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Pharmacophore modeling studies on anti-inflammatory butenolides

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ABSTRACT

In order to understand the essential structural features for COX (Cyclooxygenase) inhibitors, three-dimensional pharmacophore hypothesis were built on the basis of a set of known COX inhibitors selected from literature using PHASE program. Four point pharmacophore with one hydrogen bond acceptor (A), three aromatic rings (R) as pharmacophoric features were developed. Amongst them the pharmacophore hypothesis ARRR14 yielded a statistically significant 3D-QSAR model with 0.811 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 0.96 was observed between experimental and predicted activity values of test set molecules.

Keywords: 3D-QSAR, Pharmacophore hypothesis, Regression coefficient, Butenolides,

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INTRODUCTION

NSAIDs are widely used in the treatment of rheumatoid arthritis and inflammatory diseases. However, long term use of NSAIDs has been associated with gastrointestinal bleeding and nephrotoxicity [1]. Anti-inflammatory activity of Non-steroidal anti-inflammatory drugs (NSAIDs) is mediated by inhibition of Cyclo-Oxygenases which results in decrease production of prostanoids. This mechanism is believed to account for both therapeutic as well as adverse effects of NSAIDs. Two forms of COXs have been identified - COX-I & COX-II. While COX-I is expressed in most of body tissues, COX-II is present with low or undetectable levels in some tissues [2]. The first COX-II selective compounds were DUP697 [3] and NS398 [4]. Later on DUP697 was used as starting point for the synthesis of the diaryl heterocyclic family of selective inhibitors, which include celecoxib [5, 6] and rofecoxib [7]. The central channel of COX-II is larger than that of COX-I. the larger main channel and extra nook make the total binding site ~ 25% larger in COX-II than in COX-I. The extra size is essential for selective inhibition of COX-II. [8, 9]. The butenolide ring present in cardenolides shows a strong oral cardiotonic activity.

Butenolides is also reported to have anti-inflammatory, analgesic, antiviral, and anticancer properties [10-12].

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 [13].

Ligand-based drug design approaches like pharmacophore mapping [14] and quantitative structure-activity relationship [15,16] 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.

This paper describes the development of a robust ligand-based 3D-pharmacophore hypothesis. The pharmacophore hypothesis obtained from the pharmacophoric points is used to derive pharmacophore-based 3D-QSAR model. Such a pharmacophore model provides a rational hypothetical picture of primary chemical features responsible for activity [17]. A further important role is to develop QSAR model.

MATERIALS AND METHODS

Dataset

The *in vitro* biological data [18] of a series of 20 compounds having anti-inflammatory activity was used for the present studies. The anti-inflammatory activity was expressed as % inhibition. . 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. Fifteen molecules forming the training set were used to generate pharmocophore models and prediction of the activity of test set (5 analogues) molecules was used as a method to validate the proposed model.

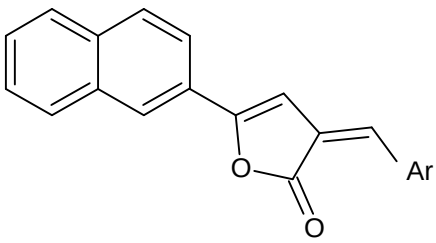
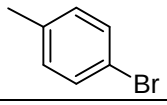
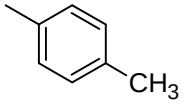
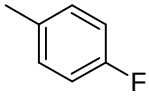
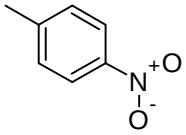
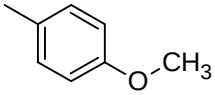
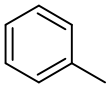
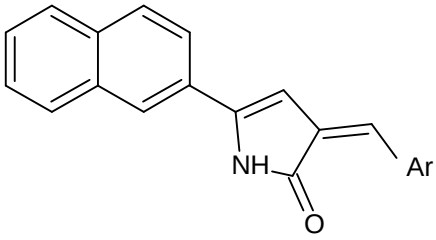


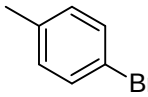
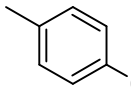
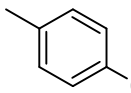
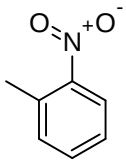
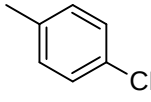
Figure 1: Basic structure of butenolides

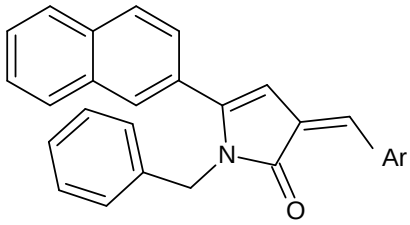
Table I: Experimental inhibitory activity of compounds

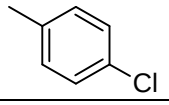
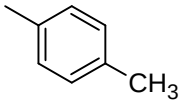
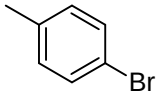
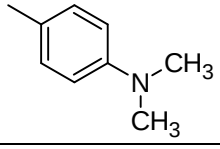
TRAINING SET

S.NO	Ar	ACTIVITY	PREDICTED ACTIVITY	FITNESS SCORE
1		43.01	43.96	**3**
2		44.08	42.53	2.49
3		47.31	44.34	2.96
4		35.48	32.93	2.02
5		47.31	48.13	2.47
6		43.01	43.74	2.47

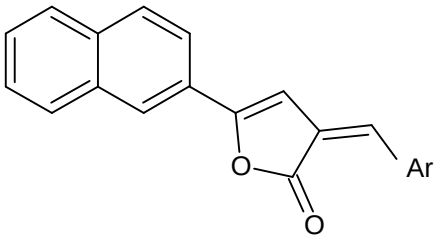


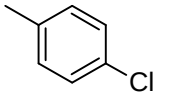
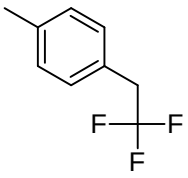
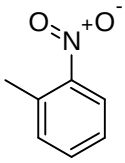
7		36.55	32.91	2.91
8		29.03	31.02	2.44
9		29.03	33.24	2.88
10		38.70	35.55	2.72
11		30.10	30.87	2.43

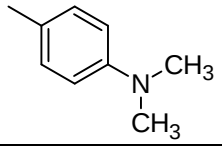


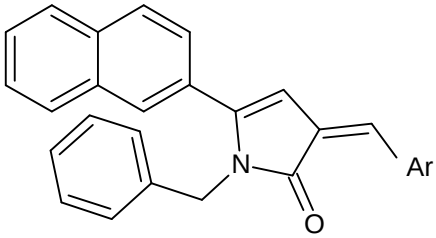
12		41.93	39.55	2.16
13		35.48	38.78	2.15
14		39.78	39.34	2.16
15		36.55	40.46	2.15

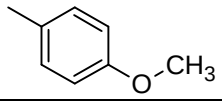
Test set



1		43.01	43.82	2.98
2		47.31	42.76	2.47
3		40.86	44.84	2.84

4		35.48	45.39	2.93



5		51.6	41.36	2.16

Pharmacophore modeling

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' [19]. To reach our research objectives we have used 'PHASE' a module of Schrödinger's software program 'MAESTRO' [20].

Ligand Preparation

The first step for pharmacophore modeling studies was ligand preparation. The chemical structures of all the compounds were drawn in maestro and geometrically refined using Ligprep module [21]. *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 the 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 MacroModel method discarding current conformers. The conformations were generated by the Monte Carlo (MCMM) [22, 23] method as implemented in MacroModel 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 iterations. For each molecule, a set of conformers with a maximum energy difference of 30 kcal/mol relative to the global energy minimum conformer was retained. The conformational searches were done for aqueous solution using the generalized born/solvent accessible surface (GB/SA) continuum solvation model [24].

Creation of Pharmacophoric Sites

The next second step in developing a pharmacophore model is to use a set of pharmacophore features to create pharmacophore sites (site points) for all the ligands. In the present study, an initial analysis revealed that three chemical feature types i.e., hydrogen-bond acceptor (A), hydrophobic group (H) 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 3 and 5 respectively. These features were selected and used to build a series of hypothesis with the find common pharmacophore option in Phase.

Searching Common Pharmacophore

In this step, pharmacophores from all conformations of the ligands in the data 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 intersite distances, i.e., the distances between pairs of sites in the pharmacophore.

Scoring Hypothesis

In this step, common pharmacophore hypothesis were 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 [25].

Generation of 3D-QSAR Model

Phase provides the means to build 3D QSAR models 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 2 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 compounds in external test series [26]. External validation is considered to be a conclusive proof for judging predictability of a model. Our priority was to develop QSAR models that were statistically robust both internally as well as externally. The main target of any QSAR modeling is that the developed model should be robust enough to be capable of making accurate and reliable predictions of biological activities of new compounds. In the present case, the developed pharmacophore model was validated by predicting the activity of test set molecules and correlation between the experimental and predicted activities of the test set molecules was determined.

RESULTS AND DISCUSSION

The purpose of pharmacophore modeling is to perform *in silico* screening searches in a three dimensional database of a virtual or real compound library to find diverse structures with desired binding activity and selectivity [27]. In the present study, a series of butenolides was considered for molecular modeling studies. The studies were aimed at developing a ligand based pharmacophore model relating the anti-inflammatory of butenolides .

Fifteen molecules forming the training set were used to develop the pharmacophores. The pharmacophoric features selected for creating sites were hydrogen bond acceptor (A), three aromatic rings (R). Pharmacophore models containing three, four and five sites, i.e., features were generated. The three featured pharmacophore hypotheses was rejected due to low value of survival score, as they were unable to define the complete binding space of the selected

molecules. Five featured pharmacophore hypothesis was also rejected due to non-availability of common pharmacophore.

The results of four featured pharmacophore hypothesis, labelled ARRR14. The hypothesis ARRR14 is the best hypothesis in this study, characterized by highest survival score. The ARRR14 pharmacophore hypothesis is presented in Figure II. The features represented by this hypothesis are one hydrogen bond acceptor (A), three aromatic rings (R). The angles and distances between different sites of ARRR14 are given in Table III and IV respectively.

Table II: Parameters of best pharmacophore hypothesis

S. No.	Hypothesis	Survival Score	R^2	F
1	ARRR14	3.543	0.8114	25.8

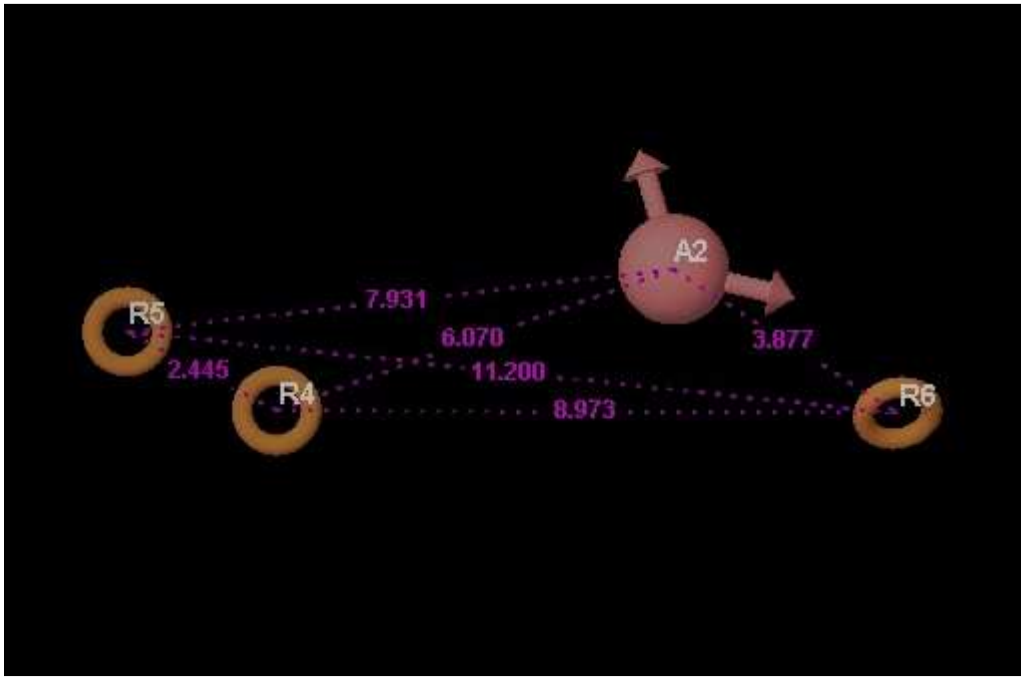


Figure II: PHASE generated pharmacophore model ARRR14 illustrating hydrogen bond acceptor (A2; pink), aromatic rings (R4; R5; R6; orange) .

Table III: Angles between different pharmacophoric sites of model ARRR14

Site1	Site2	Site3	Angle
R4	A2	R5	13.1
R4	A2	R6	127.5
R5	A2	R6	140.6
A2	R4	R6	132.6
A2	R4	R6	20.1
R5	R4	R6	152.6
A2	R5	R4	34.3
A2	R5	R6	12.7
R4	R5	R6	21.7
A2	R6	R4	32.5
A2	R6	R5	26.7
R4	R6	R5	5.8

Table IV: Distances between different pharmacophoric sites of model ARRR14

Site1	Site2	Distance
A2	R4	6.070
A2	R5	7.931
A2	R6	3.877
R4	R5	2.445
R4	R6	8.973
R5	R6	11.200

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 hypothesis. Then fitness scores for all ligands were observed on the best scored pharmacophore model. 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. Figure III shows the ARRR14 aligned with the most active compound 1 (max.fitness score=3) of the training set.

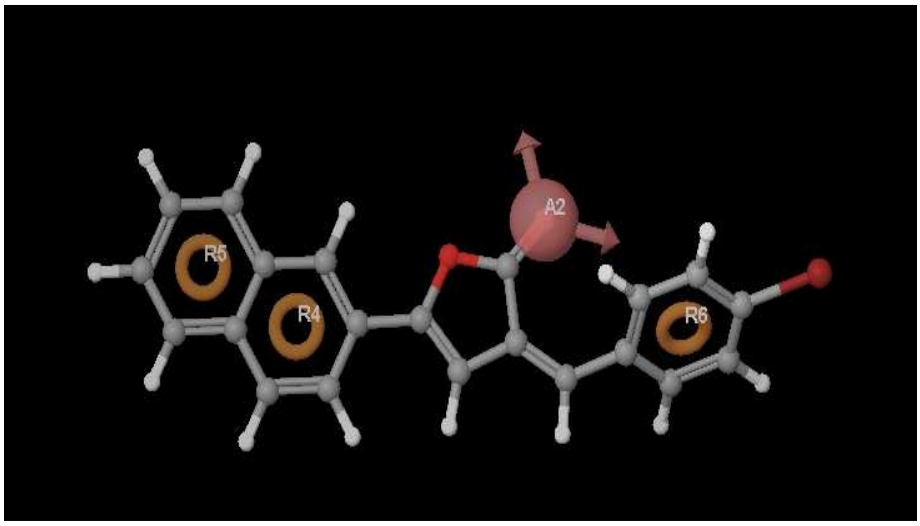


Figure III: Best pharmacophore model ARRR14 aligned with the most active compound 1 (maximum fitness score = 3) of the training set. Pharmacophore features are color coded: hydrogen bond acceptor (A2; pink), aromatic rings (R4; R5; R6 (orange).

Besides this survival score analysis, the validity and predictive character ARRR14 were further assessed by predicting the activity of test set molecules. A test set having five 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 ARRR14 and compared with the actual activity. Actual and predicted activity values of training & test set molecules are given in Table V, VI respectively. The predicted anti-inflammatory activity exhibited a correlation of 0.96 using model ARRR14 (Figure V). For a reliable model, the squared predictive correlation coefficient should be >0.60 [28, 29]. The results of this study reveal that model ARRR14 can be used for the prediction of anti-inflammatory activity. Good and consistent external predictivity was observed for ARRR14 as compared to the other hypothesis. ARRR14 showed a good r^2 value, i.e. 0.811 and squared predictive correlation coefficient of 0.960 was also observed between experimental and predicted activity values of test set molecules.

Table V: Experimental and predicted activity of training molecules based on hypothesis ARRR14

S.NO	Experimental activity (% inhibition)	Predicted activity (% inhibition)
1	43.01	43.96
2	44.08	42.53
3	47.31	44.34
4	35.48	32.93
5	47.31	48.13
6	43.01	43.74
7	36.55	32.91
8	29.03	31.02
9	29.03	33.24
10	38.70	35.55
11	30.10	30.87
12	41.93	39.55
13	35.48	38.78
14	39.78	39.34
15	36.55	40.46

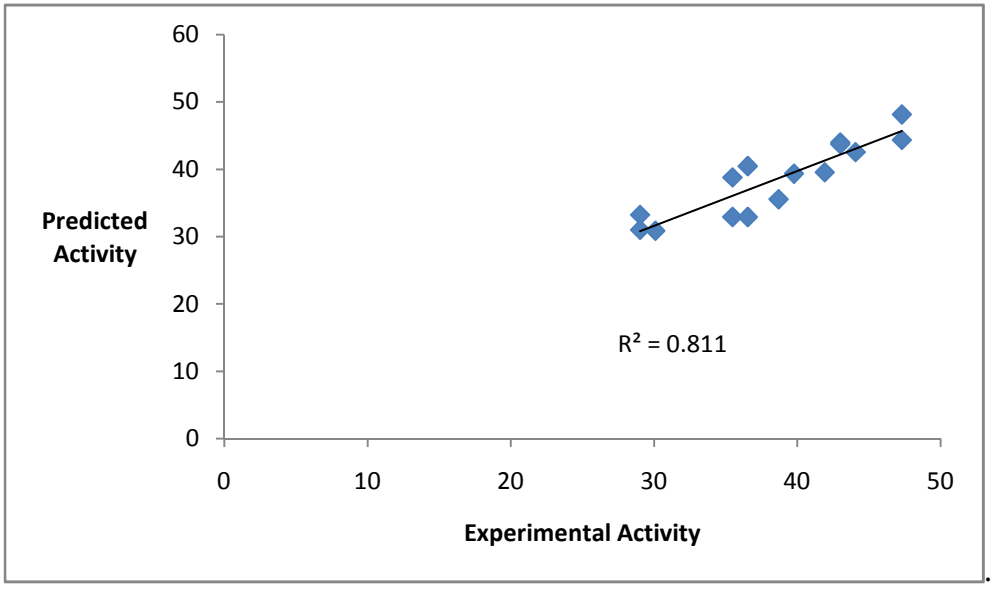


Figure IV: Relation between experimental and predicted anti-inflammatory activity of training set molecules using model ARRR14

Table VI: Experimental and predicted activity of test molecules based on hypothesis ARRR14

S. No.	Experimental activity (%inhibition)	Predicted activity (%inhibition)
1	43.01	43.82
2	47.31	42.76
3	40.86	44.84
4	35.48	45.39
5	51.6	41.36

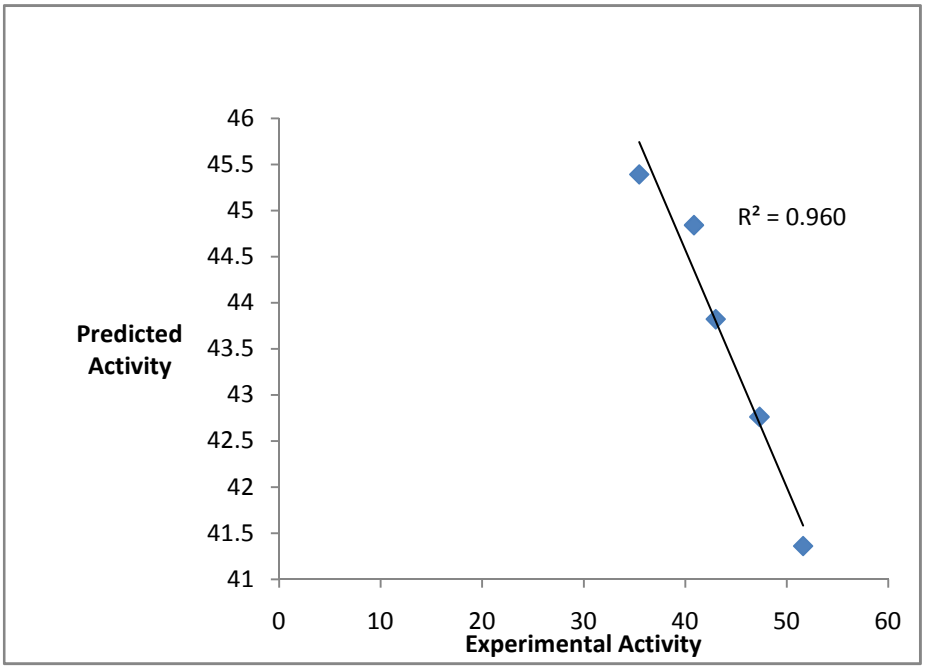
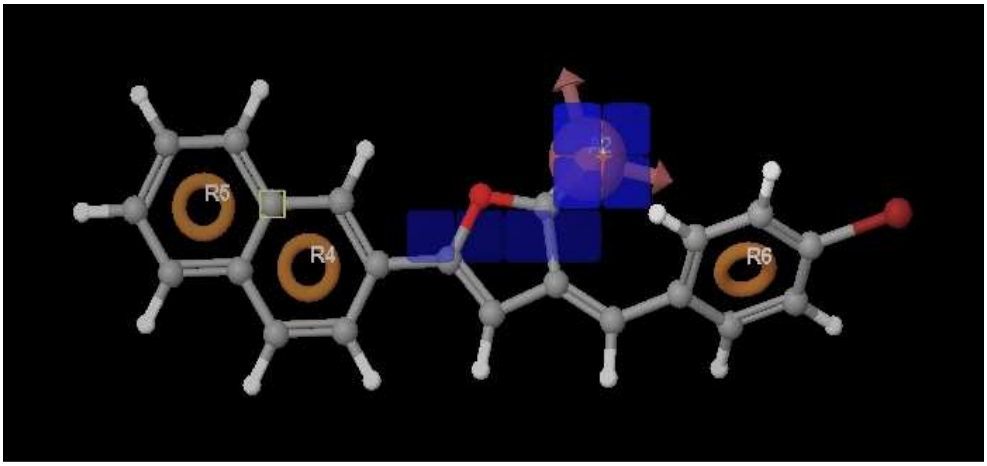


Figure V: Relation between experimental and predicted anti-inflammatory activity of test set molecules using model ARRR14

Interpretation of QSAR model

Additional insights into the inhibitory activity can be gained by visualizing the 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 and more active analogues. A pictorial representation of the 3D QSAR models based on compound 1 of the training set using hydrogen bond acceptor and hydrophobicity features is shown in Fig VI-VII. In these representations, the blue cubes indicate favorable regions while red cubes indicate unfavorable regions for activity.



FigureVI: 3D QSAR model based on compound 1 of the training set illustrating hydrogen bond acceptor features

. Figure VI shows the 3D QSAR model illustrating the hydrogen bond acceptor feature. The blue region at the site of butenolide moiety shows any substitution by hydrogen bond acceptor in this region causes increase in activity.

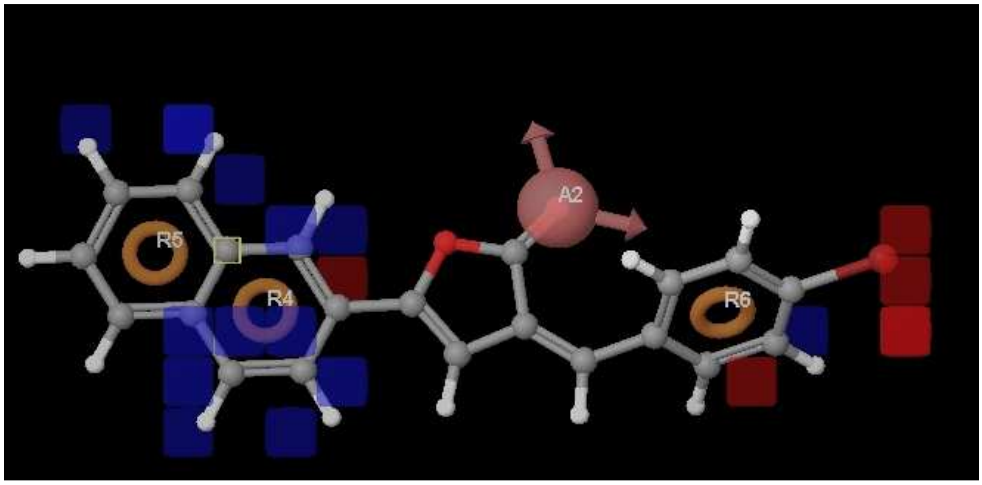


Figure VII: 3D QSAR model based on compound 1 of the training set illustrating hydrophobic features

The blue region around naphthalene shows any group attached to naphthalene which increases hydrophobicity increases the activity. Red region at 4 position (bromo) of benzene ring shows that any hydrophobic group at this position cause decrease in activity.

The studies show the generation of a pharmacophore model ARRR14 for butenolides acting as COX inhibitors. Pharmacophore modeling correlates activities with the spatial arrangement of various chemical features. Hypothesis ARRR14 represents the best pharmacophore model for determining anti-inflammatory activity. ARRR14 consists of one hydrogen bond acceptor, three aromatic ring features. This pharmacophore model was able to accurately predict anti-inflammatory activity and the validation results also provide additional confidence in the proposed pharmacophore model.

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