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# Evaluating scoring functions for docking and designing β-secretase inhibitors

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**Abstract**—Several simple scoring methods were examined for 2 series of β-secretase (BACE-1) inhibitors to identify a docking/scoring protocol which could be used to design BACE-1 inhibitors in a drug discovery program. Both the PLP1 score and MMFFs interaction energy ($E_{inter}$) performed as well or better than more computationally intensive methods for a set of substrate-based inhibitors, while the latter performed well for both sets of inhibitors.


β-Secretase (also known as β-APP cleaving enzyme or BACE-1) is one of two proteases responsible for processing the membrane-bound amyloid precursor protein (APP) to the 40/42 residue β-amyloid peptide (Aβ), the primary constituent of the amyloid plaques observed in the brains of Alzheimer's patients.[1] Since BACE-1 cleavage of APP appears to be the rate-limiting step[2] in the production of Aβ and BACE-1 knockout mice show complete absence of Aβ with no significant side effects,[3] BACE-1 appears to be an attractive therapeutic target in the treatment of Alzheimer's disease.

BACE-1 has been characterized as the first known example of a pepsin-like aspartyl protease that is membrane-tethered.[4] A crystal structure of the soluble domain reveals a high degree of similarity to the tertiary structures of other mammalian and fungal aspartyl proteases.[5] Given the availability of coordinates for BACE-1, as well as prior experience with renin[6] and HIV-1 protease inhibitors,[7] we were interested in identifying a rapid docking/scoring protocol that could aid in the design of BACE-1 inhibitors. A series of peptidomimetic inhibitors (**1**–**13**, Table 1) reported by Ghosh et al.[8] served as an initial training set. Several scoring functions were evaluated and compared to a previous study using the more computationally intensive LIE[9] and LIECE[10] methods on the same set of inhibitors. As **1**–**13** are large substrate-based inhibitors spanning a wide range of activity (five orders of magnitude), a second training set of smaller hydroxyethylamine (HEA) inhibitors (**14**–**25**, Table 4) with a narrower activity range (2 orders of magnitude) was also examined to determine how sensitive the methods were to small structure/activity variations.

Models for inhibitors **1**–**11** were derived from the X-ray structures of **12**[11] and **13**[12] as bound in the BACE-1 active site. Models of inhibitors **14**–**25** were derived from the X-ray structure of a nonpeptide BACE-1 inhibitor.[13] The flexibility of the R groups shown in Tables 1 and 4 was explored by generating 25–50 conformers using a distance geometry algorithm.[14] The crystal structures of BACE-1 complexed with **12**[11] and with a nonpeptide BACE-1 inhibitor[13] were employed for minimization of inhibitors **1**–**13** and **14**–**25**, respectively. The active site was defined by selecting all residues within 10 Å of any atom of the cognate ligand. All titratable enzyme residues were charged with the exception of one of the catalytic

*Keywords*: β-Secretase; BACE-1; Alzheimer's disease; MMFF; PMF; PLP; LIGSCORE; LUDI; X-score.
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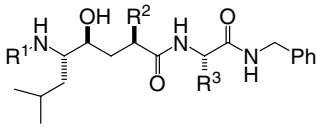
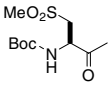
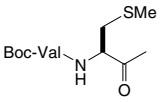
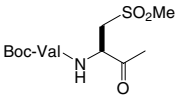
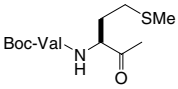
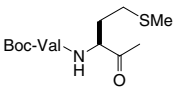
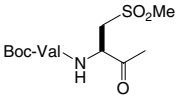
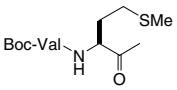
**Table 1.** Substrate-based BACE-1 inhibitors designed by Ghosh et al.[8]



| Compound | $R^1$ | $R^2$ | $R^3$ | $K_i$ (nM) | $\Delta G$ (kcal/mol) |
|---|---|---|---|---|---|
| **1** | Boc-Asn | Me | Me | 22423.0 | −6.38 |
| **2** | Boc-Asn | Me | $CHMe_2$ | 3134.0 | −7.55 |
| **3** |  | Me | $CHMe_2$ | 1129.0 | −8.16 |
| **4** | Boc-Val-Asn | Me | Me | 61.4 | −9.90 |
| **5** | Boc-Val-Asn | Me | $CHMe_2$ | 5.9 | −11.30 |
| **6** |  | Me | $CHMe_2$ | 50.1 | −10.02 |
| **7** |  | Me | $CHMe_2$ | 9.4 | −11.02 |
| **8** |  | Me | $CHMe_2$ | 5808.0 | −7.19 |
| **9** |  | Me | $CHMe_2$ | 2.5 | −11.81 |
| **10** |  | Me | $CHMe_2$ | 8.0 | −11.11 |
| **11** |  | $CH_2CHMe_2$ | $CHMe_2$ | 10491.0 | −6.84 |
| **12** | Glu-Val-Asn-Leu-Ψ-Ala-Ala-Glu-Phe | | | 1.6 | −12.06 |
| **13** | Glu-Leu-Asp-Leu-Ψ-Ala-Val-Glu-Phe | | | 0.3 | −13.05 |

aspartic acids, $Asp_{228}$, which was protonated on the inner oxygen, OD1. All inhibitors were neutral. All poses were energy-minimized using the MMFFs force field[15] in the rigid BACE-1 active site. The pose which resulted in the lowest conformational energy for the ligand was selected and the corresponding enzyme–ligand energy ($E_{inter}$) was compared to the observed $pK_i$ ($-\log K_i$) or $pIC_{50}$ ($-\log IC_{50}$). Two distance-dependent ($\varepsilon = 2r$, 4r) and a large constant ($\varepsilon = 50$) dielectric were examined as a simple means to approximate solvation effects. The energy-minimized geometries which led to the best correlation with experimental activity (i.e., $\varepsilon = 50$ for **1**–**13** and $\varepsilon = 2r$ for **14**–**23**) were subsequently rescored using LUDI,[16] PLP,[17] PMF,[18] LIGSCORE,[19] and X-Score.[20]

Models of inhibitors **1**–**11** and the energy minimized structures of **12**–**13** are shown overlaid in Figure 1. Only minor differences were observed between the X-ray and energy-minimized structures of **12** and **13**, with the exception of the $P_3'$ and $P_4'$ groups in **12** which have relatively high B factors in the crystal structure and do not make specific interactions with the enzyme.

A plot of the computed MMFFs $E_{inter}$ versus observed $pK_i$ (see Fig. 2) reveals that compound **11** is an outlier. This behavior was previously observed using the LIE method.[9] It is unclear whether this is due to the experimental observation or the computational approach, for example, solvation may play an important role, as suggested by a subsequent LIECE study[10] which employed

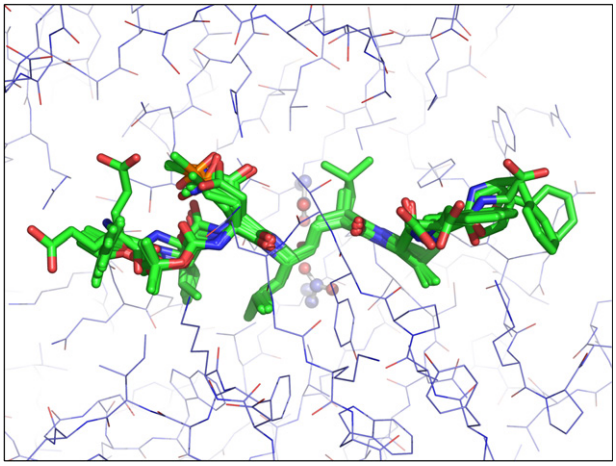


**Figure 1.** Overlay of the modeled structures for compounds **1**–**11** and the energy-minimized X-ray structures for **12** and **13** in the BACE-1 active site. The catalytic aspartates, $Asp_{32}$ and $Asp_{228}$, are shown in ball-and-stick mode. Image created using PyMol v0.98, DeLano Scientific LLC.

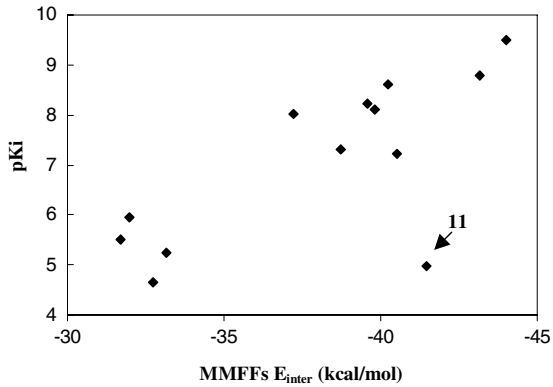



**Figure 2.** Plot of observed $pK_i$ versus MMFFs $E_{inter}$ ($\varepsilon = 50$) for compounds **1**–**13**. Note that **11** is clearly an outlier.

a more accurate electrostatic solvation term. However, as this compound was an outlier with the LIE method and with all methods employed here, it has been excluded from further consideration in the correlations reported in Table 2.

Note that the scores from several methods correlated well with the observed $pK_i$ values. The best were MMFFs energy ($\varepsilon = 50$), LIGSCORE1, and PLP1/PLP2 with $R^2$ values of 0.85, 0.88, and 0.97/0.93, respectively.

Table 3 displays the predicted versus observed $\Delta G$ values for the MMFFs and PLP1 methods compared to the previous LIE[9] and LIECE[10] study and experimental results. The MMFFs $E_{inter}$ alone leads to an rms difference of 0.79 kcal/mol, as compared to 1.10 and 0.92[21] kcal/mol for LIE[9] and LIECE.[10] Subsequent rescoring of the MMFFs energy-minimized inhibitors using PLP1 leads to an improved rms of 0.34. It is apparent that, for this series of peptidomimetic inhibitors, energy minimization (± a scoring function) yields as good, if

**Table 2.** Correlation between computed score and inhibitor $pK_i$ for inhibitors **1**–**13**

| Scoring method | Ligand geometry | $R^2$ |
|---|---|---|
| MMFFs, $\varepsilon = 2r$ | Minimized | 0.73 |
| MMFFs, $\varepsilon = 4r$ | Minimized | 0.76 |
| MMFFs, $\varepsilon = 50$ | Minimized | 0.85 |
| LIGSCORE1 | MMFFs, $\varepsilon = 50$ | 0.88 |
| LIGSCORE2 | MMFFs, $\varepsilon = 50$ | 0.55 |
| PLP1 | MMFFs, $\varepsilon = 50$ | 0.97 |
| PLP2 | MMFFs, $\varepsilon = 50$ | 0.93 |
| PMF | MMFFs, $\varepsilon = 50$ | 0.62 |
| LUDI | MMFFs, $\varepsilon = 50$ | 0.03 |
| X-Score | MMFFs, $\varepsilon = 50$ | 0.31 |

**Table 3.** A comparison of the free energy of binding ($\Delta G_{bind}$) predicted for inhibitors **1**–**10**, **12**–**13** using the MMFFs $E_{inter}$ ($\varepsilon = 50$), the PLP1 score, and the LIE[9] and LIECE[10,21] methods

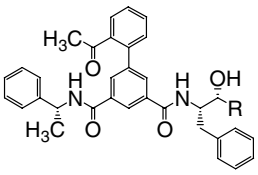
| | $\Delta G_{bind}$ | | | | |
|---|---|---|---|---|---|
| Compound | MMFFs | PLP1 | LIE | LIECE | Exp. |
| **1** | −7.6698 | −6.2047 | −8.14 | −6.47 | −6.38 |
| **2** | −7.1919 | −7.4791 | −8.92 | −7.96 | −7.55 |
| **3** | −7.3160 | −7.9636 | −7.74 | −7.67 | −8.16 |
| **4** | −11.2312 | −10.0680 | −11.17 | −9.48 | −9.90 |
| **5** | −10.8084 | −11.1181 | −10.26 | −11.53 | −11.30 |
| **6** | −10.4178 | −10.4430 | −10.38 | −10.20 | −10.02 |
| **7** | −9.7331 | −10.9074 | −10.25 | −11.12 | −11.02 |
| **8** | −7.8537 | −7.7045 | −8.31 | −8.80 | −7.19 |
| **9** | −11.1163 | −11.1349 | −9.99 | −10.21 | −11.81 |
| **10** | −10.9233 | −11.5078 | −9.94 | −11.64 | −11.11 |
| **12** | −12.4489 | −11.7321 | −11.96 | −10.05 | −12.06 |
| **13** | −12.8395 | −13.2867 | −12.98 | −12.99 | −13.05 |
| rms | 0.79 | 0.34 | 1.10 | 0.92 | |

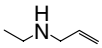
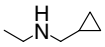
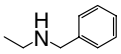
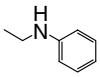
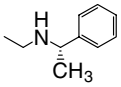
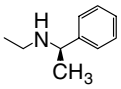
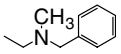
The rms deviation for each method relative to the experimental[8a] $\Delta G_{bind}$ is displayed below each column.

not better, agreement with experiment than the more computationally expensive LIE[9] and LIECE[10] methods. However, this set of inhibitors is large, substrate-based, and spans a wide range of activity. In order to assess how well the scoring protocol would perform on a typical drug-design problem, we employed a second test set of smaller, more drug-like HEA inhibitors **14**–**25**, shown in Table 4. This series of inhibitors, shown overlaid in Figure 3, varies solely in the substituent at the P1′ position. The activity range[22] is ~100-fold and includes **22** and **23** (*S* and *R* enantiomers, respectively) whose activity varies only 2-fold.

Table 5 shows the correlation between computed score and observed $pIC_{50}$ for HEA inhibitors **14**–**23**. Inhibitors **24** and **25** were excluded from the correlations since they have indeterminate $pIC_{50}$ values (i.e., >10,000 nM). In this instance, the MMFFs $E_{inter}$ correlated best with experimental activity. There was little change in $R^2$ as the MMFFs dielectric constant was varied, possibly due to fewer H-bond interactions for **14**–**25** as compared to **1**–**13**. However, the best $R^2$ of 0.82 was achieved with a 2r distance-dependent dielectric. The MMFFs $E_{inter}$ ($\varepsilon = 2r$) scores for **14**–**25** are listed in Table 4. Note that the $E_{inter}$ score was able to correctly rank the 2 enantiomeric inhibitors, **22** and **23**, with the former predicted,

**Table 4.** Peptidomimetic HEA inhibitors **14**–**25**, with variation at P1′ and their corresponding MMFFs ($\varepsilon = 2r$) $E_{inter}$ values in kcal/mol



| Compound | R | $IC_{50}$ (nM) | $E_{inter}$ |
|---|---|---|---|
| **14** | H | 9900 | −39.96 |
| **15** |  | 223 | −46.38 |
| **16** |  | 674 | −43.98 |
| **17** |  | 317 | −45.42 |
| **18** |  | 418 | −45.83 |
| **19** |  | 1400 | −45.66 |
| **20** |  | 115 | −50.07 |
| **21** |  | 110 | −49.79 |
| **22** |  | 115 | −50.36 |
| **23** |  | 348 | −49.00 |
| **24** |  | >10,000 | −42.55 |
| **25** |  | >10,000 | −41.95 |

and observed, to be the more potent isomer. In addition, the calculated energy values for **24** and **25** are low (similar to R = H, **14**), in qualitative agreement with their poor activity. This can be rationalized by examining the inhibitors in the BACE-1 active site. As depicted in Figure 4, substitution of the HEA nitrogen causes a steric bump with the outside oxygen of one of the catalytic aspartates, $Asp_{228}$, which typically participates in an H-bond with the secondary amine.

In summary, we have demonstrated that simple scoring methods can perform as well or better than more computationally intensive methods for a series of substrate-based BACE-1 inhibitors. Additionally, one of the methods, MMFFs $E_{inter}$, also performed well for a series of HEA BACE-1 inhibitors. The MMFFs $E_{inter}$ appears to be a simple, rapid scoring function which may be useful in a drug discovery program for rank-ordering virtual inhibitors in a high-throughput mode prior to synthesis.

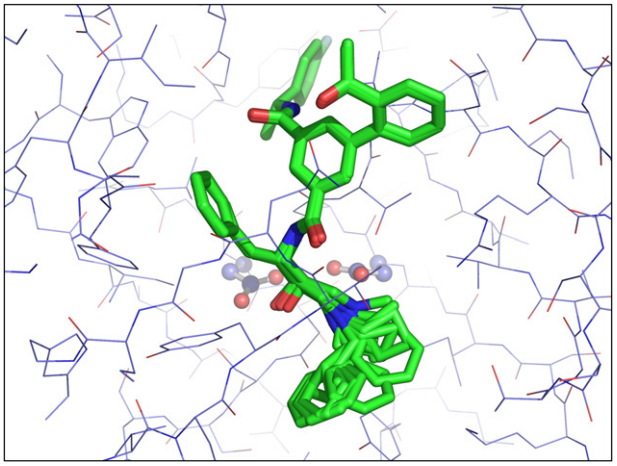


**Figure 3.** Models of HEA inhibitors **14**–**25** bound in the BACE-1 active site.[13] The catalytic aspartates, $Asp_{32}$ and $Asp_{228}$, are shown in ball-and-stick mode. Image created using PyMol v0.98, DeLano Scientific LLC.

**Table 5.** Correlation between computed score and inhibitor $pIC_{50}$ for HEA inhibitors **14**–**23**

| Scoring method | Ligand geometry | $R^2$ |
|---|---|---|
| MMFFs, $\varepsilon = 2r$ | Minimized | 0.82 |
| MMFFs, $\varepsilon = 4r$ | Minimized | 0.81 |
| MMFFs, $\varepsilon = 50$ | Minimized | 0.76 |
| LIGSCORE1 | MMFFs, $\varepsilon = 2r$ | 0.26 |
| LIGSCORE2 | MMFFs, $\varepsilon = 2r$ | 0.74 |
| PLP1 | MMFFs, $\varepsilon = 2r$ | 0.69 |
| PLP2 | MMFFs, $\varepsilon = 2r$ | 0.70 |
| PMF | MMFFs, $\varepsilon = 2r$ | 0.75 |
| LUDI | MMFFs, $\varepsilon = 2r$ | 0.49 |
| X-Score | MMFFs, $\varepsilon = 2r$ | 0.38 |

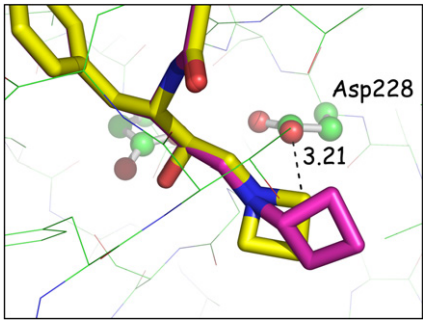



**Figure 4.** Models of HEA inhibitors **19** and **25** bound in the BACE-1 active site.[13] Note the close contact between the azetidine ring and the outside oxygen of $Asp_{228}$. Image created using PyMol v0.98, DeLano Scientific LLC.

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