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Pharmacophore modeling studies on aryl thioxothiazolidinones as ADAMTS-5 (Aggrecanase-2) inhibitors

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ABSTRACT

Pharmacophore mapping studies were undertaken for a set of 36 aryl thioxothiazolidinones as a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS-5) inhibitors. Four point pharmacophore with three hydrogen bond acceptors and one aromatic ring as pharmacophoric features was developed. Amongst them the pharmacophore hypothesis AAAR-1 yielded a statistically significant 3D-QSAR model with 0.841 as R^2 value and was considered to be the best pharmacophore hypothesis. The developed pharmacophore model was externally validated by predicting the activity of test set molecules. The squared predictive correlation coefficient of 0.687 was observed between experimental and predicted activity values of test set molecules. The geometry and features of pharmacophore were expected to be useful for the design of selective ADAMTS-5 inhibitors.

Keywords- A disintegrin and metalloproteinase with thrombospondin motifs 5, aryl thioxothiazolidinones, pharmacophore hypothesis, regression coefficient, squared predictive correlation coefficient.

INTRODUCTION

Osteoarthritis (OA) is the most common of all joint diseases. It is a major cause of morbidity in industrialized countries, where it ranks second among causes of disability, after cardiovascular disease (1, 2). This disease is characterized by progressive articular cartilage breakdown leading to chronic pain, inflammation and reduced mobility in the affected joint. The prevalence of OA increases with age and is estimated to affect 40% of people over the age of 70 years (3). Current treatment provides only symptomatic relief (NSAIDs, intra-articular injections of hyaluronic acid conjugates, and surgical joint replacement) with no therapy available to halt the progression of the disease (4).

ADAMTS-5 is a member of a disintegrin and metalloproteinase with thrombospondin motifs 5 protein family. Aggrecanase-2 has been designated ADAMTS-5 and shown to be responsible for cleavage of aggrecan (a multidomain proteoglycan, is a major component of cartilage that interacts with hyaluronic acid and link proteins to provide compressive resistance to articular cartilage) at the physiologically relevant Glu373-Ala374 peptide bond (5). ADAMTS-5 knock-out mice have been shown to have significantly reduced OA severity in a surgically induced instability model (6). The inhibition of ADAMTS-5 may therefore protect cartilage from damage and provide the first potential therapy to halt the progression of OA.

In ADAMTS-5 HTS screening efforts several inhibitor scaffolds possessing zinc chelating groups (7). These included hydroxamic acids, carboxylic acids, hydantoins and the most important thioxothiazolidinones.

The pharmacophore modeling is a well established approach to quantitatively explore common chemical features among a considerable number of structures and qualified pharmacophore model could also be used as a query for searching chemical databases to find new chemical entities. Pharmacophore modeling correlates activities with the spatial arrangement of various chemical features (8). Ligand-based drug design approaches like pharmacophore mapping (9) and quantitative structure-activity relationship (10) can be used in drug discovery in several ways, e.g. rationalization of activity trends in molecules under study, prediction of the activity of novel compounds, database search studies in search of new hits and to identify important features for activity (11).

In the present study, pharmacophore models has been generated and validated for prediction of ADAMTS-5 inhibitory activity using aryl thioxothiazolidinone derivatives. Such a pharmacophore model provides a rational hypothetical picture of primary chemical features responsible for activity (12).

MATERIALS AND METHODS

Dataset

The in vitro biological data of a series of 36 aryl thioxothiazolidinones (13) having ADAMTS-5 inhibitory activity was used for the present studies. The ADAMTS-5 inhibiting activity was expressed as IC_{50} i.e., concentration of compound in μm required for 50% inhibition of the enzyme activity. The IC_{50} values were converted to pIC_{50}. The dataset was divided randomly into training set and test set by considering the 75% of the total molecules in the training set and 25% in the test set. Twenty seven molecules forming the training set were used to generate pharmacophore models and prediction of the activity of test set (09 analogues) molecules was used as a method to validate the proposed models. The basic structures for these analogues are depicted in figure 1 and various substituents enlisted in table 1.

Pharmacophore modeling

Pharmacophore modeling, including ligand and structure-based approaches, has become an important tool in drug discovery. However, the ligand-based method often strongly depends on the training set selection, and the structure-based pharmacophore model is usually created based

on *apo* structures or a single protein–ligand complex, which might miss some important information (14).

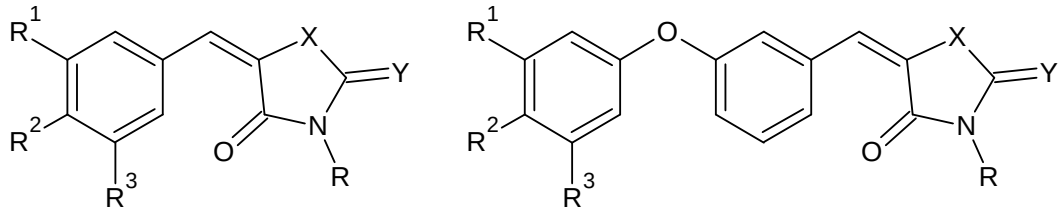

Figure 1: Basic structures of aryl thioxothiazolidinone series

Pharmacophore modeling based virtual screening of compounds is a ligand based approach and is useful when 3D structure of the target is not available but a few known active compounds are available. Pharmacophore modeling and 3-D database searching are now recognized as integral components of lead discovery and lead optimization. The continuing need for improved pharmacophore based tools has driven the development of 'PHASE' (15). To reach our research objectives we have used 'PHASE': a module of Schrödinger's drug design software (16).

Ligand Preparation

The first step for pharmacophore mapping was ligand preparation. The chemical structures of all the compounds were drawn in maestro and geometrically refined using LigPrep module. *LigPrep* is a robust collection of tools designed to prepare high quality, all-atom 3D structures for large numbers of drug-like molecules, starting with 2D or 3D structures in SD or Maestro format. The simplest use of LigPrep produces a single, low-energy, 3D structure with correct chiralities for each successfully proposed input structure.

While performing this step, chiralities were determined from 3D structure and original states of ionization were retained. Tautomers were generated using Macro Model method discarding current conformers. The conformations were generated by the Monte Carlo (MCMM) method as implemented in Macro Model version 9.6 using a maximum of 2,000 steps with a distance-dependent dielectric solvent model and an OPLS-2005 force field. All the conformers were subsequently minimized using Truncated Newton Conjugate Gradient (TNCG) minimization up to 500 interactions. For each molecule, a set of conformers with a maximum energy difference of 30kcal/mol relative to the global energy minimum conformers were retained. The conformational searches were done for aqueous solution using the generalized born/solvent accessible surface (GB/SA) continuum solvation model (17).

Creation of Pharmacophoric Sites

The next step in developing a pharmacophore model is to use a set of pharmacophoric features to create pharmacophore sites (site points) for all the ligands. In the present study, an initial analysis revealed that two chemical feature types i.e., hydrogen-bond acceptor (A) and aromatic ring (R) could effectively map all critical chemical features of all molecules in the data set. The minimum and maximum sites for all the features were kept 4 and 5 respectively and used to build a series of hypothesis with find common pharmacophore option in Phase.

Searching Common Pharmacophore

In this step, pharmacophores from all conformations of the ligands in the training set were examined and those pharmacophores that contain identical sets of features with very similar spatial arrangements were grouped together. If a given group is found to contain at least one pharmacophore from each ligand, then this group gives rise to a common pharmacophore. Any single pharmacophore in the group could ultimately become a common pharmacophore hypothesis. Common pharmacophores are identified using a tree-based partitioning technique that groups together similar pharmacophores according to their inter site distances, i.e., the distances between pairs of sites in the pharmacophore.

Table 1: Various substituents attached to basic structure of aryl thioxothiazolidinones.

Comp No.	Series	R_1	R_2	R_3	X	Y	R	Set
1	A	a-O-$CH_2C_6H_5$	H	H	S	S	H	Tr.
2	A	a-O-$CH_2C_6H_5$	a-$OCH_2C_6H_5$	H	S	S	H	Tr.
3	A	a-O-$CH_2C_6H_5$	H	a-$OCH_2C_6H_5$	S	S	H	Tr.
4	A	a-OCH_3	a-$OCH_2C_6H_5$	H	S	S	H	Tr.
5	A	a-OCH_3	a-$OCH_2C_6H_4$ (p-Cl)	H	S	S	H	Ts.
6	A	a-OCH_3	a-$OCH_2C_6H_4$ (p- OCH_3)	H	S	S	H	Tr.
7	B	H	H	H	S	S	H	Tr.
8	B	H	Cl	H	S	S	H	Tr.
9	B	H	a-OCH_3	H	S	S	H	Tr.
10	B	H	CH_3	H	S	S	H	Ts.
11	B	H	t-Bu	H	S	S	H	Tr.
12	B	Cl	Cl	H	S	S	H	Tr.
13	B	Cl	H	Cl	S	S	H	Tr.
14	B	CF_3	H	H	S	S	H	Tr.
15	B	H	Cl	H	S	O	H	Ts.
16	B	Cl	Cl	H	S	O	H	Tr.
17	B	Cl	H	Cl	S	O	H	Tr.
18	B	H	t-Bu	H	S	O	H	Tr.
19	B	H	Cl	H	NH	O	H	Tr.
20	B	Cl	H	Cl	NH	O	H	Tr.
21	B	H	t-Bu	H	NH	O	H	Tr.
22	B	H	Cl	H	NCH_3	O	H	Tr.
23	A	a-$OCH_2C_6H_5$	a-$OCH_2C_6H_5$	H	S	S	-CH_3	Ts.
24	A	a-$OCH_2C_6H_5$	a-$OCH_2C_6H_5$	H	S	S	-C_2H_5	Tr.
25	A	a-$OCH_2C_6H_5$	a-$OCH_2C_6H_5$	H	S	S	-C_6H_5	Tr.
26	A	a-$OCH_2C_6H_5$	a-$OCH_2C_6H_5$	H	S	S	-$CH_2C_6H_5$	Tr.
27	A	a-$OCH_2C_6H_5$	a-$OCH_2C_6H_5$	H	S	S	-$CH_2CH_2C_6H_5$	Ts.
28	A	a-$OCH_2C_6H_5$	a-$OCH_2C_6H_5$	H	S	S	-CH_2COOH	Tr.
29	A	a-OCH_2C_6H5	a-$OCH_2C_6H_5$	H	S	S	-CH_2CH_2COOH	Tr.
30	A	a-$OCH_2C_6H_5$	a-$OCH_2C_6H_5$	H	S	S	-$CH(C_6H_5)COOH$	Ts.
31	A	a-$OCH_2C_6H_5$	a-$OCH_2C_6H_5$	H	S	S	-CH(i-Pr)COOH	Tr.
32	A	a-$OCH_2C_6H_5$	a-$OCH_2C_6H_5$	H	S	S	-CH(i-Bn)COOH	Tr.
33	B	H	Cl	Cl	S	S	-CH_2COOH	Tr.
34	B	H	Cl	Cl	S	S	-$CH(C_6H_5)COOH$	Ts.
35	B	H	Cl	Cl	S	S	-CH(i-Pr)COOH	Ts.
36	B	H	Cl	Cl	S	S	-CH(i-Bn)COOH	Ts.

i-Pr; iso-propyl, i-Bn; iso-benzyl, t-Bu; tertiary-butyl, Tr; training set molecule, Ts; test set molecule

Scoring Hypothesis

In this step, common pharmacophore hypothesis was examined using a scoring function to yield the best alignment of the active ligands using an overall maximum root mean square deviation (RMSD) value of 1.2 Å for distance tolerance. The quality of alignment was measured by survival score (18).

Generation of 3D-QSAR Model

Phase provides the means to build 3D-QSAR model for a set of ligands that are aligned to a selected hypothesis. The Phase 3D-QSAR model partitions the space occupied by the ligands into a cubic grid. Any structural component can occupy part of one or more cubes. A cube is occupied by a feature if its centroid is within the radius of the feature. We can set the size of the cubes by changing the value in the Grid spacing text box. The regression is done by constructing a series of models with an increasing number of PLS factors. In present case, the pharmacophore based model was generated by keeping 1Å grid spacing and 3 as maximum number of PLS factors.

Validation of Pharmacophore Model

Validation is a crucial aspect of pharmacophore design, particularly when the model is built for the purpose of predicting activities of molecules in external test series (19). In the present case, the developed pharmacophore model was validated by predicting the activity of test set molecules. The correlation between the experimental and predicted activities of the test set molecules was determined.

RESULTS AND DISCUSSION

In the present study a series of aryl thioxothiazolidinone compounds were considered for molecular modeling studies. The ADAMTS-5 inhibitors protect cartilage from damage by inhibiting the cleavage of aggrecan at the physiologically relevant Glu373-Ala374 peptide bond (5) and provide the first potential therapy to reverse the progression of osteoarthritis. NSAIDs and Glucosamine salt are currently used clinically in combination with either diet or other supplements such as chonroitin sulfate & methylsulfonylmethane. A main drawback of the current drugs is the presence of side effects such as rashes, diarrhea, constipation, stomach upset & allergy (20). To either avoid or decrease the adverse effects of current agents and also to provide more candidates of drug choices, it is still necessary to search for new ADAMTS-5 inhibitors for further drug development.

Ligand-based drug design relies on knowledge of other molecules that bind to the biological target of interest. These molecules may be used to derive a pharmacophore which defines the minimum necessary structural characteristics which a molecule must possess in order to bind to the target (21). In other words, a model of the biological target is built based on the knowledge of what binds to it and this model in turn may be used to design new molecular entities that interact with the target.

Twenty seven molecules forming the training set were used to develop the pharmacophore model. The pharmacophoric features selected for creating sites were hydrogen bond acceptor (A) and aromatic ring (R). Pharmacophore models containing four to five features were generated.

The five featured pharmacophore hypothesis was rejected due to low value of survival score, as they were unable to define the complete binding space of the selected molecules. The four featured pharmacophore hypothesis was selected and subjected to stringent scoring function analysis.

The results of four featured pharmacophore hypothesis labelled AAAR-1 to AAAR-3 with their survival scores is listed in Table 2. The first hypothesis AAAR-1 is the best hypothesis in this study, characterized by highest survival score (3.543), the best regression coefficient (0.841) and Pearson-R (0.6311). The pharmacophore hypothesis AAAR-1 is presented in Figure 2. The features represented by this hypothesis are three hydrogen bond acceptor (A) and one aromatic ring (R). The distances and angles between different sites of AAAR-1 are given in table 3 and 4 respectively.

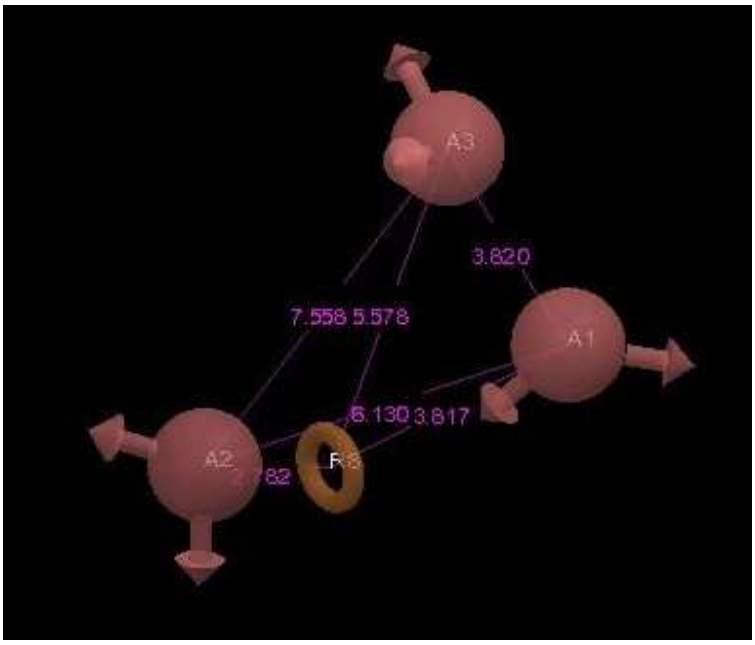

Figure 2: PHASE generated pharmacophore model AAAR-1 illustrating hydrogen bond acceptor (A1, A2, A3; pink) and aromatic ring (R8; orange) features with distances (in Å) between different sites of AAAR-1.

TABLE 2: Parameters of four featured hypothesis

Sr. No.	Hypothesis	Survival Score	R^2	F-value	Pearson-R
1	AAAR.1	3.543	0.841	68.1	0.6311
2	AARR.2	3.353	0.8376	50.6	0.4908
3	AAAR.3	3.335	0.868	64.1	0.8696

TABLE 3: Distances between different sites of model AAAR-1

Sr. No.	Site-1	Site-2	Distance (Å)	Sr. No.	Site-1	Site-2	Distance (Å)
1	A2	A1	6.13	4	A1	A3	3.82
2	A2	A3	7.558	5	A1	R8	3.817
3	A2	R8	2.782	6	A3	R8	5.578

TABLE 4: Angle between different sites of model AAAR-1

Sr. No.	Site1	Site2	Site3	Angle	Sr. No.	Site1	Site2	Site3	Angle
1	A1	A2	A3	30.2	7	A2	A3	A1	53.8
2	A1	A2	R8	25.7	8	A2	A3	R8	17.3
3	A3	A2	R8	36.6	9	A1	A3	R8	43.1
4	A2	A1	A3	96.1	10	A2	R8	A1	135.9
5	A2	A1	R8	18.4	11	A2	R8	A3	126
6	A3	A1	R8	93.8	12	A1	R8	A3	43.1

For each ligand, one aligned conformer based on the lowest RMSE of feature atom coordinates from those of the corresponding reference feature was superimposed on best hypothesis AAAR-1. Then fitness scores for all ligands were observed on the best scored pharmacophore model AAAR-1. The greater the fitness score, the greater the activity prediction of the compound. The fit function does not only check if the feature is mapped or not, it also contains a distance term, which measures the distance that separates the feature on the molecule from the centroid of the hypothesis feature. Table 5 shows the fitness score for all the molecules of training set. Figure 3 shows the AAAR-1 aligned with ligand having maximum fitness score, i.e. molecule 10 (IC_{50} = 3.2 μm) of the training set.

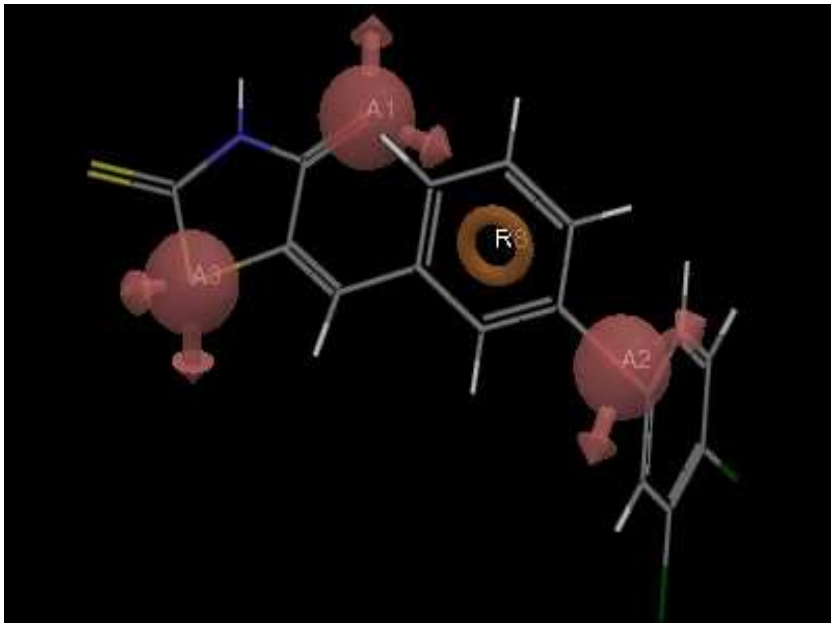

Figure 3: Best pharmacophore model AAAR-1 aligned with molecule 10. Pharmacophore features are color coded: hydrogen bond acceptor (A1, A2, A3; pink) and aromatic ring (R8; orange).

Beside this survival score analysis, another validation method to characterize the quality of AAAR-1 is represented by its capacity for correct activity prediction of training set molecules. AAAR-1 was regressed against the training set compound. Table 5 shows the actual and estimated inhibitory activities of the 29 molecules from the training set based on the pharmacophore hypothesis AAAR-1. The predicted ADAMTS-5 inhibitory activity of training set molecule exhibited a correlation of 0.841 with reported ADAMTS-5 inhibitory activity using model AAAR-1 (Figure 4).

Table 5: Experimental and predicted IC_{50} values of training set molecules based on hypothesis AAAR-1.

Comp. No.	Exp. IC_{50}(µM)	Pred. IC_{50}(µM)	Fitness Score	Comp. No.	Exp. IC_{50}(µM)	Pred. IC_{50}(µM)	Fitness Score
1	20.1	21.33	2.59	15	6.8	13.78	2.81
2	1.8	12.54	2.47	16	67	63.24	1.92
3	1.4	11.35	2.44	17	67	65.5	1.87
4	22	18.75	2.54	18	67	64.19	1.88
5	11.6	14.18	2.38	19	22	44.61	1.87
6	22	12.14	2.89	20	67	73.21	2.41
7	4.9	8.43	2.95	21	67	47.77	2.36
8	16.2	12.42	2.92	22	67	59.55	2.3
9	1.7	8.47	2.84	23	6.7	29.4	2.37
10	3.2	5.36	3	24	5.3	15.38	2.4
11	2.5	11.64	2.9	25	1.1	25.29	2.32
12	5.3	8.41	2.84	26	1.3	21.78	2.27
13	10	10.9	2.95	27	11.1	26.82	1.46
14	9.2	14.96	2.86				

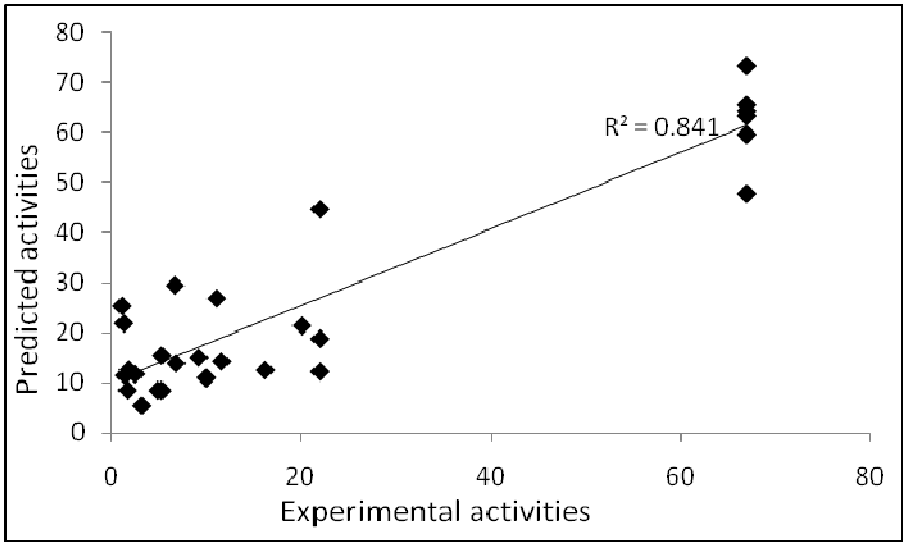

Figure 4: Relation between experimental and predicted ADAMTS-5 inhibitory activity values of training set molecules using model AAAR-1

The validity and predictive character of AAAR-1 were further assessed by using the test set prediction. The test set having nine molecules was analyzed. All the test set molecules were built and minimized as well as used in conformational analysis like all training set molecules. Then the activities of test set molecules were predicted using AAAR-1 and compared with the actual activity. Actual and predicted activity values of test set molecules are given in Table 6. The predicted ADAMTS-5 inhibitory activity of test molecule exhibited a correlation of 0.687 with reported ADAMTS-5 inhibitory activity using model AAAR-1 (Figure 5). For a reliable model, the squared predictive correlation coefficient should be >0.60 (22, 23). The results of this study reveal that model AAAR-1 can be used for the prediction of ADAMTS-5 inhibitory activity.

Table 6: Experimental and predicted IC50 values of test set molecules based on hypothesis AAAR-1

Comp. No.	Exp. IC_{50}(µM)	Pred. IC_{50}(µM)	Fitness Score	Comp. No.	Exp. IC_{50}(µM)	Pred. IC_{50}(µM)	Fitness Score
1	3.5	14.55	2.39	6	0.9	16.07	2.34
2	14.8	8.21	2.94	7	1.3	2.94	2.67
3	22	12.85	2.91	8	1.8	7.34	2.71
4	67	23.97	2.43	9	1.6	-2.73	2.64
5	67	42.8	2.32				

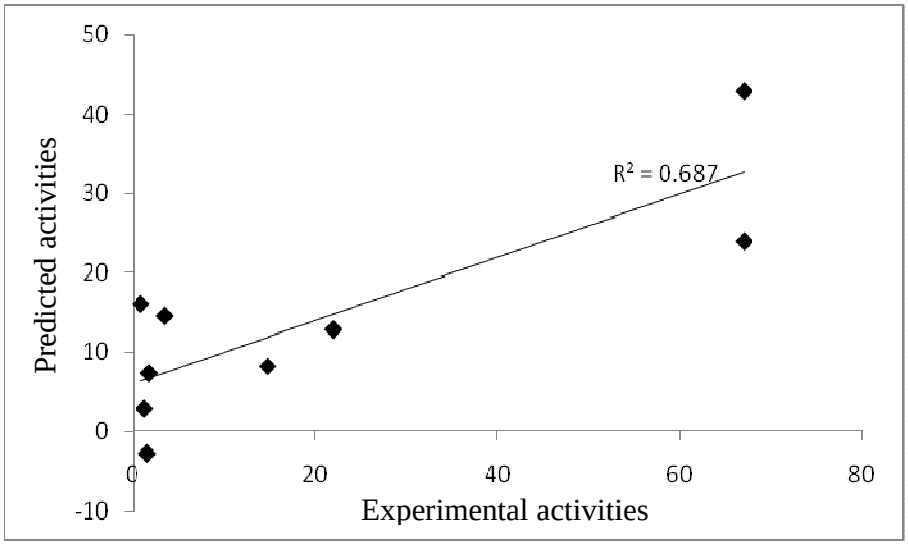

Figure 5: Relation between experimental and predicted ADAMTS-5 inhibitory activity values of test set molecules using model AAAR-1

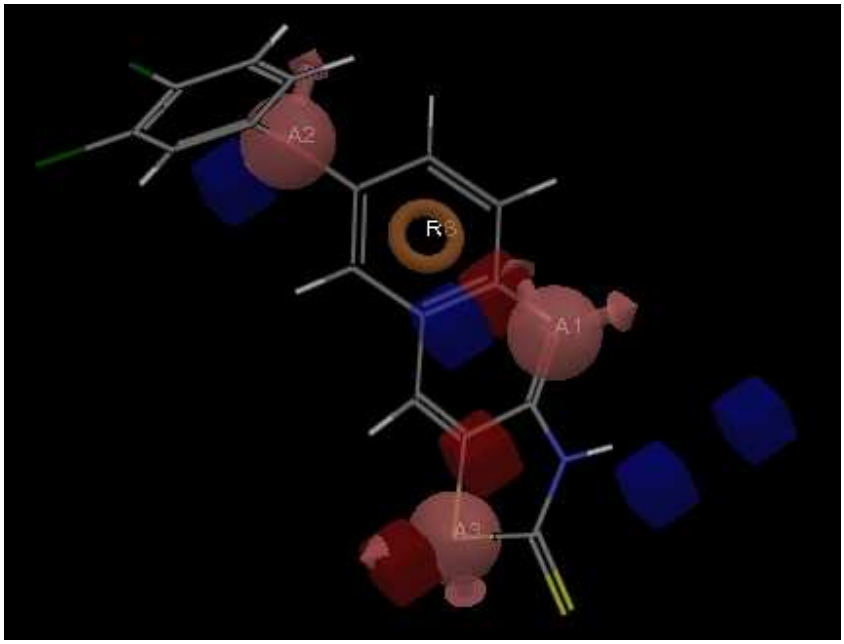

Figure 6: 3-D QSAR model based on molecule 10 of training set illustrating hydrogen bond acceptor feature

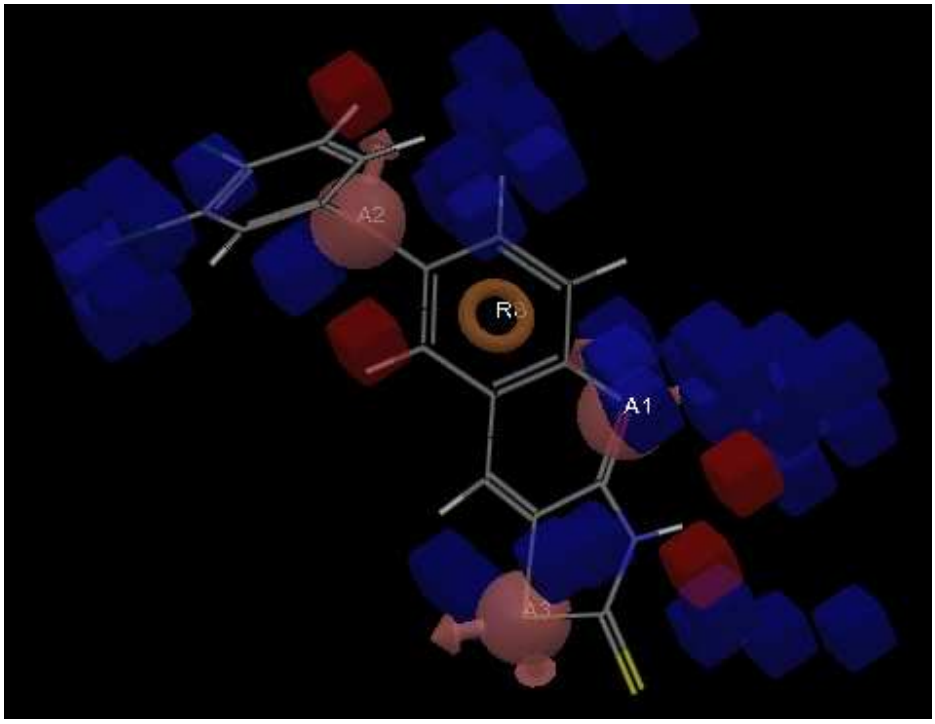

Figure 7: 3-D QSAR model based on molecule 10 of training set illustrating hydrophobic feature

3-D QSAR analysis

Additional insight into the ADAMTS-5 inhibitory activity can be gained by visualizing the 3-D QSAR model in the context of one or more ligands in the series with varying activity. This information can then be used to design new or more active analogues. 3-D QSAR models based on the molecules of training and test set using two features, i.e., hydrogen bond acceptors and aromatic ring has been studied.

***Hydrogen bond acceptor field prediction*:** The 3-D QSAR model based on molecule 10 of the training set using hydrogen bond acceptor feature is shown in Figure 6. Red region around the sulphur at position 1 of thioxothiazolidinone favours the ADAMTS-5 inhibitory activity. Replacement of this S group by any electron withdrawing group such as NH, NCH_3 and O will results in decrease in ADAMTS-5 inhibitory activity. For example- the replacement of S at position 1 in molecule 15 of training set (IC_{50}=6.8 μM) with NH in molecule 18 (IC50= 67 μM) leads to decrease in activity. Blue region around oxygen in biphenyl ether indicates that the substitution at this position by groups having more hydrogen bond acceptor property do not favours the ADAMTS-5 inhibitory activity.

***Hydrophobicity field prediction*:** The 3-D QSAR model based on molecule 10 of the training set using hydrophobicity feature is shown in Figure 7. Blue region around hydrogen or any other substituent such as chlorine as in this molecule at position 3, 4 of benzyloxy ring and hydrogen at the ortho position of the 5-benzylidene ring do not favours ADAMTS-5 inhibitory activity and substitutions at these positions by more hydrophobic groups will result in decrease in ADAMTS-5 inhibitory activity. For example- the replacement of Cl (IC_{50} = 3.2 μM) in molecule 10 at position 3 in benzyloxy ring with t-Butyl as in molecule 15 (IC_{50} = 6.8μM) of training set results

in decrease in ADAMTS-5 inhibitory activity. However this is inconsistent with the observation that the replacement of hydrogen at position 4 of benzyloxy ring by more hydrophobic groups result in good ADAMTS-5 inhibitory activity as exemplified by the observation that molecule 11 of training set (IC_{50} = 2.5 μM) having chlorine group at position 1, 4 of benzyloxy are more active as compared to the molecule 6 (IC_{50} = 22 μM) of training set.

CONCLUSION

The study shows the generation of a pharmacophore model AAAR-1 for aryl thioxothiazolidinones acting as ADAMTS-5 (A disintegrin and metalloproteinase with thrombospondin motifs 5) inhibitors. Pharmacophore modeling correlates activities with the spatial arrangement of various chemical features. Hypothesis AAAR-1 represents the best pharmacophore model for determining ADAMTS-5 inhibitory activity. AAAR-1 consists of three hydrogen bond acceptors and one aromatic ring features. This pharmacophore model was able to accurately predict ADAMTS-5 inhibitory activity and the validation results also provide additional confidence in the proposed pharmacophore model. Results suggested that the proposed 3-D QSAR model can be useful to rationally design new aryl thioxothiazolidinone molecules as ADAMTS-5 inhibitors and also to identify new promising molecules as ADAMTS-5 inhibitors in large 3-D database of molecules.

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