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Monte Carlo Algorithm in Structure-Activity Relationship of Beta-secretase 1 Inhibitors: Application in Alzheimer's Disease

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ABSTRACT

Computational approaches are widely used almost in all discipline including in drug discovery research. Monte Carlo (MC) algorithm is a randomized algorithm and widely used to solve scientific problems. In the current study, the MC algorithm was used to develop quantitative stricture-activity relationship (QSAR) models from a set of beta-site amyloid precursor protein cleaving enzyme 1 (BACE1) inhibitors to explore important chemical attributes for therapeutic application in Alzheimer's disease (AD). AD is the neuro-degenerative syndrome and more than 24 millon people affected worldwide. Till date there is no curative drug for AD in the market. It's accepted and proved that the BACE1 is mainly responsible for AD development. Therefore, successful inhibition of BACE1 will be an effective treatment of AD. In this regard, crucial chemical attributes were derived from the BACE1 inhibitors through QSAR modeling to design improved chemical agents for the treatment of AD. The entire collected set of BACE1 inhibitors was divided into training, calibration, test and external sets. Two approaches such, with- and without considering the impact of cyclic rings on inhibitory activity were adopted. QSAR models were generated from the training set and remaining set used to validate the developed models. Both models were found to be statistically robust and consist of mechanistic interpretation. Hence, derived chemical attributes in both models can be used to design and discover improved potential BACE1 inhibitors.

Keywords: Alzheimer's disease; Monte Carlo algorithm; BACE1; APP; QSAR.

1. INTRODUCTION

Alzheimer's Disease (AD) is a neurodegenerative disease that regarded as pathologically by hallmarks of a number of cognitive impairments including dementia, language skills and memory loss, severe personal activity and personality changes, problems in judgment making and ultimately ensuring the death of the suffered individual. It is illustrated that suffering from AD develops dementia along with afflicted memory. More than 24 million people are affected by AD and projected that it will double in every 20 years until at least 2050[1,2]. It is evident that human beings can be easily being affected by AD if suffering from hypertension in the mid-age of life[3,4]. It is also reported that people with a history of depression showed up approximately two times increased risk of dementia[5]. A number of research outcomes claimed that depression is directly associated with the risk of dementia and AD[6-8]. Therefore, it can be postulated that the risk factors related to AD are age, depression, genetics, lifestyle factors such as obesity, cardiovascular factors, etiological factors such as inflammation, toxic exposure, traumatic brain injury, and psychological factor such as stress. Although more than years passed the discovery of AD still it is not the absolute clear reason behind the AD development. A number of hypotheses are reported explaining the reason behind the AD accumulation. But still, the exact cause of the AD raised several confusion among the scientific community. Among several hypotheses, three are more accepted pathogenesis of AD[9-11] such as (a) the metal ion hypothesis[12-16] (b) the amyloid cascade hypothesis[9,10,17] and (c) the oxidative stress hypothesis[18-20]. Among all above the amyloid cascade hypothesis is most accepted in the scientific research community. In amyloid cascade hypothesis, the β-amyloid precursor protein (APP) is usually cut by

ISSN:0971-1260

Vol-22-Issue-40-December-2019

alpha-secretase and unevenness between yield and production of Aβ peptide found due to abnormally processed by β- and γ-secretases[21]. The produced insoluble Aβ peptides repeatedly deposited into the oligomers that ultimately dumped in diffuse senile plaques[22]. The APP can be handled into slighter peptides by either the α-secretase or the β-site amyloid precursor protein cleaving enzyme 1 (BACE1). Therefore, the successful inactivation of BACE1 can be one of the crucial options to stop the production of insoluble Aβ and stop the progression of AD. It is a matter of great concern that to date there is no proper treatment option for AD except the management of the symptoms. Therefore, the urgent need should be taken to design and identify potent and safer molecules to treat and stop the AD progression. The BACE1 is a transmembrane aspartic protease. This protein consisting of a total of 501amino acids. BACE1 is corroborated with other protease family proteins included pepsin and retroviral aspartic protease[23-26]. The BACE1 comprises of an NH_2-terminal protease domain, a transmembrane region, and a cytosolic domain[23-27]. BACE1 is the perfect target for the development of a potential drug to treat AD.

The computational drug discovery process is one of the important approaches in drug discovery research. A number of approaches including molecular docking, quantitative structure-activity relationship (QSAR), virtual screening etc. are widely used and applied methods to design and discover the promising molecules against the specific target. The QSAR is the approach in which the statistically mathematical relationship established between chemical functionalities in terms of descriptors with the biological activity of a set of mall molecules. QSAR model can be used to develop the novel drug candidates. In the current research, the QSAR method was applied to a set of BACE1 inhibitors collected from the binding database to explore the important chemical functionalities crucial to design new potentially improved BACE1 inhibitors.

2. MATERIALS AND METHODS

2.1 Dataset

A set of more than a thousand BACE1 inhibitors with respective experimental inhibition constant (K_i) was obtained from the Binding DB (http://www.bindingdb.org/). The downloaded dataset was curated by the removal of duplicate and without activity molecules. Unique molecular dataset obtained from the previous step was further used to check the LoF (Lipinski's rule of five)[28] and the Viber's rule[29] and only considered molecules those passed the above two rules. After successful curation by the above procedure, a total of 373 molecules were found to be appropriate for the QSAR study. To reduce the range and use for the QSAR model development the K_i value of the selected molecules was converted into the pK_i ($\log((1/K_i)x10^7)$). Molecules of the entire dataset were randomly divided into four sets *viz.* training, test, calibration and external sets. Different sets play a specific role in the QSAR model development. The QSAR model is developed with the help of the training set molecule. The test and calibration sets are used to verify the quality of the model during model development. The external set is used for assessing the predictive potential of the developed QSAR model. It is also important to note that molecule belongs to the test and calibration sets are present during model development i.e. visible. The external set finally is used to calculate the final predictive capability of the developed model. Molecules of the external sets are remained invisible during the model development.

2.2 Descriptors of Correlation Weights

The simplified molecular-input line-entry system (SMILES) is an important and information-rich molecular file format. In the current study, a number of molecular descriptors were retrived from the SMILES format of selected BACE1 inhibitors. The "Descriptors of Correlation Weights" (DCW) was derived from the entire set of BACE1 inhibitors. The DCW is the mathematical function of correlation weights (CW). In order to retrieve the DCW the Monte Carlo (MC) algorithm was used. Two approaches such as without and with the influence of cyclic rings on the inhibitory activity were used to calculate the DCW. The following expression (1) was used to calculate the DCW by not considering the impact of cyclic rings on the inhibitory activity.

ISSN:0971-1260

Vol-22-Issue-40-December-2019

$$DCW_1(SMILES,T,N_{epoch}) = \alpha\sum CW(S_k) + \beta\sum CW(SS_k) + \gamma\sum CW(SSS_k) + \mathrm{x}\cdot CW(NOSP) + \mathrm{y}\cdot CW(HALO) + z\cdot CW(BOND) + t\cdot CW(PAIR) \quad (1)$$

The optimal descriptors with influence of cyclic rings on inhibitory activity was calculated using following equation (2).

$$DCW_2(SMILES,T,N_{epoch}) = \alpha\sum CW(S_k) + \beta\sum CW(SS_k) + \gamma\sum CW(SSS_k) + \mathrm{x}\cdot CW(NOSP) + \mathrm{y}\cdot CW(HALO) + z\cdot CW(BOND) + t\cdot CW(PAIR) + CW(\mathrm{C3}) + CW(C4) + CW(C5) + CW(C6) + CW(C7) \quad (2)$$

The T is the threshold which explains the classification of attributes derived from the molecules based on coefficient. Based on the derived molecular attributes the molecular features are explained into two forms i.e. active and rare. It is well explained that attributes under the active category directly associated with the model development but rare molecular attributes do not involve in the model development. The N_{epoch} represents the epoch number in the Monte Carlo optimization which gives optimal statistical values of the calibration set. In equation (1) symbols S_k, SS_k and SSS_k denote the one, two and three symbols molecular attributes derived from the SMILES representation of the molecules. In addition to the above, a number of elemental molecular attributes are also extracted for the model development. The presence or absence of atomic attributes can be represented by the NOSP, HALO, BOND and PAIR. In NOSP describes the presence or absence of atoms included nitrogen, oxygen, sulphur and phosphorus. Halogen atoms can be denoted by the HALO. Different bonds including double (=), triple (#) or stereochemical bonds (@ or @@) can be represented with BOND attribute. The PAIR explains the association of two attributes or atoms. In equation (2) the C3, C4, C5, C6 and C7 are represented by three-, four-, five-, six- and seven-membered cyclic rings. The coefficients, α, β, γ, x, y represent the discrete coefficient and it should be either 1 or 0.

In order to calculate the CW from the molecules, the MC algorithm was used. To select the best epoch number and corresponding threshold value all datasets were used to generate the statistical parameters for epoch number 1 to 30 and threshold for each epoch as 1 to 10. On details analyses of statistical parameters, the best (N*, T*) were selected for final model development. End point can be calculated with the help of correlation weight as given below.

$$\text{Endpoint} = C_0 + C_1 \times \text{DCW}(\text{SMILES}, N_{epoch,} T) \quad (3)$$

The endpoint represents the biological activity and, C_0 and C_1 are constant. A number of statistical parameters from training, test, calibration and external sets were retrieved to adjudge the quality of the model. These parameters included, correlation coefficient (R^2), cross-validated correlation coefficient (Q^2), the error of estimation (s), modified correlation coefficient (R_m^2), degree of freedom (F) and coefficient of determination ($^cR^2_p$). Details about all the above parameters can be found in our previous publication[30].

3. RESULTS AND DISCUSSION

A diverse set of BACE1 inhibitors consisting of 373 molecules was downloaded from the online Binding database to develop robust QSAR models using the CORAL software (http://www.insilico.eu/coral/). The well-known MC algorithm was used to identify the molecular attributes from the BACE1 inhibitors and subsequent the QSAR model developed. The QSAR models were developed using the optimal number of epoch and thresholds. Each model was validated and interpreted based on the statistical parameter. The molecular attributes were used to explain the mechanistic interpretation of each model. On details analyses of statistical parameters from the training set finally set of epoch number and threshold was selected. In case of without considering the influence of cyclic rings on the inhibitory activity, the optimal set of epoch number and threshold was found to be (5, 7). On the other hand, the optimal set of epoch number and threshold was found to be (9, 9) when the impact of cyclic rings on inhibitory activity was considered.

ISSN:0971-1260

Vol-22-Issue-40-December-2019

3.1 Without considering the influence of cyclic rings

In this approach, the impact of the cyclic rings was not considered on inhibitory activity and the DCW calculated. The selected set of epoch optimal number and threshold was used to develop the model and given below (Model 1).

$$pKi = 0.726(\pm 0.008) + 0.024(\pm 0.001) \times DCW(7,5)$$

(**Model 1**)

Training set: n = 192; R^2 = 0.691; s = 0.621; F = 425; Q^2 = 0.686; R_m^2 = 0.672; ${}^{C}R^2_p$ = 0.689; $R^2 - Q^2$ = 0.005

Calibration set: n = 62; R^2 = 0.703; s = 0.781; F = 142; Q^2 = 0.688; R_m^2 =0.631; ${}^{C}R^2_p$ = 0.696; $R^2 - Q^2$ = 0.093

Test set: n = 64; R^2 = 0.592; s = 0.791, F = 91; Q^2 = 0.565; R_m^2 =0.589; ${}^{C}R^2_p$ = 0.577; $R^2 - Q^2$ = 0.027

External set: n = 54; R^2 =0.532; s = 0.775; F = 221; Q^2 = 0.528; R_m^2 = 0.511, $R^2 - Q^2$ = 0.004

A number of statistical parameters were calculated for training, calibration and test sets using the Model 1 and given above. The correlation between experimental and calculated inhibitory activity were used to calculate the R^2 value. The R^2 of the training, calibration, test and external sets was found to be 0.691, 0.703, 0.592 and 0.532 respectively. The cross-validation correlation coefficient (Q^2) was also found to be more than 0.5 for all sets. One of the validation parameters of known as modified correlation (R_m^2) was obtained as 0.672, 0.631, 0.589 and 0.511 for the training, calibration, test and external sets respectively.

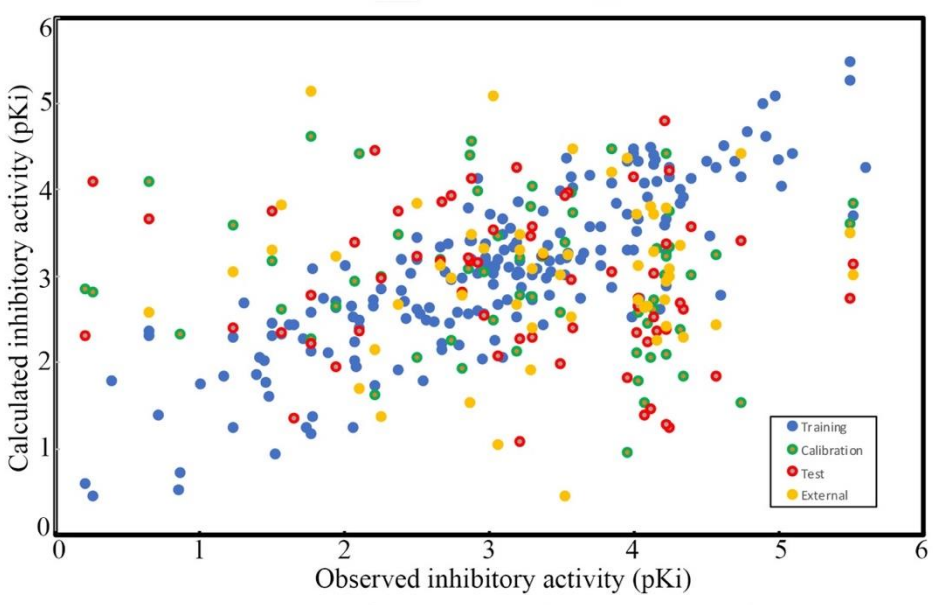

Figure-1: Experimental and predicted inhibitory activity calculated through Model 1

The coefficient of determination (${}^{c}R^2_p$) can explain the predictive capability of the model. ${}^{c}R^2_p$ was found to be 0.6.89, 0.696 and 0.577 for the training, calibration and external sets respectively. It can be noted that all of the statistical parameters showed high correlation coefficient between the experimental and calculated inhibitory activity. Therefore, from above discussion it is clear that the Model 1 statistically robust and well predictive in nature. The inhibitory activity all sets was calculated through Model 1 and given in Figure 1.

3.2 With considering the influence of cyclic rings

ISSN:0971-1260

Vol-22-Issue-40-December-2019

The DCW was calculated by considering the influence of cyclic rings on inhibitory activity and subsequently QSAR model developed (Model 2). The best model was developed with an epoch number and threshold of 9 and 9 respectively. The statistical parameters were also calculated using the developed Model 2 and given in followed by the model expression.

$$pKi = 0.657(\pm 0.008) + 0.034(\pm 0.001) \times DCW(9,9)$$

(**Model 2**)

Training set: n = 192; R^2 = 0.720; s = 0.591; F = 488; Q^2 = 0.714; R_m^2 = 0.652; ${}^{C}R^2_p$ = 0.717; $R^2 - Q^2$ = 0.006

Calibration set: n = 62; R^2 = 0.748; s = 0.718; F = 178; Q^2 = 0.736; R_m^2 =0.611; ${}^{C}R^2_p$ = 0.743; $R^2 - Q^2$ = 0.012

Test set: n = 65; R^2 = 0.556; s = 0.824, F = 79; Q^2 = 0.529; R_m^2 =0.552; ${}^{C}R^2_p$ = 0.547; $R^2 - Q^2$ = 0.027

External set: n = 54; R^2 =0.558; s = 0.713; F = 258; Q^2 = 0.541; R_m^2 = 0.524, $R^2 - Q^2$ = 0.017

On close inspection of the above statistical parameters it can be seen that R^2 and Q^2 of all training, calibration, test and external sets shown to have more than 0.500. Another two correlation coefficient value between experimental and calculated K_i such as modified correlation coefficient and coefficient of determination were also found to be high (> 0.5). It is reported that close prediction of R^2 and Q^2 is one of the important validation methods for the robustness of the model. Difference between R^2 and Q^2 (R^2 - Q^2) was found to be 0.006, 0.012, 0.027 and 0.017 for the training, calibration, test and external tests respectively. The above data and discussion undoubtedly explained that Model 2 showed predictiveness and robustness. The inhibitory activity of all four sets was calculated and given in Figure 2. From Figure 2 it can be seen that calculated K_i close to the experimental K_i that clearly portrayed good predictiveness of the Model 2.

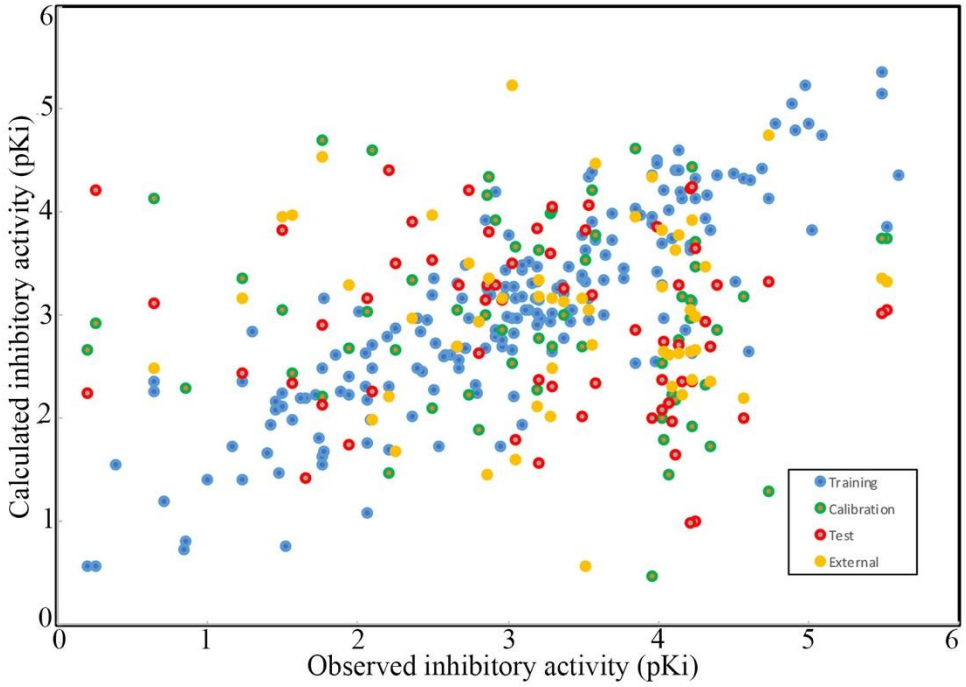

Figure-2: Experimental and predicted inhibitory activity calculated through Model 2

3.3 Mechanistic interpretation

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Vol-22-Issue-40-December-2019

Followed by the model development and exploration of the statistical robustness it is crucial to check the mechanistic interpretation in regards to the CW of SMILES attributes (SA_k) of BACE1. The importance of any SA_k can be checked by the increase or decrease respective CW. It can be assessed four ways; i) If CW(SA_k) is positive then pK_i of BACE1 will increase; ii) If CW(SA_k) < 0 then pK_i of BACE1 will decrease; iii) If CW(SA_k) is not clear that possesses both positive and negative value then molecular features play unclear role; and, iv) if the CW(SA_k) has '0' value then structural attribute rare or blocked. Keep in mind the above assumptions, important molecular attributes and respective CW value were obtained from both Models 1and 2 and given Table 1.

In the case of Model 1, the molecular attribute 'NOSP11000000' which explains the presence of nitrogen and oxygen was found to give a positive impact on BACE1 inhibition. Moreover, presence of combined double and stereochemical bonds ('BOND10100000'), combined effect of nitrogen and oxygen ('++++N---O==='), presence of two stereochemical bonds ('@@.....'), etc. were crucial to impart the inhibitory activity of BACE1 inhibitors. On the other hand, absence of halogen atoms ('HALO00000000'), combination of oxygen with double bond ('++++O---B2=='), combined nitrogen and double bond ('++++N---B2=='), etc. were found to have negative influence to enhance the K_i of BACE1 inhibitors.

Table-1: Important SMILES attributes and corresponding correlation weight (CW) as per Model 1 and 2

Model 1		**Model 2**	
Attribute	CW	Attribute	CW
NOSP11000000	0.4966	NOSP11000000	10.9987
HALO00000000	-0.5009	HALO00000000	-2.4981
BOND10100000	1.4966	BOND10100000	4.0031
++++N---O===	5.0045	++++N---O===	-2.4974
++++O---B2==	-2.9962	++++O---B2==	-4.4962
++++N---B2==	-2.0032	++++N---B2==	5.2481
@@.....	2.5023	C.....	-0.2488
O...=.....	-1.2460	O...=.....	3.5043
C...=.....	-1.2500	C...=.....	-1.9958
C...1.....	-6.0019	C...1.....	-5.9962
O...1.....	-8.9968	O...1.....	0.0
O...C.....	-2.5024	N...(.....	3.5035
C...(.....	0.7454	N...(.....	3.5035
[...(.....	-0.0015	[...(.....	1.0040
[...H.....	1.0000	O...(.....	1.5013
[...H.....	1.0000	N...(.....	3.5035
O...=.....	-1.2460	N...(.....	3.5035
O...=...C...	9.9985	O...=...C...	6.0007
[...(...C...	0.7504	=...C...1...	0.2519
[...2...=...	8.5000	O...1...C...	0.0

A number of important molecular attributes were analysed to check the mechanistic interpretation of Model 2 and given in table 1. In Table 2, it can be seen that presence of nitrogen with oxygen ('NOSP11000000'), double bond with stereochemical bond ('BOND10100000'), nitrogen with double bond ('++++N---B2=='), etc., were found to be crucial to increase the inhibitory activity of BACE1 inhibitors. A number of chemical attributes derived in Model 2 were found to negative influence on K_i of BACE1 including absence of halogens ('HALO00000000'), nitrogen with oxygen ('++++N---O==='), oxygen with double bond ('++++O---B2=='), etc. Above findings of important chemical attributes responsible to increase or decrease the CW undoubtedly explained that both Models 1 and 2 showed mechanistic interpretation.

ISSN:0971-1260

Vol-22-Issue-40-December-2019

4. CONCLUSION

The Monte Carlo algorithm was used to develop the statistically robust QSAR models from a set of BACE1 inhibitors. The entire dataset was divided into training, calibration, test and external sets. QSAR model was developed from the training set and the remaining sets were used to validate the model. Two approaches, with- and without the influence of cyclic rings on inhibitory activity were considered to develop the model. Statistical analyses undoubtedly explained that both models were statistically robust in nature. Further, in comparison of statistical data was clearly shown that Model 2 was developed with better statistical parameters which clearly suggested that presence of cyclic rings in BACE1 inhibitors crucial for the inhibitory activity. The chemical attributes were checked and found that both models explain mechanistic interpretation. Hence, it can be postulated that important molecular fragmanets can play crucial protagonist to design new promising BACE1 inhibitors for the therapeutic applications in Alzheimer's disease.

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