

## Human Leukocyte Elastase Inhibitors: Analog Based Studies to Design Novel Lead Compounds for the Treatment of COPD

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### Abstract:

Human Leukocyte Elastase is a serine protease that is released from leukocytes upon inflammatory stimulus. HLE is thought to aid in the migration of neutrophils to extra vascular compartments through degradation of a number of structural proteins including elastin. Normally this enzyme is kept in check by indigenous inhibitors, most notably alpha-1 proteinase. However alpha-1 p1 may be damaged by cigarette smoking, or because of genetic defect, produced in insufficient quantities. In either case, the balance between HLE and alpha-1 p1 is upset which can lead to tissue damage which manifests itself in diseases such as chronic obstructive pulmonary diseases, asthma, emphysema, cystic fibrosis, and adult respiratory distress syndrome. Consequently there has been concerted effort to develop low molecular weight inhibitors of HLE to treat these disease states. We have performed Pharmacophore and 3D-QSAR studies for developing novel HLE inhibitors using the Catalyst 4.7 and Cerius2 program suite respectively. QSAR equations have been generated for 58 HLE inhibitors employing Molecular Field Analysis (MFA) as well as Receptor surface Analysis (RSA) using Genetic function approximation (GFA) as regression method. The best equations with training set consisting 40 molecules, produced r<sup>2</sup> value of 0.845 and r<sup>2</sup><sub>cv</sub> value of 0.839 in MFA-model and r<sup>2</sup> value of 0.880 & r<sup>2</sup><sub>cv</sub> of 0.856 in the RSA-model. For the 18 test set molecules predicted activities have correlation of 0.845 and 0.880 for MFA and RSA with observed activities. Pharmacophore models were generated using 21 molecules as training set. The best quantitative pharmacophore model consists of hydrogen bond acceptor, hydrophobic aliphatic and hydrophobic aromatic feature. For the training set the accuracy in predicting active and inactive compounds was 80%. The best pharmacophore hypothesis yielded a RMS deviation of 1.06 and a Correlation coefficient of 0.943 with a Cost difference (Null cost minus Total cost) of 79.02. The obtained pharmacophore models were validated on 50 test molecules to give correlation value of 0.846.

**Keywords:** QSAR, CAT B, MFA, RSA, Catalyst, Pharmacophore.

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### Introduction:

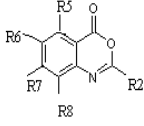
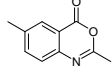
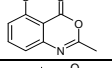
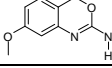
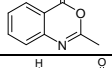
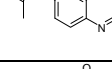
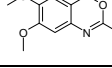
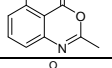
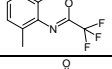
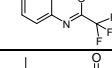
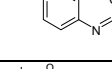
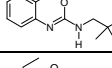
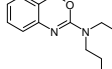
Human Leukocyte Elastase (HLE) is a serine protease stored in and secreted from neutrophils that plays a determinant role in the pathogenesis of several lung diseases. It is also known as human neutrophil elastase, and has been assigned a unique number by the Enzyme Commission of the International Union of Biochemistry based on its activity [1-3]. We have performed analog based design, i.e. MFA and RSA studies along with Pharmacophore hypothesis development on a series of Human Leukocyte Elastase (HLE) inhibitors [4-8]. We intend to utilize the pharmacophore information to undertake 3D searches on large databases to identify Novel Lead candidate against HLE inhibitors for treatment of chronic obstructive pulmonary diseases [9-15].

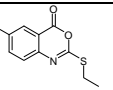
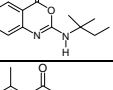
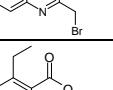
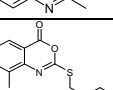
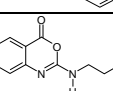
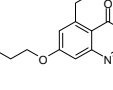
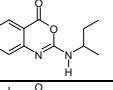
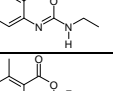
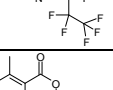
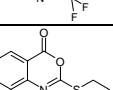
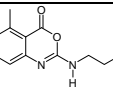
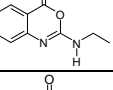
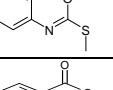
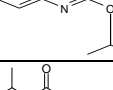
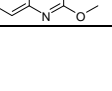


### Experimental work:

All molecular modeling works were performed on a Silicon Graphics Octane2 R12000 computer running Irix 6.5.12 (SGI, 1600 Amphitheatre Parkway, Mountain View, CA 94043) using Cerius2 4.6 and Catalyst 4.7 version. 40 molecules forming the training set were used to generate the QSAR equation. For MFA studies molecular field was created using proton and methyl groups as probes, which represent electrostatic and steric fields respectively. For RSA studies chemical properties namely charge, electrostatic potential, hydrogen bonding propensity and hydrophobicity associated with each surface point were calculated. For generating equations, only 10% of the total descriptors whose variance was highest were considered for further analysis. Regression analysis was carried

out using G/PLS method consisting of over 50,000 generations with a population size of 100. (Table.1)

**Table 1 :** Human leukocyte elastase inhibitors with observed and calculated biological activities possessing the following scaffold.

Scaffold		$\mu\text{M}$ $\text{IC}_{50}$	Predicted activity	
			MFA RSA	
Training set molecules				
1		4.0460	4.909	4.133
2		4.840	5.564	5.609
3		5.100	5.572	5.969
4		5.320	5.572	5.971
5		5.840	6.667	6.872
6		5.920	5.992	5.692
7		6.000	6.115	7.076
8		6.270	5.983	6.627
9		6.290	6.310	6.321
10		6.470	5.992	6.560
11		6.620	6.116	7.135
12		6.750	7.15	6.916

13		6.770	6.777	6.777
14		6.810	7.7002	7.640
15		6.820	6.3452	7.158
16		6.920	6.6382	7.181
17		6.960	6.323	7.203
18		7.080	7.609	7.436
19		7.280	7.618	6.744
20		7.360	6.349	6.247
21		7.470	8.062	7.322
22		7.510	7.952	6.796
23		7.540	7.358	7.671
24		7.550	7.618	6.991
25		7.640	8.005	7.508
26		7.670	8.265	7.915
27		7.680	7.618	6.950
28		7.940	7.609	8.085
29		8.000	8.115	7.103

30		8.090	7.618	7.846
31		8.110	7.610	7.559
32		8.240	7.618	8.005
33		8.310	7.619	8.015
34		8.380	8.540	8.323
35		8.880	7.928	9.208
36		9.0300	9.008	8.425
37		9.330	8.443	9.646

#### Test set molecules

1		5.670	5.991	6.037
2		6.380	5.816	6.446
3		6.660	6.133	6.417
4		6.770	5.979	6.495
5		6.950	7.608	6.919
6		6.960	7.611	6.857
7		7.040	7.730	6.331
8		7.406	6.444	6.891

9		7.420	7.832	7.481
10		7.680	7.618	7.139
11		8.000	9.026	9.407
12		8.190	8.726	8.114
13		8.850	9.012	8.164
14		9.40	9.055	9.057
15		9.720	9.374	9.547

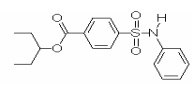
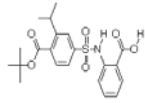
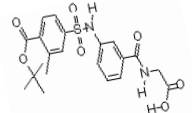
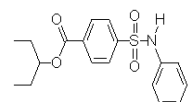
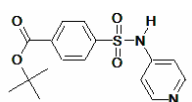
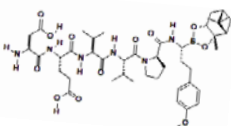
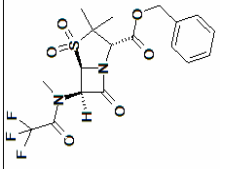
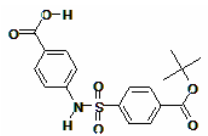
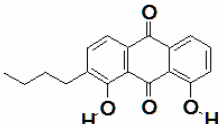
Catalyst version 4.7 was used to generate Pharmacophore models. 21 molecules forming the training set were used to generate Hypogen hypothesis.

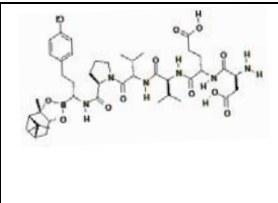
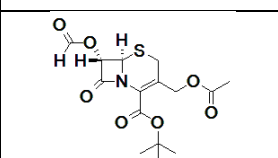
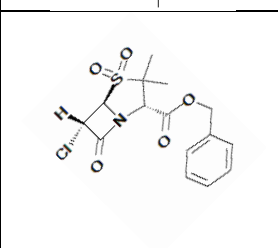
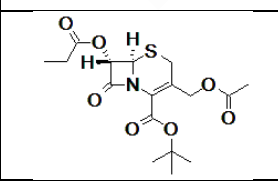
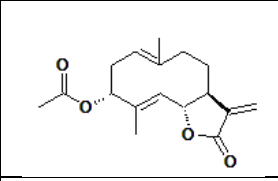
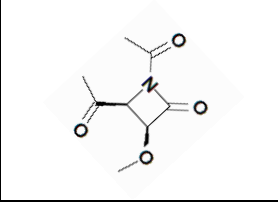
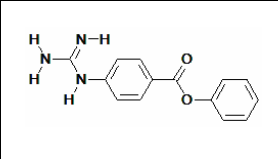
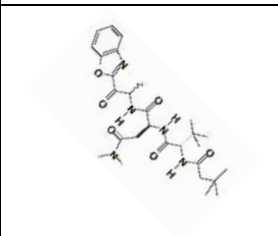
**Table 2:** Statistical Parameters for MFA and RSA

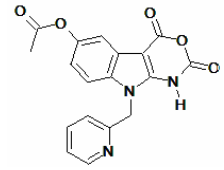
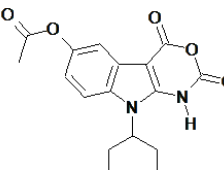
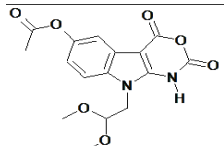
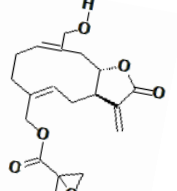
Statistical Parameters	MFA RSA	
R <sup>2</sup>	.839	.856
XVR <sup>2</sup>	.793	.739
Outliers	2	2
BSR <sup>2</sup>	.845	.798
BSR <sup>2</sup> ERROR	.002	.013
PRESS	10.56	9.26

All structures were built and minimized within the Catalyst software package, and conformational analysis of each molecule was implemented using the poling algorithm. Hypotheses were generated from a collection of conformational models of compounds spanning activities of 4-5 orders of magnitude.

**Table 3.1:** Training Set with Experimental and Predicted Activity

M.. No	Structure	IC50		Error F	it Value	Activity Scale	
		Experimental Pr	redicted			Exp	Pre
1		0.001	0.013	1.3	9.42	+++	+++
2		0.092	0.22	2.4	7.59	+++	+++
3		0.12	0.14	1.2	7.87	+++	+++
4		0.27	0.22	1.2	7.48	+++	+++
5		0.4	2.9	7.3	6.66	+++	++
6		0.56	0.366	1.5	7.4	+++	+++
7		0.7	2.3	3.2	7.35	+++	++
8		0.97	2.5	2.6	6.80	+++	++
9		1.4	1.2	1.2	6.89	++	++

10		6.4	2.2	2.9	6.25	++	++
11		10	15	1.5	5.95	++	++
12		14	6.4	2.2	5.47	++	++
13		36	23	1.5	5.79	++	++
14		70	73	1	5.70	++	++
15		94	1100	12	4.10	++	+
16		150	310	2.1	4.21	+	+
17		180	13	14	6.25	+	++

18		290	350	1.2	4.21	+	+
19		450	300	1.5	4.45	+	+
20		740	320	2.3	4.21	+	+
21		1000	100	9.9	5.73	+	+

## Results and discussion:

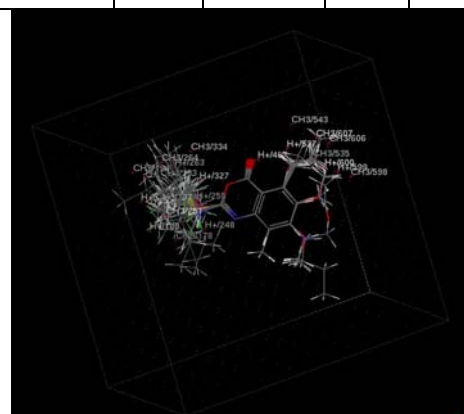
### Molecular field analysis (MFA)

The regression analysis on training set molecules produced a QSAR model and is shown in eq. 1

$$\text{Activity} = -0.046748 * \text{"CH3/334"} + 0.03696 * \text{"H+/467"} + 0.04704 * \text{"CH3/543"} - 0.027816 * \text{"H+/600"} + 0.042922 * \text{"CH3/193"} + 0.064957 * \text{"CH3/187"} - 0.025432 * \text{"CH3/606"}$$

### Receptor surface analysis (RSA)

The QSAR model generated by RSA is represented as eq 2



**Fig 1:** Molecular Field Analysis Grid (3D-QSAR studies)

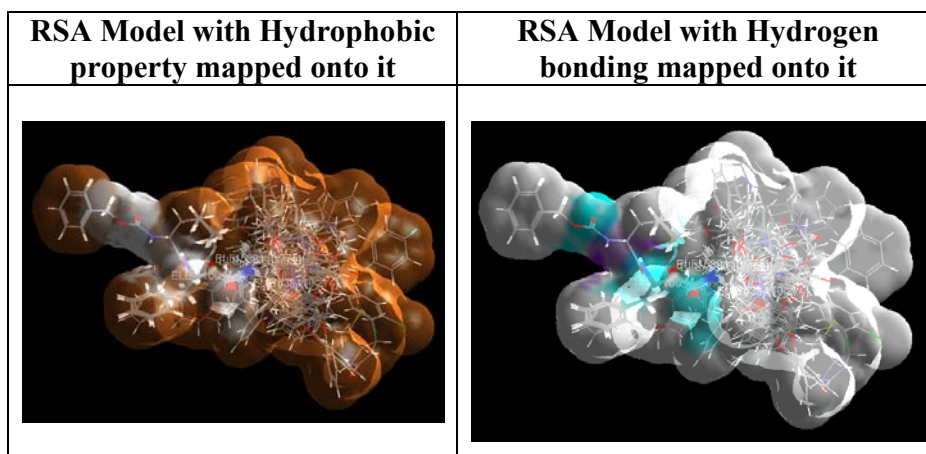


Fig 2: Receptor Surface Models

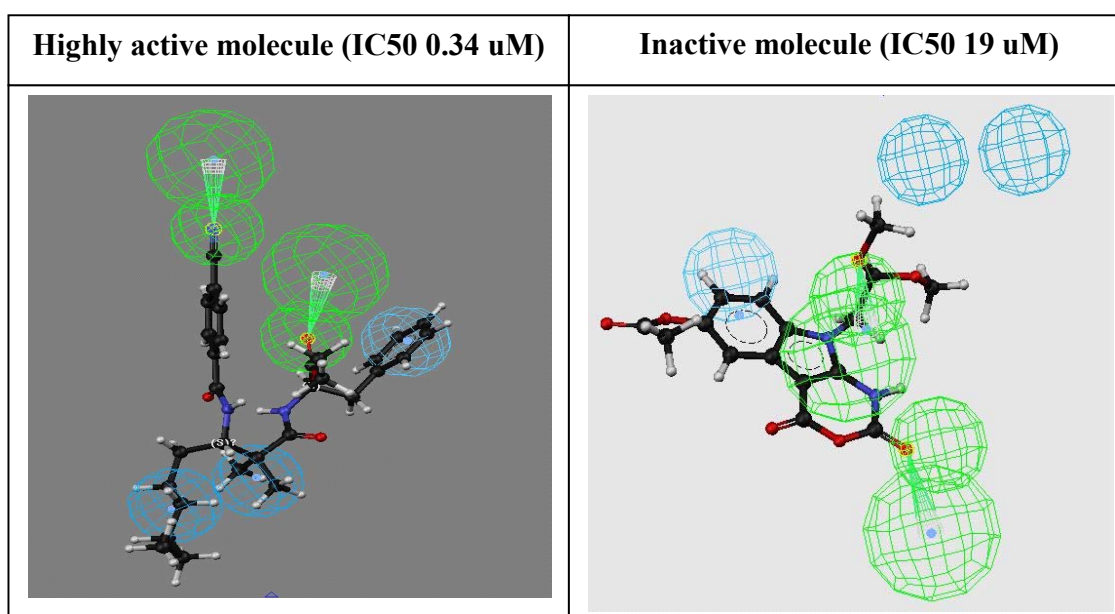


Fig 3: Pharmacophore Mapping

$$\begin{aligned} \text{Activity} = & -0.90135 - 34.8009 * \\ & \text{"ELE/1380"} + 36.9685 * \text{"VDW/1104"} \\ & - 1.6223 * \text{"ELE/2615"} - 1.73236 * \\ & \text{"VDW/1618"} - 4.48241 * \text{"ELE/1974"} \\ & + 9.67656 * \text{"VDW/2150"} - 4.50458 * \\ & \text{"ELE/2344"} \end{aligned}$$

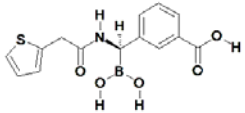
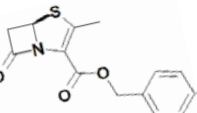
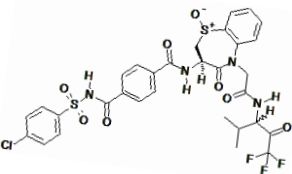
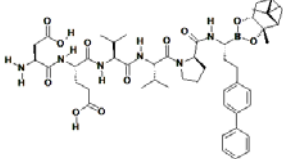
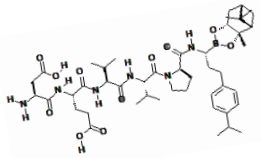
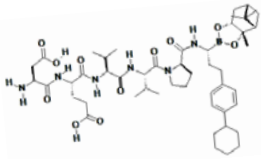
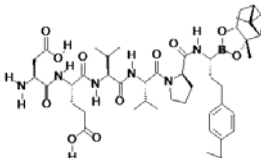
The statistical parameters for MFA and RSA are given in Table 2. The Stereo view of rectangular molecular field surrounding aligned molecules and receptor surface which represents the virtual active site is shown in Fig 1 and 2 respectively.

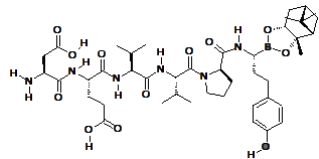
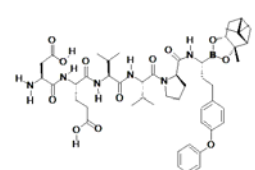
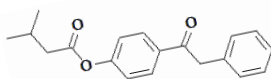
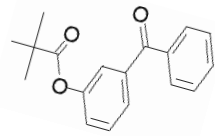
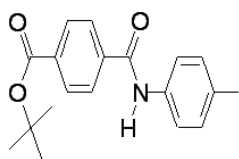
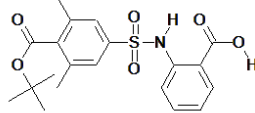
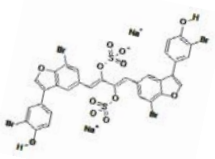
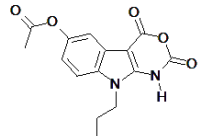
### Pharmacophore Hypothesis Generation:

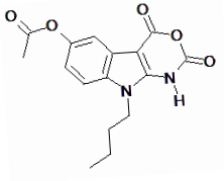
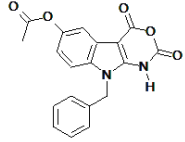
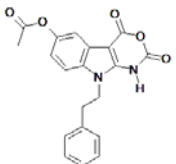
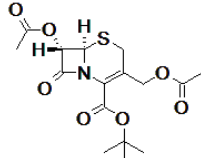
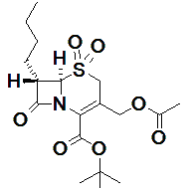
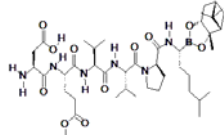
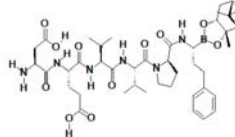
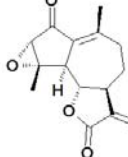
Training set consists of 21 compounds tested against HLE was used to develop Pharmacophore hypotheses. A total of 10 hypotheses were generated and its different cost values, correlation coefficients (r), RMS deviations, and pharmacophore feature definitions are listed in Table 3.

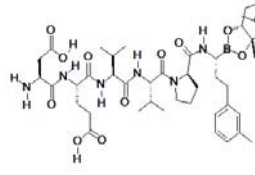
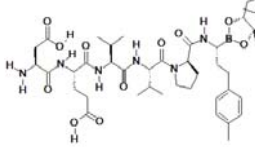
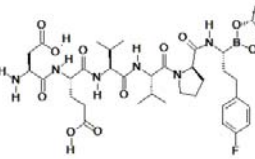
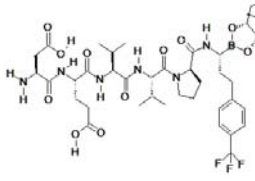
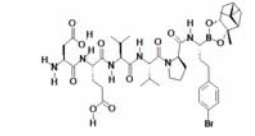
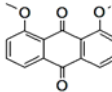
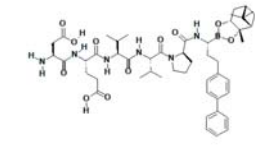
For the training set the accuracy in predicting active and inactive compounds was 80%.

**Table 3.2:** Test Set with Experimental and Predicted Activity

M.No S	structure	IC50 $\mu$ M		Error	Activity Scale	
		Exp P	redicted		Exp	Predicted
22		500	350	-1.4	+	+
23		15	28	1.9	++	++
24		6.8	14	21	+++	++
25		0.9	4.6	5.2	+++	+++
26		0.45	4.1	9.2	+++	+++
27		0.4	4.6	1.2	+++	+++
28		0.34	4.2	12	+++	+++

29		0.9	5	5.6	+++	+++
30		0.22	4.4	20	+++	+++
31		1.9	18	9.6	+++	++
32		6.66	29	4.4	+++	++
33		100	13	-7.6	++	++
34		0.96	6.8	7	+++	+++
35		0.47	7.3	16	+++	+++
36		17.5	12	-1.5	++	++

37		665	410	-1.6	+	+
38		810	400	-1.6	+	+
39		75	510	6.8	+	+
40		92	650	7.1	++	+
41		40	24	-1.7	++	++
42		40	64	-1.6	++	++
43		7.3	23	3.1	+++	++
44		3.5	6.6	1.9	+++	+++

45		110	49	-1.2	+	++
46		5.7	4.9	-1	+++	+++
47		5	4.8	-1	+++	+++
48		0.8	9.9	12	+++	+++
49		1.8	4.5	2.5	+++	+++
50		1.6	5	3.1	+++	+++
51		19	6.2	-3.1	++	++

+ = Low active    ++ = Moderately active    +++ = Highly active

**Table 3.3:** 10 Pharmacophore Hypotheses Generated Using 21 Training Set Molecules

Hypothesis number	Total Cost	Cost diff (null-Total)	RMS deviation	Training set correlation (r)	Features	Test set Correlation (r)
1	100.857	79.028	1.06899	.9435567	A ,A ,H ,H ,Z	.819387
2	106.066	73.819	1.18453	.931674	A ,A ,H ,Z	.809371
3	107.143	72.742	1.32568	.911664	A ,A ,H ,H ,Z	.757693
4	107.506	72.379	1.34072	.909527	A ,A ,H ,H ,Z	.71806
5	108.263	71.662	1.36745	.905694	A ,A ,H ,H ,Z	.65848
6	108.772	71.113	1.38425	.903245	A ,A ,H ,H ,Z	.62456
7	109.26	70.625	1.33177	.911934	A ,A ,H ,Z	.618473
8	109.551	70.334	1.40963	.899473	A ,A ,H ,H ,Z	.795995
9	110.854	69.031	1.42659	.897321	A ,A ,H ,H	.759223
10	111.13	68.775	1.46328	.891099	A ,A ,H ,H ,Z	.661418

A = hydrogen bond acceptor    H = hydrophobic aliphatic    Z = hydrophobic aromatic

extremely grateful to Dr. J. A. R. P. Sarma, GVK Biosciences for providing

The selected pharmacophore hypothesis yielded a RMS deviation of 1.06 and a correlation coefficient of 0.943 with a cost difference of 79.02. The best pharmacophore model was validated on 50 test molecules to give correlation value of 0.846. For the test set, the accuracy in predicting active compounds was greater than 10 %, while 16% and 8% representing both false positive and negative respectively. The mapping of Hypothesis model onto an active and inactive training set compound is shown in Fig 3.

#### Conclusion:

The 3D QSAR studies show that hydrophobic groups are responsible for increase in activity, which shows that RSA model predicts better than MFA. Further, the knowledge of this four-feature pharmacophore hypothesis for Human Leukocyte Elastase inhibitors can be very useful for virtual screening to design more potent lead moieties for the treatment of various types of COPD.

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