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QSAR studies—potent benzodiazepine γ-secretase inhibitors

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Abstract—A series of benzodiazepine compounds, which act as γ-secretrase inhibitors were subjected to QSAR studies. A correlation between the physicochemical properties $Q\log P$, SMR and the inhibitory activity was obtained and a model equation was generated to predict the best possible pharmacophore for treating Alzheimer's disease. The inhibitory activity of the compound depends on the lipophilicity positively and is sensitive to small changes in its SMR. The compound with a high lipophilicity (around 9.31) and low SMR gives a potent activity.

1. Introduction

Alzheimer's disease (AD) affects more than 12 million patients worldwide, accounting for most dementia diagnosed after the age of 60. At present there is no effective cure for the disease. The current standard care for mild to moderate AD includes treatment with acetyl choline esterase inhibitors to improve cognitive functions.[1,2] These drugs are effective to some extent but do not tackle the root cause of the disease.

Much of the research done so far points to the accumulation of the sticky peptide β amyloid (βA) as the cause of the disease.[3,4] It is actually the βA42 residue that has a higher affinity to polymerize and accumulate, causing neuronal death.[3,5] The amyloid precursor protein (APP), which is coded on the chromosome 21 is the substrate for the α-, β-, γ-secretases and anomalous processing of the APP leads to increased $A\beta_{42}$ production.[6] Mutations on APP at the cleavage sites of α-, β-, γ-secretases and /or mutations within Aβ sequence of APP are responsible for the abnormal $A\beta_{42}$ accumulations.[6–10]

Drugs so far have targeted the secretases α and β but not γ owing to the enigmatic nature of γ-secretase. γ-secretase generally cleaves the hydrophobic integral membrane domain of its substrates, resulting in the release of protein fragments at the extracellular and cytoplasmic side of the membrane.[11] γ-Secretase also plays an important role in gene transcription regulation and other signaling processes.[11] Now active γ-secretase complex is thought to be composed of Aph-1, Pen-2 and Nicastrin with the presenilins.[11] The presenilins seem to provide the active core of the protease.[12] All the above mentioned proteins are required for the active proteolytic activity of the γ-secretase. Another interesting observation in case of γ-secretase is its action on two markedly different proteins Notch (involved in cell signaling) and APP. The site of cleavage in Notch[13–16] is markedly different in nature as compared to the site of cleavage in APP.[12]

This makes γ-secretase even more amusing. Efforts are in progress to target drugs onto γ-secretase so that its signaling properties are preserved and its proteolytic activity on APP is inhibited.[17] This might require compounds that block γ-secretase through allosteric effects rather than interaction with active site.[18] γ-Secretase is a complex drug target, which challenges classical thinking about proteolytic processing and cell signaling. This is just one of the many efforts being made to investigate a possible pharmacophore for γ-secretase inhibition employing the quantitative structure activity relationship (QSAR) technique.

Keywords: Alzheimer's disease; γ-Secretrase; Benzodiazepine; QSAR; $Q\log P$; SMR.
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2. Experimental

2.1. Computational methods

The Inhibitory activity data of a series of benzodiazepine (BDP) variant molecules for the present study was selected from the work of Ian Churcher et al.[18] The IC_{50} values, which represent the minimum concentration of the inhibitor expressed in ppm required for 50% inhibition of γ-secretase is taken up and the biological activity $\log(1/IC_{50})$ is calculated.

2.2. Molecular 3D structure building

A series of compounds tested for Biological activity were selected for the present study and the program of Window Chem Software Inc.[19] was used for the modeling studies. The molecules were generated and energy was minimized by using Molecular Modeling Pro. The window version software[20] SPSS 10.0 was used in regression analysis.

2.3. Building of QSAR models

QSAR technique was applied to a series of compounds by introducing structural modifications solely at the N position (Fig. 1). Appropriate descriptors or parameters for the compounds, $Q\log P$ and SMR were correlated to the observed inhibitory activity on γ-secretase and were used as the explanatory variables in the multiple regression analysis. The regression models are the QSAR molecular models that were used to predict and design a compound with best possible inhibitory property.

2.4. Chemical descriptors

2.4.1. Lipophilicity parameter ($Q\log P$). The lipophilicity factor P ($Q\log P$) is the most used property where P is defined by 1-octanol/water partition coefficient. All the $Q\log P$ values used were calculated as per Bodor and Buchwald method[21] in ChemSW.

2.4.2. Steric factor (molar refractivity, SMR). This parameter gives a measure of the steric factors and bulkiness of the given base molecule with various substituents. It is the molar volume corrected by the refractive index and represents size and polarizability of a fragment or molecule.

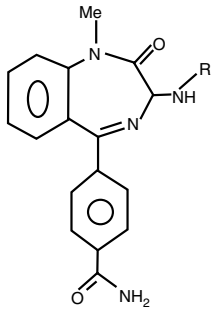


Figure 1. Benzodiazepine.

2.5. Correlation analysis

Relationship between biological activity, expressed as $\log 1/IC_{50}$ and the physicochemical parameters X_i ($Q\log P$ and SMR) were analyzed statistically by fitting the data to correlation equations consisting of various combinations of these parameters.

$$\mathbf{Log(1/IC_{50})} = \Sigma\ \mathbf{a_i X_i}$$

The statistical optimization is used to propose the best correlation model. The constant and the correlation coefficient, a_i for each term were determined by the least squares method.

3. Results and discussions

The activity data log ($1/IC_{50}$) and the physicochemical properties $Q\log P$ and SMR of the BDP variants 1–18 are presented in Table 1.

The data from Table 1 was subjected to regression analysis and the corresponding correlation matrices, predicted-residual values were generated using SPSS 10.0 software and are shown in Table 2, Tables 3 and 4.

From the correlation matrix M1, (Table 2) it is seen that the activity may be a function of $Q\log P$ and $Q\log P^2$. Hence carrying out regression for $Q\log P$ and $Q\log P^2$ the correlation equation is obtained is shown in Table 5.

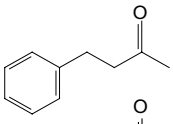
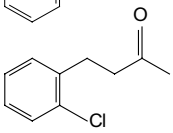
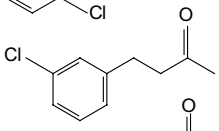
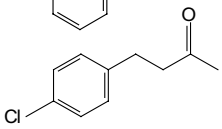
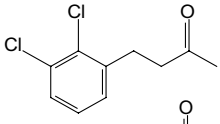
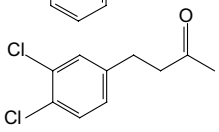
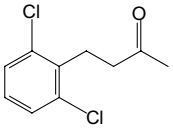
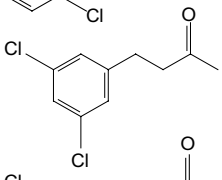
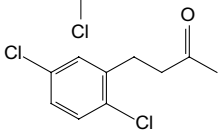
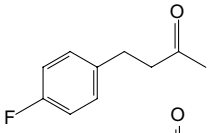
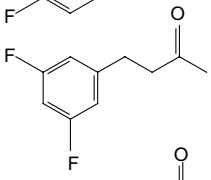
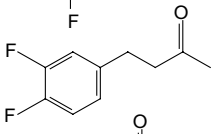
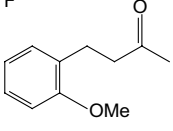
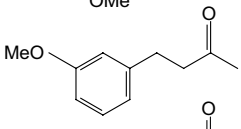
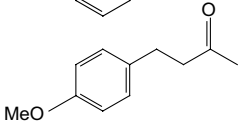
Eq. 1 (Table 5) shows a large error in the coefficients hence outliers were sought and eliminated. In addition, the plot of the observed activity versus the predicted activity was not satisfactory (Fig. 2). After the elimination of the outliers ($BDP_{4,6,7,12,13,18}$) a second model was developed, the correlation matrix M2 for which is presented in Table 3.

Eq. 2 (Table 5) represents a good regression analysis with $R^2 = 0.966$ but the error in the coefficient of $Q\log P$ is large and further a perusal of Table 1 shows that the activity is dependent on SMR too and the magnitude of SMR being large its effect on activity cannot be neglected. Hence, model equation is developed by including SMR^2 term in the correlation Eq. 3 (Table 5).

Since this is a second-degree equation in $Q\log P$ an optimum $Q\log P$ that corresponds to a maximum activity can be evaluated by finding the maxima of the curve (Fig. 3). Eq. 3 was partially differentiated with respect to $Q\log P$ the first derivative was set to zero and a value of $Q\log P_{opt} = 9.31$ was obtained Figure 4.

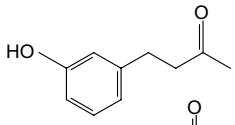
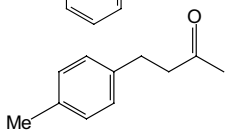
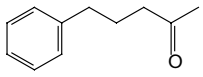
Therefore the inhibitor with $Q\log P$ around 9.31 is expected to give maximum activity. The activity of the compounds is positively dependent on its lipophilicity and this is evident from Table 1 where an increase in $Q\log P$ results in an increase in activity of the inhibitors BDP_{1-6}.

Table 1. Activity[18] and physicochemical properties of the BDP variants

Inhibitor	Structure of R	IC_{50} (ppm)	Log(1/IC_{50})	SMR	$Q\log P$
BDP_1		340	1.4685	126.4330	2.51108
BDP_2		305	1.5157	131.2378	2.95534
BDP_3		93	2.0315	131.2378	2.95234
BDP_4*		15	2.8239	131.2378	2.95266
BDP_5		22	2.6576	136.0426	3.40109
BDP_6*		12	2.9208	136.0426	3.39208
BDP_7*		3200	0.4949	136.0426	3.40002
BDP_8		40	2.3979	136.0426	3.39413
BDP_9		60	2.2218	136.0426	3.39692
BDP_{10}		110	1.9586	126.6494	2.67053
BDP_{11}		110	1.9586	126.8658	2.82970
BDP_{12}*		29	2.5376	126.8658	2.83073
BDP_{13}*		5100	0.2924	132.8962	2.53543
BDP_{14}		1600	0.7959	132.8962	2.53439
BDP_{15}		1340	0.8729	132.8962	2.53356

(*continued on next page*)

Table 1 (*continued*)

Inhibitor	Structure of R	IC_{50} (ppm)	$Log(1/IC_{50})$	SMR	$Q\log P$
BDP_{16}		6800	0.1675	128.1271	2.02964
BDP_{17}		180	1.7447	131.4742	3.00474
BDP_{18}*		5000	0.3010	131.0340	2.99544

* The outliers considered for model Eqs. 2 and 3.

Table 2. Correlation matrix (M1) of the BDP variants*

	Activity	$Q\log P$	SMR	$Q\log P^2$	SMR^2
Activity					
Pearson correlation	1.000	0.559	0.110	0.543	0.115
Sigma 2 tailed	—	0.016	0.663	0.020	0.651
Q log P					
Pearson correlation		1.000	0.689	0.997	0.693
Sigma 2 tailed		—	0.002	0.000	0.001
SMR					
Pearson correlation			1.000	0.715	1.000
Sigma 2 tailed			—	0.001	0.000
$Q log P^2$					
Pearson correlation				1.000	0.719
Sigma 2 tailed				—	0.001
SMR^2					
Pearson correlation					1.000
Sigma 2 tailed					—

* $N = 8$ (inhibitors $BDP_{1–18}$).

Table 3. Correlation matrix (M2) of BDP variants*

	Activity	$Q\log P$	SMR	$Q\log P^2$	SMR^2
Activity					
Pearson coefficient	1.000	0.900	0.382	0.886	0.388
Sigma 2 tailed	—	0.000	0.220	0.000	0.213
Q log P					
Pearson coefficient		1.000	0.703	0.997	0.706
Sigma 2 tailed		—	0.011	0.000	0.010
SMR					
Pearson coefficient			1.000	0.728	1.000
Sigma 2 tailed			—	0.007	0.000
$Q log P^2$					
Pearson coefficient				1.000	0.732
Sigma 2 tailed				—	0.007
SMR^2					
Pearson coefficient					1.000
Sigma 2 tailed					—

* $N = 12$ (compounds $BDP_{1–3}$, BDP_5, $DP_{8–11}$ and $BDP_{13–17}$).

The steric factor SMR is a measure of the bulkiness of the inhibitor. The correlation Eq. 3 shows a negative coefficient for SMR^2 and hence contributes negatively to the activity. Eq. 3 predicts a decrease in activity with increase in SMR. The experimental data in Table 1 shows that for inhibitors BDP_1 and BDP_{13} (which have a more or less constant $Q\log P$) a 5% increase in SMR brings about an 80% decrease in activity. This shows the sensitivity of activity towards SMR. The activity is actually a combination of the positive effect of $Q\log P$ and the negative effect of SMR. This can clearly be seen in the compounds BDP_{10} and BDP_{11} (Table 1) where the SMR and $Q\log P$ are increasing but the activity remains the same showing that the positive effect of the increase in $Q\log P$ has been nullified by the negative effect of SMR.

Table 4. Observed and predicted activity values of BDP variants (model Eq. 3)

Inhibitor	Observed activity	Predicted activity	Residual value
BDP1	1.4685	1.3622	−0.1063
BDP2	1.5157	1.9002	0.3845
BDP3	2.0315	1.8938	−0.1376
BDP5	2.6576	2.3611	−0.2964
BDP8	2.3979	2.3479	−0.0499
BDP9	2.2218	2.3532	0.1314
BDP10	1.9586	1.6886	−0.2699
BDP11	1.9586	2.0060	0.0474
BDP14	0.7959	0.8713	0.0754
BDP15	0.8729	0.8695	−0.0033
BDP16	0.1675	0.1330	−0.0344
BDP17	1.7447	1.9803	0.2356

Further more polar functionality in the substituent was poorly tolerated, that is, they had less activity. The higher the lipophilicity, the greater the expected activity. This is supported by the fact that the halogen substituted BDPs have high activity.[18]

The statistical validity and the predictive ability of the models were assessed for the compounds in the data set using the quality factor (Q) and cross validation approach (q^2_{cv}) given in Table 5.

The quality factor,[22] Q is defined as the ratio of regression coefficient (R) to the standard error estimate (SEE), that is, $Q = R/SEE$. This indicates that the higher the

Table 5. Model equations generated for the benzodiazepine compounds

Eq. no	Equations	n	R^2	SEE	F-ratio	Q
1	$\text{Log}(1/\text{IC}_{50}) = 0.245(0.180)Q\log P_2 - 0.164(0.554)Q\log P$ $\text{PRESS} = 10.3284;\ q^2_{cv} = 0.300$	18	0.833	0.803	39.895	1.137
2	$\text{Log}(1/\text{IC}_{50}) = 0.334(0.095)Q\log P_2 - 0.391(0.283)Q\log P$ $\text{PRESS} = 1.3037;\ q^2_{cv} = 0.776$	12	0.966	0.361	142.553	2.723
3	$\mathbf{Log(1/IC_{50}) = -0.165(0.129)Q\log P^2 + 3.013(0.805)Q\log P}$ $\mathbf{-0.000323(0.000)SMR^2}$ $\mathbf{PRESS = 0.4232;\ q^2_{cv} = 0.927}$	12	0.989	0.216	269.656	4.602

n = no. of data points; SEE = standard error estimate; Q = quality factor; PRESS = predictive sum of squares; q^2_{cv} = cross-validated.

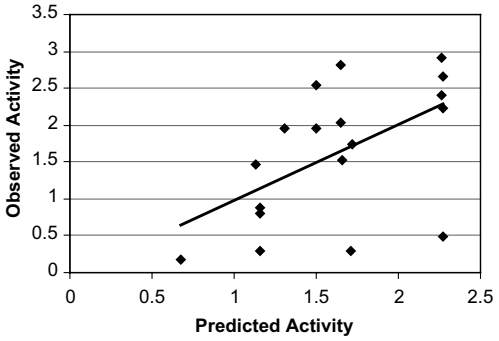


Figure 2. Plot of observed versus predicted activities using model 1.

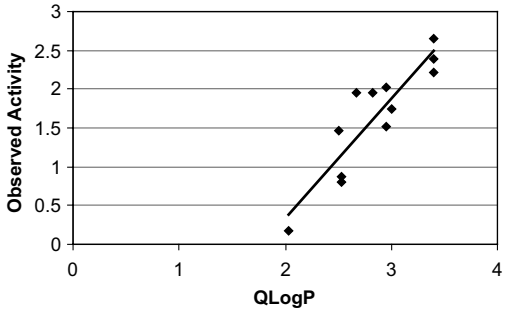


Figure 3. Plot of activity versus $Q\log P$ using model 3.

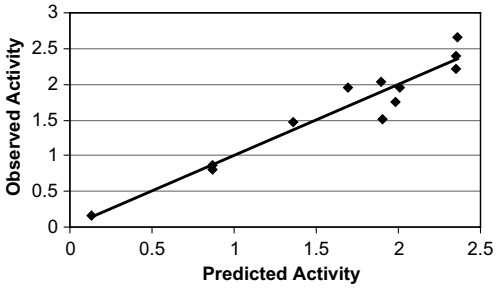


Figure 4. Plot of observed versus predicted activities using model 3.

value of R, and the lower the value of SEE, higher is the magnitude of Q and the better is the correlation.

$$q^2_{CV} = \frac{(\text{SD} - \text{PRESS})}{\text{SD}}$$

Where the PRESS (predictive residual sum of squares) and SD (standard deviation) values are obtained as

$$\text{PRESS} = \Sigma(\text{Property}_{\text{observed}} - \text{Property}_{\text{predicted}})^2$$

$$\text{SD} = \Sigma(\text{Property}_{\text{observed}} - \text{Property}_{\text{mean}})^2$$

Eq. 3 gives a good q^2 value of 0.927. q^2 will always be smaller than R^2. A model is considered significant when $q^2 > 0.3$.

The generated model from the present study projects the best pharmacophore to be the inhibitor BDP with $Q\log P = 9.31$ and a minimum SMR.

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