformations, but the allowed range is so restricted that its binding would be entropically unfavorable. These modeling results are consistent with the experimental data mentioned above.

The position of the ammonium nitrogen was shifted by 1.51 Å toward the peripheral site compared with **3**. In series 5, the maximum activity was observed when there were five atoms between C=O and the ammonium nitrogen. This is not consistent with the data for series 1-3, for which six was the best. This difference might be attributed to the difference in the position of the nitrogen atom mentioned above. In **34** (n = 3), the hydrogen bond between C=O and Tyr 121 OH cannot be formed, at least with a local minimum conformation, and this could be the cause of its lower activity. Compound **32** (n = 1) cannot adopt a conformation similar to the active conformation of **33** (n = 2), and when the piperidine ring was superimposed on that of **33** in its complex with AChE, bad contacts between the benzoyl moiety and the enzyme was unavoidable. This is in agreement with its significantly low activity.

Some of 1-benzazepine and indoline derivatives (**39**-**48**) are also potent AChE inhibitors, and **39** (TAK-147) is now under clinical investigation. Compound **39** can adopt an orientation nearly identical to that of **33**

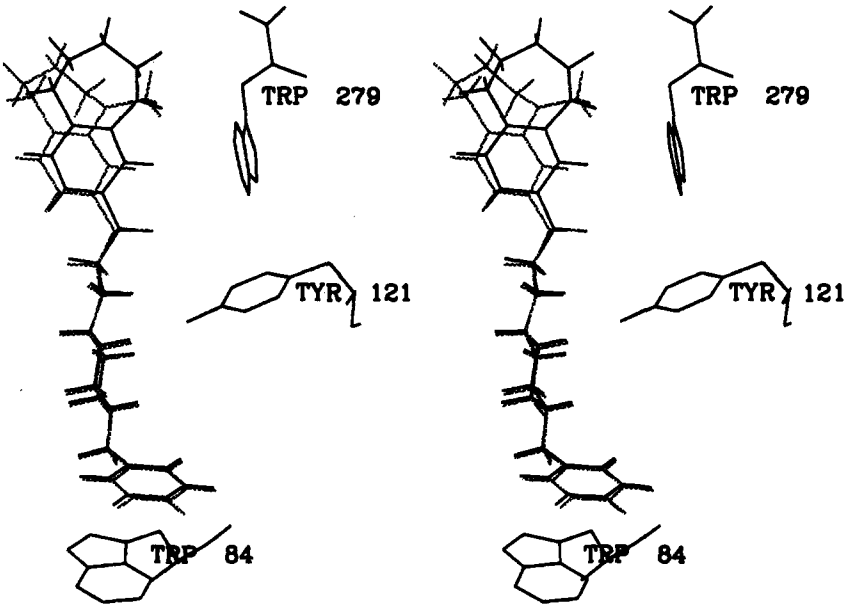


Figure 10. Comparison of the binding mode of **39** (solid lines) and **40** (dotted lines).

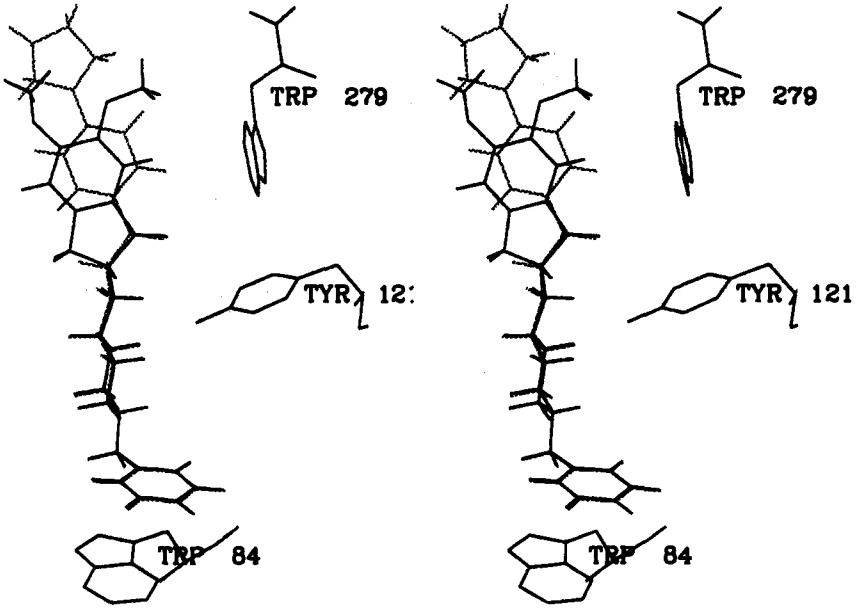


Figure 11. Comparison of the binding mode of **33** (dotted lines) and **50** (solid lines).

(Figure 10). Its *N*-methyl derivative (**40**) can also bind to the enzyme with a slight conformational change (Figure 10). However, if the substituent is larger than methyl, bad contacts with the enzyme (around Trp 279) cannot be avoided. These modeling results are compatible with the experimental data that bulky substituents decreased the activity significantly. In the case of indoline derivatives with the acyl moiety at a position para to the ring nitrogen (**44-48**), bulky substituents are acceptable at the nitrogen atom. In our model for **44**, there is sufficient space around this atom, and the substituents at this position are assumed to be exposed to the solvent (data not shown). This is consistent with the data showing that introduction of a large substituent does not alter the activity significantly.

(**7**) **Series 6.** A model for **50** was easily built from the active conformation of **33** (Figure 11). A similar model was obtained for **49** (data not shown), in agreement with the data that **49** and **50** are equally active. When the indanone ring was replaced with phthalimide (**53**), the activity was significantly decreased. This can be attributed to the difference in the hybridization state of the atom next to C=O: sp^3 for indanone and sp^2 for phthalimide. In **53**, the phthalimide ring cannot be directed the same way as the indanone ring of **50** and cannot make favorable contacts with the enzyme. The corresponding compound in series 1 (**7**) is more flexible and can fit in the pocket with a reasonable conformation (Figure 6). In other words, compound **53** was fixed in a bad conformation by cyclization. When another methylene was inserted between the phthalimide and piperidine (**51** and **54**), the activity was high. A reasonable model for **51** that is consistent with other active compounds was constructed on the basis of the compound **33** model (Figure 12). On the other hand, the corresponding compound in series 5 (**34**), which has lower activity, cannot adopt a similar orientation without internal strain. Namely, inclusion of C=O in the ring structure helped **51** and **54** adopt the active conformation.

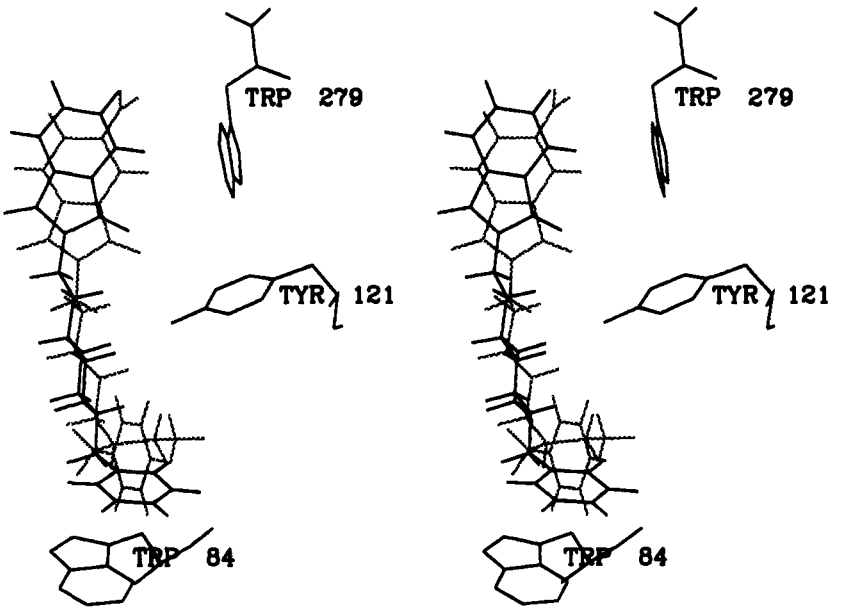


Figure 12. Comparison of the binding mode of **2** (dotted lines) and **51** (solid lines).

Cardozo *et al.*[17] have speculated on the active conformation of this series of compounds without considering the enzyme structure. However, their active conformation is different from ours. We docked their model into AChE using DOCK, but no high-score orientation formed a hydrogen bond between C=O and the enzyme. On the other hand, when our model conformer was docked with DOCK, high-score orientations were similar to the model orientation, and the hydrogen bond between C=O and Tyr 121 was well reproduced. Therefore, our model seems more reasonable, although there is a possibility that a slight conformational change of their model could improve the result. They have also suggested that C=O forms a hydrogen bond with Ser 200. However, considering the spatial relationship between Ser 200 and the quaternary group binding site, such a binding mode seems impossible.

Conclusions

By using (directed-)DOCK and the three-dimensional structure of AChE, we have succeeded in obtaining reasonable binding models that are, at least qualitatively, consistent with the structure–activity data for more than 50 compounds. In our model, one of the two aromatic groups of the inhibitor interacts with Trp 84, which is located near the bottom of the binding pocket, and the other one with Trp 279 at the peripheral hydrophobic site. These Trp residues would correspond to HBS-1 and HBS-2, which Ishihara *et al.* have assumed previously without the knowledge of the AChE structure.[2] In addition, the hydrogen-bonding interaction between the C=O group and Tyr 121 OH also seems important for tight binding. DOCK alone is not sufficient for determining the most reasonable orientation, but its use in combination with structure–activity data and manual modeling greatly helps define the active orientation. Although X-ray analysis of the complex is essential for very accurate study, the approach as described in this paper would still be very powerful for the design of more potent inhibitors when crystal structures of enzyme–inhibitor complexes are not easily available.

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