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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 14 (2004) 4843–4846

Modeling the binding affinities of β-secretase inhibitors: application to subsite specificity

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Received 12 May 2004; revised 21 July 2004; accepted 22 July 2004
Available online 7 August 2004

Abstract—A new linear binding affinity model has been developed for hydroxyethylene based inhibitors of β-secretase (BACE). This model is an improvement over a previously published model, and has been applied to a series of analogs not included in the training set. The linear model has been used to study subsite specificity for the P_2 through P'_2 positions, and to evaluate a small number of C-terminal analogs. The predicted rankings are in good agreement with experiment and support using this model for structure-based design of BACE inhibitors.

Accumulation of the 40–42 residue Aβ peptide has been implicated as a primary cause of AD.[1–3] Aβ results from sequential cleavage of β-amyloid precursor protein (APP) by β-secretase(BACE) and γ-secretase.[4] The initial cleavage of APP by β-secretase has been determined to be a critical step in the formation of Aβ,[5] and is an attractive therapeutic target for the treatment of AD. A number of potent BACE inhibitors derived from transition-state analogs (TSA) such as hydroxyethylene (HE), statine, and hydroxymethylcarbonyl have been reported in literature.[6–9] The crystal structure of the soluble portion of BACE complexed with two potent inhibitors OM99-2 and OM00-3 (K_i's of 1.6 and 0.32 nM) have been reported recently by Tang and co-workers.[8,10] Additionally, a number of related inhibitors have also been reported by Gosh et al.[11] The crystal structure and published inhibitors were used by Tounge and Reynolds[12] to derive a linear interaction energy (LIE) model for BACE. We report an improved model using a slightly different functional form of the computed interaction energies, and the application of this model to a series of analogs outside the original training set. A comparative analysis of the difference between the two models will be described elsewhere.

The crystal structure of the complex, BACE with OM99-2, was used as the starting configuration for model construction. Based on previous studies, we adjusted the active site aspartates so that Asp32 is deprotonated (charge = −1) and Asp228 is protonated at the inner oxygen. The MAESTRO, PPREP, and HBUILD programs (available from Schrodinger Inc.)[13] were used to set the protonation state of the ionizable residues and add the hydrogens. This was followed by a limited minimization protocol to optimize the hydrogen atom positions. All calculations were carried out using the OPLS-AA forcefield[14] and GB/SA continuum water model.[15] A subsection of the protein was defined by selecting all the amino acid residues within 15 Å from any ligand atom in OM99-2. The residues outside the selected range were then deleted and the dangling ends were capped using methyl groups. The initial positions for the various ligands were constructed by using the backbone co-ordinates of OM99-2. Initial relaxation of ligands in the complex was accomplished with 50 steps of minimization (steepest descent) followed by conjugate gradient minimization (0.05 convergence criteria). The optimized ligand conformation from the ligand–protein complex was used to minimize the free ligand. The relative difference in the computed electrostatic and van der waals energy contributions in the bound and free states of the ligand were extracted and used to create a binding energy model.

A linear regression fit to the electrostatic and van der Waals terms for the 12 ligands reported by Ghosh

Keywords: β-Secretase; BACE; Structure based design; Binding affinity; Modeling.
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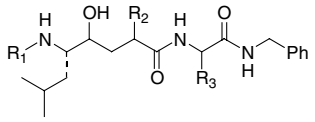
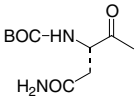
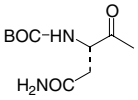
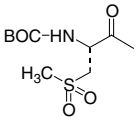
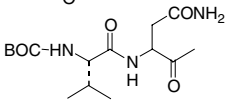
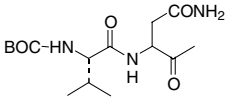
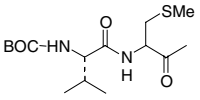
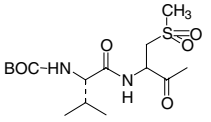
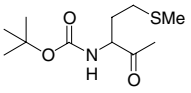
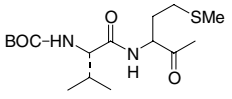
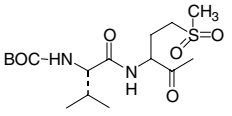
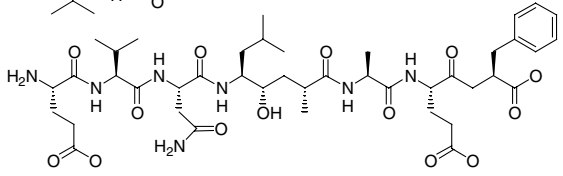
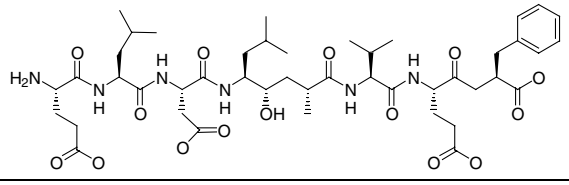
0960-894X/$ - see front matter © 2004 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2004.07.044

Table 1. Experimental and computed binding energies for the training set



	R_1	R_2	R_3	K_i (nM)	ΔG^{Expt} (kcal/mol)	ΔG^{Comp} (kcal/mol)
1		Me	Me	22,423.0	−6.38	−6.83
2		Me	$CHMe_2$	3134.0	−7.55	−6.72
3		Me	$CHMe_2$	1129.0	−8.16	−6.54[a]
4		Me	Me	61.4	−9.90	−10.11
5		Me	$CHMe_2$	5.9	−11.30	−10.51
6		Me	$CHMe_2$	50.1	−10.02	−10.03
7		Me	$CHMe_2$	9.4	−11.02	−9.3[a]
8		Me	$CHMe_2$	5808.0	−7.19	−8.06
9		Me	$CHMe_2$	2.5	−11.81	−11.04
10		Me	$CHMe_2$	8.0	−11.11	−11.80
OM99-2				1.6	−12.06	−12.28
OM00-3				0.32	−13.05	−13.00

[a] Values are based on the linear regression fit to all 12 ligands.

et al. was derived using the statistical program S-PLUS.[16] This led to a model with an RMSD of 1.2 kcal/mol (compared to 1.101 kcal/mol for the previous model).[12] The optimized LIE coefficients were $\alpha = 0.2043$ and $\beta = 0.0137$. The electrostatic coefficient is smaller than the theoretical range of 0.33–0.5,[17] but compares well with the Tounge and Reynolds model.[12] Statistical analysis of the data using Cooks distance (evaluates leverage and residual distance) and the residuals (normal q–q plots) revealed that ligands 3 and 7 might be considered

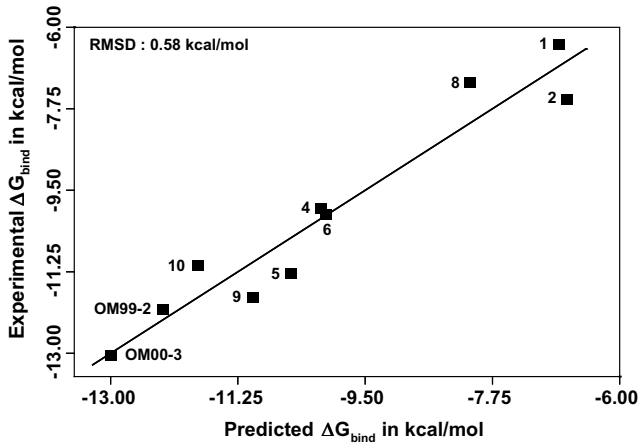


Figure 1. Plot of experimental and LIE computed ΔG of binding for the training set.

outliers (Table 1). Omission of 3 and 7 provided a much improved model with an RMSD of 0.58 kcal/mol. This final model is given in Eq. 1.

$$\Delta G^{\mathrm{Bind}} = 0.33\langle \Delta U_{\mathrm{VDW}} \rangle + 0.016\langle \Delta U_{\mathrm{Elec}} \rangle + 10.93 \quad (1)$$

The predicted versus experimental binding affinity plot for the final set of 10 ligands (Table 1), used to derive the model, is given in Figure 1. It is interesting to note that the binding affinities of the charged ligands are well reproduced. This was a problem in the previously reported LIE model. The weaker binders do have a slightly greater spread, but this is probably to be expected. The two ligands that were omitted based on statistical considerations both contained sulfone groups. It is impossible to determine from these results whether this is due to a limitation of the forcefield, electrostatics, experimental errors, or some other source of error. However, we feel justified in omitting these compounds in light of the statistical tests, and also the results that will be described later for compounds outside the training set.

Tang and co-workers[18] performed a systematic amino acid scan of all eight subsites (P_4–P'_4) in an eight-peptide inhibitor of BACE. This provided the relative residue preferences for each position in the peptide represented by EVNLAAEF. The residue specificities were identified through substrate kinetic studies and confirmed by follow-up screening of a combinatorial library of inhibitors. The library of inhibitors and the calculations reported here, are based on the prototype inhibitor given in Figure 2. In general, the P subsites were found to be more specific compared to the P′ subsites, with P_1 being most critical. To test the performance of our binding affinity model, the residue preferences for subsites P_1, P'_1, P_2, and P'_2 were probed computationally (Fig. 2).

Five residues were selected from the reported literature data for each of the subsites listed above. Specifically, we chose LEU, PHE, MET, TYR, THR for subsite P_1, ASP, ASN, LEU, ALA, THR for subsite P_2, MET, ALA, SER, THR, ASN for subsite P'_1 and VAL, ALA, TYR, SER, ASN for subsite P'_2. These residues were selected because they exhibit a range of activities at each subsite. The residues at each site were ranked by their relative computed binding affinities (Table 2). The residue preferences at P_1, P_2, and P'_1 sites are reproduced reasonably well, with the top two residues correctly identified for the subsites. For the P_2 site the order of the first and second ranked residues is reversed, and the third and fifth ranked residues are also reversed with respect to the experimental values. In general, greater variance is seen for the weak binders, and for sites that show less selectivity. Analysis of the experimental subsite preferences shows that binding sites near the catalytic site display greater selectivity than sites that are more remote. The P_1 site is particularly stringent. Consistent with this observation, the model is able to rank ligands in the P_1 and P'_1 pockets more accurately than P_2 and P'_2. The P'_2 results are particularly poor. Nevertheless, considering the fact that some of the subsite selectivities are small the agreement between computed and experimental rankings is quite good for P_2–P'_1. It must also be noted that the experimental data are for peptide substrates while the calculations are for the corresponding hydroxyethylene inhibitors.

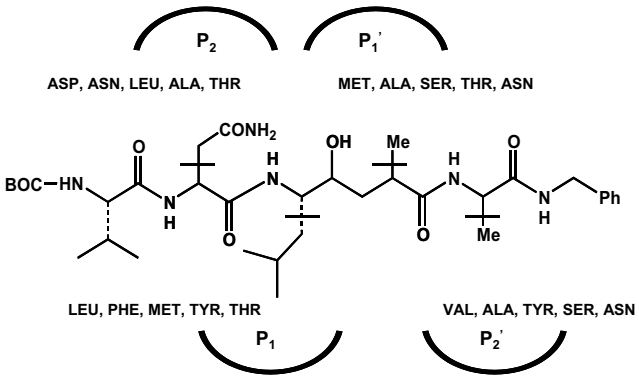
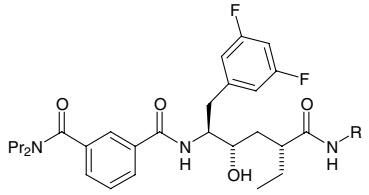


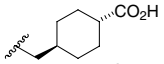
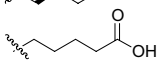
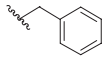
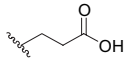
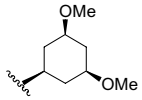
Figure 2. Residue substitutions at P_2–P'_2.

Table 2. Experimental and computed activity ranking for P_2–P'_2

		LEU	PHE	MET	TYR	THR
P_1	Experimental	1	2	3	4	5
	Computed	1	2	4	3	5
		MET	ALA	SER	THR	ASN
P'_1	Experimental	1	2	3	4	5
	Computed	1	2	5	3	4
		ASP	ASN	LEU	ALA	THR
P_2	Experimental	1	2	3	4	5
	Computed	2	1	5	4	3
		VAL	ALA	TYR	SER	ASN
P'_2	Experimental	1	2	3	4	5
	Computed	4	2	5	3	1

Table 3. Computed rank and experimental IC_{50}



R	IC_{50} (nM)	LIE rank
	50	1
	400	2
	1400	4
	2800	3
	6600	5

We also examined a series of analogs synthesized by Hom et al. where the C-terminal site was varied.[7] A set of 5 C-terminal analogs, as shown in Table 3 were ranked using our linear interaction energy model. The computed rankings were compared to the experimental binding affinities, as shown in Table 3. The overall performance of the model is quite good. The top two inhibitors are ranked correctly. The only difference between the model and experiment is that predicted order of the third and fourth most potent inhibitors is reversed. This is not a serious error given that the IC_{50} values only differ by a factor of two. The weakest inhibitor in the set was also correctly identified.

In conclusion, we have developed an improved LIE model for computing the binding affinities of hydroxyethylene-based β-secretase inhibitors. Application of this model to analogs outside the original training set shows that it is a predictive model. The model provided a good accounting of the subsite specificity of the P_2–P'_2 binding sites in BACE, and the relative inhibitory ability of a series of published C-terminal analogs.

Acknowledgements

Ramkumar Rajamani acknowledges a Johnson and Johnson Excellence in Science postdoctoral fellowship sponsored by the Corporate Office of Science and Technology. We thank Ellen Baxter, Douglas Brenneman, Kelly Conway, Allen Reitz, Brett Tounge, and the Spring House CNS Team for their helpful comments and insights.

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