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3D QSAR, PHARMACOPHORE INDENTIFICATION STUDIES ON SERIES OF 4-SUBSTITUTED BENZOTHIOPHENE ANALOGS AS FACTOR IXA INHIBITORS

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ABSTRACT

Factor IXA now a day is becoming attractive target for many researchers for the designing novel anticoagulants. The advances in the computational chemistry can be use full for designing of new chemical entities. The pharmacophore identification and QSAR studies on reported 51 factor IXa inhibitors have been carried out. QSAR model developed considering training and test set approaches with stepwize variable selection method. QSAR models which were further validated for statistical significance and predictive ability by internal and external validation. The hydrogen bond accepter, hydrogen bond donor, positively charged and aromatic carbon are the important features which are contributing towards the activity. The selected best QSAR model A has a training set of 40 molecules and test set of 10 molecules with correction coefficient of 0.9622.

Keywords: Factor IXA, Anticoagulant, 3D QSAR, PLS.

INTRODUCTION

Factor IXA is a vitamin k- dependent coagulation factor playing key role maintaining internal homeostasis in the intrinsic pathway of the clotting cascade. Now a day with the increase of mortality rate due to ischemic diseases and thrombosis, development of safer anticoagulant is current area of interest for the medicinal chemists. Factor IXa can act as attractive target from the other serine protease in the coagulation cascade because of inhibition of factor IXa can provide the safe and clinically effective anticoagulants. Factor IX is single chain glycoprotein which is synthesised by the hepatocytes as precursor protein.[1-13] Quantitative structure-activity (QSAR) studies are of great importance in modern chemistry. The goal of QSAR is to transform searches for compounds with desired properties using chemical knowledge and experience into a mathematically quantified form. Once a correlation between structure and activity is found, number of compounds with desired properties can be synthesised. Thus, the QSAR approach accelerates the process of development of new molecules for use as drugs. In Partial least squares regression is an extension of the multiple linear regression models. In its simplest form, a linear model specifies the (linear) relationship between a dependent (response) variable Y, and a set of predictor variables X's. PLS regression, the X block of independent variables (descriptors) is correlated with the y vector (activities) in such a way that the projected coordinates, T, are good predictors of y. partial least squares regression is probably the least restrictive of the various multivariate extensions of the multiple linear regression model. This flexibility allows it to be used in situations where the use of

traditional multivariate methods is severely limited, such as when there are fewer observations than predictor variables. Furthermore, partial least squares regression can be used as an exploratory analysis.[14-21] We present here our Pharmacophore identification and 3D-QSAR studies using PLS method on a training set of 4 substituted benzothiophene derivatives as Factor IXa inhibitors by considering the steric and electrostatic influences. The model derived from this investigation having good predictive ability, which could aid new Factor IXa inhibitors prior to their synthesis.

Computational Details

Dataset

A dataset of 51 compounds was taken from the published factor IXa inhibitors by wang et.al.[22] The structures and their inhibitory activities are listed in Table 1. The whole dataset was ra2ndomly divided into a training set of 40 compounds and a test set 11 of compounds (asterisked molecules in Table 1). The training set was used to construct 3D-QSAR models and the test set was used for the models validation.

MATERIALS AND METHODS

Ligand Preparation

The structure of benzothiophene was used as template to built the molecules in the dataset in Vlife MDS 3.5. All the structure was minimized using the standard Merck molecular force field (MMFF) with distance dependant dielectric function and energy gradient of 0.001 kcal/mol A^0.

Molecular Alignment

The molecules of the dataset were aligned by the template based technique, using common structure of benzothiophene. The alignment of all the molecules on the template is shown in figure.no.2.

Descriptor Calculation

Like many 3D QSAR methods, a suitable alignment of given set of molecules was performed using the Vlife MDS 3.5 Engine. This was followed by generation of a common rectangular grid around the molecules. The hydrophilic, steric and electrostatic interaction energies which are computed at the lattice points of the grid using a methyl probe of charge +1.

3D QSAR Studies Using Partial Least Squares Regression

A relationship between independent and dependent variables (3D fields and biological activities, respectively) were determined statistically using regression analysis. Linear regression is achieved by fitting a best-fit straight line to the data using the least squares method. The quality of fit for a regression equation was assessed relative to its correlation coefficient and standard deviation. The F value represents the level of statistical significance of the regression. Quality of selected models was further ascertained to select the best model from cross-validated squared correlation coefficient (q2).For a regression model, r2 was used to describe the fitness of data and fitness is considered to improve as r2 approaches 1. Thus models having correlation coefficient above 0.7 were used to check the external predictivity while the significance of the model was decided on the basis of F value. Models showing q2 below 0.6 were discarded. The selected models are shown in Table 2.

Pharmacophore Modeling

Pharmacophore modeling was carried out using the mol sign module of Vlife Mds 3.5 software and ligand scout 3.02. Series of factor IXa inhibitors were first aligned on the active molecule. The software was set to generate minimum 4 pharmacophoric features obtained keeping the tolerance limit at 10 A^0.

RESULTS

In the present study, 40 molecules were used in the training set (Table 1) to derive 3D QSAR models with the number of field grid points being not more than five per model. To evaluate the predictive ability of

generated 3D-QSAR models, and test set of 11 molecules with regularly distributed biological activities was used (Table 1). On successful run of PLS two models were selected they are shown in table 2.

DISCUSSION

Interpretation of 3QSAR Model

The optimum structural properties of benzothiophene analogs for factor IXA inhibition were obtained in the form of the 3D descriptors of model A. The r^2 value for model A was 0.9622 while that of model B was 0.9430 (figure 2). Model A shows the first model which is selected on the basis of statistical coefficient like r2 (0.9622) and Pred r2 (0.8123). The contributing descriptor for model A are S_652, S_389, E_800, E_944 which are nothing but the electrostatic and steric interaction at that lattice point. The electrostatic interaction at lattice point E_800 is negatively contributing means substitution of electron withdrawing groups can yield potent factor IXa inhibitors. The derivatives which are having the halogen substitution on the aryl ring attached to amide bridge are showing more activity than other derivatives. The Electrostatic interaction at the lattice point E_944 and steric interaction at lattice points S_652, S_389 are positively contributing so the substitution favoring this interaction at that lattice point could yield active molecule. Model B r2 (0.9430) and Pred r2 (0.6154) is not as good as model A in terms of correlation (figure 3 & 4) of the selected field descriptors with biological activity as well as its predictive ability (table no2).

Pharmacophore Identification Studies Using Vlife MDS 3.5

A set of pharmacophore hypothesis was generated using the mole sign module of V life MDS 3.5 on the reported inhibitors of factor IXa. Each hypothesis was found to contain common features like hydrogen bond doner, hydrogen bond acceptor, positive ionizable and aromatic.

The pharmacophore hypothesis generated in V life MDS 3.5 (figure no 5) indicated the significance of presence of two aromatic features for the factor IXa inhibition, these features are contributed by the benzothiophene nucleus, which are separated by 1.2 $A^{0.}$ The positive ionizable is also important feature for factor IXa inhibition, in present data set the amidine group is contributing this feature. The ester oxygen is contributing the hydrogen bond acceptance while the hydrogen bond donation is contributed by the substitution on the aromatic rings.

The pharmacophore hypothesis generation also carried out by the Ligand scout 3.2(figure 6), the hyphothesis generated showed significance of hydrogen bond doner, hydrogen bond acceptor, positive ionizable and aromatic. The amidine group contributing the positive ionizable feature which can be responsible for interaction with acidic amino acids like aspartic acid. The amino group present in the amidine also found to contributing the hydrogen bond donation. The carbonyl oxygen's are acting as hydrogen bond acceptors while amide nitrogen is acting as hydrogen bond donor. The aromatic features which are required for PI- PI interaction with the receptors are contributed by the two aromatic rings and benzothiophene ring in the structure.

CONCLUSION

The present communication is an attempt to indentify the structural requirement of benzothiophene analogs for inhibition of factor IXA. The pharmacophoric requirement of factor IXA inhibition are also been identified by the generation of two different hyphothesis, having similar results thus The model derived from this investigation having good predictive ability, which could aid new Factor IXA inhibitors prior to their synthesis.

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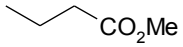
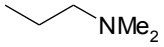
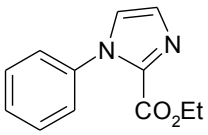
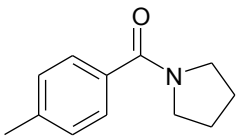
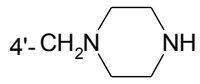
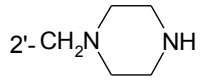
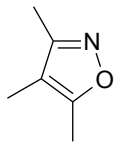
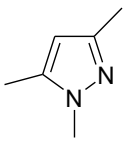
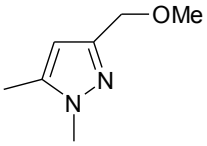
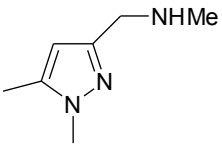
Table 1: Showing derivatives under study with observed and predicted activity

Sr. No.	Compound code	R	Observed activity	Predicted activity
1.	16	----	0.27	0.06
2.	26	----	5.15	5.03
3.	27		0.5	0.93
4.	28		8.4	8.49
5.	29		1	0.69
6.	30	Ph	0.17	0.03
7.	31	Bn	0.39	0.99
8.	32	2'-MeO-Ph	0.14	-0.01
9.	33	3'-MeO-Ph	0.13	0.32
10.	34	4'-MeO-Ph	0.14	-0.01
11.	35		0.42	-0.03
12.	36	4'-(Me_2NCO)-Ph	0.37	0.22
13.	37		0.29	0.24
14.	38	NH_2	0.16	0.35
15.	39	NHCOMe	0.34	0.23
16.	40	NHBn	0.1	0.30
17.	41	$NHCO_2Ph$	0.05	0.15
18.	42	NHCONHPh	0.029	-0.07
19.	43	OH	0.27	0.32
20.	44	OCONHMe	0.097	0.10
21.	45	OCONHEt	0.028	-0.18
22.	46	OCONHPh	0.01	0.29
23.	47	OCONHBn	0.061	0.41
24.	146	H	0.01	0.02
25.	48	2'-F	0.005	0.09

26.	49	3'-F	0.025	0.44
27.	50	4-F'	0.008	0.10
28.	51	2'-Me	0.008	0.15
29.	52A(R)	4'-MeO	0.003	0.08
30.	52B(S)	4'-MeO	0.0035	0.16
31.	52	4'-MeO	0.005	0.002
32.	53	4'-O(CH_2)2NMe_2	0.004	0.13
33.	54		0.01	0.045
34.	55	2'-CH_2NH_2	0.004	0.001
35.	56	2'-CH_2NHMe	0.003	-0.01
36.	57	2'-CH_2NHBu-i	0.002	0.007
37.	58	2'-CH_2NMe_2	0.008	-0.20
38.	59		0.003	0.001
39.	60	2',4'-F,F	0.007	0.002
40.	61*	2',6'-F,F	0.035	0.026
41.	62*		0.012	-0.019
42.	63*		0.01	0.21
43.	64*		0.009	0.31
44.	65*		0.005	-0.004

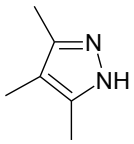
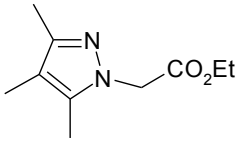
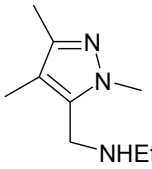
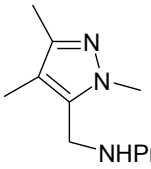
45.	66*		0.008	0.09
46.	67*		0.011	0.008
47.	68*		0.005	0.060
48.	69*		0.003	-0.007
49.	70*	H	0.0155	0.029
50.	71*	2'-CH_2NH_2	0.0044	0.001
51.	72*	2'-CH_2NMe_2	0.0081	0.003

Table 2: Showing the selected MLR QSAR equations along with statistical parameters employed for model selection.

Model No.	QSAR model	N	r2	q2	F value	Pred r2
A	Ki= 0.0546+0.3583 S_652+0.1586 S_389-0.0357 E_800+0.0310 E_944	51	0.96	0.94	54.13	0.81

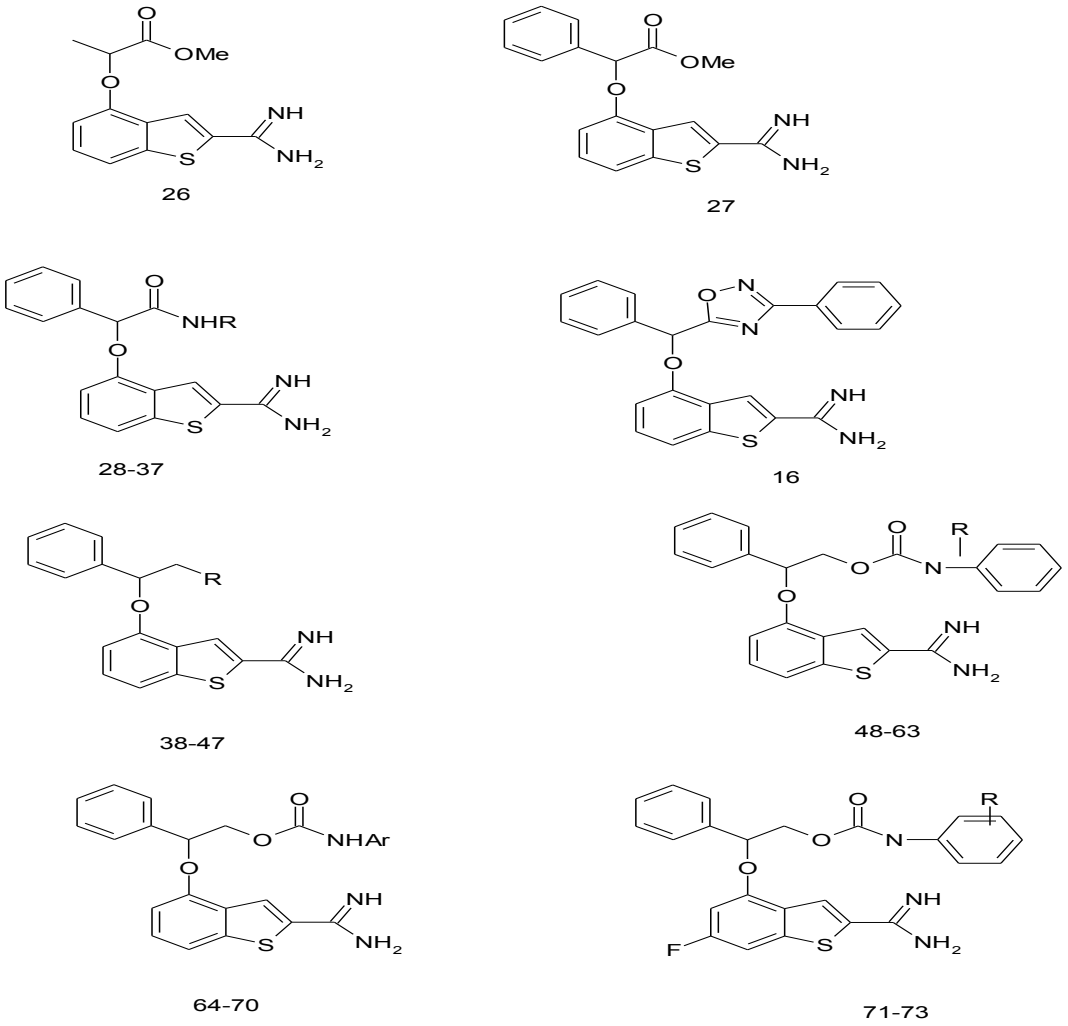


Figure 1: Showing molecules under study

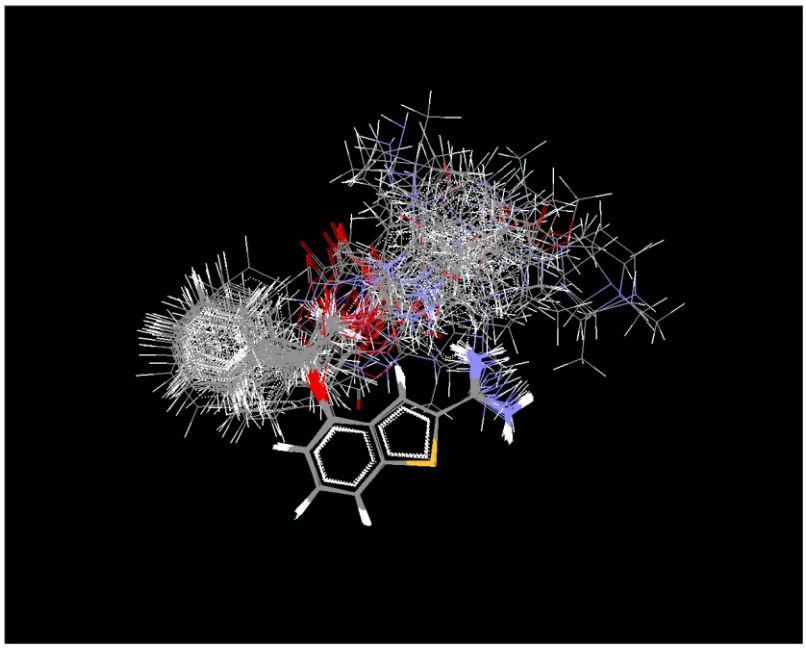


Figure 2: Showing the alignment of the molecules

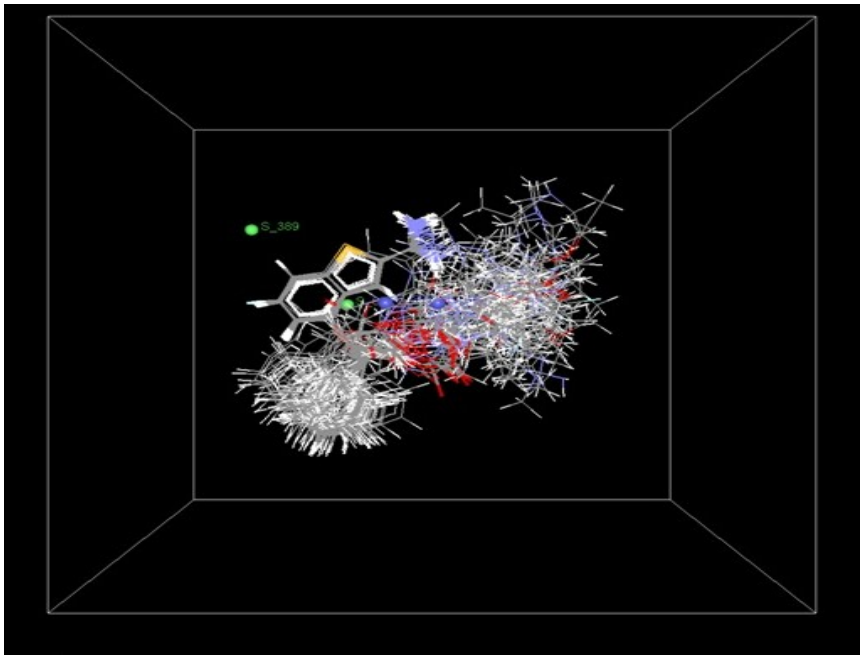


Figure 3: Showing the field point of selected QSAR model A

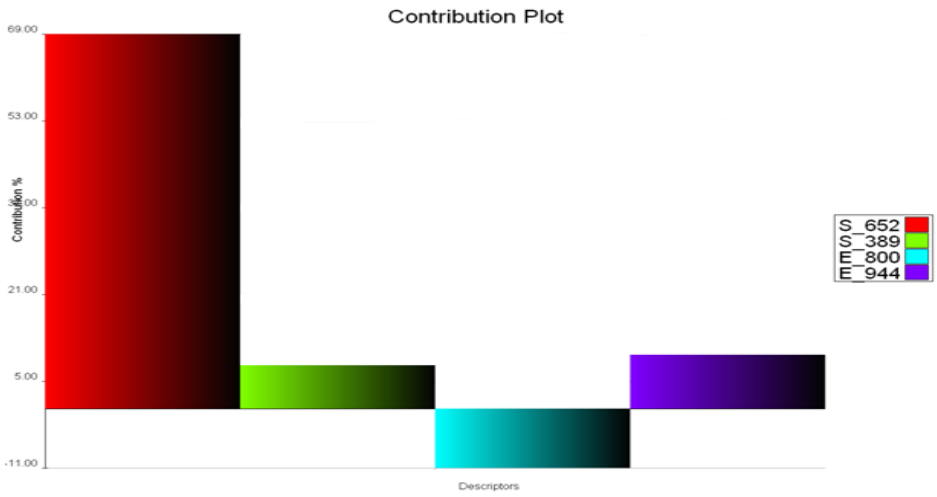


Figure 4: Showing contribution plot of selected QSAR model A

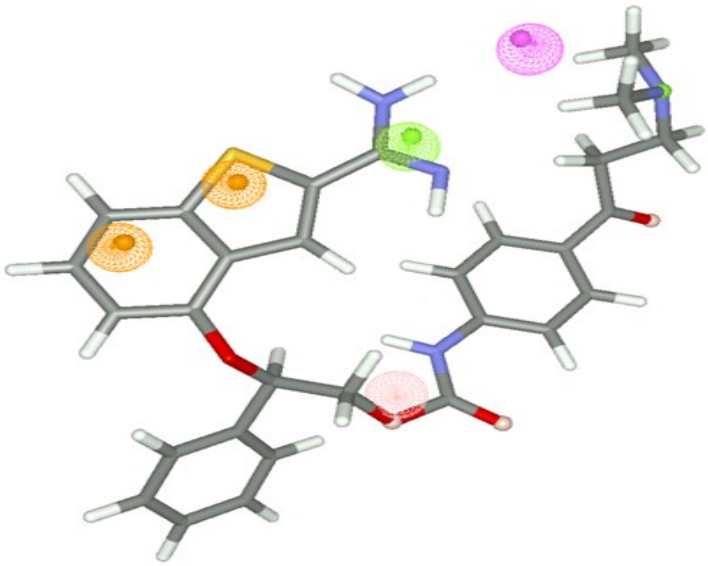


Figure 5: Showing selected pharmacophore model generated through Vlife MDS

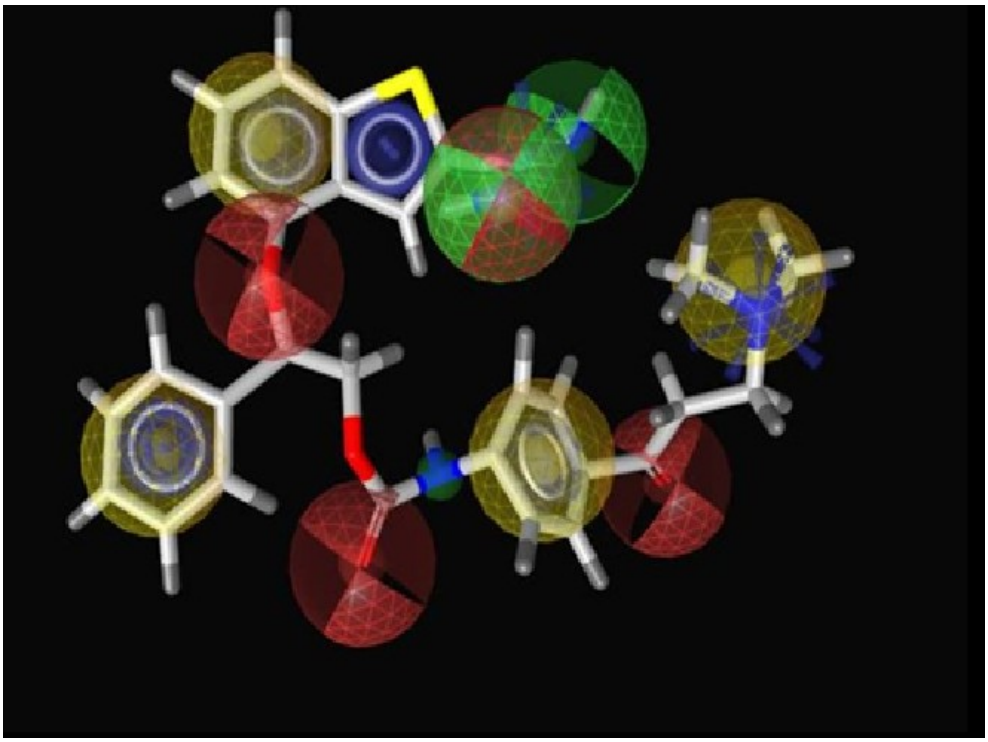


Figure 6: Showing selected pharmacophore model generated through Ligand Scout 3.2

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