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General Unrestricted Structure Activity Relationships based evaluation of quinoxaline derivatives as potential influenza NS1A protein inhibitors

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ABSTRACT

In present work, we have performed GUSAR analysis of quinoxaline derivatives, previously reported as potential influenza NS1A protein inhibitors. A robust, statistically sound and thoroughly validated consensus model is obtained. The four parametric model has following statistical characteristics R^2 = 0.746, F = 12.897, SD = 0.283, Q^2 = 0.645. GUSAR analysis provides idea regarding contribution of each atom in deciding binding with protein. The analysis could be very useful in designing better influenza NS1A protein inhibitors.

Keywords: GUSAR, Quinoxaline derivatives, anti-influenza, Drug Designing.

INTRODUCTION

Influenza, a respiratory disease, caused by virus killed thousands of peoples in 2009. The influenza virus is of three types: influenza A, influenza B, and influenza C [1,2]. Among the three types, type A is very dangerous and one of its subtype H1N1 caused the 2009 flu pandemic. There are some commercial drugs which can be used effectively against the virus, but with rise of resistance against the marketed drugs there is urgent need either to modify the existing drugs or to develop a new drug to combat influenza. The very successful idea in modern drug designing is to target a highly conserved protein to develop a new drug. Such target specific drugs usually have high biologic activity and low toxicity. One such highly conserved influenza

virus encoded protein is NS1 which play crucial role in replication of virus [3]. Modern drug designing techniques such as QSAR, molecular docking etc have been used successfully in developing new drugs [4-6].

In modern QSAR studies, different modeling techniques for example multi linear regression (MLR), partial least squares (PLS), artificial neural networks (ANN) and support vector machine (SVM) are widely practiced [7-10]. The ANN and SVM are able to generate nonlinear relationships between descriptors and biological activity and many times create better QSAR models compared to models derived by the conventional approaches MLR and PLS. However, ANN and SVM also have some serious drawbacks like their ‘‘concealed’’ nature, large computational requirements, susceptible to over-fitting and the empirical nature of model development. In SVM and ANN, the model is implicit and does not give clear knowledge representation in the form of rules, or some other easily interpretable form. In addition, there are increasing evidences that variable selection is also vital for thriving SVM analysis and the inappropriate variable selection can also spoil the SVM performance [11-13].

Herein we report the use of General Unrestricted Structure Activity Relationships (GUSAR) [14-16], a relatively novel approach in QSAR, as a modeling technique. Some of the advantages associated with GUSAR are: (1) Self-Consistent Regression (SCR) method is used which provides the selection of the optimal number and set of descriptors for creation of a reliable QSAR model. (2) Utilizes Quantitative Neighbourhoods of Atoms (QNA) descriptors which are better than conventional descriptors to reveal the nature of intermolecular interactions. (3) It predicts the quantitative values of biological activity of chemical compounds on the basis of their “structural formulae” only and there is no need to have information about the 3D structure of ligands and/or target proteins. (4) GUSAR gives output, which is in the form a diagram, revealing the atoms suitably colored according to their specific role in deciding the activity (5) during model building, GUSAR performs cross validation and Y-randomization and checks the various statistical characteristics to build consensus model. It has been verified that the GUSAR is a useful tool for QSAR modeling.

MATERIALS AND METHODS

2. Experimental/Computational protocol:

2.1 Data set:

A set of 30 quinoxaline derivatives reported as potential influenza NS1A protein inhibitors [3] was used to test the performance of the GUSAR in QSAR. The set consists of diverse substituents from electron donating to electron withdrawing groups located at several positions in the bicyclic core as shown in Fig. 1. The activities of these compounds have been reported elsewhere[3]. For the sake of convenience, the data reported in the form of %Binding at 50µm was converted to p(Binding at 50µm) i.e. $-\log_{10}$(%Binding at 50µm). These are listed in table 1.

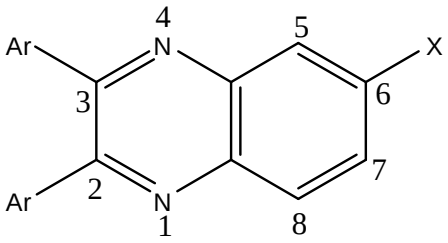


Fig. 1. Structure of 2,3,6-substituted quinoxaline derivatives

2.2. Preparation of the structures:

The 30 molecules were drawn in ChemSketch 12 freeware [17] followed by optimization and biologic data addition before further analysis in GUSAR. For better analysis, following settings were used: Y-randomization = 20 iterations, Leave Many Out (LMO) = 20 iterations, No. of leave out = 10%, leverages = 0.99, Similarity = 0.70, kNN RMSE/ Average RMSE = 1, No. of Models = 36. Hyperchem 8.05 was used to get the charges on each atom using Semi-empirical calculations based on PM3 module.

RESULTS AND DISCUSSION

3.1 Theory of GUSAR:

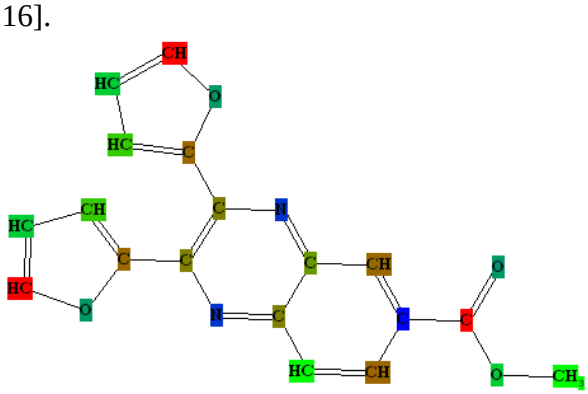
GUSAR originally proposed by Filimonov *etal*[14-16], is based on the novel approach of Quantitative Neighbourhoods of Atoms (QNA) and Multilevel Neighbourhoods of Atoms (MNA) descriptors as well as on Self-Consistent Regression (SCR) algorithm. In GUSAR, calculations of QNA involve kNN for better and accurate results. The basic difference between conventional QSAR and GUSAR lies in the representation of molecule in the space of calculated descriptors. In conventional QSAR approach, any molecule is represented as a single point in a many-dimensional space of molecular descriptors whereas in GUSAR any molecule is represented as a set of points in two-dimensional (2D) space of QNA descriptors. In GUSAR, QNA and MNA descriptors are used to build the consensus model, since the calculations of these descriptors are well documented in the literature[13-16] , it is not necessary to duplicate the same here.

3.2 Analysis of GUSAR output:

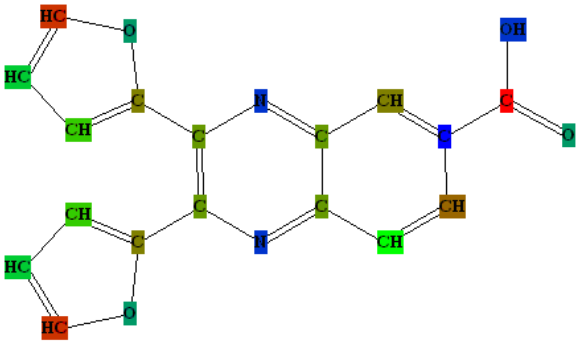
The output of GUSAR is in the form of a diagram in which the atoms are colored according to their contribution towards biological activity along with various statistical characteristics used to arrive at the consensus model. The obvious limitation of GUSAR is that it neither provides the QSAR model as MLR in interpretable form nor any knowledge about the descriptors that are used to build the consensus model. If QSAR model(s) was (were) produced on the basis of QNA descriptors the involvement of every atom into the predicted value is showed for a studied compound. The contribution is a calculation of activity value for a single atom from the structure of the studied molecule. Explanation of the colours is as following:

“Green” means that the impact of the atom approximately corresponds to the predicted activity value for a whole molecule. “Blue” means that the particular atom may decrease the activity. “Red” means that the particular atom may increase the activity. Thus, if one would like to increase the activity, the number of “blue” atoms should be reduced, and the number of “red”

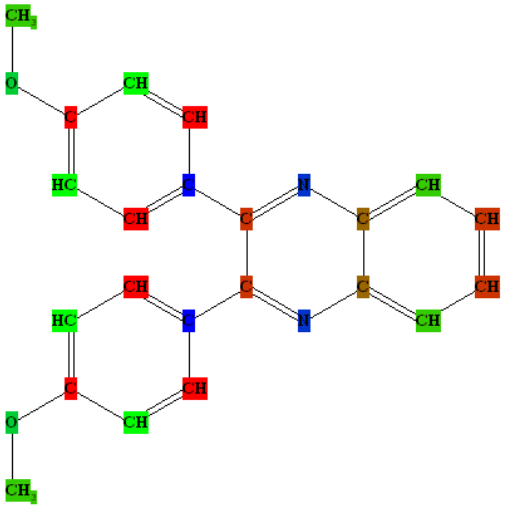
atoms should be increased. One can analyze how many fragments have "red" and "blue" colors for finding the most important fragments [14-16].



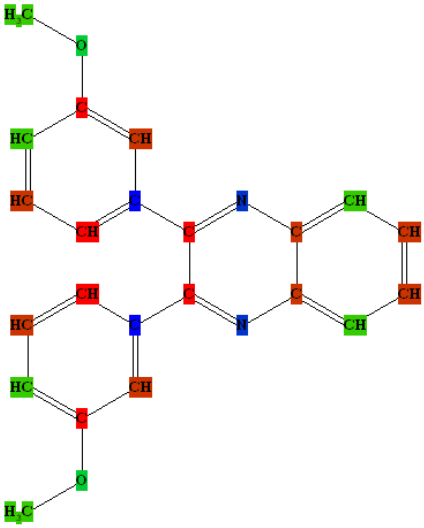
Molecule No. 29



Molecule no. 28



Molecule No. 9



Molecule No. 5

Fig. 2: Contribution of each atom towards biological activity. (Red-Positive, Blue-Negative and Green- No Effect)

To have a better idea, compound no. 29, 28 and 33, 5 were used for analysis purpose as representative examples of the same series having higher binding and less binding with enzyme (see figure 2).

The consensus QSAR model based on four descriptors for quinoxaline derivatives used in present study has following statistical characteristics along with the interpretation of QSAR model in terms of the specific contribution of atom and other molecular features to the modeled activity: Name of Activity: p(% Binding at 50 µM)

Statistical Characteristics of Consensus model based on QNA descriptors from 36 models.

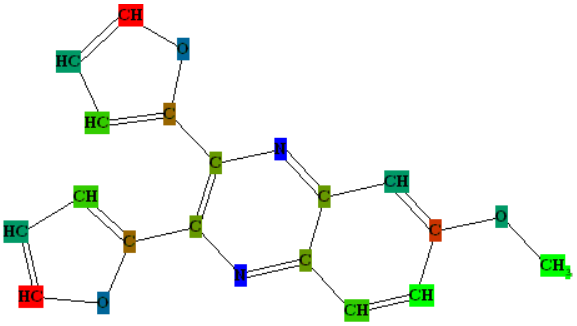
N = 30 R^2 = 0.746 F = 12.897 SD = 0.283 Q^2 = 0.645 V = 4

Where N is total number of molecules used, R is correlation coefficient, F is value of Fischer's parameter, SD is standard deviation, the cross-validated R^2 and V is no. of variables used in the model building. The high value of R^2, Q^2, F and low value of SD indicates that the model is statistically very sound and could be used for future drug designing. To analyze the contribution of atom towards binding with receptor, we examined the output which is in the form a diagram showing atoms suitably colored according to their specific role in deciding the activity.

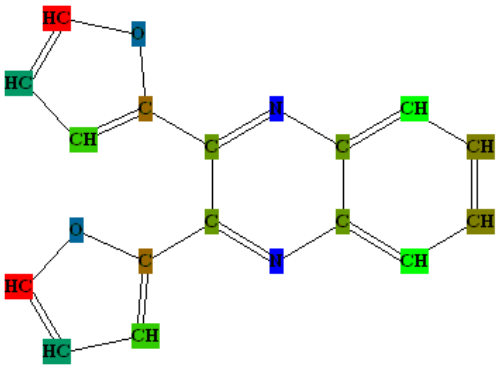
Table 1: Comparison between observed and predicted values for binding with enzyme

Sr. No.	Predicted Value p(% Binding at 50 µM)	Observed Value p(% Binding at 50 µM)	Reidual (Exp-pred)
1.	-0.45082	-0.2552700	0.21222
2.	-0.69068	-0.6532100	0.04723
3.	-1.4521	-1.0374000	0.432
4.	-0.86471	-1.0374000	-0.17984
5.	-1.4532	-1.3979000	0.0549
6.	-1.3716	-1.4579000	-0.0915
7.	-1.2987	-1.6314000	-0.3473
8.	-0.71344	-1.0086000	-0.28388
9.	-0.27706	-0.6989700	-0.42263
10.	-1.3259	-1.5888000	-0.2478
11.	-1.3107	-1.1239000	0.1972
12.	-1.25	-1.2900000	-0.0395
13.	-0.92619	-0.1760900	0.73938
14.	-1.2627	-1.3655000	-0.1141
15.	-1.1661	-1.7528000	-0.6009
16.	-1.0857	-0.8633200	0.23448
17.	-1.2541	-1.7348000	-0.4596
18.	-1.664	-1.7846000	-0.1069
19.	-1.5383	-1.8808000	-0.3338
20.	-0.54778	-0.6434500	-0.09136
21.	-1.6582	-1.4065000	0.2546
22.	-1.2129	-1.1959000	0.0068
23.	-1.1935	-0.8920900	0.27971
24.	-0.85639	-0.4471600	0.4092
25.	-0.76664	-0.8260700	-0.07081
26.	-0.27411	-0.1461300	0.11924
27.	-0.45049	-0.8573300	-0.40728
28.	-0.74105	-0.9590400	-0.22593
29.	-0.78525	-0.1760900	0.59595
30.	-0.21079	0.3010300	0.52855

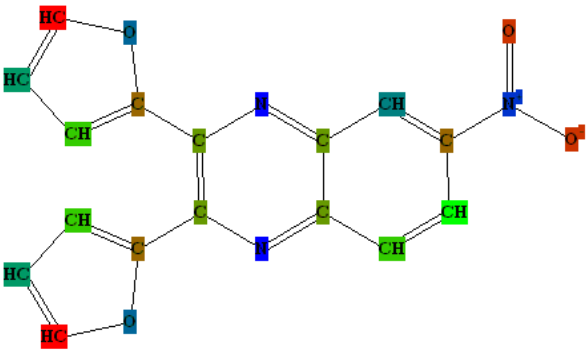
In order to assess the role of various fragments and specific atoms like furanyl oxygen, subtituents etc., we compared compound no. 26-30 (see fig. 2 and 3). From figure 2 and 3, it is evident that contribution of oxygen atom in furanyl ring to binding with protein is negligible. The presence of electron withdrawing substituents on carbon number 6 of quinoxaline ring play negative role, in future drug designing the position of substituent need to be changed for better activity, in contrast, electron donating groups at this position enhance its role in binding with receptor.



Molecule no. 27



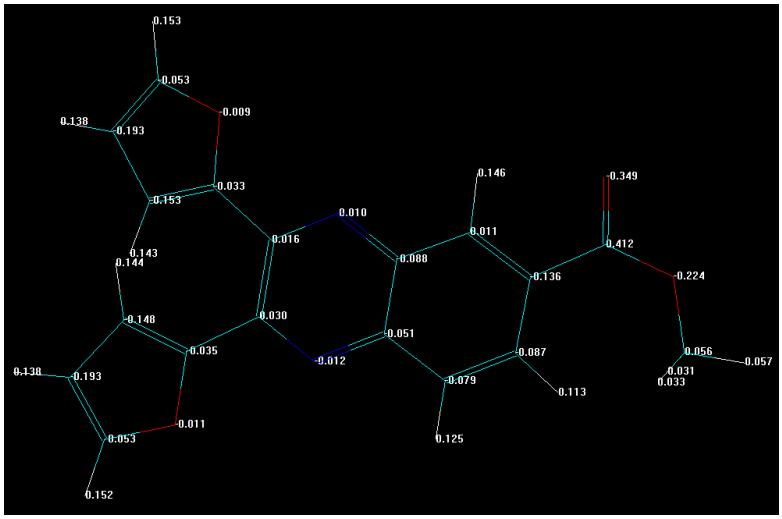
Molecule no. 26



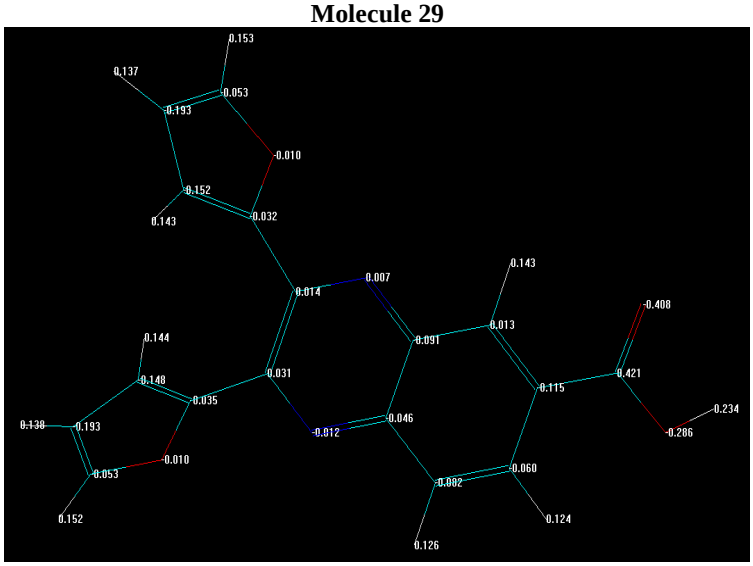
Molecule no. 30

Fig. 3: Contribution of various atoms/fragments in governing binding with enzyme NS1. (Red-Positive, Blue-Negative and Green- No Effect)

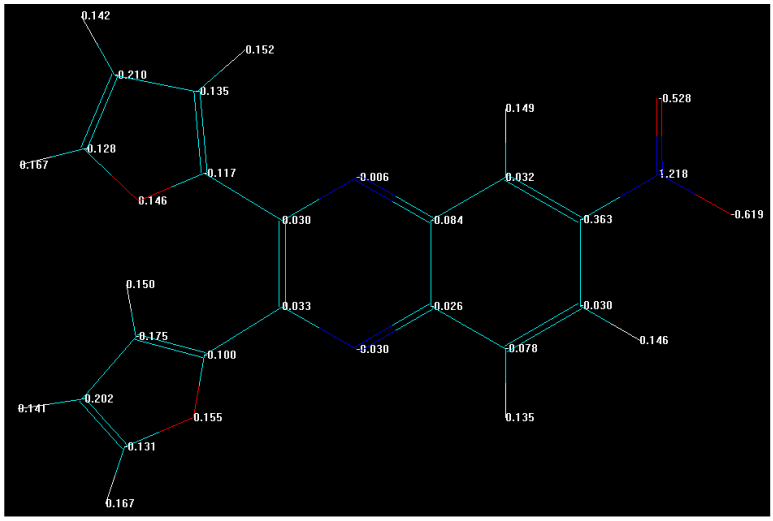
Appearance of very few “red” labeled atoms in quinoxaline ring indicates that this bicyclic ring do not play crucial role in binding with receptor NS1. An electrophilic atom directly attached to carbon no. 6 of quinoxaline rings is in favour of binding with protein. The carbon atom adjacent to furanyl oxygen contributes positively. Interestingly, the –OH group of carboxylic group attached to carbon no. 6 of quinoxaline ring, play negative role. One very important factor, which is in favour of binding, is the presence of “electrophilic centre” in direct contact with carbon no. 6 of quinoxaline ring, but the electrphilic centre should not be either too soft or too hard as suppoted by the PM3 semi- empirical based calculation of charges (fig.4).



Molecule 29



Molecule 28



Molecule 30

Fig.4: Charges on each atom as calculated by PM3 semi-empirical method for molecule 29, 28 and 30.

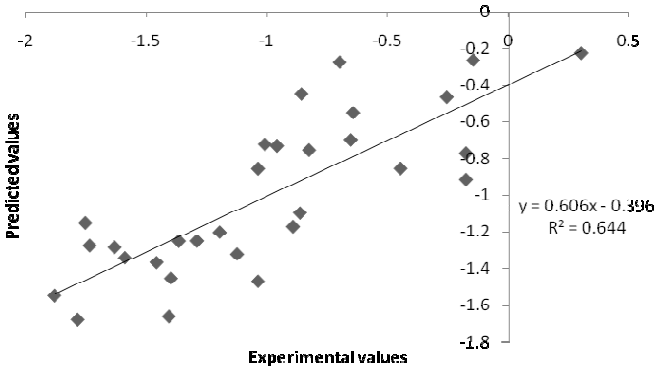


Fig. 5. Graph between observed and predicted values for biologic activity.

CONCLUSION

In conclusion, the oxygen atom in furanyl ring has insignificant influence on binding with enzyme NS1. The presence of electron withdrawing substituents on carbon number 6 of quinoxaline ring play negative role whereas electron donating groups have reverse effect. The bicyclic quinoxaline ring plays negligible role in binding with protein. The carbon atom adjacent to furanyl oxygen contributes positively. The –OH group of carboxylic group, attached to carbon no. 6 of quinoxaline ring, play negative role. GUSAR shows good predictive performance and has ability to provide some insight into the relative importance of the individual atoms involved in determining the biologic activity or binding with receptor.

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