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Hologram Based QSAR Analysis of Xanthine Oxidase Inhibitors

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Abstract

Xanthine Oxidase is an enzyme, which oxidizes hypoxanthine to xanthine, and xanthine to uric acid. It is widely distributed throughout various organs including the liver, gut, lungs, kidney, heart, brain and plasma. It is involved in gout pathogenesis. Hence, in the present study, Hologram based Quantitative Structure Activity Relationship Study was performed on a series of Xanthine Oxidase antagonist named 2-(indol-5-yl) thiazole derivatives. The best HQSAR model was obtained using Atoms, Bonds, Connection, Hydrogen, Chirality and Donor Acceptor as fragment distinction parameter using hologram length 71 and 4 components with fragment size of minimum 2 and maximum 5. Significant cross-validated correlation coefficient (q^2= 0.563) and non cross-validated correlation coefficients (r^2= 0.967) were obtained. The model was then used to evaluate the six external test compounds and its r^2_{pred} was found to be 0.798. Contribution map show that presence of propyl ring in indole thiazole makes big contributions for improving the biological activities of the compounds. We hope that our HQSAR model and analysis will be helpful for future design of xanthine oxidase antagonists.

Keywords: Xanthine Oxidase; HQSAR

1. Intoduction

Xanthine oxidase is an enzyme that generates reactive oxygen species. The enzyme oxidizes hypoxanthine to xanthine, and xanthine to uric acid, producing hydrogen peroxide. XO is widely distributed throughout various organs such as the liver, gut, lung, kidney, heart, brain and plasma[1]. High level of XO secretion is found in the gut and the liver[2]. It is localized to the capillary endothelial cells, in the myocardium[3]. It is capable of catalyzing the formation of urate in man[4]. Xanthine oxidase is involved in gout pathogenesis. In gout, defective metabolism of uric acid causes arthritis, especially in the smaller bones of the feet and deposition of chalkstones. This causes episodes of acute pain in patients. According to the Third National Health and Nutrition Examination Survey (1988–1994), gout is a prevalent disease with occurrence of >2% in men older than 30 years and in woman older than 50 years[5]. It occurs in individuals who have high serum uric acid levels, in response to precipitation of monosodium urate monohydate crystals in various tissues, followed by an inflammatory response. Typical symptoms include acute recurrent gouty arthritis, a tophinodular collection of monosodium urate crystals and uric acid urolithiasis[6]. XO also plays an important role in various forms of ischemic and other types of tissue and vascular injuries, inflammatory diseases, and chronic heart failure[7]. Xanthine oxidase inhibitor is any substance that inhibits the activity of xanthine oxidase. There are two kind of Xanthine oxidase inhibitors: purine analogues and others. Purine analogues include allopurinol, oxypurinol, and tisopurine. Others include febuxostat, topiroxostat, and inositols (phytic acid and myo-inositol). These commercially available drugs have certain disadvantages[8-10]. Hence, the discovery of structurally diverse XO inhibitors and analyzing the important structural properties required for their biological activity becomes important.

Hologram Quantitative Structure Activity Relationship (HQSAR) is the novel 2D fragment-based QSAR method that employs specialized molecular fingerprints[11,12] and eliminates the need for 3D structure, molecular alignment and conformational search[13,14]. In HQSAR, each molecule in the training set is divided into several structural fragments, which are arranged to form a molecular hologram, assigned by a cyclic redundancy check

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(CRC) algorithm. Although HQSAR uses two dimensional information of a molecule, it also utilizes some three dimensional information such as chirality and molecular hybridization. In the present study, HQSAR has been employed to study the activity of 2-(indol-5-yl) thiazole derivatives as xanthine oxidase antagonist. Many HQSAR models were generated with different combinations of parameters and based on statistical values of the model, the best model was selected and its contribution map was also analyzed. We also identified the important features of the compounds for improving the activity.

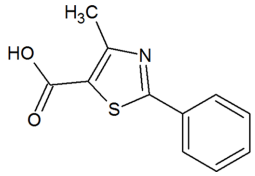
2. Materials and Methods

2.1. Data Set

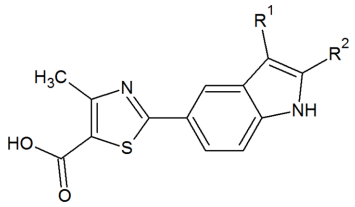
The structure of the 2-(indol-5-yl) thiazole derivatives and their biological activities of 21 compounds were

Table 1. Structures and biological activities (pIC_{50}) of Xanthine Oxidase inhibitors

The Xanthine Oxidase inhibitor scaffold

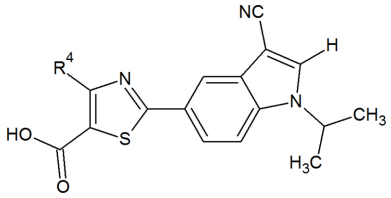


a) Compound 1-7



Compound	R^1	R^2	pIC50 values
1	H	H	6.9586
2	Cl	H	7.9706
3	Cl	H	8.5529
4	Cl	CH_3	8.0458
5	NO_2	H	7.9101
6	NO_2	H	8.2518
7	CN	H	8.2218

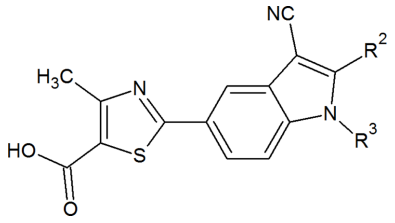
b) Compound 8-11



Compound	R^4	pIC50 values
8	H	8.4089
9	CF_3	8.0655
10	OCH_3	7.0457
11	CH_3	8.2441

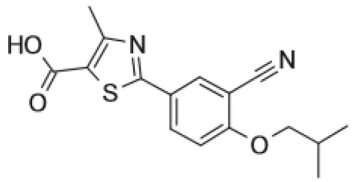
Table 1. Continued

c) Compound 12-21



Compound	R^2	R^3	pIC50 values
12	H	2-methylpropane	7.7959
13	CH_3	1-fluoro-2-methylpropane	8.3767
14	H	2-methylpropan-1-ol	7.9208
15	H	1-methoxy-2-methylpropane	8.2596
16	H	1-(methylsulfonyl)propane	8.3565
17	H	N-propylacetamide	6.0482
18	H	N-propylmethanesulfonamide	7.0458
19	H	ethylbenzene	8.4559
20	H	1-ethyl-2,4-difluorobenzene	8.320

d) Febuxostat

Compound	Structure	pIC50 values
21		8.2596

taken from the literature[15]. Biological activities i.e. IC_{50} values of each inhibitor was converted into pIC_{50} (-log-IC_{50}) and the dataset (26 compounds) were segregated into test (6 compounds) and training set (20 compounds). The training and test sets were classified to ensure that both sets could completely cover the whole range of biological activity and structural diversity. The structures and their activity values are displayed in Table 1.

2.2. HQSAR

HQSAR is a two dimensional computational technique that uses a fragmenting approaches that relates sub structural components of compounds to their biological activity. In this method, each molecule is divided into a series of unique structural fragments that are counted in the bins of a fixed length array to form the molecular hologram[16]. The parameters such as hologram length, fragment size and fragment distinction affect the HQSAR model. The hologram length (HL) determines the number of bins in the hologram into which the fragments are hashed. The optimal HQSAR model was derived from screening through the default HL values, which were set of prime numbers ranging from 53 to 401 to avoid fragment collisions. Fragment size controls the minimum and maximum length of the fragments to be included on the hologram fingerprint with the default values as 4 and 7 respectively. Molecular fragment generation utilizes the following fragment distinctions: atoms (A), bonds (B), connections (C), chirality (Ch), hydrogen atoms (H) and donor/acceptor (DA). To evaluate the hologram generation, numerous models with the various combinations of the parameters were developed. The validity of the model depends on the statistical parameters such as cross-validated r^2 (q^2),

non cross-validated r^2 by Leave-One-Out (LOO), r^2_{pred} and standard error.

2.3. Predictive Correlation Coefficient (r^2_{pred})

The predictive power of CoMFA model was determined from six test molecules which were excluded during model development. The predictive correlation coefficient (r^2_{pred}) based on the test set molecules, is defined as:

$$r^2_{pred} = \frac{(SD - PRESS)}{SD}$$

where PRESS is the sum of the squared deviation between the predicted and actual activity of the test set molecules, and SD is defined as the sum of the square deviation between the biological activity of the test set compounds and the mean activity of the training set molecules[17].

3. Results and Discussion

3.1. HQSAR Analysis

HQSAR model generation was performed on 2-(indol-5-yl) thiazole derivatives derivatives using three distinct parameters namely fragment size, hologram length and fragment distinction. Five HQSAR models were generated using the different fragment distinction with the fragment size 4-7. The models were generated using different combination of Atoms, Connections, Hydrogens, Chirality and Donor Acceptor parameters gave better results. We found that the model which includes the parameter Bond didn't yield a HQSAR model. The statistical results of the generated HQSAR models are shown in Table 2. These models was further investigated to see the influence of length of fragment sizes (2-5, 3-6, 4-7, 5-8, 6-9, 7-10 and 8-11) and its results are summarized in Table 3. The statistical parameters showed that there is was an improvement by changing the fragment size for this dataset. We had chosen the best model with higher q^2 values as summarized in Table 3 for examining the predictive ability r^2_{pred} of test set molecules. Based on better q^2 and r^2_{pred} values the final model was selected (q^2 =0.563, r^2 =0.967, SEE =0.140, r^2_{pred} =0.798) which was built using parameters A/B/C/H/Ch/DA as fragment distinction, fragment size set to min 2 and max 5 with hologram length 71 and 4 components. The detailed predicted versus actual activities along with the residual values for training and test set was depicted in Table 4. Low residual values obtained for developed HQSAR model indicates its reliability and can be used to predict the biological activity of novel compounds.

3.2. HQSAR Contribution Map Analysis

The HQSAR results gave direct evidence about the individual atomic contributions to the biological activity through the use of different color codes. The contributions of the different fragments for the activity of the molecules are displayed in Fig. 1. The colors at the red end of the spectrum indicates the poor contributions (red, red orange and orange), while colors at the green end reflect favorable contributions (yellow, green blue and green). Atoms with intermediate contributions are colored in white. In the contribution map we found that the common scaffold indole thiazole is represented in white in all compounds which depicts the intermediate contribution to the activity of all molecules. The generated HQSAR model for few compounds is shown in Fig. 1 where the atoms colored in cyan color indicates the common substructure and it contributes to the inhibitory

Table 2. Statistical results obtained from HQSAR analysis for various fragment distinctions with default fragment size (4-7)

Model no	Fragment Distinction	q^2	r^2	SEE	N	HL
1	A/C/H/DA	0.507	0.940	0.179	3	59
2	A/B/H/DA	0.442	0.952	0.168	4	53
3	A/B/C/H/DA	0.427	0.939	0.182	3	71
4	A/C/H/Ch/DA	0.476	0.948	0.167	3	59
5	A/B/C/H/Ch/DA	0.459	0.970	0.132	4	151

The model chosen is highlighted in bold.

q^2 –cross validated correlation coefficient; r^2 –non cross validated correlation coefficient; SEE –standard error of estimate; N – number of statistical components; HL –hologram length; A–atoms; B –bonds; C –connections; H –hydrogen atoms; Ch –chirality; D/A donor and acceptor.

Table 3. Influence of various fragment size using in HQSAR analysis

Model no	Fragment Size	q^2	r^2	SEE	N	HL	r^2_{pred}
	2-5	0.444	0.959	0.155	4	71	
	3-6	0.224	0.593	0.434	1	53	
	4-7	0.507	0.940	0.179	3	59	
1	5-8	0.228	0.541	0.461	1	61	
	6-9	**0.562**	**0.960**	**0.154**	**4**	**59**	**0.726**
	7-10	0.390	0.965	0.144	4	307	
	8-11	0.444	0.910	0.230	4	53	
	2-5	0.454	0.844	0.279	2	71	
	3-6	**0.563**	**0.984**	**0.103**	**5**	**61**	**0.639**
	4-7	0.442	0.952	0.168	4	53	
2	5-8	0.240	0.517	0.473	1	61	
	6-9	0.421	0.962	0.150	4	151	
	7-10	0.190	0.410	0.523	1	59	
	8-11	0.167	0.378	0.537	1	61	
	2-5	**0.551**	**0.959**	**0.155**	**4**	**59**	**0.629**
	3-6	0.530	0.970	0.134	4	71	
	4-7	0.427	0.939	0.182	3	71	
3	5-8	0.417	0.967	0.139	4	71	
	6-9	0.543	0.972	0.128	4	53	
	7-10	0.443	0.971	0.131	4	53	
	8-11	0.388	0.970	0.134	4	71	
	2-5	0.425	0.919	0.209	3	61	
	3-6	0.253	0.626	0.416	1	53	
	4-7	**0.476**	**0.948**	**0.167**	**3**	**59**	**0.535**
4	5-8	0.321	0.883	0.241	2	61	
	6-9	0.376	0.923	0.204	3	61	
	7-10	0.405	0.940	0.188	4	61	
	8-11	0.349	0.860	0.275	3	53	
	2-5	**0.563**	**0.967**	**0.140**	**4**	**71**	**0.798**
	3-6	0.522	0.971	0.130	4	71	
	4-7	0.459	0.970	0.132	4	151	
5	5-8	0.443	0.961	0.152	4	53	
	6-9	0.413	0.965	0.143	4	151	
	7-10	0.558	0.973	0.127	4	53	
	8-11	0.338	0.889	0.245	3	71	

The best models are highlighted in bold.

activity of the compound. In the highly active compounds (03, 19 and 8), indole thiazole is white in color and its combination with propanyl group helps in improving the activity of the molecule. In the least active compounds 01 the presence of isobutyl group attached to indole thiazole is responsible for its lower activity. In compound 17 the presence of acetylamino ethyl and in compound 18 the presence of sulfonamide might be responsible for the low activity of that molecule.

4. Conclusion

This study was conducted to rationalize the 2-(indol-5-yl) thiazole derivatives by HQSAR analysis. All the

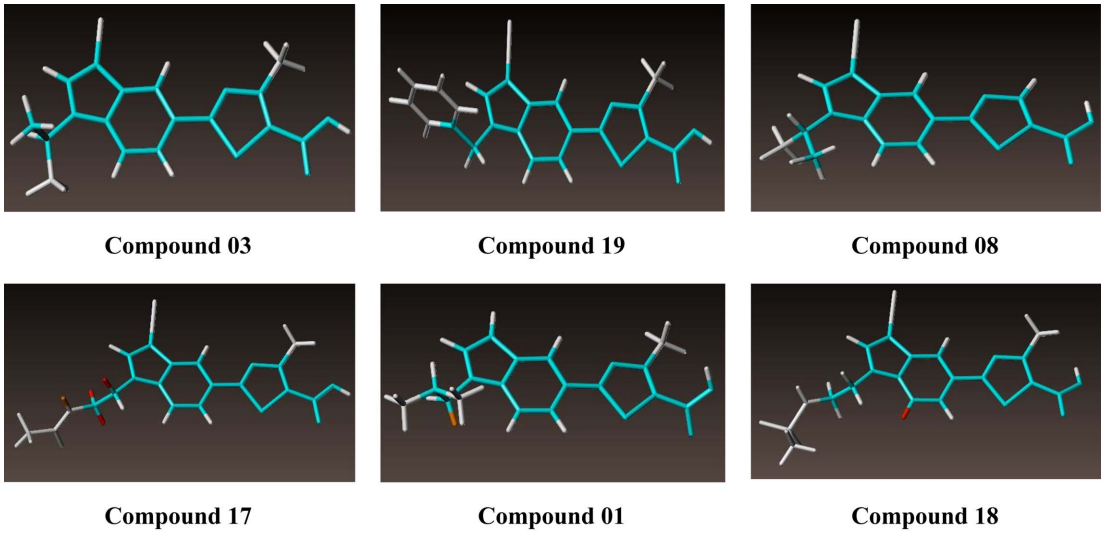


Fig. 1. HQSAR contribution map of highly active compound (compound 03, 19 and 08) and least active molecule (compound 17, 01and 18).

Table 4. Experimental and predicted pIC_{50} values of training and test set compounds

Compound no	Actual pIC_{50}	HQSAR	
		Predicted (pIC_{50})	Residual
1*	6.959	8.208	-1.249
2	7.971	8.203	-0.232
3	8.553	8.417	0.136
4	8.046	7.918	0.128
5	7.910	8.097	-0.187
6	8.252	8.237	0.015
7	8.222	8.061	0.161
8	8.409	8.252	0.157
9	8.065	7.984	0.081
10*	7.046	7.189	-0.143
11	8.244	8.276	-0.032
12*	7.796	7.980	-0.184
13*	8.377	9.030	-0.653
14	7.921	7.995	-0.074
15	8.260	8.323	-0.063
16	8.356	8.337	0.019
17	6.048	5.999	0.049
18	7.046	7.028	0.018
19*	8.456	8.513	-0.057
20	8.320	8.351	-0.031
21*	8.260	8.360	-0.100

enerated models showed different statistical results in terms of q^2 and r^2 values when different combinations of fragment distinction were used. The best model was selected based on high q^2 (0.563) and r^2_{pred} (0.798) values. Contribution map show that presence of propyl ring in indole thiazole ring makes favorable contributions in the highly active compounds. Hence, this study is useful for the discovery of new antagonists for xanthine oxidase receptor.

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