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3D-QSAR CoMFA studies on bis-coumarine analogues as urease inhibitors: A strategic design in anti-urease agents

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Dedicated in the memory of late Latief Ebrahim Jamal, a great philanthropist.

Abstract—A 3D-QSAR study has been performed on thirty (30) bis-coumarine derivatives to correlate their chemical structures with their observed urease inhibitory activity. Due to the absence of information on their active mechanism, comparative molecular field analysis (CoMFA) was used in the study. Two different properties: steric, electrostatic, assumed to cover the major contributions to ligand binding, were used to generate the 3D-QSAR model. Significant cross-validated correlation coefficients q^2 (0.558) and r^2 (0.992) for CoMFA were obtained, indicating the statistical significance of this class of compounds. The red electrostatic contour map highlighting those portion of compounds which may be interacting with nickel metal center in the active site of urease; while the blue contour map indicates positively charged groups in the ligands have improved biological activity and thus lower the IC_{50}s. The steric contour map shows that bulkier substitutions at the 'R' position are detrimental to ligand receptor interaction. Actual urease inhibitory activities of this class and the predicted values were in good agreement with the experimental results. Moreover, from the contour maps, the key features vital to ligand binding have been identified, which are important for us to trace the important properties and gain insight into the potential mechanisms of intermolecular interactions between the ligand and receptor.
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1. Introduction

Activities of ureases (E.C 3.5.1.5) have been shown to be an important virulence determinant in the pathogenesis of many clinical conditions, which is detrimental to human and animal health as well as agriculture. Urease is directly involved in the formation of infection of stones and contributes to the pathogenesis of urolithiasis, pyelonephritis, ammonia and hepatic encephalopathy, hepatic coma, and urinary catheter encrustation.[1,2] It is also known to be a major cause of pathologies induced by *Helicobacter pylori* (HP), which allows bacteria to survive at acidic pH of the stomach during colonization and, therefore, plays an important role in the pathogenesis of gastric and peptic ulcer (including cancer).[2] In agriculture, high urease activity causes significant environmental and economic problems by releasing abnormally large amounts of ammonia into the atmosphere during urea fertilization. This further induces plant damage primarily by depriving them of their essential nutrients and second ammonia toxicity, which increases the pH of the soil.[1,3] Therefore, strategies based on urease inhibition are now considered as the first line of treatment for infections caused by urease-producing bacteria. The important aims of a 3-QSAR model are to correlate the three-dimensional (3D) structures of drug molecules with their biological activities and to be able to predict the activity of new molecules prior to synthesis.[4] At present, one of the most widely used tools for QSAR and drug design is comparative molecular field analysis (CoMFA).[5] In

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order to make use of CoMFA, the following four procedures are required: (1) superposition of a set of molecules whose activities have been measured; (2) computation of the interaction energy fields with various probes; (3) statistical analyses to correlate the fields with activities; and (4) interpretation of the coefficients of the resulting QSAR equations. A multivariate data analysis method, e.g., partial least squares (PLS), is applied for the CoMFA models in order to derive QSAR equations from the results of descriptor field calculations.[6,7] We have previously reported a number of novel synthetic and natural inhibitors of urease and their inhibition kinetics and structure–activity relationship studies.[8,13–15] In continuation of our efforts to discover potent inhibitors and their mechanism of inhibition, the present effort is one of them. In a rational drug design, identification of the 3D-QSAR analysis is one of the important steps, especially when the active mechanism of the ligand and target protein remains unclear. Below, we present a CoMFA study on bis-coumarines, which are urease inhibitors. In the current study, we are presenting, for the first time, CoMFA study on bis-coumarine class of compounds.

2. Computational methods applied in 3D-QSAR modeling

2.1. Compounds and activities

The structures of these 30 compounds and their activities against *jack bean* urease have been taken from the literature which has been recently published by our research group.[8] The molecular structures of 30 compounds derivatives were modeled with the SYBYL 6.8 molecular modeling program (Tripos Associates, Saint Louis, MO) using the sketch approach. The fragment libraries in SYBYL database were used as building blocks for the construction of larger ones. Each structure was first energy minimized using the standard Tripos force field (Powell method and 0.05 kcal/(mol Å) energy gradient convergence criteria) and electrostatic charge was assigned by the Gasteiger–Hückel method. The values which are expressed as the pIC_{50} (log $1/IC_{50}$) are listed in Tables 1 and 2.

3. Computer 3D-QSAR modeling and structure alignment

Structural alignment is one of the most sensitive parameters in 3D-QSAR analyses. The accuracy of the prediction of a CoMFA model and the reliability of the contour models strongly depend on the structural alignment of the molecules. Since no X-ray crystallographic data of molecules were available, two different alignment methods, superimposition and field fit, were performed. The superimposition of molecules was based on trying to minimize root-mean-squares (rms) differences in the fitting of selected atoms with those of a template molecule. Compound **1** with the highest percent inhibition was used as a template molecule. All atoms of the benzene moiety of the bis-coumarine structural element were selected for superimposition. Conformations, which exhibited a minimum of rms after superimposition procedure, were selected and stored in the database for the next step. All molecules were aligned based on the assumption that they bind to urease in the same manner. Geometry optimization was carried out using MAXIMIN molecular mechanics and Tripos force field supplied within SYBYL. Structure alignment is shown in Figure 1.

3.1. CoMFA methodology

CoMFA was performed with the usually used steric and electrostatic fields in SYBYL (Tripos Associate Ltd., St. Louis, MO (1999). The Gasteiger–Hückel charges were applied in the determination of the electrostatic field. All CoMFA calculations were perform using electrostatic fields with Lennard-Jones and Coulomb-type potentials, dielectric constant $1/r$, and cutoff 30 kcal mol^{-1}) using an sp^3 carbon atom with a charge of +1.0 $|e|$. A lattice of 2 Å was generated to surround the whole molecular aggregates after the alignment. The surrounding lattice was selected with a sufficiently large margin (4 Å) to enclose all aligned molecules, and the grid spacing was set to 2 Å. All calculations in this study were performed in SYBYL. On AMD Athlon dual processor PC using Linux operating system.

3.2. CoMFA main features

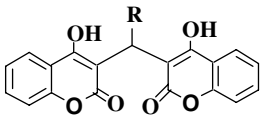
(1) Develop quantitative structure–activity relationships.
(2) Predict the properties and activities of untested molecules.
(3) Compare different QSAR models statistically and visually.
(4) Optimize the properties of a lead compound.
(5) Validate models of receptor binding sites.
(6) Generate hypotheses about the characteristics of a receptor binding site.
(7) Prioritize compounds for synthesis or screening.
(8) Determine key structural requirements for high affinity receptor ligands.

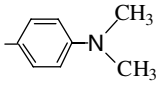
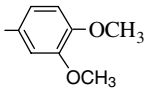
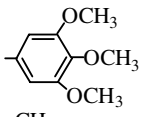
4. PLS analysis

Partial least squares (PLS), the statistical method used in deriving the 3D-QSAR models, is an extension of multiple regression analysis in which the original variables are replaced by a small set of their linear combinations.[9,10] These latent variables (components) so generated are used for multivariate regression, maximizing the commonality of explanatory and response variable blocks. PLS has been useful in cases in which the number of descriptors is greater than the number of samples (compounds) as is the case with comparative molecular field analysis (CoMFA).

Leave-one-out (LOO) cross-validated PLS analyses were used to check the predictive ability of the models

Table 1. Chemical structures of the bis-coumarine **1**–**30**[8]



Compound	R
1	H
2	–CH_3
3	
4	
5	
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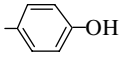
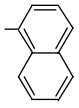
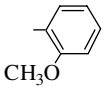
7	
8	
9	–$(CH_2)_2CH_3$
10	
11	
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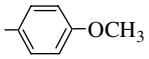
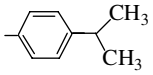
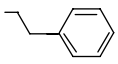
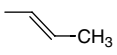
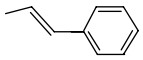
13	
14	–$(CH_2)_4CH_3$
15	
16	
17	
18	
19	–$(CH_2)_3CH_3$
20	

Table 1 (*continued*)

Compound	R
21	
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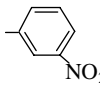
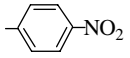
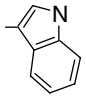
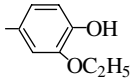
23	
24	
25	
26	
27	
28	
29	
30	

Table 2. Experimental activities arid predictive activities of CoMFA, all the values are in μM quantity

Compound	Experimental activities	Predictive activities
1	4.82	4.81
2	4.20	4.16
3	4.09	4.10
4	4.10	4.09
5	4.08	4.08
6	4.28	4.28
7	4.13	4.14
8	4.07	4.09
9	4.17	4.20
10	4.07	4.06
11	4.08	4.07
12	4.25	4.22
13	4.22	4.24
14	4.06	4.06
15	4.13	4.13
16	4.13	4.09
17	4.08	4.05
18	4.17	4.17
19	4.19	4.19
20	4.36	4.38
21	4.22	4.23
22	4.23	4.22
23	4.45	4.47
24	4.14	4.12
25	4.12	4.12
26	4.09	4.10
27	4.15	4.13
28	4.21	4.21
29	4.04	4.03
30	4.33	4.30

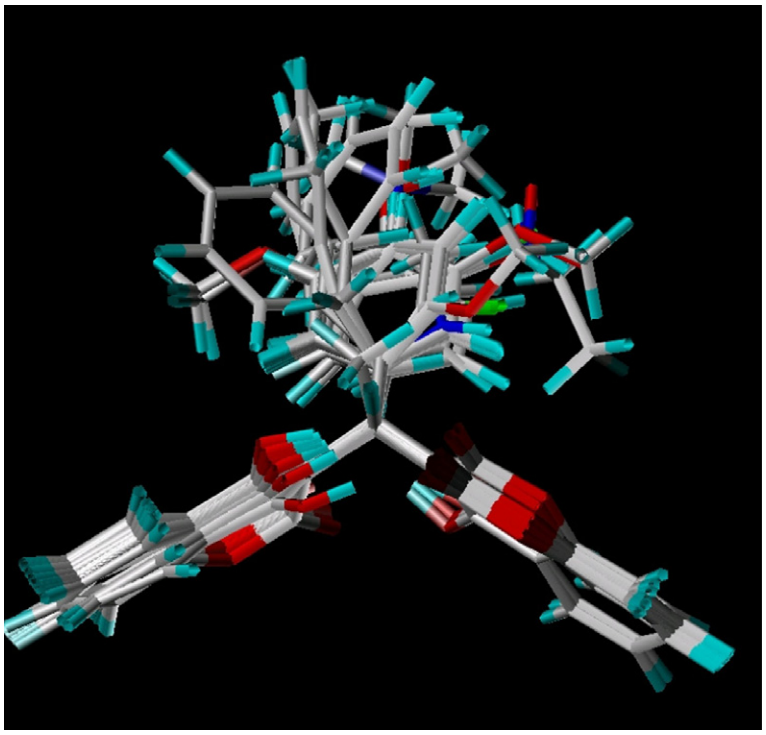


Figure 1. Plot of all 30 overlapped compounds used for the 3D-QSAR analysis. During the alignment, all of the other molecules were fitted to the template molecule, while maintaining their molecular geometry. Oxygen is shown in red, carbon in silver, hydrogen in cyan, nitrogen in blue, and chlorine in green.

and to determine the optimal number of components to be used in the final QSAR models. PLS analysis based on least-squares fit gave a correlation with a cross-validated q^2 of 0.344, with the maximum number of components being four. Upon detailed examination of the residuals for this calculation it becomes apparent that compounds **2**, **9**, **12**, **13**, **17**, and **23** were at least partially responsible for the medium q^2. Omission with the compounds **2**, **9**, **12**, **13**, **17**, and **23** as outliers from the aligned compounds and recalculation resulted in a much better q^2 of 0.558 at five components in the case of CoMFA. The non-cross-validated PLS analysis of these compounds was repeated with the optimum number of components, as determined by the cross-validated analysis, to give a r^2 of 0.992. The results of the analyses are shown in Tables 2 and 3. The chemical structures of (**30**) bis-coumarine compounds are shown in Table 1 and results are graphically shown in Figure 2 and contour map in Figure 3.

5. CoMFA contour maps

The QSAR produced by CoMFA, with its hundreds or thousands of terms, was usually represented as 3D 'coefficient contours.' Colored polyhedra in the maps represent those areas in 3D space where changes in the field values of those compounds correlate strongly with concomitant change in activities. The CoMFA steric and electrostatic fields for the analysis based on alignments of these bis-coumarines are presented as contour plots in Figure 1.

Table 3. Results of the CoMFA analysis

	CoMFA
q^2	0.558
r^2	0.992
Standard error of estimate	0.018
F	367.045
No. of compounds	30
Fraction	
Steric	0.711
Electrostatic	0.289
Grid spacing (Å)	2.0

6. Results and discussion

6.1. CoMFA using the inhibition of urease

Urease is a large heteropolymeric enzyme that catalyzes the hydrolysis of urea to yield ammonia and carbamate. Carbamate is unstable and spontaneously decomposes to yield a second molecule of ammonia and carbonic acid. The enzyme accelerates the rate of urea hydrolysis by at least 10^{14} over the spontaneous reaction by using a bimetallic nickel center. The active site contains two nickel (II) atoms which, as shown by X-ray analysis, are linked by a carbamate bridge; furthermore, two imidazole nitrogen atoms are bound to each nickel atom, and a carboxylate group and a water molecule fill the remaining coordination site of the metal ion.[11] In order to discriminate among the inhibition capacities of various compounds, it is important to understand the coordination mechanism between the active site of the

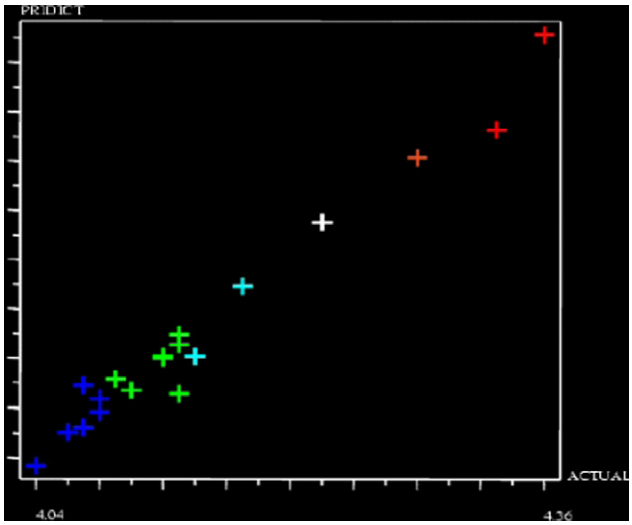


Figure 2. Plot of experimental versus calculated pIC_{50} values of biscoumarine **1**–**30**. Plot showing actual urease inhibitory activities of this class, and the predicted values were in good agreement with the experimental results.

enzyme and the inhibitor. With the urease inhibitory activity as response variable, initial CoMFA PLS analyses of the 30 compounds gave low q^2 values. Removal of the outliers based on residual values afforded models with a q^2 value of 0.558. All compounds were initially aligned by atoms 3, 4, 9, 12, and 13, 14 of the main skeleton which are common in all compounds. Removal of **2** and **17**, the compounds resulted in a vastly improved model, with a cross-validated q^2 of 0.484. This dramatic improvement suggests that these two compounds may be interacting with the receptor in a different way or interacting with receptor other than the active site. The graph of the actual pIC_{50} versus the predicted pIC_{50} values for 30 analogs by the CoMFA models based on urease inhibition is shown in Figure 2.

According to CoMFA results, the CoMFA contour maps were created by using the data from PLS analysis. These maps help us to explain the steric and electrostatic features of the compounds included in our analysis. The electrostatic contour maps are shown as red and blue contours in Figure 3, with the red indicating regions in which the electronegative groups in the ligands are associated with increased inhibition activities. The portions of compounds under red contour may be that position of compounds which interact with nickel atoms; this is because of the reason that only electronegative groups that can interact favorably with the positively charged nickel ions in the active site. These CoMFA results support our previously published results of these compounds.[8] Both lactones and both hydroxyl moieties are located in the same red region in the CoMFA contour map; these moieties are common in all compounds of this class which are included in the study. Blue contour map indicates that positively charged groups in the ligand have improved biological activity and thus lower the IC_{50}s, those electron-rich groups which are attached at the substituent 'R' decrease the activity like com-

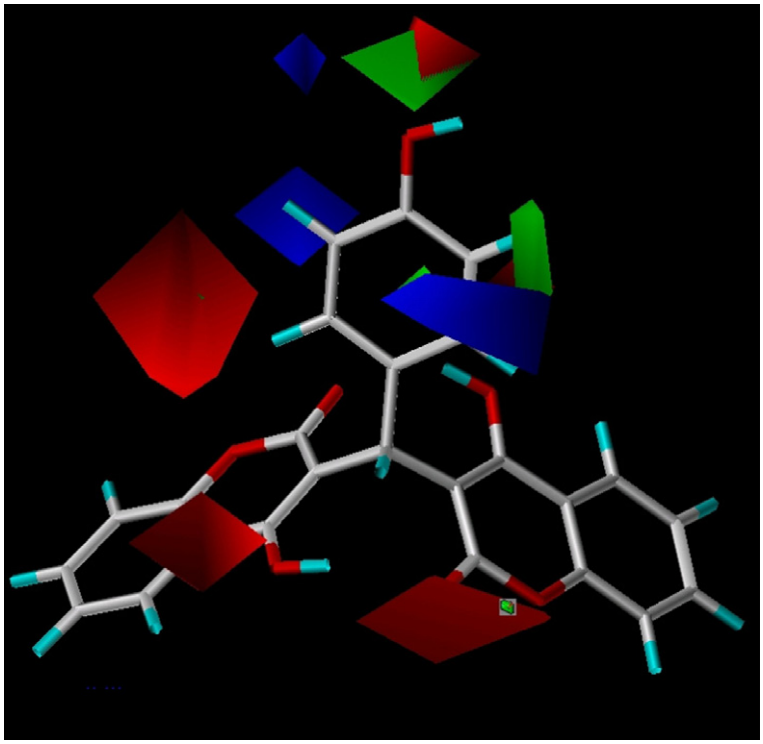


Figure 3. CoMFA electrostatic field contour map. Red regions indicate where biological activity is improved by a negative charge, while the blue colour indicates that electropositively charged moieties in this regions enhance activity. CoMFA steric field contour map. Yellow contours beyond the ligand indicate regions where steric bulk will be decreasing the biological activity. The very small green contours indicate regions where steric bulk might increase activity.

pounds 3, 4, 5, etc., with pIC_{50} values 4.09, 4.10, and 4.08, respectively. This indicates that more positive charge is favored in this region, which also supports previously published results[12] in which benzohydroxamic acid at pH 5.0 shows more potent activity than pH 8.0 this is because of the reason that at acidic pH 5.0 the benzohydroxamic acid is in a positively charged form.

The steric CoMFA map (Fig. 3) also indicates the areas in which steric bulk on an inhibitor is favored (green contours). The yellow contour represents regions in which steric bulk is detrimental to binding and hence increases the IC_{50}s by decreasing the overall inhibitory activity. Very small green contour may be hydrophobic site in the receptor, which can accommodate hydrophobic groups, for example compounds 2, 9, 12, 13, 17, and 13, etc., with pIC_{50} values 4.21, 4.17, 4.22, 4.06, 4.08, and 4.45, respectively; this also support, all other previously published results about the SAR of urease that highly bulky hydrophobic moieties are responsible for decreasing the activity.[13] This CoMFA analyses can be extremely useful in the design of new chemical moieties based on a prediction of their urease inhibitory activities.

7. Summary and conclusion

In the absence of previous information about the binding site in urease of these bis-coumarine analogues, the final CoMFA models provide a guide for the design of more active and potent analogs. CoMFA model has good cross-validated q^2 values, suggesting good predictive ability. The characteristics of the CoMFA 3D contour plots derived in this study helped us to understand the underlying mechanism of receptor–drug interaction. The present CoMFA study revealed that the benzene ring region and lactone moieties under the red contour possibly can bind with positively charged nickel metal ions in the active site of urease and furthermore bulkier substitutions at 'R' position are responsible for decrease of activity. We are synthesizing several bis-coumarine derivatives which have electron-donating and electron-withdrawing ability on substitutions at the benzene ring region to further elucidate the role of electron density requirement for binding to be evaluated against urease in vitro through co-crystallization and STD NMR studies to help further refine our model. This study of 3D-QSAR of the bis-coumarine derivatives is expected to provide rational information for designing new potential drugs.

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