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A Computational View On the Significance of E-Ring in Binding of (+)-Arisugacin A to Acetylcholinesterase

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Abstract

A computational docking study of a series of *de novo* structural analogs of the highly potent, non-nitrogen containing, acetylcholinesterase inhibitor (+)-arisugacin A is presented. In direct comparison to the recently reported X-ray single-crystal structure of (+)-territrem B bound *h*AChE, the modeling suggests that there is a unique conformational preference for the E-ring that is responsible for the superior inhibitory activity of (+)-arisugacin A against *h*AChE relative to (+)-territrem B, and that substitutions on the E-ring also play an important role in the protein-ligand interaction.

Graphical abstract

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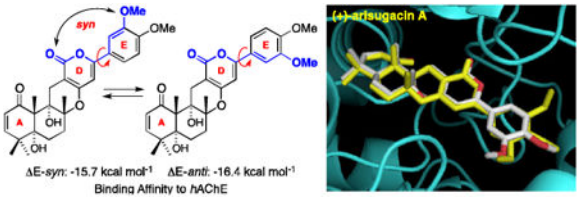

Keywords

(+)-Arisugacin A; E-ring conformation; E-ring substitutions; Territrems; Acetylcholinesterase; Alzheimer's disease; Computational modeling

We recently disclosed a computational study of the highly potent, non-nitrogen atom containing, acetylcholinesterase [AChE] inhibitor (+)-arisugacin A,[1] which was isolated

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Supplementary Material: Supplementary material presenting AChE docking model development and validation has been provided.

from *Penicillium Sp. Fo-425* with an impressive IC_{50} of 1.0 nM against AChE [Figure 1].[2,3] Our modeling results obtained using *AutoDock Vina*[4] reveal that (+)-arisugacin A is a potential dual binding site and possibly a covalent inhibitor of AChE, thereby representing a novel mode of action. As highlighted in Figure 1, a set of predicted non-covalent interactions (a)–(e) between (+)-arisugacin A and AChE [*Torpedo Californica* AChE or *tc*AChE numbering] are quite consistent with the only available SAR data reported by Peng[5a] using (+)-territrem B,[5b] a close structural relative of (+)-arisugacin A [*vide infra*].[5b-1] More significantly, we identified two additional key and likely critical features that render (+)-arisugacin A unique in its mode of binding.

Firstly, a close view of the catalytic site near the classical serine protease triad, His440, Ser200, and Glu327, evokes the potential for the engagement of Ser200 with the A-ring enone through either 1,2-addition of Ser200-OH to the C1-carbonyl or 1,4-addition to C3, thereby suggesting a possible reversible covalent inhibition as the mode of action.[1,6-8] Although this possibility may be dependent on the ability of (+)-arisugacin A to move within the binding pocket leading to C1 or C3 proximity to Ser200, it is consistent with Peng's observation[5a] that the α,β-unsaturated ketone motif in the A-ring is significant. Secondly, we demonstrated that (+)-arisugacin A mimics donepezil [Aricept™],[9] which is a dual binding site inhibitor of AChE. These two ligands share a H-bonding interaction between the Phe288-NH and the respective ligand carbonyls, a π-stacking interaction between Trp279 and aromaticity in the respective ligands, and H-bonding between Tyr70 and the respective methoxy-aryl groups [Figure 2B].

These findings can be significant in the context of developing disease-modifying therapeutics targeting symptomatic aspects of dementia diseases with a combined long-term efficacy in neuroprotection. Alzheimer's disease [AD][10] affects well over 24 million people worldwide[11,12] mostly in the geriatric population. With the exact pathogenic mechanism of this degenerative and devastating syndrome remaining unknown, the current leading theory in disease-modifying therapeutic approaches is the *amyloid cascade hypothesis*. It focuses on β-amyloid peptides [Aβ], which is a primary component of the neurotoxic senile plaques, with specific intents of (a) inhibiting its production,[13] (b) preventing its aggregation, and (c) altering its metabolism and clearance.[14] On the other hand, the long standing *cholinergic deficiency hypothesis*[12,15] represents almost the only rational design that has led to FDA approved drugs such as tacrine, rivastigmine, galanthamine, and huperzine A [Figure 3] as well as donepezil [see Figure 2B].[16-18]

While the two hypotheses appear to be parallel, recent findings believe that they are in fact not mutually exclusive but strongly interconnected. Firstly, acetylcholine [ACh] activation of the muscarinic receptors leads indirectly to increases in the non-amyloidogenic α-secretase processing of Aβ precursor protein (APP),[19] thereby linking simple ACh-hydrolysis inhibition to the diversion of APP away from Aβ production. Secondly, pro-aggregating ability of AChE on Aβ formation has been recognized[20] to involve the enzyme's peripheral anionic site (PAS) located at the entrance of the active site gorge. Thus, AChE inhibitors able to bind to PAS have been found to inhibit this secondary enzyme activity.[20a] Consequently, there has been a re-emerging interest in AChE inhibitor-based drug development, leading to a new paradigm of developing disease-modifying drugs that target

multiple pathways associated with AChE with modulations of both the cholinergic and amyloid targets through dual binding site inhibition. The goal is not only impeding ACh-hydrolysis, but also (a) aiding or improving PAS binding, leading to enhanced inhibition of Aβ pro-aggregating activity of AChE,[21,22] and/or (b) concomitantly inhibiting β-secretase (BACE-1) activity, the enzyme involved in the rate-limiting step of APP processing into Aβ.[23]

In light of these findings, our earlier modeling suggests that designing dual binding site ligands based on (+)-arisugacin A can be significant in identifying novel AD therapeutics capable of targeting early stages of the neurotoxic cascade while mitigating the cholinergic deficiency. Recently, our computational evidence of the mode of binding of (+)-arisugacin A in *tc*AChE was confirmed by Cheung *et al*.'s seminal X-ray crystal structure of human AChE [*h*AChE] bound with (+)-territrem-B [TB].[24] Their reported structure is almost in exclusive agreement with our model [*vide infra*].[1] Encouraged by this success, we expanded our modeling studies to the arisugacin and territrem families of natural products as well as a series of *de novo* analogs focusing on the E-ring. We wish to report here computational evidence in which the E-ring plays a significant role in the binding of (+)-arisugacin A to *h*AChE.

Cheung's published X-ray crystal structure of (+)-territrem B-bound-*h*AChE [TB–*h*AChE] provided the foundation for the study of the binding affinities of the arisugacins and territrems. Specifically, the reported TB–*h*AChE[24] bound structure appears to better accommodate arisugacin-like structures with a large degree of reorganization around the catalytic active site and protein backbone in the peripheral site that led to an altered gorge shape.[24] Our modeling study employed *AutoDock v4.2* and the more recent version in *AutoDock Vina*,[4] made available by The Scripps Research Institute under a General Public License. While these models do allow for flexibility in selected protein residues, these degrees of freedom do not account for the conformation changes seen between the donepizil-*tc*AChE (protein structure source in our 2011 study[1]) and TB–*h*AChE[24] active site gorge conformations. As shown in Figure 4, our docking of TB in *h*AChE matched perfectly the reported structure.

When examining the predicted binding of (+)-arisugacin A with *h*AChE and comparing these docking events with the X-ray structure of TB–*h*AChE, we uncovered an intriguing as well as significant observation. As shown in Figure 5, while the X-ray structure of TB-*h*AChE nails the predicted binding of (+)-arisugacin A to *h*AChE, there is a minor structural difference between (+)-arisugacin A and TB: (+)-Arisugacin A has one less *m*-OMe group on the E-ring. This minor difference disrupts the E-ring symmetry seen in TB, thus rendering two possible E-ring conformations for (+)-arisugacin A. The D and E-rings of (+)-arisugacin A could be regarded as being *syn* when the *m*-OMe group on the E-ring is *syn* to the α-pyrone-carbonyl of D-ring, while being *anti* when the *m*-OMe group is *anti* to the α-pyrone-carbonyl group. Intriguingly, these two conformations pose a real difference in their respective binding energies. Specifically, we found that the conformer (+)-arisugacin A-DE-*syn* has a binding affinity of -15.7 kcal mol^{-1}, which is inferior to that of TB [-16.1 kcal mol^{-1}]; while (+)-arisugacin A-DE-*anti has a* superior binding affinity at -16.4 kcal mol^{-1}

This difference in binding affinities aroused our interest because TB is reported by Õmura,[2a,2d] to possess an inferior inhibitory power against AChE versus that of (+)-arisugacin A. We recognized that our observation appears to be more than just a coincidence, and that binding of (+)-arisugacin A to *h*AChE may very well favor the *anti*-conformation with respect to the DE-ring. Consequently, we proceeded to obtain binding affinities of all known members from both arisugacin and territrem families. As shown in Table 1, we compare the predicted binding affinities with their respective IC_{50} values reported by Õmura,[2a,2d] and tabulated Δ affinities against the analog we named as (+)-arisugacin-Ph in which the E-ring contains no OMe substitutions [entry 1].

Our tabulated analysis distinctly reveals potential significance of not only the *meta*- but also the *para*-OMe group on the E-ring of arisugacins and territrems. Lack of an OMe substitution at the *meta* position, as shown with (+)-arisugacin B, appears to be associated with inferior binding affinity as well as IC_{50} value [entry 4 versus 2 or 3]. On the other hand, although the experimental IC_{50} value of (+)-arisugacin-Ph is not known, it appears that the binding affinity suffers when devoid of the *p*-OMe group [entry 1 versus 4]. Most notably, as we had eluded to, TB has an IC_{50} value of 7.6 nM[2a,2d] [entry 13] versus that of 1 nM for (+)-arisugacin A [entry 2 and 3].[2a] However, only when the DE-ring is in the *anti* conformation is the predicted binding affinity consistent with this lower IC_{50} value [entry 3].

In addition, intriguingly, (+)-territrem A [TA] also exhibits a binding affinity difference between DE-*syn* and *anti* [entries 11 and 12], although to the best of our knowledge, the IC_{50} value of TA against AChE has not been documented. Lastly, our predicted binding affinities should correlate to much lower IC_{50} values for (+)-arisugacins C-H [entries 5-10 – all in blue]. These analogs involve structural alterations on the A-ring[5] and/or deletions of the angular OH groups. Discrepancies in IC_{50} values and predicted binding affinities may be due to physical properties differences (*ex*. solubility, compare Arisugacin B to G with one less degree of unsaturation and two less hydroxyl groups), although we are not certain at this point as to the real rationale behind these differences.

Intrigued by these observations, we attempted through modeling to identify possible interactions that would be consistent with the DE-ring conformational preference as well as benefit of the *p*-OMe group. As shown in Figure 7, we found three key interactions that could rationalize our finding. Firstly, the presence of an *m*-OMe group at the *syn* position has an energetic cost of 0.1 to 0.3 kcal mol^{-1}. This cost is likely due to possible steric interactions between the *syn m*-OMe group with the TYR341 and SER293 carbonyl groups. Secondly, the presence of *m*-OMe at the *anti* position actually provides an energetic benefit of 0.5 kcal mol^{-1}. Possible H-bonding between the *anti m*-OMe group and the TYR72-OH group could lead to such benefit. Thirdly, the presence of *p*-OMe appears to have an energetic benefit of 0.4 kcal mol^{-1}. This could be a result of possible interaction between the *p*-OMe group and the TRP286 methylene unit through van der Waal forces.

Recognizing the potential significance of our finding, we proceeded to examine a series of *de novo* arisugacin structural analogs with a focus on the E-ring substitution patterns through modeling and found striking evidence that further accentuates the impact of the E-ring on binding.[25] Firstly, we observed with the arisugacin analog (+)-arisugacin-*m*-OMe with the

E-ring containing just one OMe group at the *meta* position, the *anti* conformer again possesses superior binding affinity than its respective *syn* conformer [Figure 8]. On the other hand, the analog with bis-*m*-OMe groups provides no further improvement on binding affinity than (+)-arisugacin-*m*-OMe-DE-*anti* – until an OMe group is re-enlisted at the *para* position, which is of course TB itself! This study is in agreement with the three key interactions described above.

The second study stems from the observation that (+)-territrem B and (+)-territrem C [TC] differ only by the group at the *para* position on the E-ring: OMe in B versus OH in C. Their binding to *h*AChE and IC_{50} values appear to be very comparable with TC being slightly lower. This prompted us to systematically examine if changes from OMe to OH would improve or diminish binding. As shown in Table 2, a general trend with some additive value can be summarized as following: (a) When the *syn m*-OMe group is replaced by an OH group, an enhancement in binding was found [entry 4 vs. 3; 12 vs. 11; and 15 vs. 13]. This enhancement is likely very much related to diminished steric interaction between the *syn m*-OMe group and the TYR341 and SER293 carbonyl groups. (b) When an *m*-OMe group is replaced with OH group at the *anti* position, diminish binding is observed [entry 8 vs. 7 and 16 vs. 13]. One exception is shown in entry 14 in comparison to TB in entry 11. (c) A *p*-OH group also leads to diminish binding affinities [entry 2 vs. 1; 5 vs. 3; and 9 vs. 7].

Overall, we seem to have hit a plateau at -16.5 kcal mol^{-1} for binding affinities through simple changes of OMe groups on the E-ring. This however unveils a structure that represents the best arisugacin analog [entry 4]. Another plateau is at -16.4 kcal mol^{-1} for the best TB structural analog [entry 12]. It is noteworthy that with a switch of the *m*-OMe group to OH, the respective arisugacin analogs tend to have a higher binding affinity in the *syn* DE-ring conformation based on our definition rather than the *anti* conformer, a reversal from that observed with (+)-arisugacin A [compare entries 3-7 with 4-8, 12 to 14, and 15 to 16]. This improvement is likely associated with both relief of steric interactions between the OMe group and the TYR341 and SER293 carbonyl groups, and introduction of at least a new H-bonding interaction between the *m*-OH and the SER293 carbonyl with maintained proximity to the TYR341 carbonyl [Figure 9].

We note here that we are aware of the fact that binding affinity differences may not arise solely from positioning of E-ring substituents, as any substitution change could impact the position of the entire scaffold.[24] In addition, as much as one would expect to gain in binding affinity by improving or building in new non-covalent interactions, there is an empirical basis to expect non-additive and/or overall diminishing returns. This relates to the empirical limits of achievable binding affinities of small molecule ligands.[26,27] However, while Table 2 may be presenting a simplistic view, its general trend, as summarized, can serve as a useful aid for designing potent inhibitors of AChE based on how presence or lack of a substituent could impact binding. Our current study demonstrates that it is possible to identify more powerful inhibitors than (+)-arisugacin A.

In summary, we have examined computational dockings of a series of *de novo* structural analogs of the highly potent, non-nitrogen containing, acetylcholinesterase inhibitor (+)-arisugacin A. Aided by the X-ray crystal structure of (+)-territrem B bound *h*AChE, our

modeling suggests that there is a unique conformational preference for the E-ring that is responsible for the superior affinity of (+)-arisugacin A to *h*AChE in comparison to (+)-territrem B. In addition, substitutions on the E-ring appear to play a critical role in binding to *h*AChE. These computational assessments present useful parameters for future designs of potential disease-modifying therapeutics based on (+)-arisugacin A. Synthetic efforts toward some of these computationally designed structural analogs are underway.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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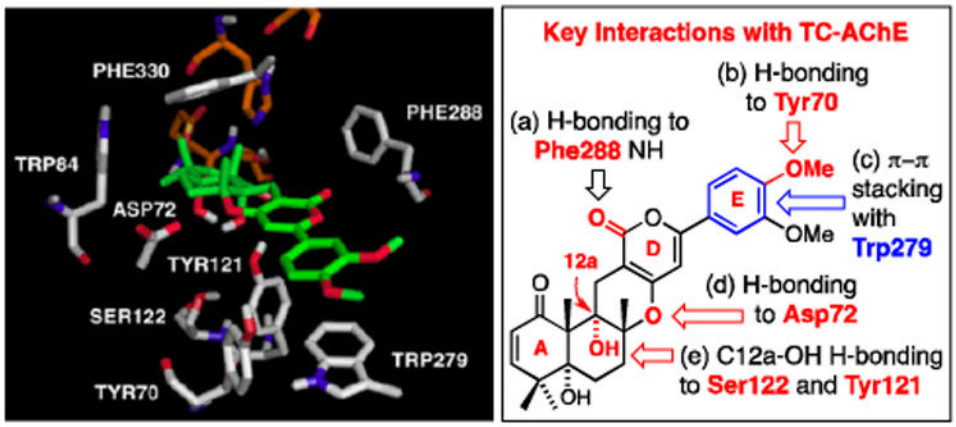


Figure 1.

Predicted Signature Interactions in the Binding of (+)-Arisugacin A to *Torpedo Californica* AChE (*tc*AChE) Using *AutoDock Vina*.

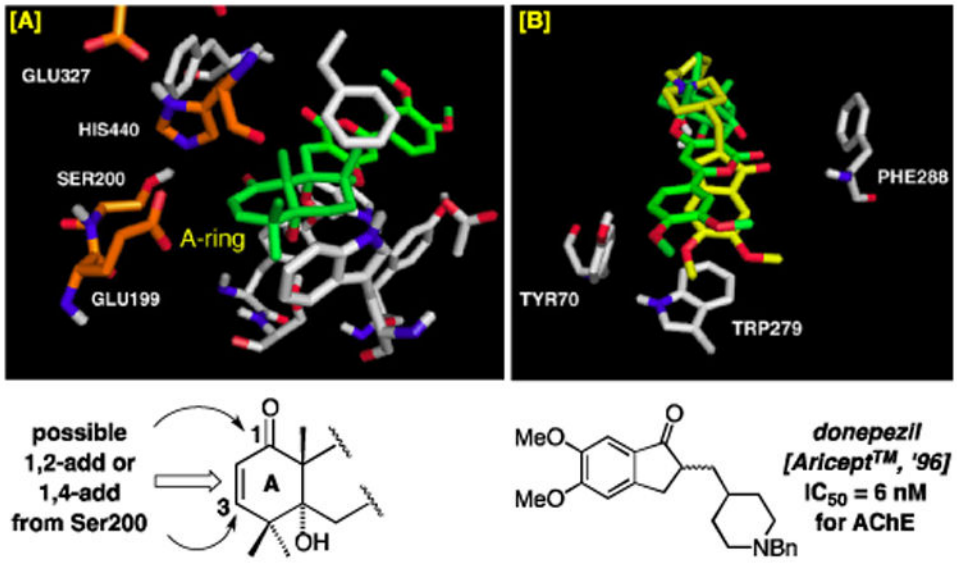


Figure 2.

[A] Possible Covalent Interactions Between *tc*AChE and the A-Ring of (+)-Arisugacin A. [B] Overlay of Binding of (+)-Arisugacin A (in Green) to *tc*AChE with Donepezil (in Yellow).

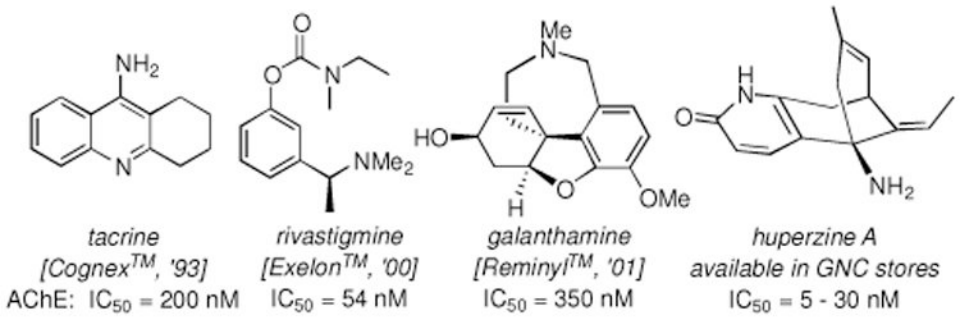


Figure 3.

AChE Inhibitors Approved as Therapeutic drugs for Combating AD. Respective Trade Names and the Year of Release are in Brackets.

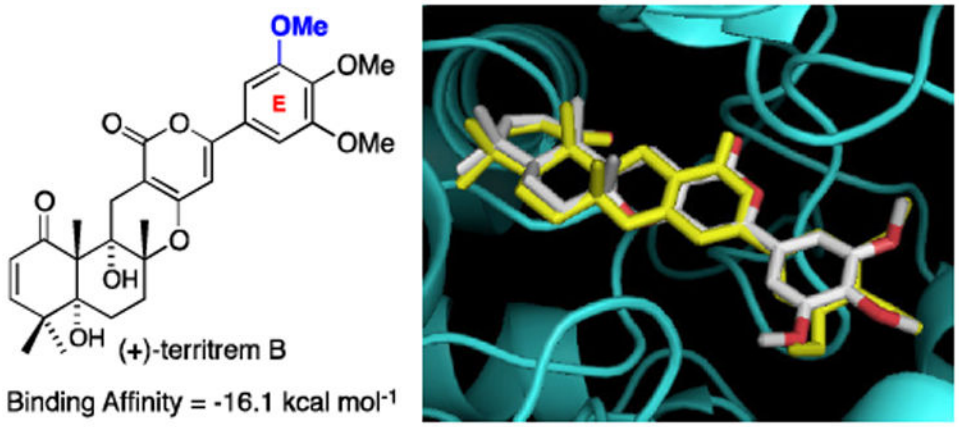


Figure 4.

An Overlay of the Predicted Binding of TB to *h*AChE using *AutoDock* (in White) and the Observed Binding of TB to *h*AChE in the Actual X-Ray Structure obtained by Cheung *et al*. (in Yellow).

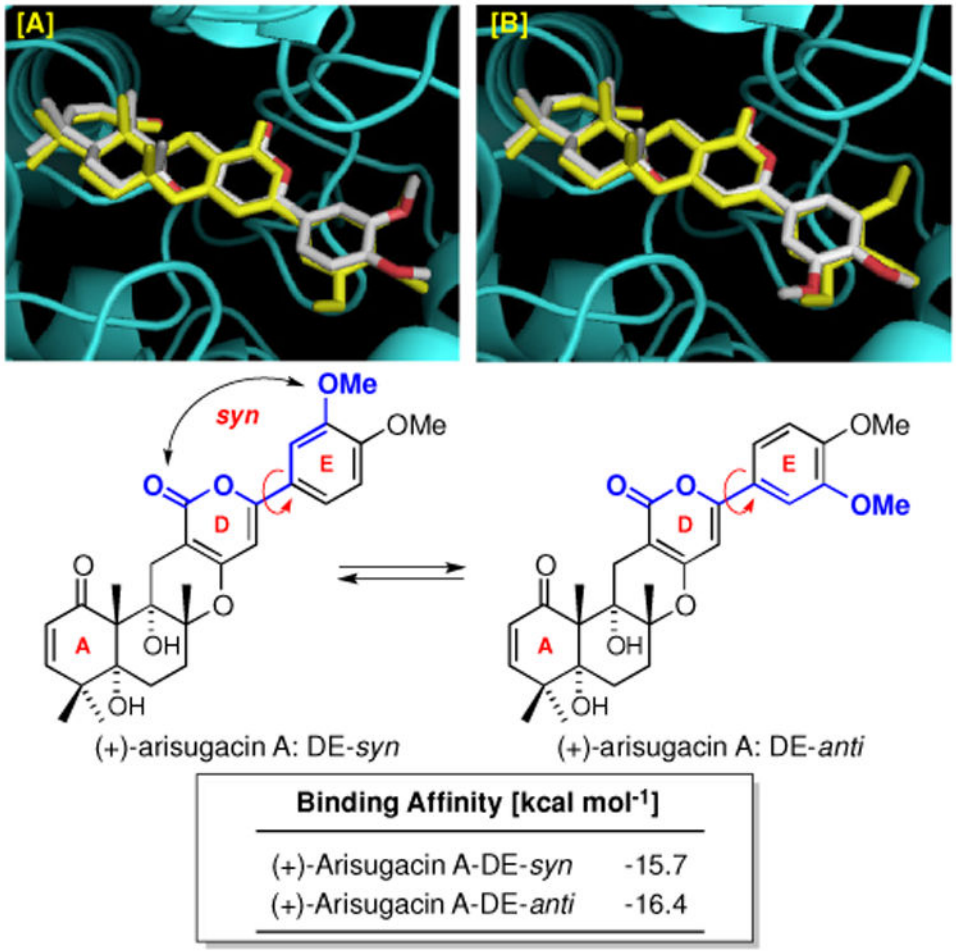
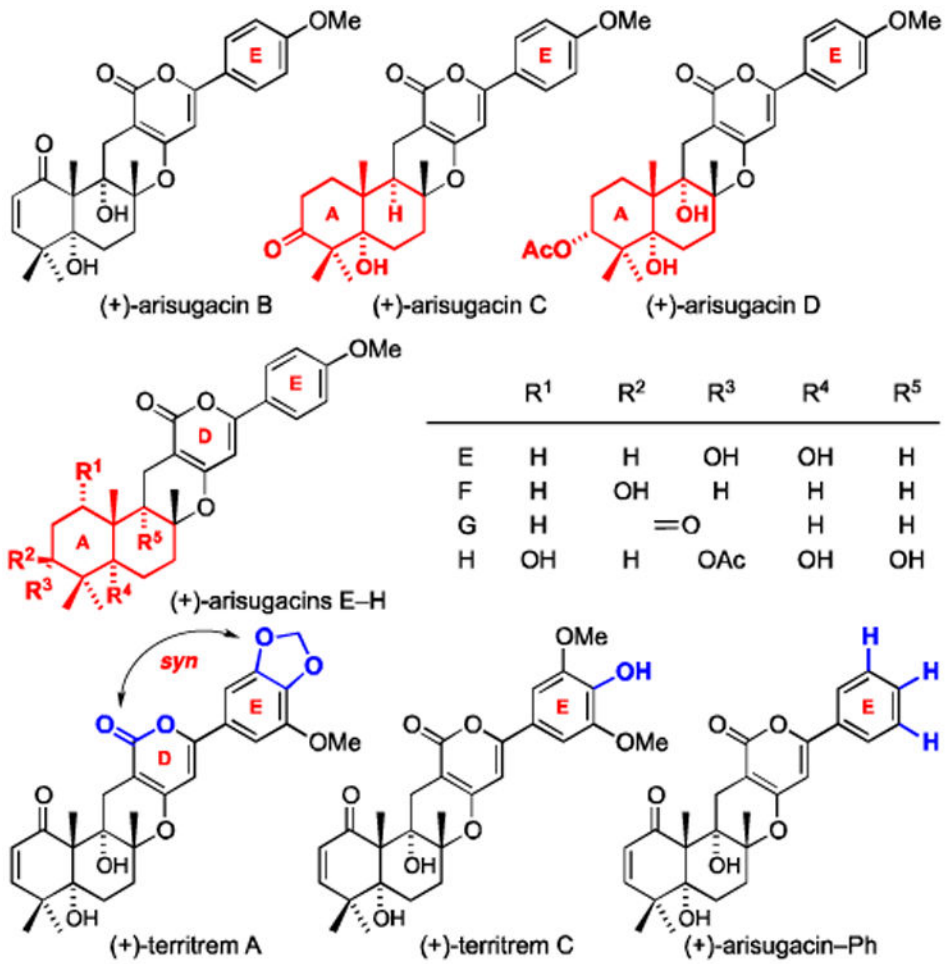


Figure 5.

[A] An Overlay of the Predicted Binding of (+)-Arisugacin A-DE-*syn* to *h*AChE using *AutoDock* and the Observed Binding of TB–*h*AChE in the X-Ray Structure. [B] An Overlay of the Predicted Binding of (+)-Arisugacin A-DE-*anti* with the Observed Binding of TB.



	R^1	R^2	R^3	R^4	R^5
E	H	H	OH	OH	H
F	H	OH	H	H	H
G	H	=O		H	H
H	OH	H	OAc	OH	OH

Figure 6.
Arisugacins A-H and Territrems A-C.

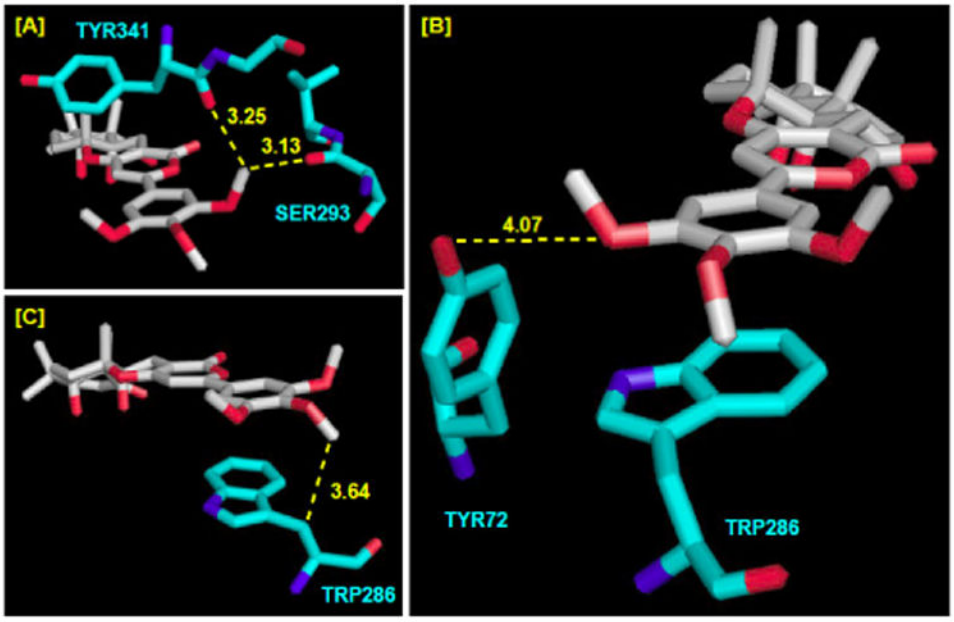


Figure 7.

Close Views of [A] TB's *m*-OMe-*syn*. [B] TB's *m*-OMe-*anti*. [C] TB's *p*-OMe. Interatomic distances (Å) in yellow dotted lines. All are *h*AChE numberings.

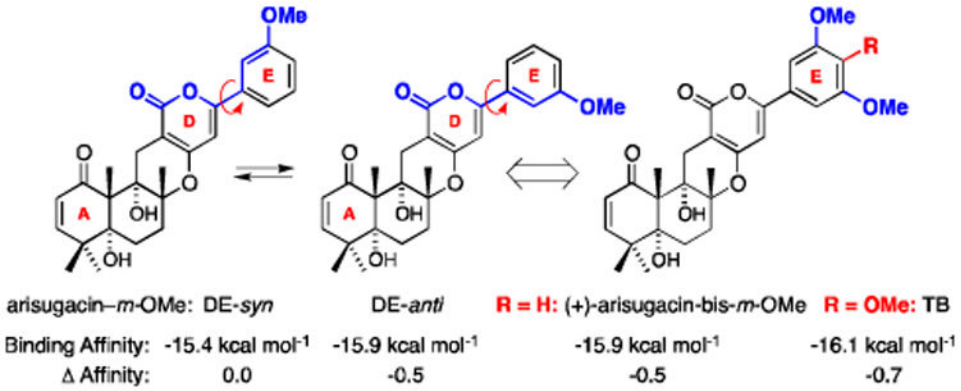


Figure 8.

(+)-Arisugacin Analogs Containing Either Mono *m*-OMe Group or Bis-*m*-OMe Groups on the E-Ring. Δ Affinity = Binding Affinity of the Analog Subtracting Affinity of Arisugacin-Ph [-15.4 kcal mol^{-1}].

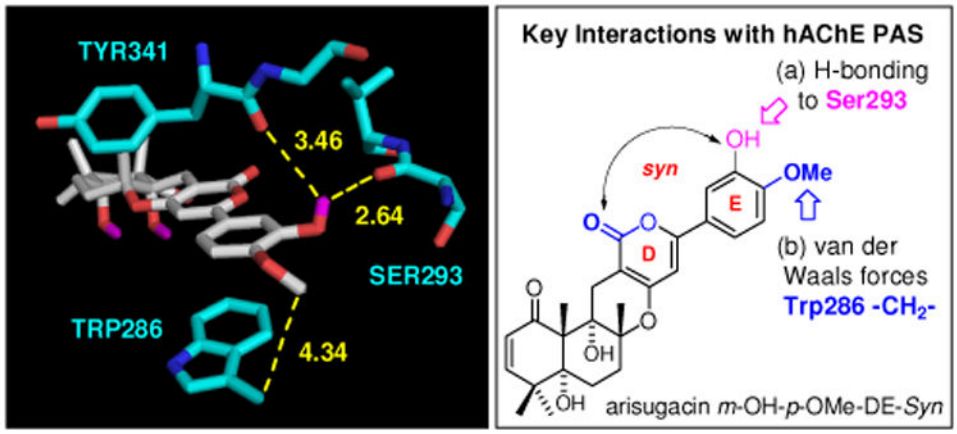


Figure 9.

Predicted Interactions of Arisugacin *m*-OH-*p*-OMe-DE *Syn* with the *h*AChE PAS. Interatomic distances (Å) in yellow dotted lines. All are *h*AChE numberings.

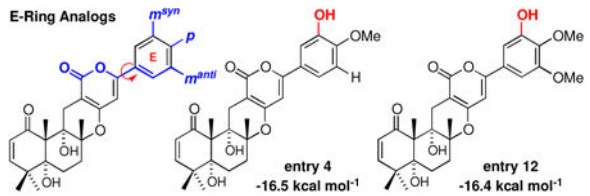
Table 1

A Comparison of Reported IC_{50} Values [All are taken from reports by Õmura: See References 2a and 2d] against AChE with Predicted Binding Affinity Values using *AutoDock*. Δ Affinity = Binding Affinity of the Inhibitor Subtracting Affinity of Arisugacin-Ph [entry 1].

entry	AChE Inhibitors	IC_{50} [in μM]	binding affin it y [kcal mol^{-1}]	Δ affinity
1	Arisugacin–Ph	--	-15.4	0.0
2	**(+)-Arisugacin A-DE-*syn***	**0.001**	**-15.7**	**-0.3**
3	**(+)-Arisugacin A-DE-*anti***	**0.001**	**-16.4**	**-1.0**
4	**(+)-Arisugacin B**	**0.0258**	**-15.8**	**-0.4**
5	(+)-Arisugacin C	2.5	-15.6	-0.2
6	(+)-Arisugacin D	3.5	-12.8	+2.6
7	(+)-Arisugacin E	>100.0	-13.9	+1.5
8	(+)-Arisugacin F	>100.0	-15.0	+0.4
9	(+)-Arisugacin G	>100.0	-15.6	-0.2
10	(+)-Arisugacin H	>100.0	-12.4	+3.0
11	**(+)-Territrem A-DE-*syn***	--	**-16.4**	**-1.0**
12	**(+)-Territrem A-DE-*anti***	--	**-16.3**	**-0.9**
13	**(+)-Territrem B**	**0.0076**	**-16.1**	**-0.7**
14	**(+)-Territrem C**	**0.0068**	**-16.1**	**-0.7**

Table 2

Impact of E-Ring Substitutions on Predicted Binding Affinities. Δ Affinity = Binding Affinity of the Analog Subtracting Affinity of Arisugacin-Ph [-15.4 kcal mol^{-1}].



entry	NPs and Analogs	m^{syn}	p	m^{anti}	binding affinity [kcal mol^{-1}]	Δ affinity
1	(+)-Arisugacin B	H	OMe	H	-15.8	-0.4
2	(+)-Arisugacin B-OH	H	OH	H	-15.5	-0.1
3	(+)-Arisugacin A-DE-*syn*	OMe	OMe	H	-15.7	-0.3
4		**OH**	**OMe**	**H**	**-16.5**	**-1.1**
5		OMe	OH	H	-15.6	-0.2
6		OH	OH	H	-16.1	-0.7
7	(+)-Arisugacin A-DE-*anti*	H	OMe	OMe	-16.4	-1.0
8		H	OMe	OH	-16.3	-0.9
9		H	OH	OMe	-16.1	-0.7
10		H	OH	OH	-15.9	-0.5
11	(+)-Territrem B	OMe	OMe	OMe	-16.1	-0.7
12		**OH**	**OMe**	**OMe**	**-16.4**	**-1.0**
13	(+)-Territrem C	OMe	OH	OMe	-16.1	-0.7
14		**OMe**	**OMe**	**OH**	**-16.3**	**-0.9**
15		**OH**	**OH**	**OMe**	**-16.3**	**-0.9**
16		OMe	OH	OH	-15.9	-0.5
17		**OH**	**OMe**	**OH**	**-16.3**	**-0.9**
18		OH	OH	OH	-15.9	-0.5