Turk J Pharm Sci 2021;18(2):151-156
DOI: 10.4274/tjps.galenos.2020.45556

ORIGINAL ARTICLE

QSAR Studies on Neuraminidase Inhibitors as Anti-influenza Agents

Anti-influenza Ajanları Olarak Nöraminidaz İnhibitörlerinin QSAR Çalışmaları

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ABSTRACT

Objectives: The present study aimed to establish significant and validated quantitative structure-activity relationship (QSAR) models for neuraminidase inhibitors and correlate their physicochemical, steric, and electrostatic properties with their anti-influenza activity.

Materials and Methods: We have developed and validated 2D and 3D QSAR models by using multiple linear regression, partial least square regression, and k-nearest neighbor-molecular field analysis methods.

Results: 2D QSAR models had q^2: 0.950 and pred_r^2: 0.877 and 3D QSAR models had q^2: 0.899 and pred_r^2: 0.957. These results showed that the models werere predictive.

Conclusion: Parameters such as hydrogen count and hydrophilicity were involved in 2D QSAR models. The 3D QSAR study revealed that steric and hydrophobic descriptors were negatively contributed to neuraminidase inhibitory activity. The results of this study could be used as platform for design of better anti-influenza drugs.

Key words: QSAR, neuraminidase inhibitors, thiazolidine-4-carboxylic acid derivatives, anti-influenza activity

ÖZ

Amaç: Bu çalışma nöraminidaz inhibitörlerinin belirgin ve valide nicel yapı-aktivite ilişkisi (QSAR) modellerini kurmayı ve bu bileşiklerin fizikokimyasal, sterik ve elektrostatik özelliklerini anti-influenza aktiviteleriyle korele etmeyi amaçlamıştır.

Gereç ve Yöntemler: Çoklu regresyon, parsiyel en düşük kare regresyon ve k-en yakın komşu moleküler alan analizi yöntemlerini kullanarak 2D ve 3D QSAR modellerini geliştirdik ve valide ettik.

Bulgular: Geliştirilen 2D QSAR modeli için q^2: 0,950 ve pred_r^2: 0,877 bulunurken, 3D QSAR modeli için q^2: 0,899 ve pred_r^2: 0,957 bulundu. Bu sonuçlar modellerinin tahmin gücünün olduğunu gösterdi.

Sonuç: Hidrojen sayısı ve hisrofilisite gibi parametreler 2D QSAR modellerine dahil edildi. 3D QSAR modelleri sterik ve hisrofobik tanımlayıcıların nöraminidaz inhibitör aktivitesine negatif etki ettiği belirlendi. Bu çalışmanın sonuçları influenzaya karşı ilaç tasarlamak için bir platform olarak kullanılabilir.

Anahtar kelimeler: QSAR, nöraminidaz inhibitörleri, tiyazolidin-4-karboksilik asit deriveleri, anti-influenza aktivitesi

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Received: 07.06.2019, **Accepted:** 12.03.2020

INTRODUCTION

Quantitative structure-activity relationship (QSAR) is a technique of indirect drug designing. It is a method of quantification of the relationship of structure with biological activities of a set of molecules having common parent structure and useful in lead optimization. Magnitudes of particular physical properties are considered in classical QSAR. Steric, electrostatic, and hydrophobic properties are covered in 3D QSAR. The objective of the current study was to utilize the reported biological data of a series of anti-influenza compounds to develop predictive QSAR models and to explore the relationship between the ligand properties and biological activity.

Influenza virus is the causative agent for the contagious respiratory infectious disease influenza. Influenza A, B, and C are the three types of flu virus. The worst influenza pandemic occurred in 1918, and it caused 40-100 million deaths worldwide.[1] Recently new subtypes such as H7N7 and H7N2, H9N2, and H7N9 have also been identified to cause human infection.[2-4] The four major classes of anti-influenza drugs available now are inhibitors of hemagglutinin, M2 ion channel blockers, inhibitors of viral RNA polymerase, and inhibitors of neuraminidase.[5]

The number of QSAR studies[6-12] has been reported for various classes of influenza inhibitors. In the current study, we had selected thiazolidine-4-carboxylic acid derivatives to provide structural insight responsible for selectivity of these derivatives toward influenza by QSAR analysis. Due to their high structural diversity and broad-type biological activity, these compounds were selected for the present study. The developed 2D and 3D QSAR models could be used to design new anti-influenza compounds.

MATERIALS AND METHODS

CS Chem Office 2004 (Cambridge Soft Corp., Cambridge, USA) and Vlife MDS 4.3 (VLife Sci. Tech. Lim, Pune, India) are the modeling software used in the present study. The neuraminidase inhibition activity (pIC_{50}: -log10 IC_{50}) of 28 thiazolidine-4-carboxylic acid derivatives (Table 1) was taken from the research reported by Liu et al.[13]

Energy minimization

Using CS Chem Office, the structure of thiazolidines was sketched by, and the 3D structure optimization was done in Vlife MDS by following the method reported by Veerasamy et al.[14] Merck molecular force field energy minimized stable structure of individual compound was stored as Sybyl.mol2 files and used to compute various 2D independent descriptors.

2D QSAR analyses

An auto-scaling method was used to reduce the number of descriptors to 200.[14] The data set was split into training set and prediction set by adopting the sphere exclusion (dissimilarity values 2 and 2.5) and random selection methods (10 trials, 70%, 75%, 80%, and 85%).[15] 2D QSAR equations using multiple linear regression (MLR) and partial least squares regression (PLS) methods were built as per the method reported by Veerasamy et al.[14]

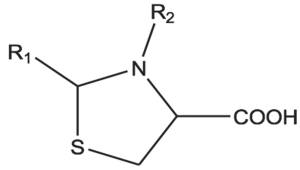
Model quality and validation

The methods compiled by Veerasamy et al.[14] were used to check the model quality and validation of the QSAR models.[16]

3D QSAR analyses

Compound 23 was used as a scaffold to align the molecules using template alignment method. The method reported by

Table 1. The structures of neuraminidase inhibitor derivatives with their activities



Compound no	R_1	R_2	pIC_{50}
1	C_6H_5-	H	4.672
2	(2-OH) C_4H_4-	H	4.695
3	(2-COOH) C_4H_4	H	4.742
4	(4-CN) C_4H_4	H	4.631
5	(2-NO_2) C_4H_4-	H	4.648
6	(2-OH, 3-CH_3O) C_4H_3-	H	4.91
7	C4H3O-	H	4.366
8	C_6H_5-	H	5.123
9	(2-OH) C_4H_4-	$ClCH_2CO$-	5.234
10	(2-COOH) C_4H_4	$ClCH_2CO$-	4.971
11	(4-CN) C_4H_4	$ClCH_2CO$-	5.063
12	(2-NO_2) C_4H_4-	$ClCH_2CO$-	5.116
13	(2-OH, 3-CH_3O) C_4H_3-	$ClCH_2CO$-	5.101
14	C_4H_3O-	$ClCH_2CO$-	4.889
15	C_6H_5-	$PhCH_2CO$-	5.917
16	(2-OH) C_4H_4-	$PhCH_2CO$-	6.187
17	(2-COOH) C_4H_4	$PhCH_2CO$-	5.717
18	(4-CN) C_4H_4	$PhCH_2CO$-	5.607
19	(2-NO_2) C_4H_4-	$PhCH_2CO$-	5.728
20	(2-OH, 3-CH_3O) C_4H_3-	$PhCH_2CO$-	5.790
21	C_4H_3O-	$PhCH_2CO$-	5.539
22	C_6H_5-	NH_2CH_2CO-	6.276
23	(2-OH) C_4H_4-	NH_2CH_2CO-	6.678
24	(2-COOH) C_4H_4	NH_2CH_2CO-	6.553
25	(4-CN) C_4H_4	NH_2CH_2CO-	6.092
26	(2-NO_2) C_4H_4-	NH_2CH_2CO-	5.991
27	(2-OH, 3-CH_3O) C_4H_3-	NH_2CH_2CO-	6.854
28	C_4H_3O-	NH_2CH_2CO-	6.009

Veerasamy et al.[14] was used to generate rectangular grid around the aligned molecules. The selected field descriptors were electrostatic, steric, and hydrophobic. For the electrostatic and steric field, 10.0 and 30.0 kcal/mole were used as the cutoff values.[14]

"A methyl probe of charge +1 at the lattice points of the grid was used to compute steric, electrostatic and hydrophobic interaction energies".[14] The k-nearest neighbor (kNN)-molecular field analysis and PLS methods with each one of the following variable selection methods (stepwise forward-backward) or (genetic algorithm) or simulated annealing) were used to generate 3D QSAR models. The variable selection methods were discussed somewhere else.[14] The methods compiled by Veerasamy et al.[14] were used to validate the 3D QSAR models.[16]

RESULTS AND DISCUSSION

2D QSAR

A data set of 28 thiazolidines and their influenza neuraminidase inhibitory (pIC_{50}) activity in Table 1 was utilized in the present *in silico* study. Two of the best and significant models obtained by using various feature selection and development methods were equations (1) and (2). The used criteria were 80% random selection, stepwise forward-backward variable selection, and MLR.

pIC_{50}: 2.704+0.157 (±0.004) hydrogen count - 6.833 (±0.477) SK average

Hydrophilicity + 0.314 (±0.047) SsssNE-index - 0.279 (±0.050) SsCH3E-index equation (1)

Test set compounds: 1, 6, 16, 21, 26, 27

n=22, r^2: 0.968, r^2 se: 0.132, q^2: 0.950, q^2 se: 0.165, $F_{4,17}$: 128.955, pred r^2: 0.877, pred_r^2 se: 0.308, Z score r^2: 6.941, Z score Q^2: 4.150, best rand R^2: 0.518, best rand Q^2: 0.158

pIC_{50}: 3.139+1.0825 T_N_N_3 - 0.1862 SsCH3E-index + 0.1566 hydrogens count equation (2)

Test set compounds: 3, 11, 16, 19, 22, 27

n=22, r^2 = 0.945, r^2 se: 0.161 q^2: 0.923, q^2 se: 0.190, $F_{3,18}$: 164.005, pred r^2: 0.908, pred_r^2 se: 0.281, Z score r^2: 8.915, Z score Q^2: 7.203, best rand R^2: 0.526, best rand Q^2: 0.340

Equation (1) could explain 96.8% and predict 87.7%, and equation (2) could explain 94.5% and predict 90.8% of the variance of the influenza virus neuraminidase inhibitory data. Thus, the selected good model was equation (1). The absence of intercorrelation between the descriptors was also observed. The parameters (hydrogen count, SK average hydrophilicity, SsssNE-index, SsCH3E-index) were involved, and the calculated influenza virus neuraminidase inhibitory activity by equation (1) is given in Table 2. The correlation between descriptors in 2D QSAR model equation (1) is given in Table 3. The correlation of experimental and predicted activities is graphically represented in Figure 1.

The good internal prediction of selected model was exhibited by q^2: 0.950, and the external prediction power was also confirmed by pred r^2: 0.877 (pred r^2 >0.6). The low randomized r^2 (0.118) and q^2 (0.158) values confirmed the robustness of the model, and the results were not due to a chance correlation.

The positive contribution of descriptor hydrogen count in the selected model clearly suggests that influenza virus neuraminidase inhibitory activity could be increased with an increase in the number of hydrogen atoms in a compound. The SK average hydrophilicity is influencing the activity variation and is indirectly proportional to the activity. The SK average hydrophilicity reveals the importance of average hydrophilic value on the Van der Waals surface. The SsssNE-index has positive effect on the activity. It is an electrotopological state index descriptor. The SsssNE-index highlights the significance of the number of nitrogen atoms connected with three single bonds in a molecule. The SsCH3E-index has a positive effect on the activity, and it shows the importance of the number of -CH_3 groups connected with one single bond in a molecule.

3D QSAR

The criteria used were 80% random training and test selection method, stepwise forward-backward variable selection, and kNN method.

pIC_{50}: S_775 (-0.229-0.163); H_582 (-0.056-0.011) equation (3)

Test set compounds: 1, 3, 10, 11, 26

k-nearest neighbour: 4

n=22, q^2: 0.896, q^2 se: 0.232, pred r^2: 0.931, pred_r^2 se: 0.182

pIC_{50}: H_682 (-0.060-0.016); S_775 (-0.229-0.128) equation (4)

Test set compounds: 1, 3, 10, 11, 26

k-nearest neighbour: 4

n=23, q^2: 0.899, q^2 se: 0.224, pred r^2: 0.957, pred_r^2 se: 0.153

Equation (3) could predict 93.1%, and equation (4) could predict 95.7% of the variance of the influenza virus neuraminidase inhibitory data. Thus, the selected good model was equation (4). The parameters involved in the selected model (steric and

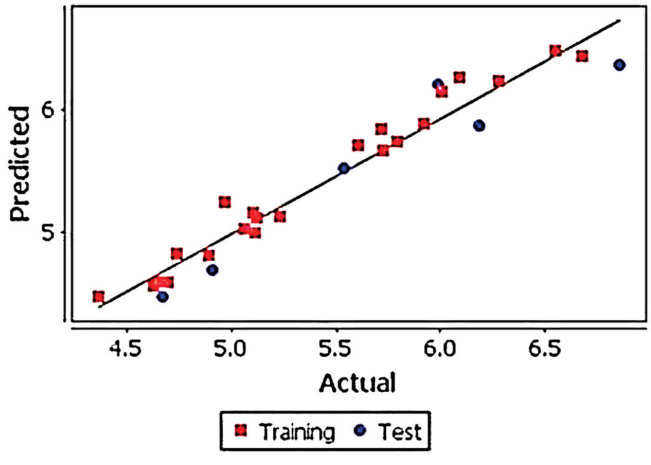


Figure 1. Fitness plot between the experimental and predicted activities for 2D QSAR model equation (1)

QSAR: Quantitative structure-activity relationship

Table 2. Descriptors and predicted activity of 2D QSAR model equation (1)

Compound no	Hydrogen count	SK average hydrophilicity	SsCH3E-index	SsssNE-index	Actual activity (pIC_{50})	Predicted activity (pIC_{50})
1a	11	-0.007	0	0	4.672	4.477
2	11	-0.025	0	0	4.695	4.598
3	11	-0.059	0	0	4.742	4.833
4	10	-0.044	0	0	4.631	4.573
5	10	-0.048	0	0	4.648	4.602
6a	13	-0.057	1.573	0	4.91	4.694
7	9	-0.053	0	0	4.366	4.475
8	12	0	0	1.675	5.123	5.112
9	12	-0.008	0	1.587	5.234	5.137
10	12	-0.027	0	1.51	4.971	5.248
11	11	-0.015	0	1.605	5.063	5.033
12	11	-0.015	0	1.469	5.116	4.993
13	14	-0.029	1.539	1.552	5.101	5.156
14	10	-0.01	0	1.492	4.889	4.806
15	17	0	0	1.655	5.917	5.889
16a	17	0	0	1.567	6.187	5.862
17	17	0	0	1.49	5.717	5.837
18	16	0	0	1.585	5.607	5.711
19	16	0	0	1.449	5.728	5.668
20	19	0	1.536	1.532	5.79	5.735
21a	15	0	0	1.472	5.539	5.518
22	14	-0.125	0	1.509	6.276	6.228
23	14	-0.159	0	1.421	6.678	6.43
24	14	-0.169	0	1.344	6.553	6.477
25	13	-0.156	0	1.44	6.092	6.263
26a	13	-0.153	0	1.303	5.991	6.198
27a	16	-0.167	1.513	1.386	6.854	6.364
28	12	-0.167	0	1.326	6.009	6.140

a: Indicates test set compounds, QSAR: Quantitative structure-activity relationship

Table 3. Correlation matrix for descriptors in 2D QSAR model equation (1)

	pIC_{50}	Hydrogen count	SK average hydrophilicity	SsssNE-index	SsCH3E-index
pIC_{50}	1	-	-	-	-
Hydrogen count	0.685	1	-	-	-
SK average hydrophilicity	-0.584	0.066	1	-	-
SsssNE-index	0.623	0.567	-0.004	1	-
SsCH3E-index	0.111	0.360	-0.061	0.006	1

QSAR: Quantitative structure-activity relationship, SK: S- SlogP, K- Kellog

hydrophobic) and the calculated influenza virus neuraminidase inhibitory activity by equation (4) are given in Table 4 and correlation in Table 5. Figure 2 shows the contribution plot for steric and hydrophobic interactions in lattice. The good internal prediction of the model was confirmed by q^2: 0.899. The external prediction power of the model was confirmed by pred r^2: 0.957 (pred r^2 >0.6).

Hydrophobic descriptors like H_682 with a negative range around the chemical structure of neuraminidase inhibitor indicate that more hydrophobicity is not favorable on those sites for the influenza virus neuraminidase inhibitory activity of the compounds. Steric descriptor like S_775 with a negative range around the chemical structure of neuraminidase inhibitor indicates that the bulky groups are not favorable on those sites for the influenza virus neuraminidase inhibitory activity. Figure 3 shows the plots of predicted vs. observed values of pIC_{50}.

Table 4. Descriptors and predicted activity of 3D QSAR model equation (4)

Compound no	S_775	H_682	Actual activity (pIC_{50})	Predicted activity (pIC_{50})
1a	-0.049	0.075	4.672	4.585
2	-0.045	0.039	4.695	4.638
3	-0.035	0.015	4.742	4.723
4	-0.05	0.033	4.631	4.653
5a	-0.036	0.026	4.648	4.650
6	-0.042	0.015	4.91	4.585
7	-0.052	0.034	4.366	4.720
8	-0.14	0.107	5.123	5.364
9a	-0.131	0.078	5.234	5.057
10	-0.129	0.047	4.971	5.089
11a	-0.134	0.064	5.063	5.144
12a	-0.127	0.058	5.116	5.087
13	-0.136	0.049	5.101	5.092
14	-0.102	0.088	4.889	5.144
15	-0.124	0.176	5.917	5.762
16	-0.076	0.169	6.187	5.758
17	-0.103	0.14	5.717	5.874
18	-0.127	0.14	5.607	5.621
19	-0.171	0.141	5.728	5.589
20	-0.067	0.126	5.79	5.602
21	-0.152	0.211	5.539	5.744
22	-0.164	-0.016	6.276	6.331
23	-0.229	-0.029	6.678	6.429
24	-0.163	-0.06	6.553	6.261
25	-0.122	-0.034	6.092	5.993
26a	-0.171	-0.05	5.991	6.246
27	-0.26	-0.062	6.854	6.386
28	-0.128	-0.046	6.009	6.018

a: Indicates test set compounds, QSAR: Quantitative structure-activity relationship

CONCLUSION

Significant and predictive QSAR models were developed for thiazolidine neuraminidase inhibitor. 2D QSAR model evidenced the influence of structural properties and neuraminidase inhibitory activity of thiazolidines. The engendered 3D QSAR contour maps evidenced the influence of ligand features on the enzyme neuraminidase. It is concluded that modifications in the

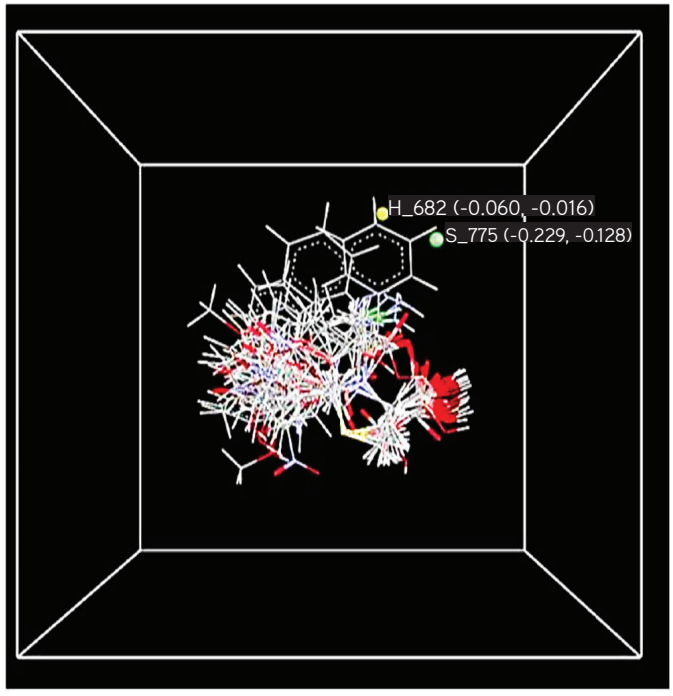


Figure 2. Contribution plot for steric and hydrophobic interactions in lattice for 3D QSAR model equation (4)

QSAR: Quantitative structure-activity relationship

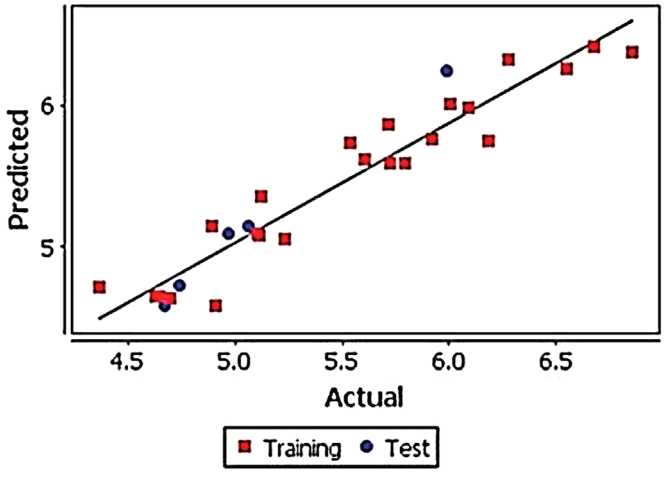


Figure 3. Fitness plot between the experimental and predicted activities for 3D QSAR model equation (4)

QSAR: Quantitative structure-activity relationship

Table 5. Correlation matrix for descriptors in 3D QSAR model equation (4)

	pIC_{50}	H_682	S_775
pIC_{50}	1	-	-
H_682	-0.219	1	-
S_775	-0.756	0.232	1

QSAR: Quantitative structure-activity relationship, pIC_{50}: Negative logarithmic concentration of 50% inhibition, H_682: Hydrophobic descriptor at point 682, S_775: Steric descriptor at point 775

structure of thiazolidines based on the information obtained from the present study could lead to new thiazolidines with potent neuraminidase inhibitory activity. Further in silico tests, such as molecular docking, and kinetic and dynamic studies can be carried out for a better understanding of the mechanism of action. The field is also further open for designing, synthesis, and biological evaluation of potent anti-influenza virus compounds, pharmacokinetic studies, and clinical studies to establish those molecules as drug.

ACKNOWLEDGMENTS

Authors are thankful to VLife Sciences, Pune, India for the software.

Conflicts of interest: No conflict of interest was declared by the authors. The authors alone are responsible for the content and writing of the paper.

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