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Galanthamine as *bis*-functional ligand for the acetylcholinesterase

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Abstract Acetylcholinesterase plays a key role in the development of Alzheimer's disease as this enzyme is responsible for cleavage of the neurotransmitter acetylcholine, and, according to recent investigations, also promotes aggregation of β-amyloid peptides, which causes plaque formation in synaptic areas. We have performed a molecular modeling study to investigate *bis*-galanthamine derivatives connected by a methylene spacer of varying length as dual acting acetylcholinesterase ligands. Our results suggest that such ligands indeed can interact simultaneously with both biological functions of the enzyme and should therefore serve as the basis for a further development of *bis*-functional Alzheimer drugs.

Keywords Acetylcholinesterase · Alzheimer's dementia · Docking · Galanthamine · Geometrical scoring

Introduction

Alzheimer's dementia is a neurodegenerative disease that is presently treated exclusively by inhibitors of the enzyme acetylcholinesterase (AChE), following the cholinergic hypothesis. [1] AChE is responsible for degradation of the neurotransmitter acetylcholine (ACh) in the synaptic cleft of neuromuscular junctions and of neuronal contacts in the central nervous system. This enzyme has been studied extensively since the pioneering work of Nachmansohn and Neumann. [2] The 3D structure of AChE and the structural details of a number of AChE–inhibitor complexes are largely known from the work of Sussman and coworkers [3, 4, 5, 6] and from the recent contributions by Bartolucci et al. [7] These studies prove the cleavage site for acetylcholine and accordingly also the binding site of competitive inhibitors like galanthamine [8] to be located 20 Å deep in the AChE protein at the end of narrow gorge.

Galanthamine was first isolated from snowdrops [9] and has long been known to exhibit esterase blocking activity. [10] Recently, Jordis and Froehlich [11] have developed a stereospecific synthesis for a large scale production of galanthamine that opened the door for therapeutic use of this compound, so that galanthamine is now commercially available as the fourth anti-Alzheimer drug. We have studied the interaction of galanthamine with AChE in terms of its orientation in the gorge by molecular modeling techniques and were able to predict the geometry of the complex successfully. [12]

Unlike the other commercial Alzheimer therapeutics tacrin, [5, 13] E2020, [6, 14] and rivastigmin, [15] galanthamine is known not only to block the esterase activity, but also to display an allosteric potentiating effect on the nicotinic acetylcholine receptor (nAChR). [16] This finding makes galanthamine a most valuable drug for Alzheimer treatment as this dual interaction enhances signal transmission by acetylcholine twofold.

Inestrosa and coworkers [17, 18] have recently demonstrated that AChE, besides its established esterase cleavage activity, is also involved in the formation of β-amyloid plaques, which are known to play an essential role in the development of Alzheimer's dementia. [19, 20] Aggregation of this peptide seems to occur specifically at the peripheral anionic site (PAS) [21, 22] of AChE, which is located at the mouth of the gorge [23] around amino acid Trp279 (numbering of *Torpedo* AChE, PDB [24] Code: 2ACE). More specifically, a 35 amino acid *Torpedo* derived peptide fragment corresponding to AChE-sequence position 274–308 is capable of amyloid complex formation. [25, 26]

Combining on the one hand this dual activity of AChE and on the other hand the established *bis*-functional effect of galanthamine, we are investigating galanthamine derivatives that perhaps bear a third therapeutic potential for the cholinergic synapse, which is directed against the formation of β-amyloid plaques. In our

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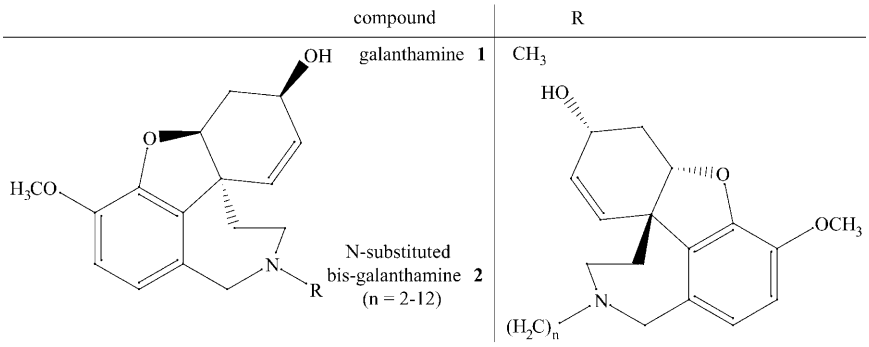

Fig. 1 Galanthamine and derivatives investigated

model systems we can show that galanthamine derivatives containing specific substituents on the nitrogen atom can interact favorably with Trp279, thereby possibly preventing deposition of β-amyloid peptides at this site. Here we describe the development of a *bis*-galanthamine type ligand for the cholinergic synapse that has the potential of being a *tris*-functional Alzheimer drug, which (i) will be an excellent inhibitor of AChE activity, (ii) could additionally interfere with plaque formation and (iii) should still maintain the allosteric potentiating function at the nAChR.

Methods

Enzyme structure

The crystal structure of TcAChE complexed with (–)-galanthamine was retrieved from the Protein Data Bank (PDB Code 1QTI). *Torpedo Californica* AChE (TcAChE) rather than the structure of the human AChE (hAChE) was used because of the availability of *Torpedo* crystallographic data and the lack of activity data of ligands against hAChE. Due to the high sequence homology (77%) between the hAChE and TcAChE in the active site area it seems reasonable to use the TcAChE instead of the human enzyme. Moreover, the catalytic triade and the PAS are conserved. [23]

The inhibitor and all water molecules were removed in the preparation of the enzyme. The water molecules were removed because the docking programs are unable to treat them flexibly with all their degrees of freedom. Therefore putting any water molecule inside the pocket would add some bias to the structure. Furthermore, the results justify this treatment, because the core of one of the two galanthamine structures is still well aligned with the X-ray structure. The residue numbering throughout this paper refers to the numbering of *Torpedo* acetylcholinesterase (TcAChE).

Compounds

Bis-galanthamines with varying lengths of a methylene spacer between the two galanthamine ring systems were used as inhibitory ligands (see Fig. 1). Furthermore, the established AChE inhibitor BW284c51 [22] was used for comparison. All inhibitors were examined as non-protonated (uncharged) compounds.

The substituent on the amine moiety of galanthamine can adopt either an axial or an equatorial orientation, which have to be considered independently in the docking studies as the offline version of FlexX cannot discriminate between these two “pro chiral” configurations. Results are therefore referred to as (eq)- and (ax)-conformers respectively.

Docking

QXP [27] and FlexX [28] were used in this study because they allow for docking of flexible ligands to a rigid target. In addition, in a comparative docking study with galanthamine derivatives, these two docking/scoring algorithms showed good reliability in reproducing the crystal structure of AChE complexes (unpublished results). The major difference between these two programs lies in the algorithm for sampling the conformational space and in the empirically determined parameters for their scoring functions. QXP statistically scans the conformational space with an implementation of a Monte Carlo procedure, which makes the sampling more general but also more time consuming. In contrast, FlexX is faster, due to its use of ligand fragmentation, base fragment placement and incremental reconstruction, but might not examine placements where the reconstruction of the ligand starts at an unfavorable position. For scoring QXP uses a modified version of the AMBER force field and FlexX has determined its own parameters for a simple analytical function by fitting those parameters with a manually selected training set of known ligand–protein complexes.

All torsion angles within the ligands were kept flexible. The active site was defined by all complete amino acids which have atoms no further away than 7.5 Å from the galanthamine location within the active site.

Independent of the axial or equatorial orientation of the methylene spacer at the galanthamine nitrogen atom, only the highest scoring conformers are reported for all docking results. While the docking programs treat the three possible conformers (aa, ae, ee) separately, rapid inversion is expected in vivo.

Lipo score

Besides the conventional scoring implemented in the docking programs, which is based on complex energy criteria, a particular scoring procedure was needed that evaluates selected interactions between the ligand and specific amino acid side chains of the peripheral anionic site of AChE. Due to the fact that FlexX exports a detailed description of what makes up the final score for a particular placement, we were able to extract just the lipophilic term for interaction between the ligand and Trp 279. These actually are calculated by scaling a constant energy linearly with respect to any deviation from the expected interaction distance. The respective constant energy is determined by fitting the scoring function of FlexX against experimental data.

Geometric score

Independently from the FlexX scoring methods we developed a scoring procedure for the interaction of galanthamine derivatives with the PAS, more specifically with the indole system of Trp279. In this respect we looked at four parameters: (i) the centroid distance (*d* in Fig. 2) between the galanthamine a-ring and the unweighted geometric center of the nine indole ring atoms, (ii) the

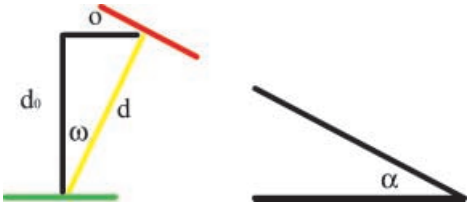

Fig. 2 Definition of the geometric parameters. Shown in *yellow* is the distance *d* between the centroid of the indole from Trp279 (*green*) and the centroid from the aromatic a-ring of galanthamine (*red*). The angle α describes the angle between the two ring planes. ω describes the angle between the normal on Trp279 and the distance vector. d_0 and *o* are parameters of interest for π–π interactions, the distance of the (assumed parallel) planes and the offset of the centroids within a plane

angle (α in Fig. 2) that is spanned between these two aromatic ring planes, (iii) the angle between the normal on Trp279 and the vector between the two centroids (ω in Fig. 2) which corresponds to a co-latitude with respect to polar coordinates, and (iv) the space fill value which determines the relative overlapping volume between a sphere of 5 Å radius around the centroid of Trp279 with the atomic van der Waals radii of the ligand atoms.

For π–π interactions there a two different geometric parameters of interest, which can be calculated by trigonometric operations. The first parameter is the distance of the assumed parallel planes (d_0 in Fig. 2) of the aromatic groups and the second is the offset (*o* in Fig. 2) of the two centroids, which is the distance of two centroids within the planes. These two distances resemble the catheti within an orthogonal triangle, while ω is one of the angles of this triangle.

Results

The three dimensional structure of the AChE–galanthamine complex is known from our docking studies [12] and from the crystallographic resolution of the structure. [3, 7] Particularly the docking studies, which give identical results with FlexX and QXP, respectively, enable us to design new anti-AChE drugs based on galanthamine with enhanced and/or modified activity for AChE. In this respect, we have focused our attention on N-substituted galanthamine derivatives that simultaneously can block the catalytic triade and potentially can also interact with amino acid side chains of the PAS, particularly with Trp279.

Our earlier docking studies revealed only two major binding sites of galanthamine within the gorge. [12, 29] One is located at the bottom of a 20-Å deep gorge that is directed down into the protein and the other at the mouth of this gorge. These binding modes are displayed in Fig. 3 as their energetically favored orientations of galanthamine at either site. As can be seen, the nitrogen atoms of the two galanthamine moieties are directed towards each other. This suggests a *bis*-galanthamine as an AChE ligand that can potentially block the ester cleavage site at the catalytic triade and also simultaneously interact with the PAS around Trp279. Such a *bis*-galanthamine compound could be obtained by merely linking the two galanthamine cores via their nitrogen atoms by a spacer.

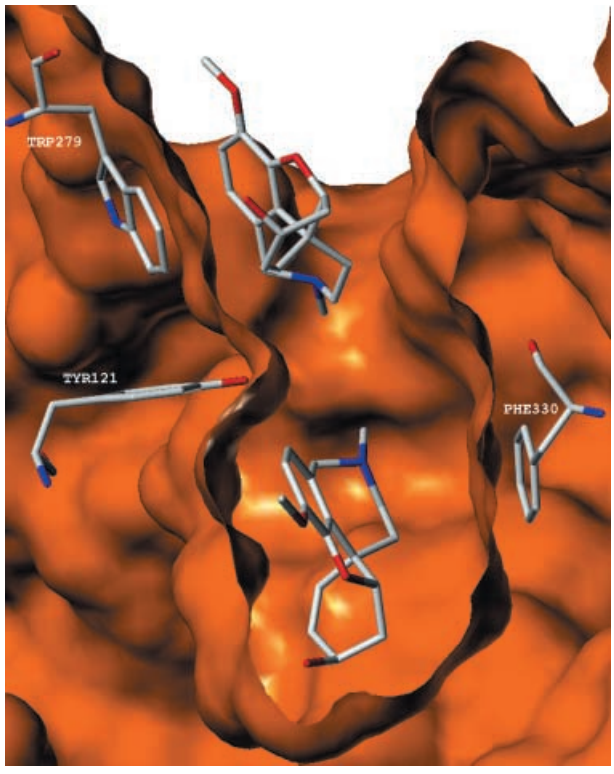

Fig. 3 The two major binding modes of (–)-galanthamine within the AChE gorge, depicted by their lowest energy conformer at the upper and lower site, respectively. The lower galanthamine binding site corresponds to the ester cleavage site of the enzyme, while the upper galanthamine ring structure is located close to the peripheral anionic site

We have chosen a methylene spacer with a length of two to twelve methylene groups for our modeling studies. This homologous series of derivatives was used to optimize the interaction of the two galanthamine moieties with the separate binding sites in the gorge. Furthermore, it should be noted that N-substituted galanthamine derivatives are already known to show in general stronger AChE inhibition than galanthamine itself. [7] Two docking procedures, QXP [27] and FlexX [28] were employed to predict *bis*-galanthamine candidates for a dual interaction with AChE independently.

Docking studies with QXP

Separate QXP docking runs were performed for the three possible conformers that result from the axial and equatorial orientation of a methylene spacer between the two galanthamine bodies, i.e. the two galanthamine systems were linked by an (eq–eq), (ax–eq) and (ax–ax) conformation of the two nitrogen atoms, respectively.

Figure 4 displays the lowest energies for each of the AChE–*bis*-galanthamine complexes with spacer lengths between 2 and 12 CH_2 groups. Considering the possible inversion at the nitrogen atom in biological systems, we have only compared the highest scoring result for each

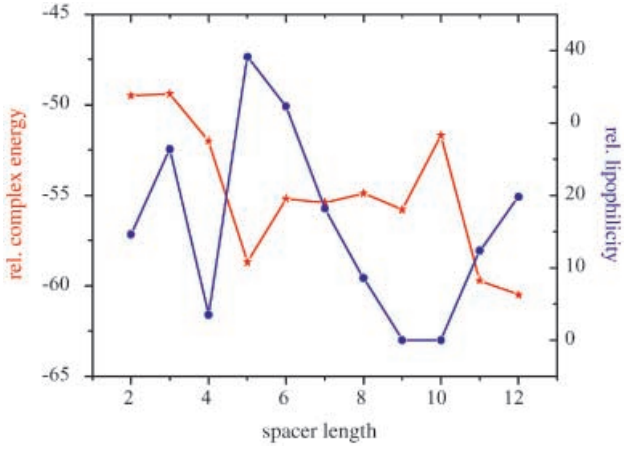

Fig. 4 Complex energies of energetically favored *bis*-galanthamine derivatives (*asterisks*) and their corresponding lipophilic affinities to Trp279 (*circles*) plotted against the spacer length

particular spacer length, regardless of their conformational nature. From these data, no clear picture results in terms of the minimum energy structure, as there is a decreasing energy with a spacer length of more than ten methylene groups, though also the 5-methylene derivative is similarly low in energy.

In order to evaluate the hydrophobic interaction of these *bis*-galanthamine ligands with the indole ring of Trp279, which is believed to be the central amino acid of the PAS, we have extracted the corresponding energy terms of the energetically favored complexes. These data can be derived by applying the FlexX scoring to structures that result from the QXP docking experiment. Figure 4 shows the resulting lipophilicity values, which show the derivative with a spacer length of five methylene group as a favorable compound.

Variation of the spacer length between two and twelve methylene groups results has three remarkable effects. First, the 4-methylene derivative shows a drastic decrease in hydrophobic interaction compared to the structurally related 3- and 5-methylene derivatives. Second, the lipophilicity value is down to zero for the 10- and 11-methylene compounds, and third these values rise steeply again with larger spacer length. A closer look at the three dimensional structure of the AChE–bis-galanthamine complexes reveals that the differences in lipophilicity are caused mainly by the differing interaction of the indole side chain of Trp279 with structural elements of the *bis*-galanthamines (Fig. 5).

Figure 5 shows that for the 5-methylene derivative there is a perfectly stacked orientation. This leads to strong van der Waals interactions. In addition, the galanthamine a-ring of the upper ring structure shows a T-shaped π–π interaction (see Fig. 6) with Tyr70, another amino acid of the PAS in the close neighborhood of Trp279.

In contrast, structures with three, four, seven, and eight methylene groups have the aromatic rings less well aligned. The galanthamine derivative with a spacer length of six shows a reasonable alignment for stacked π–π interactions with Trp279. The 3-methylene derivative gains its Trp279 lipophilicity value from an interaction of the indole with tetrahedral CH_2 and CH group of the cyclohexenol ring that are positioned at about 4 Å distance from the centroid of the indole. The *bis*-galanthamines with eleven and twelve methylenes show the same type of hydrophobic interaction, however, interacting with methylene groups of the spacer that again are positioned less than 4 Å away from the indole ring. Finally the 9-, and 10-methylene derivatives do not show any interaction with the indole ring at all, because the upper galanthamine ring in these cases is rotated away from Trp279.

In all of these structures the anchoring galanthamine core in the lower part of the gorge nicely overlays with the crystal structure with RMSD values between 0.28 and 0.77 Å. However, flexibility of the methylene spacer yields a wide variety of conformations of the alkyl chain and therefore results in varying positions of the second galanthamine moiety in the upper part of the gorge.

Nevertheless, the derivatives with a spacer length of two to eight methylene groups (with the exception of the 4-methylene compound) all result in a conformation that allows for a favorable interaction of the galanthamine a-ring with the sidechain of Trp279. The *bis*-galanthamine derivatives with eight and nine methylene groups are still bound to the same gorge area like the shorter derivatives, but their position is dominated by interactions other than with Trp279. Only the longer spacer derivatives (ten to twelve methylene groups) start to reach out of the gorge, and therefore would not be good candidates for an interaction with the AChE gorge.

Based on the hypothesis that a blockade of the PAS, particular of Trp279, is necessary for an inhibition of plaque formation, effective ligands would have to occupy the space around Trp279. Independent of the scoring procedure of FlexX, we therefore developed a scoring system that is based on the relative orientation of the galanthamine a-ring and the indole side chain of Trp279 as this seems to be the important term in the interaction of the ligand with the upper part of the gorge.

A ligand with strong interaction with the indole ring of Trp279 should then

- show a small distance between the centroids of the indole ring and the galanthamine a-ring
- either be parallel with its galanthamine a-ring to the indole (corresponding to a low α) and approximately opposite (small distance *o*) to the indole ring for van der Waals interactions
- or be parallel with a small offset (distance *o* of about 2–4 Å, depends on d_0) to lead to stacked π–π interactions
- or be perpendicular (with α about 90°) and directly opposite (small distance *o*) to the indole to contribute with T-shaped π–π interactions

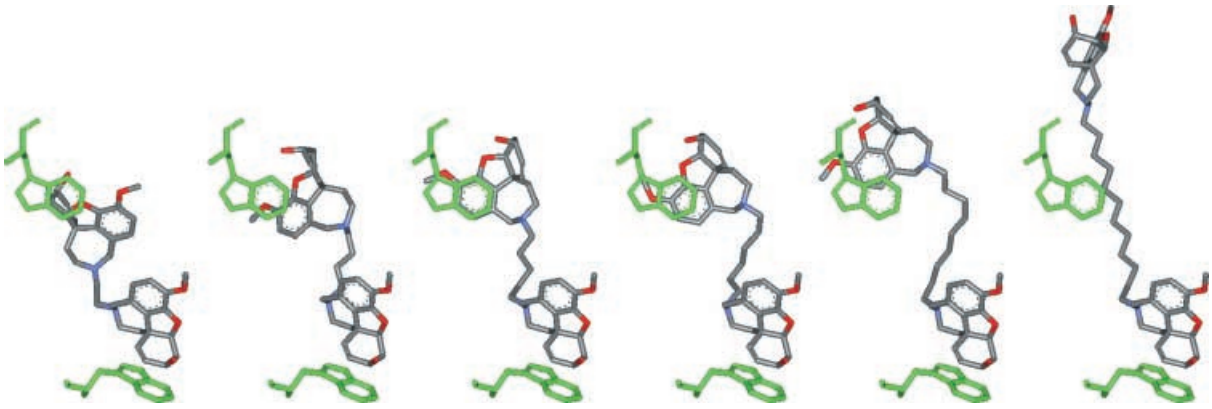

Fig. 5 Binding modes for *bis*-galanthamine with spacer length (from left to right) of two, four, five, six, nine, and twelve methylene groups. Selected residues lining the gorge ends are also shown in *green* (Trp279 and Trp84)

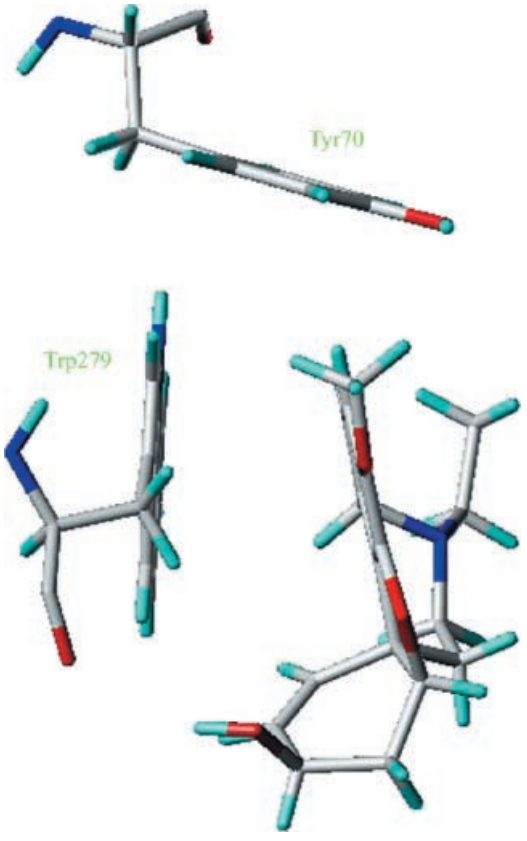

Fig. 6 T-shaped π–π interaction of the upper galanthamine core, together with selected PAS residues

- show good overlap of the upper galanthamine core with a 5 Å sphere of the indole ring

We chose a particular value of 5 Å for the sphere size because that sphere would resemble the space around the PAS while not covering the whole gorge entrance. A radius of 5 Å furthermore ensures that any atom interacting with the PAS is taken into account. Structural evaluation of the complexes according to these geometrical parameters then results in the data depicted in Fig. 7, which again show the outstanding orientation of the 5-methylene derivative.

A low distance and a high space fill value would presumably be the best indication of a close vicinity of the upper galanthamine moiety and the indole of Trp279. Furthermore, low angles α and ω would specify this interaction as a van der Waals interaction between the indole ring system and the galanthamine a-ring. On the other hand, large distances (e.g. >6 Å) and small space fill volumes (e.g. <10%) indicate no interaction of Trp279 with the upper galanthamine core.

Together with the complex energy as the most important factor that governs the formation and stability of an enzyme–ligand complex, the space filling factor then is a simple measure for the blockade of the PAS around Trp279. Furthermore, comparison of the lipophilicity curve of Fig. 4 and the space filling data of Fig. 7 clearly shows that these data closely resemble the relative strength of hydrophobic interaction of the galanthamine derivative with the PAS. *Bis*-galanthamine ligands with a good potential of blocking the PAS according to this analysis should have a low energy structure that at the same time shows a high space filling value. Furthermore, a small distance value (<5 Å) guarantees an interaction between the indole of Trp279 and the galanthamine core.

Figures 4 and 7 prove that this is especially the case for the 5-methylene ligand, but to a slightly smaller extent also for the 6-methylene-derivative. These two compounds therefore should be good candidates for galanthamine derivatives that simultaneously can block the ester-cleavage site and the plaque formation site.

Docking studies with FlexX

Independent of the QXP docking study we have also investigated the interaction of *bis*-galanthamine derivatives with AChE using the FlexX docking procedure. Again, the hydrophobic interaction of the galanthamine derivatives with Trp279 was extracted from the scoring output and compared to the total complex energy (see Fig. 8).

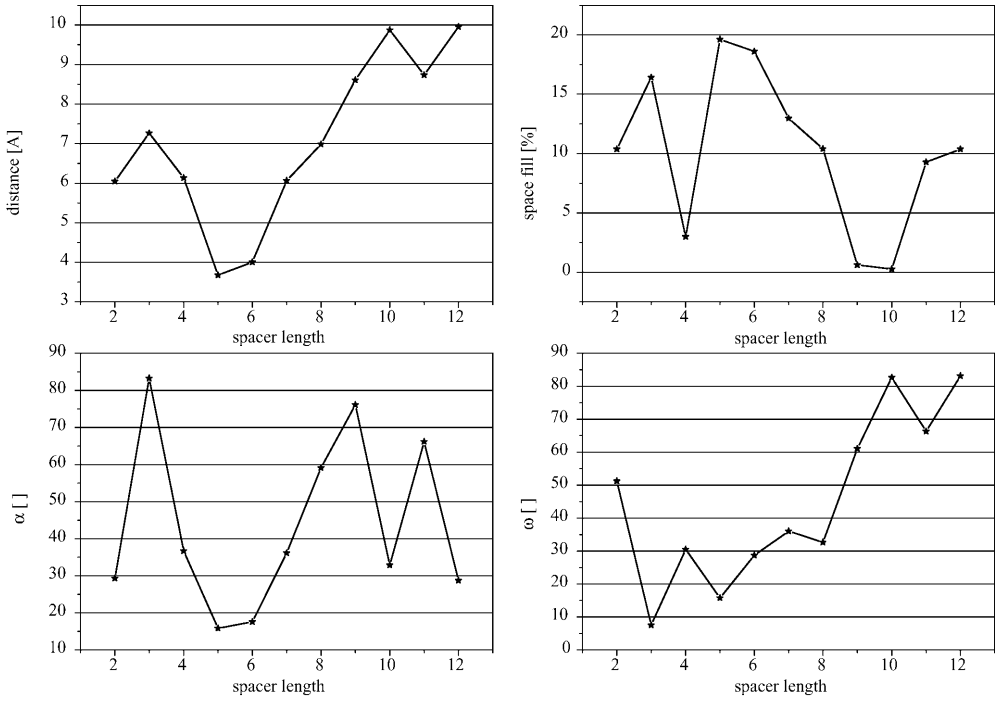

Fig. 7 Upper row: distance between the centroids of galanthamine a-ring and the indole ring of Trp279 (*left*) and relative overlapping volume between a sphere of 5 Å radius around the center of Trp279 with the atomic van der Waals radii of the ligand (*right*). Lower row: angle between the planes of galanthamine a-ring and the indole sidechain of Trp279 (*left*) and angle between the normal on the six-membered ring of Trp279 and the connecting vector between the centroid of this ring and galanthamine a-ring (*right*). For definition of distance, α, and ω see Fig. 2

In contrast to the QXP results, FlexX determines the 7-methylene derivative as the lowest energy complex structure, which at the same time also represents the strongest interaction with Trp279. Investigation of the structural parameters of these complexes shows a less obvious picture than do the QXP results (Fig. 9).

However, with the prerequisite of a 5 Å distance between the indole ring of Trp279 and the upper galanthamine core, *bis*-galanthamine structures with two and eight to twelve methylene groups can be ruled out as ligand candidates. Of the remaining alternatives the derivatives with three, four, six, and seven methylene groups show favorable geometrical values and high lipophilicity. Again, as seen with the QXP analysis, the space fill values closely resemble the lipophilicity values as extracted from the FlexX output.

Inspecting the three dimensional structure of the various complexes as described in Fig. 10 shows a perfect orientation of the 3-methylene compound in terms of closest contact and most parallel orientation of the galanthamine a-ring with respect to the indole ring of Trp279. In contrast, the energetically favored 7-methylene derivative seems to have a considerably less good alignment with the indole ring. However, the 7-methylene derivative gains its low complex energy through a stacked π–π interaction. The distance between the two aromatic planes (d_0=3.5 Å) and their parallel orientation (α below 20°) together with an offset of the two centroids (o=2.8 Å) accounts for a strong π–π interaction [30, 31, 32] (see Fig. 7). Combining the FlexX energy data of the AChE–ligand complexes with the lipophilicity values or alternatively with the geometrical parameters point to the 7-methylene derivative and also the 6-methylene derivative as the most promising candidates for a dual interaction with AChE.

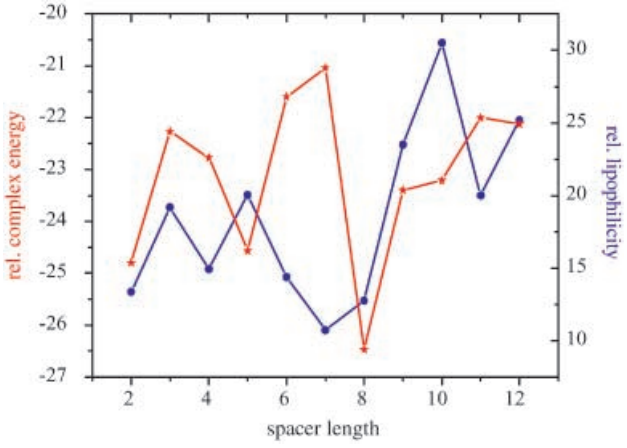

Fig. 8 Docking results with FlexX: complex energies of energetically favored *bis*-galanthamine derivatives (*asterisks*) and their corresponding lipophilic affinities to Trp279 (*circles*) plotted against the spacer length

Comparison of docking results

The employed docking procedures, QXP and FlexX, both yield a strongly preferred conformations of the *bis*-galanthamine derivatives that favorably interact with Trp279. The results are similar but are not exactly the same for the two docking procedure, which can be explained by the difference in the underlying docking algorithms of the two programs.

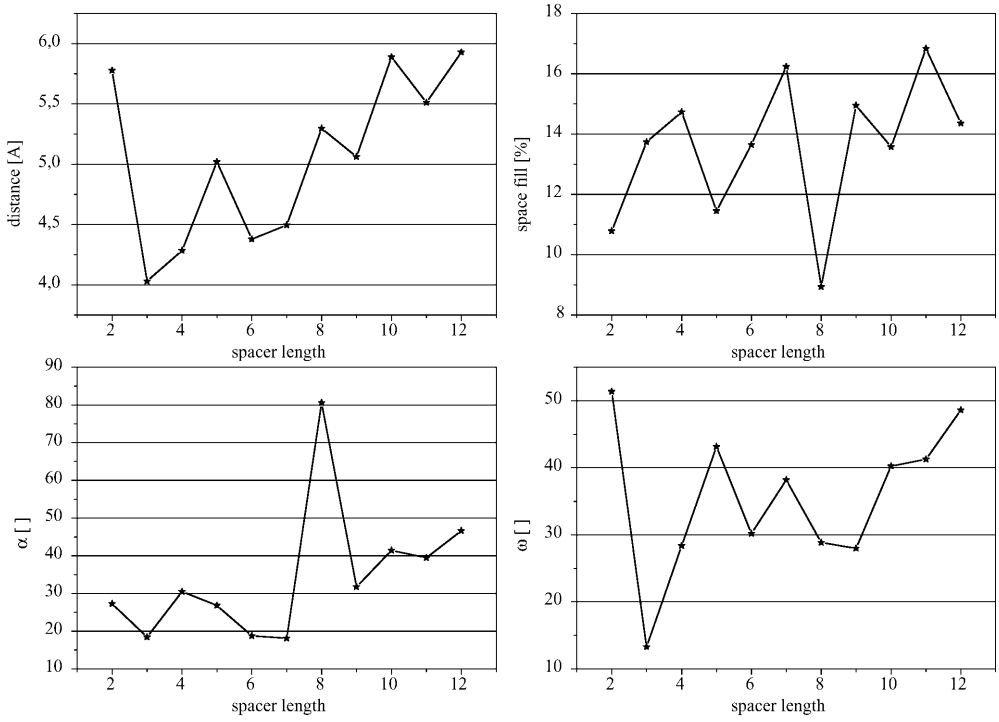

Fig. 9 Upper row: distance between the centroids of galanthamine a-ring and the indole ring of Trp279 (*left*) and relative overlapping volume between a sphere of 5 Å radius around the center of Trp279 with the atomic van der Waals radii of the ligand (*right*). Lower row: angle α between the planes of galanthamine a-ring and the indole sidechain of Trp279 (*left*) and angle ω between the normal on the six-membered ring of Trp279 and the connecting vector between the centroid of this ring and galanthamine a-ring (*right*). For definition of distance, α, and ω see Fig. 2

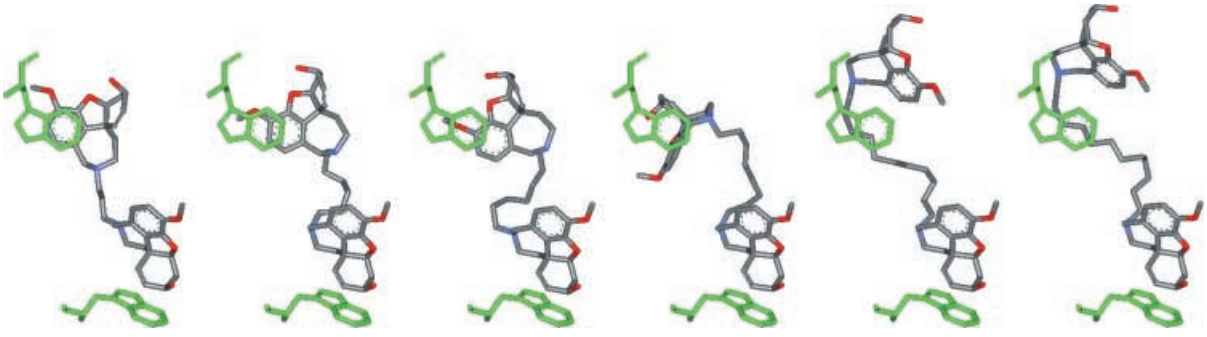

Fig. 10 Binding modes for *bis*-galanthamine with spacer length (from *left* to *right*) of three, six, seven, eight, eleven, and twelve methylene groups. Selected residues lining the gorge ends are also shown in *green* (Trp279 and Trp84)

While QXP is based on a Monte Carlo search for qualified conformations, followed by an energy minimization through a geometrical refinement, the FlexX procedure builds up the ligand step by step in the gorge and keeps track of a changing ensemble of potential candidates. [33] In contrast to QXP the FlexX procedure seeks to minimize the energy of every substructure during the build up of the ligand structure. This necessarily leads to a maximization of the interaction already during the stepwise assembling of *bis*-galanthamine fragments in the gorge and therefore to much more compact structures as compared to the QXP results. This effect can be seen by a comparison of ligand volumes in the gorge for the various *bis*-galanthamine derivatives (Fig. 11).

Particularly with the longer spacer length this effect is clearly visible and, as a result, QXP predicts a 5- or 6-methylene compound as the favored derivative, while the FlexX algorithm yields a 6- or 7-methylene derivative as the preferred candidate. With our initial assumption that β-amyloid plaque can be inhibited by ligand interaction with Trp279 at the PAS, either one of these *bis*-galanthamines with a spacer length of five to seven methylene groups should serve the purpose with sufficient efficiency. All of these ligands will occupy the space around Trp279 and thereby inhibit an aggregation of β-amyloid peptides at this site. However, a desired high affinity would require a low energy complex that simultaneously exhibits strong hydrophobic interaction with the indole side chain of Trp279.

Discussion

We have developed a modeling procedure that allows the optimization of galanthamine derivatives that can potentially interfere with more than a single function of AChE. Recent investigations [17, 18] have shown that this enzyme is not only responsible for cleaving the

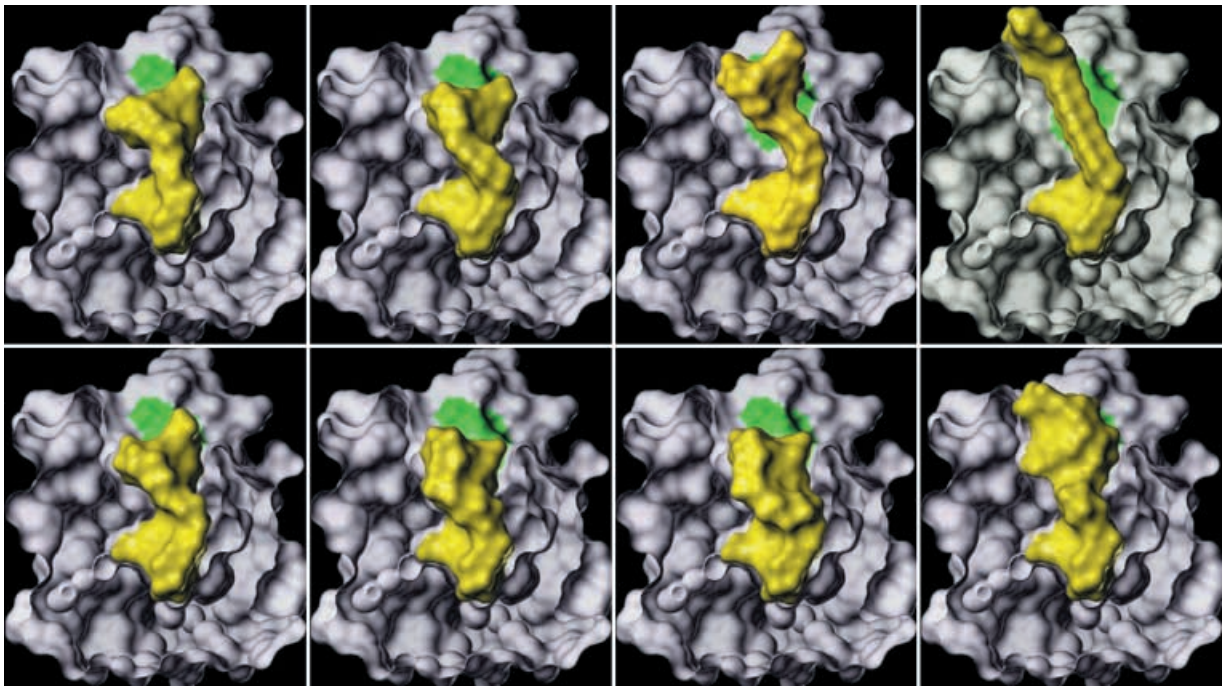

Fig. 11 Comparison of the gorge occupancy by different *bis*-galanthamine ligands with spacer lengths three, five, seven, and twelve (from *left* to *right*). The upper row shows the results of QXP-docking while the FlexX results are shown in the bottom line

neurotransmitter acetylcholine, but also for β-amyloid plaque formation by promoting the aggregation of this peptide at the peripheral anionic site. This site is located at the mouth of a gorge which penetrates 20 Å down into the AChE. At the bottom of this gorge resides the catalytic triade which is responsible for acetylcholine cleavage.

Our bisfunctional AChE ligands are based on the structure of galanthamine, a competitive AChE inhibitor that recently has been approved for Alzheimer therapy in several European countries. Docking studies with galanthamine have correctly predicted the orientation of this drug in the ester cleavage site; however, this investigation also suggested a site at the PAS which is centered around amino acid Trp279 as a further binding site for galanthamine. In addition, the galanthamine molecules at these two sites are oriented head to head in terms of the nitrogen atoms, which suggests a galanthamine dimer linked by an appropriate spacer as a potential bisfunctional ligand for AChE. Starting from this model, we have investigated *bis*-galanthamine compounds with a spacer length of two to twelve methylene groups in terms of their potential dual interaction with AChE.

Our modeling procedure involves docking studies (QXP and FlexX) of various *bis*-galanthamines in the AChE gorge and a scoring procedure that not only reflects energy criteria but also separately evaluates the hydrophobic interaction of these ligands with the PAS, particularly with the indole ring of Trp279. Taking advantage of the scoring output of FlexX, which allows us to extract the hydrophobicity term of the total complex energy, we have compared this value with geometrical structure parameters that describe the orientation and conformation of *bis*-galanthamine fragments that interact with the indole. Our results predict a *bis*-galanthamine derivative with a spacer length of five to seven methylene groups as a favorable structure that simultaneously allows interaction with the ester cleavage site and the peripheral anionic site. In this respect it should be noted that the synthesis of *bis*-galanthamines with a spacer of six, eight, and ten methylene groups has already been described in the literature. [34] Testing of these compounds on AChE from *Electrophorus Electricus* yielded a maximum inhibition by the 8-methylene compound. With a *bis*-functional ligand one might, however, have to make a compromise on the two desired activities.

In order to experimentally prove our prediction, and as a basis for further investigation of a dual AChE activity inhibition, we are presently developing a biological assay that should allow us to quantitatively measure the plaque formation in addition to the esterase blocking activity. These experimental data will serve to calibrate our geometrical scoring procedure for the interaction of AChE ligands with the PAS and should facilitate the approach towards a design of *bis*-functional AChE ligands.

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