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Simple Structure-Based Approach for Predicting the Activity of Inhibitors of Beta-Secretase (BACE1) Associated with Alzheimer's Disease

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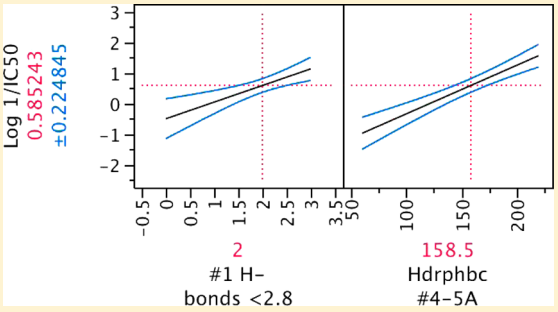
Supporting Information

ABSTRACT: Beta-site amyloid precursor protein cleaving enzyme-1 (BACE1) is a target of interest for treating patients with Alzheimer's disease (AD). Inhibition of BACE1 may prevent amyloid-ß (Aß) plaque formation and the development or progression of Alzheimer's disease. Known BACE1 inhibitors were analyzed using computational chemistry and cheminformatics techniques to search for quantitative structure−activity relationships (QSAR). A remarkable relationship was found with only two simple descriptors. The square of the linear correlation coefficient r^2 is 0.75. The main descriptor is the number of hydrophobic contacts in the range 4−5 Å between the atoms of the ligand and active site. The other descriptor is the number of short (<2.8 Å) hydrogen bonds. Our approach uses readily available structural data on protein-inhibitor complexes in the Protein Data Bank (PDB) but would be equally applicable to proprietary structural biology data. The findings can aid structure-based design of improved BACE-1 inhibitors. If an inhibitor has less observed activity than predicted by our correlation, the compound should be retested because the first assay may have underestimated the compound's true activity.



INTRODUCTION

Alzheimer's disease (AD) is a costly affliction affecting millions of mostly elderly people. AD has been called the disease with two victims: the patient and the caregiver. Indirectly, the disease expends the time and resources of the caregivers of the afflicted. Incidence of the disease keeps increasing as the population ages and fewer people die of other diseases.[1] In the United States, the disease is the sixth leading cause of death, and the only one in the top ten causes of death for which there is no preventive or cure.[2] Even after decades of earnest research effort, there are relatively few treatment options for patients. The number of drugs on the market for AD is small, and the efficacy of these compounds is disappointing.[3] Therefore better therapies are desperately needed.[4]

Amyloid precursor protein (APP) is a natural protein associated with neurons of the brain. In Alzheimer's disease, APP is cleaved by an appropriately named enzyme called beta-site APP cleaving enzyme-1 (BACE1), resulting in short 40−42 amino acid segments called amyloid-ß (Aß).[5] Aggregation of Aß into fibrils and plaques results in the death of neurons and is associated with AD. Even after years of intensive research, the exact molecular mechanism of AD is still being identified. Theories on the pathogenesis of Alzheimer's disease abound. For example, one of many hypotheses is that Aß may form channels through the membranes of neurons thereby letting calcium ions pass through and resulting in neurotoxicity.[6,7] Inhibition of the BACE1 enzyme may prevent Aß formation and thereby prevent the development or progression of the disease.

BACE1 (EC 3.4.23.46, ß-secretase, memapsin 2) is currently a prime target of interest for treating patients with AD.[8−14] Finding a useful therapeutic has been challenging. One BACE1 inhibitor appeared interesting until off-target toxicity was encountered.[15,16] A recent phase III clinical trial on the monoclonal antibody solanezumab did not achieve the desired end points, but the preliminary data were still thought to support the amyloid hypothesis.[17]

Many BACE1-inhibitor complexes are catalogued in the Protein Data Bank (PDB),[18] an online library containing the three-dimensional (3D) structures of over 80,000 biomolecules. Our goal was to find a quantitative relationship or at least a trend between 3D structural properties of known BACE1 inhibitors and their biological activity. Our strategy was to look in publicly accessible databases to identify inhibitors meeting two criteria: (1) the ligand-protein complexes have been deposited in the PDB, and (2) the bioactivities of these ligands were available. Using the 3D structures of catalogued BACE1 inhibitors cocrystallized with the enzyme (as reported by independent research groups) and biological activities reported in the literature or online databases, we were able to analyze ligand−receptor structures to determine physical descriptors that contribute to the potency of BACE1 inhibition.

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There have been surprisingly few quantitative structure−activity relationships (QSARs) studies of BACE1 inhibitors reported. This dearth could indicate it is extremely difficult to find such QSARs, or it could indicate that the field is very competitive so research groups have been reluctant to report their findings. One prior investigation found impressive correlations using descriptors from MOE in a Free-Wilson type analysis, but the compounds treated were limited to BACE1 inhibitors sharing an identical core scaffold.[19] Another prior investigation of QSARs of BACE1 inhibitors used CATALYST software to find hypothetical pharmacophoric conformations and then employed a large eclectic set of descriptors.[20] The latter included molecular connectivity indexes, quantum properties, surface areas, etc. By using both linear terms and some unusual cross-terms, the Jordanian group was able to find a very complex QSAR equation (r^2 = 0.88) when eleven descriptors were invoked. During the course of their work, the Jordanian group conceived some new compounds, but when synthesized none was reported with enough activity to warrant further investigation.

In contrast, our work leads to a simple, intuitive QSAR equation with only two descriptors that could be exploited in structure-based drug design involving a variety of molecular scaffolds.

METHODOLOGY

Selection of the ligands was random because we did not know going in to this research project what, if anything, we would discover. The initial 21 ligands were randomly selected from the PDB. They are listed in Table S1 in the Supporting Information. The compounds had been reported by various research groups. The ligands covered a wide range of activities and represented structural diversity. Based on encouraging results, additional test ligands were added to the data set to determine the predictive power of the original analysis (see Table S1).

In the last 40−50 years, thousands of molecular descriptors[21−23] have been investigated seeking QSARs for numerous classes of compounds. We wanted to investigate some new descriptors made available from the growing wealth of information provided by structural biology (a.k.a. protein crystallography). Protein Data Bank tools and applications were used in this computational chemistry study of BACE1 inhibitors. These tools include Ligand Explorer[24] and PoseView.[25,26] Ligand Explorer is a 3D molecular modeling software application associated with the Molecular Biology Toolkit. PoseView is an application linked to the PDB page of each ligand but was separately developed at and is operated by the University of Hamburg, Center for Bioinformatics. PoseView displays hydrogen bonding and hydrophobic interactions in a clear 2D scheme (see, e.g., Figure 1). (We use PoseView's terminology re hydrophobic.) The complete list of physical features investigated is given in Table S2 of the Supporting Information. Some of these structural characteristics proved useful for explaining inhibitory activity. We also tested some common descriptors from molecular modeling software, but these were not useful and were abandoned (Table S2).

Data for the structure-based descriptors were obtained using PDB Ligand Explorer 3.9.[24] Viewing each ligand in this application, we selected, for example, to view "Hydrogen Bond" interactions with the threshold set to 2.8 Å. The interactions within this range were then saved as a list of hydrogen bonds of distance less than 2.8 Å as well as the atoms participating in each bond. Atoms were specified by elemental symbol; atoms on BACE1 were further described using the amino acid to which they belong. PoseView was used to determine the interactions most relevant to the docking characteristics of each ligand. The interactions listed on PoseView were matched with those from PDB Ligand Explorer based on amino acid participating in docking. Each hydrogen bond was then assigned a bin based on the bond distance specified by PDB Ligand Explorer. The H-bond bins were categorized by distances of less than 2.8 Å, between 2.8 and 3.0 Å, and greater than 3.0 Å.

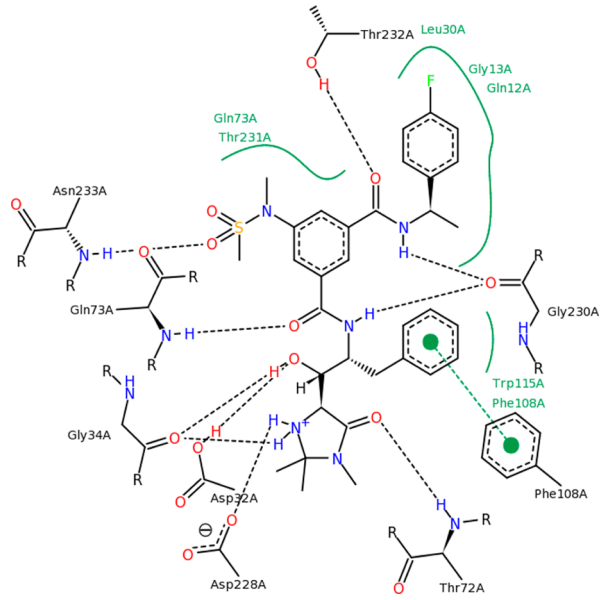

Figure 1. Example of a PoseView image showing the inhibitor in 2P8H (PDB identifier). Hydrogen bonding interactions are represented by dotted lines, hydrophobic interactions by green lines, and pi-stacking by the green dotted line between the ligand and the amino acids of BACE1.

A descriptor of major influence in the development of a QSAR is the number of hydrophobic interactions between 4.0 and 5.0 Å. These nonbonded interactions correspond to the spatial fit of a particular ligand in the active site of BACE1. To obtain the number of hydrophobic interactions, each ligand was viewed in PDB Ligand Explorer 3.9 and set for "Hydrophobic" interactions with a threshold of 3.5 Å, 4.0 Å, and 5.0 Å, consecutively. Lists of hydrophobic interactions for each of these three distances were saved into separate documents, and the number of interactions for each list was counted. Bins were created for interactions less than 3.5 Å, from 3.5 to 4.0 Å, and from 4.0 to 5.0 Å. By setting the threshold to 5.0 Å and excluding those interactions noted for the previous two bins, we were able to determine the total number of hydrophobic interactions between 4.0 and 5.0 Å. BACE1 inhibitors with better biological activity (IC_{50} < 0.05 μM) typically had 190−210 hydrophobic interactions as defined by PoseView and measured with Ligand Explorer.

To look for correlations, we used JMP,[27] a software tool for comprehensive, interactive statistical analysis. The statistical analysis first involved stepwise regression analysis to identify the most useful descriptors among all those that we tried. Then the most useful descriptors were tested in standard linear regression analysis.[28] Descriptors with t-ratios less than 2.0 were discarded because of their weak contribution in explaining

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the variance in the biological data. We sought the minimum number of descriptors that would give the statistically strongest correlation. We also wanted descriptors that would be intuitive and useful for drug design. Numerical values of some of the descriptors we employed are listed in Table S3 of the Supporting Information.

Biological data were obtained from multiple sources to confirm accuracy and minimize chances of error in online data compilations. The most common form of expressing BACE1 activity is IC_{50}, i.e., the concentration of a ligand to achieve 50% inhibition of enzymatic activity. Initial biological activities were taken from the Binding Data Base (BindingDB),[29,30] which is associated with the PDB. This database often had a range of values listed for each inhibitor. BindingDB is hosted by the Skaggs School of Pharmacy at University of California, San Diego. Their data were compared with the Binding Mother of All Databases (Binding MOAD).[31,32] Binding MOAD is hosted by the group at the University of Michigan.

During the study, it was noted that biological activities were highly variable. Some ligands displayed a 3-fold range in variability about the mean IC_{50} value for a single assay. Depending on the biological assay used to determine biological activity, ligands such as 2VJ6 had a 30-fold range in bioactivity.[33,34]

We also encountered a few errors in the databases of biological activity. In particular, the activity reported for the ligand 2QP8 was actually that for Aß40 reduction, not an IC_{50} value for BACE1 inhibition. We used the correct IC_{50} value in our analysis. Activity data reported for the ligand of 2VA7 was actually the activity of an intermediate compound not in PDB. We used the correct IC_{50} value for 2VA7. Likewise we chose to use 3K5G instead of 3K5F because the latter was an intermediate compound. The ligand of 3INF had ambiguities in the hydrogen bonding, so it was eliminated from our data set. In summary, all activity data used in our QSAR analysis were confirmed in the original literature sources where the inhibitors were reported; these sources are listed in the PDB.[18] The only ligands included in our final data set were those with no structural ambiguity. The biological data in our final data set are all IC_{50} values. Single values of mean IC_{50} rather than data ranges were employed. The activities are listed in Table S4 of the Supporting Information.

RESULTS

JMP provided insight to the most influential factors relating physical structure of an inhibitor to its biological activity. Of particular importance were the number of hydrogen bonds between a ligand and BACE1 under 2.8 Å, and the number of hydrophobic interactions between 4.0 and 5.0 Å. Figure 2 displays a plot of the predicted biological activity versus the activity published in literature sources. Activity is expressed as $\log_{10}(1/IC_{50})$. This choice was made based on the physical chemistry concept relating the logarithm of an equilibrium constant to the free energy for the association of the ligand and enzyme. The reciprocal is used so that higher activity (tighter binding) yields a larger number. The QSAR equation and associated statistics are shown in eq 1. The data points and equation are plotted in Figure 2. Note that the IC_{50} values span over 3 orders of magnitude. The regression line fits the data points quite well over the whole range. The training data set is shown in black, and the test set of compounds is shown in green. We use the notation L-ABCD to indicate the cocrystallized ligand in the complex with the PDB identifier ABCD.

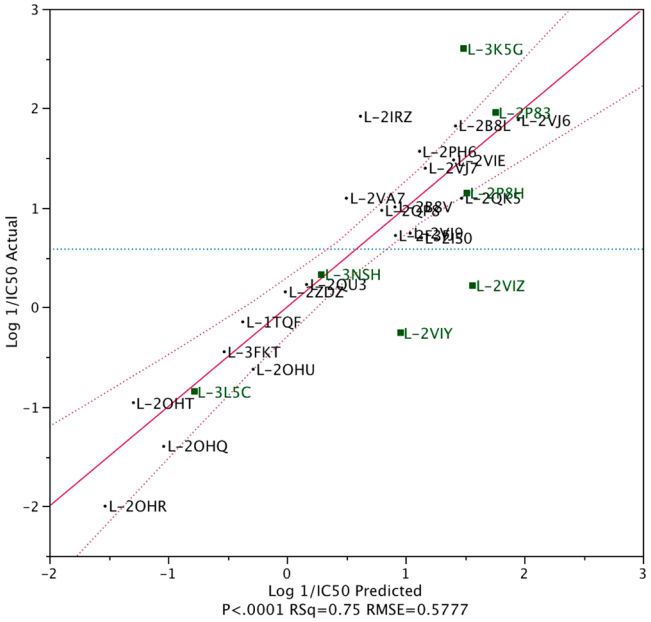

Figure 2. QSAR relationship between the actual biological activity values and those predicted by the regression equation. The regression line is shown in solid red. The dotted red lines mark the 95% confidence limits of the regression. The horizontal dotted blue line indicates the mean of the actual activity values. We use the notation L-ABCD to indicate the ligand in the protein complex with the PDB identifier ABCD. The data points marked by black dots are those in the original set of 21 compounds. The points shown as green squares are the additional seven compounds that were added to test the rigor of the correlation. For the chemical names of the inhibitors, see Table S1 of the Supporting Information.

$$\log_{10} 1/IC_{50} = -3.0324 + 0.01594\ \mathrm{hc45} + 0.5459\ \mathrm{Hb28} \quad (1)$$

where hc45 is the number of hydrophobic contacts in the range 4−5 Å, and Hb28 is the number of H-bond contacts under 2.8 Å. The standard error and t-ratio for the three terms of the equation are, respectively, 0.4287 and −7.07; 0.00234 and 6.80; and 0.1491 and 3.66. Note that all the t-ratios are well above 2.0. The standard statistics for the equation include the following: $r^2 = 0.7543$, $r^2_{adj} = 0.8685$, $n = 28$, root-mean-square error = 0.5777, and $p = <0.0001$, where r^2 is the variance explained, r is the correlation coefficient, and p is the probability of the null hypothesis.

Remarkably, 75% of the variance in the biological activity values is explained by just two descriptors. These descriptors are the number of hydrophobic contacts in the range 4−5 Å, and the number of hydrogen bond contacts under 2.8 Å with the receptor. Of the 28 ligands in our data set, 70% fall within the 95% confidence interval, indicated by the two dotted red curves in Figure 2. There is a 95% probability of the "true" correlation falling in this range.

Figure 3 qualitatively displays the leverage of the two most useful descriptors in explaining the variance in the biological activities. Both variables have a positive slope, meaning that increasing values of the variables increases activity. The slope for the hydrophobic count is greater than that for the count of H-bonds, meaning that the former is more important.

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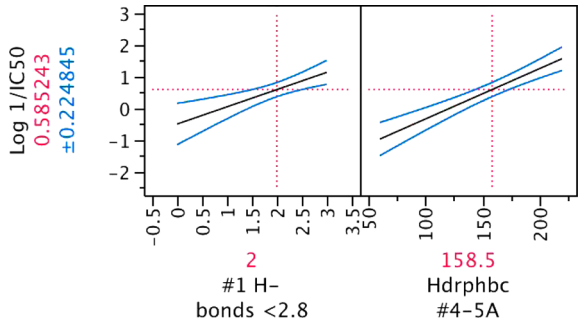

Figure 3. Leverage of the two descriptors in eq 1. The red dotted lines indicate the means of the abscissa values and of the ordinate values.

In addition to our main result above, it is instructive to look at some of our other statistical analyses. When we regress $\log_{10}(1/IC_{50})$ on only the three bins with the number of hydrophobic contacts (3−3.49 Å, 3.5−4 Å, 4−5 Å, respectively), we obtain an r^2 value of 0.65. However, the t-ratios for the bins for shorter distances are not significant (1.14 and −0.77, respectively). The lack of significance for the shorter contacts is seen qualitatively in Figure 4 where we show the leverage provided by the three bins. Only hydrophobic contacts in the range 4−5 Å have a steep slope. If we regress biological activity on only the number of hydrophobic contacts in the range 4−5 Å, then the r^2 value is 0.62. In other words, change in this variable has a large influence on the biological activity. If we regress the biological activity on only the short H-bond contacts less than 2.8 Å between the hydrogen donor and acceptor atoms, then r^2 is 0.30.

DISCUSSION AND CONCLUSIONS

A key part of our strategy was to divide the hydrophobic and hydrogen bonding contacts into bins. This strategy resulted in finding the best descriptors for understanding the inhibitory power of the compounds. The predictive power of the QSAR shown in Figure 2 may aid in drug design and could furthermore indicate compounds that should be retested for biological data because the original assay may have underestimated the compound's true activity.

The amount of variance explained by our QSAR equation, 75%, is gratifying considering the inherent uncertainty possible with biological activity data and crystallographic data. These data came from experiments performed by different research groups and could have involved conditions that differed slightly. The data values are thus not ideally comparable. Nevertheless, our results suggest that any noise in the data is not enough to prevent a correlation from emerging.

As seen in Figure 2, there are only four serious outliers: L-2IRZ, L-3K5G, L-2VIZ, and L-2VIY. Two of these compounds (L-2IRZ[35] and L-3K5G[36]) had activity greater than expected from the correlation. They may possess some unique advantageous interactions with the receptor. For example, cocrystallization of the ligand in 2IRZ resulted in the active site aspartyls being noncoplanar. Also in the case of the ligand of 2IRZ, the Merck scientists thought the compound activity was enhanced by decreased intramolecular flexibility and interactions with the flap region of BACE1. Hanessian[36] and collaborators noted that L-3K5G exhibits a new hydrogen bonding pattern in the receptor. The other two outliers (L-2VIZ and L-2VIY) are from a paper by the GlaxoSmithKline group in England.[37] These two inhibitors exhibited wide error bars. The IC_{50} values of these two compounds may be underestimated. It would be interesting if these compounds were reassayed.

With new BACE1 complexes being added to the PDB constantly, our method can be further validated. The 3D structure of any BACE1 inhibitor that cocrystallizes with the enzyme can be handled by our method. Even though we used structures from the PDB, the same software tools are applicable to proprietary BACE1 structure complexes.

An area of future work would be to determine which of the docking and scoring methods[38−43] work well with our QSAR equation. Our method could also be evaluated for solvated structures of enzyme−inhibitor complexes as generated by molecular dynamics simulations.[44,45]

In the context of a medicinal chemistry campaign, our work highlights the importance of a ligand design based on maximizing nonbonded contacts in the 4−5 Å range, which corresponds to favorable van der Waals interactions or having an influence on solvation or entropy. The greater importance of long, weak nonbonded contacts compared to short hydrophobic contacts suggests that the much greater number of the former is key. Many weak nonbonded contacts is more important than a few close contacts. (See Table S3 of the Supporting Information.) Our QSAR equation also shows that strong hydrogen bonding contacts improve activity, as expected.

BACE1 is an aspartyl protease with a relatively large, open active site.[46,47] Therefore the receptor can accommodate large ligands including polypeptides. For rational design[48,49] of better inhibitors, increasing the number of hydrophobic contacts has to be done with discretion. Just adding more groups to the ligand might increase the number of contacts, but would also

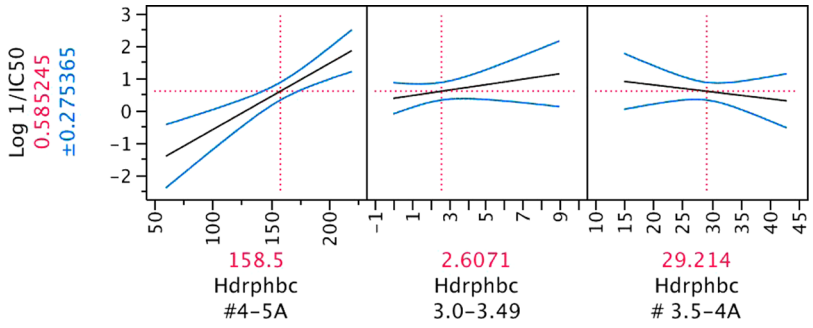

Figure 4. Leverage of the three bins of hydrophobic contacts to explain the variation in biological activities. Only the one on the left (4−5 Å) has a steep slope.

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make the molecular weight of the compound higher, and thereby be a detriment for the compound crossing the blood-brain barrier. Increasing the number of short H-bonds may be desirable, but this must be done in light of the possibility that increasing polar surface area may diminish brain penetration. Also, making an inhibitor too lipophilic would make distribution to the neurons in the brain more difficult.[49] Selectivity of the ligand for the target enzyme BACE1, rather than for other proteins, is still another factor medicinal chemists always have to consider.

Our approach of capitalizing on structural biology information to understand and predict binding affinities is being extended to other targets of interest to medicinal chemists.

■ ASSOCIATED CONTENT

Supporting Information

Tables of the compound set, descriptors, and biological activities. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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