Molecular Docking Studies of Natural Cholinesterase-Inhibiting Steroidal Alkaloids from *Sarcococca saligna*

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Alkaloids isolated from *Sarcococca saligna* significantly inhibit acetyl- and butyrylcholinesterase enzyme, suggesting discovery of inhibitors for nervous-system disorders. Studying interactions with the active site of the AChE enzyme from *Torpedo californica*, we have identified hydrophobic interactions inside the aromatic gorge area as the major stabilizing factor in enzyme−inhibitor complexes of these alkaloids. Molecular Dynamics simulation of a predicted complex indicates that ligand binding does not extensively alter enzyme structure, but reduces flexibility at the gorge.

Introduction

Alzheimer's disease (AD) is the most common cause of dementia in the elderly population. The cholinergic hypothesis of AD has provided the rationale for the current major therapeutic approach to AD. Therefore, the enzyme acetylcholinesterase (AChE) has been targeted in treatments for Alzheimer's disease.[1] However, to date, all the longer-term studies have shown clinical efficacy to decline with time as a result of either a loss of drug efficacy or the relentless progression of the disease. Thus interest in the discovery of novel AChE inhibitors is continued since the current AChE inhibitors lack perfection. The availability of several crystal structures of AChE in complex[2,3] with the inhibitors provides the possibility to apply docking protocol for the protein−inhibitor complexes. In a previous paper, we reported the isolation, characterization, and biological evaluations of a series of steroidal alkaloids **1**−**15**, isolated from *S. saligna*.[4] The work described in this paper is an attempt toward the better understanding of interaction of these alkaloids in the active site of AChE enzyme from *Torpedo californica*.

Results and Discussion

The Brookhaven Protein Data Bank[5] contains several AChE complexes with small molecules.[2,3,6−8] The major difference in these complexes is the orientation of Phe330. This side chain controls the access to the bottom of the gorge and was identified to adopt three major conformations, an open, a closed, and an intermediate access position.[9] For gorge-spanning ligands such as decamethonium, Phe330 adopts an open access position. Owing to the size and shape of our tested compounds, it is safe to assume a gorge-spanning binding mode. Therefore, the crystal structure of cocrystallized decamethonium was taken for comparison in order to control the performance of our docking approach. The detailed inspection of the AChE−inhibitor X-ray structures shows a nearly identical three-dimensional structure of the active site. The active site is located 20 Å from the protein surface at the bottom of a deep and narrow gorge.[10] However, the position of the known inhibitors in the binding pocket is quite different, indicating that more than one clearly defined binding region exists.

Table 1 summarizes the docking results of all compounds. Usually the docking position with the lowest energy was also found most often during the docking procedure. This indicates that the phase space is sufficiently sampled. Additionally we repeated the docking protocol for each ligand several times and found that the best docking positions (position with lowest energy) and their respective minimum energies are consistently reproduced. However, no correlation between our calculated binding energies and experimentally determined IC_{50} or K_i values could be observed, which could have several reasons: On one hand, the docking process and the stability of the complex does not describe all electronic interactions properly, and the influence of the neglected aqueous environment could also play a role. On the other hand, the experimental data[4] may not be precise enough and do not give consistent values for standard confidence limits.

Comparison of the different docking results of all 15 ligands shows (Figure 1) that in principle all compounds adopt the same binding mode. This similar binding mode is not surprising since all compounds contain almost identical structures with minor differences only at side chains C-3 and/or C-20 or by substitution of functional groups (Chart 1). The unflexible steroid backbone always enters the aromatic gorge from the same side. They penetrate the aromatic gorge through the six-membered ring A. Thus, ring A is placed in the bottom of the gorge, which might be due to the apparently greater hydrophobicity of ring A in comparison with that of the five-membered ring D (Figure 2). The compounds are also completely buried inside the aromatic gorge of the AChE. This might contribute highly to the stabilization of the complex since the steroid backbone of the ligands should be highly hydrophobic due to its aliphatic character and, therefore, not well

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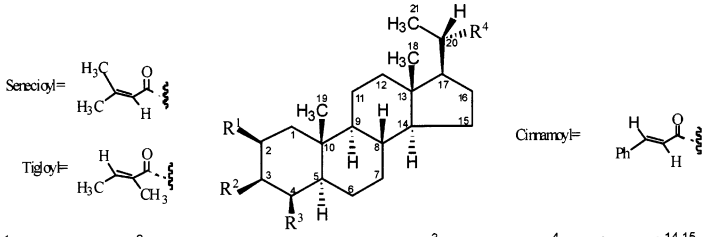
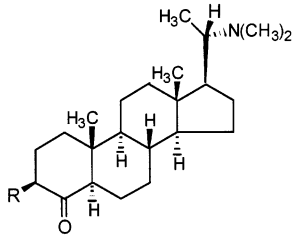
Chart 1. Chemical Structures of Steroidal Alkaloids **1**−**15**



1	R^1= OH	R^2= *HN-Tigloyl*	R^3= OAc	R^4= $N(CH_3)_2$, $\Delta^{14,15}$
2	R^1= α OH	R^2= *HN-Tigloyl*	R^3= H	R^4= $N(CH_3)_2$, $\Delta^{4,5}$ & $\Delta^{16,17}$
3	R^1= OH	R^2= *HN*-Benzoyl	R^3= H	R^4= $N(CH_3)_2$
4	R^1= H	R^2= $NCH_3COCH{=}CCH_3CH(CH_3)_2$	R^3= H	R^4= $N(CH_3)_2$ $\Delta^{16,17}$
5	R^1= H	R^2= $NCH_3COCH{=}CCH_3CH(CH_3)_2$	R^3= H	R^4= $N(CH_3)_2$
6	R^1= OH	R^2= *HN-Benzoyl*	R^3= OAc	R^4= $N(CH_3)_2$
7	R^1= OH	R^2= *HN-Tigloyl*	R^3= OAc	R^4= $N(CH_3)_2$
8	R^1= H	R^2= *NH*Ac	R^3= H	R^4= $N(CH_3)_2$
9	R^1= H	R^2= *NH*CH_3	R^3= H	R^4= *N*HCH_3Ac
10	R^1= H	R^2= CH_3*N -Cinnamoyl*	R^3= H	R^4= $N(CH_3)_2$
11	R^1= H	R^2= *NH*$COCH{=}C(CH_3)CH(CH_3)_2$	R^3= H	R^4= $N(CH_3)_2$
12	R^1= H	R^2= *HN-Senecioyl*	R^3= OAc	R^4= $N(CH_3)_2$



13	R= *HN-Benzoyl* $\Delta^{2,3}$
14	R= *HN-Tigloyl*$\Delta^{5,6}$
15	R= *HN*-Tigloyl$\Delta^{5,6}$ & $\Delta^{14,15}$

Table 1. Summary of the in Vitro Anticholinesterase Activities and Binding[a]

compd	binding energy, kcal/mol	acetylcholinesterase		butyrylcholinesterase	
		IC_{50} (μM)	K_i (μM)	IC_{50} (μM)	K_i (μM)
1	−42.47	61.3	134	38.36	26.3
2	−37.71	185.2	-	23.78	-
3	−43.07	78.2	-	28.9	16.2
4	−22.18	6.2	10.7	3.65	9.1
5	−21.02	6.3	4.1	4.07	3.4
6	−46.11	227.9	126	17.99	20.3
7	−43.03	182.4	- -	18.2	- -
8	−35.11	69.9	90.3	10.3	7.5
9	−32.71	204.2	216	16.5	8.6
10	−35.91	19.9	12.2	4.84	6.6
11	−32.71	50.6	9.05	4.63	3.25
12	−39.53	8.5	17.6	2.3	2.58
13	−38.12	5.2	3.03	2.49	2.15
14	−33.87	7.1	5.4	2.1	3.08
15	−35.74	5.8	2.65	4.29	1.6

[a] Energy obtained by docking of compounds **1**−**15**.

hydrated. The main hydrophobic interactions between the hydrocarbon skeleton of the inhibitors and the protein were observed with the residues Tyr-70, Asp-72, Gln-74, Ser-81, Trp-84, Asn-85, Tyr-118, Trp-279, Asn-280, Val-281, Leu-282, Pro-283, Phe-284, Asp-285, Ser-286, Ile-287, Phe-288, Arg-289, Phe-290, Phe-330, Phe-331, Tyr-334, and Gly-335 of the aromatic gorge (Figure 2). Since most of the residues involved in the hydrophobic interactions belong to the peripheral site and not to the active site, the docking results further support the experimental result that these compounds are noncompetitive inhibitors of AChE. The interactions of the compounds with the peripheral site at the top of the enzyme's aromatic gorge leave enough space to accommodate the substrate in the catalytic triad site and the quaternary ammonium binding site.

Due to the large hydrophobic skeleton, the molecules are thought to find a number of interaction points within the gorge of the active site. In addition, the benzoyl feature may form aromatic interactions with the aromatic residues of the enzyme. Hydrogen bonding between the polar groups of inhibitors and TRY-121, TYR-70, TYR-334 may occur as the distances and angles observed are within a suitable range. Notably, the C-4 carbonyl of compound **13** is hydrogen bonded with the hydroxyl proton of TRP-70; furthermore, in compound **14** the amidic carbonyl is hydrogen bonded with the

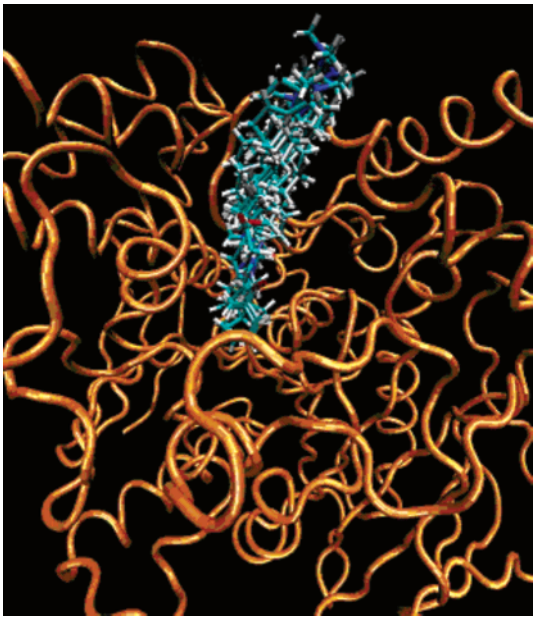


Figure 1. Superimposition of all investigated inhibitors **1**−**15** in the active site of AChE.

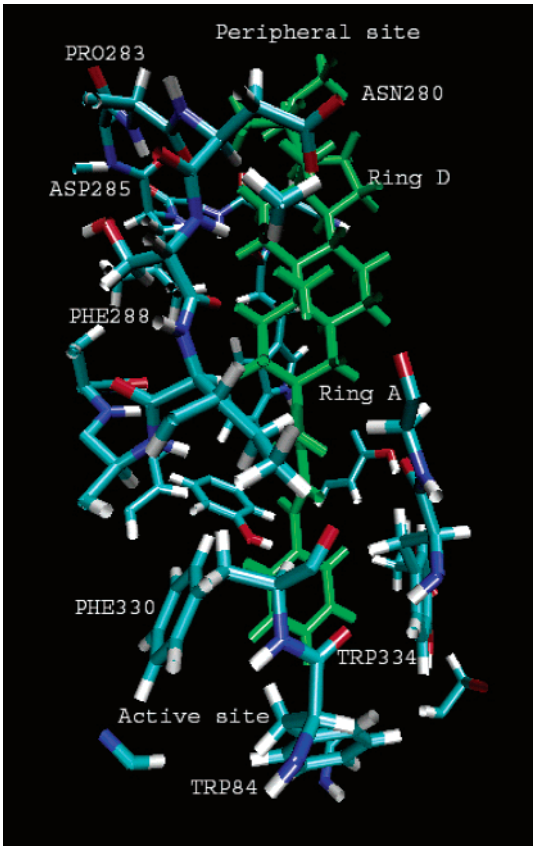


Figure 2. Docking of compound **11** and AChE, showing the dominant hydrophobic interactions and the specific rule of ring A and ring D of steroid mentioned in the text.

hydroxyl proton of TRY-121. Some relatively weaker hydrogen bonds between nonpolar hydrogens of inhibitors and carbonyl groups of Phe-283, Ser-286, Tyr-279, Tyr-334 were also observed during the docking procedure, but they should not be a major factor determining the structure of the complex. This assumption is confirmed by the observation that the binding energy of all inhibitors mainly consists of van der Waals interactions and that electrostatic contributions are almost negligible, i.e less than −0.5 kcal/mol.

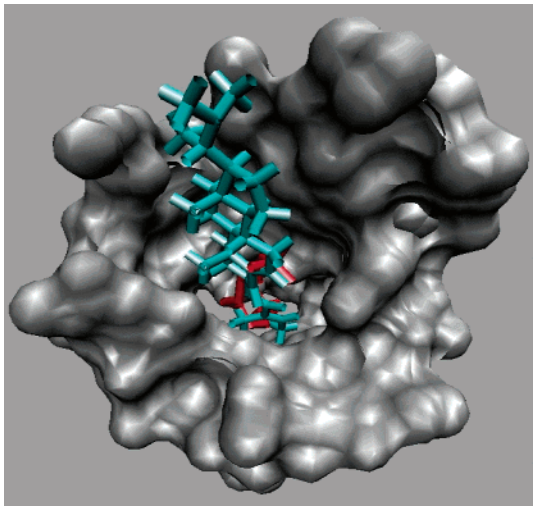


Figure 3. Comparison between the predicted position of inhibitor (magenta) and the X-ray structure of decamethonium (red) are shown. Only the amino acid residues within 15 Å are displayed for clarity.

The docking studies also show that the amino groups at C-20 and C-3 come close to Phe-281 and Tyr331, respectively. The nitrogen atoms of the inhibitors build interactions with Phe-281 and Tyr331. The average distance between the nitrogen atom and the aromatic ring system of Phe-281 and Tyr331 lies in the range between 4.0 Å and 4.5 Å. The superimposition (Figure 3) of the decamethonium ligand in the crystal structure of the complex (PDB entry 1ACL) with our results shows that the binding position in both cases is quite similar, with the only difference that decamethonium enters relatively deeper into the aromatic gorge than our investigated inhibitors.

The sarsalignenone−AChE docking complex was chosen, representatively for all complexes, as starting point of a MD simulation. The rmsd value of the protein with respect to the starting structure (which is also the X-ray structure) is stable after about 200 ps at a value of only 1.6 Å, showing that the complex stays stable and the protein does not undergo major structural rearrangements. The rms value of the binding site (defined as amino acid within 4.0 Å of the ligand) alone compared to the X-ray structure is in the range of 1.75 Å and thus again proves that the compounds do not alter the structure of the protein significantly from that of the decamethonium complex (cocrystallized ligand in the X-ray structure).[11] Although the structure of the protein is not distinctively affected by complexation, the dynamics of the gorge are changed significantly. The dynamics of the gorge has already been investigated extensively by MD simulations, proposing that the high flexibility of the gorge is necessary for enzyme activity.[11] The comparison of our simulation results of the complexed state and the respective calculation of the uncomplexed enzyme clearly show that complexation reduces the flexibility of the gorge. The distance between the center of mass of Trp279 and Gly335 was used as definition of the gorge width. In the case of the complexed state this gorge width varies between 10.9 and 13.0 Å, resulting in a mean of about 12.0 ± 0.3 Å, while in the uncomplexed state the distance is in the range

of 10.6 to 15,3 Å, resulting in a similar mean of 12.5 Å but with standard deviation of 0.8 Å, demonstrating higher flexibility.

Conclusion

The goal of this study was to explore the possible binding modes of several steroidal alkaloids which have already shown their ability to inhibit acetylcholinesterase. One major observation found from computational docking is that the ligands bind similar to that already observed for AChE inhibitors into the aromatic gorge of the enzyme. Although the ligands are completely buried in this gorge, the investigated compounds are not able to enter as deep as, for example, decamethonium. This is probably due to the bulky steroid part of the investigated compounds. The stabilizing interactions between the protein and the ligands are mainly of hydrophobic nature. All compounds exhibit a similar binding mode, and therefore they are an ideal target for a systematic variation of the substituents. Because of these docking results, we were able to understand the inhibition type of the ligands which is mainly of a noncompetitive nature. The subsequent molecular dynamics simulation showed that the complexation with these ligands does not alter the structure of the enzyme significantly, but has a considerable influence on the dynamics of the gorge width. The establishment of any quantitative relationship between inhibitory activity and molecular properties will require a detailed analysis of the electronic structure by means of quantum chemical methods and/or further docking experiments, taking into account explicity the influence of hydration on complex formation between enzyme and substrate.

Materials and Methods

Docking studies were performed, using the program AUTODOCK 2.4.[12] The enzyme conformation of the AChE−decamethonium complex (1ACL)[2] was chosen for our docking study. The affinity grids were centered at the aromatic gorge of the enzyme, with dimensions of 45 Å × 36 Å × 43 Å and a grid spacing of 0.5 Å. Several other grid centers and sizes, in total covering the whole protein, were also used, but no acceptable docking results were obtained. As charges, the standard RESP charges[13] already deduced for proteins were taken. For docking we used a Monte Carlo simulated annealing search process starting at a temperature corresponding to RT = 1200 cal/mol. The charges of the ligands were obtained using the standard RESP procedure.[13] The necessary ab initio calculations were performed with GAUSSIAN98.[14]

As starting coordinates for the Molecular Dynamics simulation, the best (lowest energy) docking structure of the sarsalignenone−AChE complex was used. The MD-calculations were performed with the AMBER program package.[15] For consistency with subsequent MD simulations, the force-field parameters of the ligand were taken in analogy from the existing all-atom force field of Cornell et al.[16] with the respective modifications of Cheatham et al.[17] Subsequent solvation of the complex with TIP3P Monte Carlo water boxes requiring 8 Å in all directions resulted in a system with the size 87 Å × 85 Å × 82 Å containing 13 942 water molecules. For simulation,[15] standard protocols were taken using a time step of 2 fs and Particle Mesh Ewald (PME).

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