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RESEARCH ARTICLE

Pharmacophore-based Virtual Screening and *In-vitro* activity Evaluation of *α*-Chymotrypsin Inhibitors.

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Abstract

Virtual screening is a high through put technique which provides useful information towards drug designing and discovery process in a timely manner. In our case of study, we used *in silico* prediction methods (structure-based and ligand-based) to identify *drug-like* candidates using our *in-house data-base* against *α*-chymotrypsin enzyme. Over expression of *α*-chymotrypsin is associated with various gastro intestinal tract (GIT) disorders such as intestinal bowel syndrome (IBS), gastro esophageal reflux disease (GERD) and Crohn's disease. Chymostatin is the most commonly available drug used for the treatment, but due to its hepatic toxicity and protein degradation of skeletal muscles, there is a strong need to develop new *α*-chymotrypsin inhibitors with improved potency and reduce toxic effects. From our current study we identified nineteen hits which were further subjected for *in-vitro* screening. Experimentally eight compounds showed inhibition against *α*-chymotrypsin receptor; while three compounds considered as good inhibitors with an IC_{50} value of 319.8, 474.8 and 481.3 μM, respectively.

Introduction

α- Chymotrypsin known as serine endopeptidase, a specific digestive enzyme belongs to the serine proteases family, involved in proteolysis. The inactive form of *α*-chymotrypsin is known as chymotrypsinogen, which is cleaved by trypsin enzyme into two portions that remain connected *via* disulphide bond, and cleave the chymotrypsinogen molecules, which subsequently activate each other by eliminating two small peptides in a *trans*-proteolysis manner. It enhances the cleavage of peptide bonds through hydrolysis reaction, The major substrates of *α*-chymotrypsin includes tryptophan, tyrosine, phenylalanine, leucine, and methionine, which hydrolyze at their carboxyl terminal [1]. *α*-chymotrypsin inhibitors have been used for the treatment of various disorders, including intestinal bowel syndrome (IBS), gastro esophageal reflux disease (GERD) and Crohn's disease. In literature natural synthetic and semisynthetic inhibitors of *α*-chymotrypsin have been reported such as, coumarins, aryl substituted enol lactones, benzohydroxamic acid derivatives [2-5].

In drug discovery process computational methods are successfully aiding in a number of ways such as target receptor identification, structure determination and prediction of *drug-likeness* of compounds. Virtual screening is progressing rapidly in the recent years for lead identification in the pharmaceutical industry. In computational techniques, various filters have been used at the time of compounds selection, to avoid unstable, toxic and metal-complexes, based on Lipinski's rule of five which provides a simple and logical criteria regarding the bioavailability of compounds, which is mainly based on molecular weight, [in terms of octanol / water partition coefficient log P], hydrophilicity [presence of hydrogen donor and hydrogen acceptor [6]. Initial careful filtering of *data-set* reduce the risk of false positive, downstream ADME / Tox failures, and subsequently increases the probability of true positive hit rates [7].VS strategies can be divided into two categories, Structure-based VS [SBVS] [8] and Ligand-based VS

[LBVS] [9, 10] depends upon the availability of receptor / protein bound-ligand information. SBVS and LBVS have been considered almost mutually exclusive, suggesting SBVS to be used when the 3D structure of the target receptor / protein with high atomic resolution is available [11]. While LBVS is the choice of method in the absence of target receptor structure. Furthermore, some current studies showed that LBVS offers a strong suitable alternative towards SBVS even in the presence of protein structural information. It is successfully being used generally through expansions of available chemical libraries, published bioassay data of compound, and the search of techniques of new molecular features and similarity of pharmacophore. This approach helps to reduce biologically non-active molecules, therefore increases the chances of sensible and biologically targeted compounds synthesis [12]. Pharmacophore based virtual screening is an alternative strategy in the absence of required protein information. Ligand-based pharmacophore can be classified into two main classes, first, common features based pharmachophore model, while second is based on the biological activity and chemical structure of molecule used for pharmacophore model generation [13]. It focuses on specific protein-ligand binding to disclose binding pattern. Therefore, it has great importance to use methods that make optimal use of both docking as well as pharmacophore and improve the selection of active molecules by calculating enrichment factors.

Docking of *in-house data-base* against *α*-chymotrypsin:-

Experimental methodology:-

Target Selection:-

The 3D structure of bovine α-chymotrypsin bound with benzohydroxy vanadium metal complex ligand retrieved from Brook Haven Protein Data Bank PDB I.D: 2P8O with 1.50 Å resolution, it was selected for molecular docking.

Data-Set preparation:-

95 known inhibitors of α-chymotrypsin from diverse classes of compounds like, lactones, coumrins, oxazolone and easter type of coumarin's were retrieved from literature. [15-17]. Structure preparation was done by ChemDraw further converted into 3D format using OpenEye Babel. MOE was used to correct the atom type, Hydrogen atoms, minimization and partial charge MMFF94 assigned for each ligand.

***In-house* decoy set of compounds:-**

9,303 compounds which comprises of synthetic, semi-synthetic and natural product-based compounds retrieved from *In-house* database of International Center for Chemical and Biological Sciences [ICCBS] compound bank were used to conduct pharmacophore based virtual screening; ninety five known active inhibitors from literature against α-chymotrypsin were also added in the decoy set of molecules. Filtration was applied by using OpenEye Filter Program. Minimization and MMF94 Charges were employed to the whole dataset.

Docking with FRED [Fast Rigid Exhaustive Docking] software:-

Molecular docking of 8,262 filtered compounds was performed by using FRED docking software. Initially software was validated with the co-crystallize structure of ligand bound receptor complex and root mean square deviation (RMSD) was calculated, which comes less than 1Å. The software comprises of eight scoring functions. During molecular docking unfortunately fourteen molecules were failed. Therefore, 8,248 molecules were successfully docked on the active site with different binding affinities. Docked results were analyzed for each scoring function, and then enrichment factor was calculated. PLP scoring function was found to be the most enriched in the whole *in-house* data set. However, for 5% of the *data-base* shape gauss scoring function was the dominant one. After completion of docking experiment various scoring functions were used to calculate the enrichment factor by using known actives. We did not get appreciable enrichment factor value, so we changed the protein X-ray structure, and used Apo form without ligand bound protein structure. By using *FRED* with cluster machine we conducted docking experiment. Similarly, we calculated enrichment factor. At this time, although the enrichment factor value increased comparative to docking with GOLD, but again not appreciable. The maximum value of PLP [Piecewise Linear Potential] scoring function was obtained and found to be as 1.05. Therefore, we performed an experiment on entirely different strategy. New strategy was based on the ligand-based Pharmacophore mapping and virtual screening through similarity search [20].

Pharmacophore Model Generation:-

Pharmacophore model was generated by Ligand Scout version 3.0. For this purpose a reference drug chymostatin was selected on the basis of its IC_{50}, 8.24 ± 0.11 µM, [22]. While the biotin was aligned over it, which generates common feature between these molecules. Pharmacophore structural features are usually explained in terms of lipophilic centers, hydrophobic region, proton donor, proton acceptor, aromatic ring centroids and so on [23].

Molecules Selection for Pharmacophore Generation:-

The major goal of drug designing is to identify and develop new ligands with more therapeutic potential with its high affinity towards the binding site of receptor / protein. One of the effective and useful methods for achieving this goal is a pharmacophore modeling [24]. The most important step for pharmacophore model generation is the selection of training set of compounds [25]. For this purpose we selected two drug molecules chymostatin and biotin on the basis of their potent biological activitiy having IC_{50} value, 7.8µ M, which is the more potent drug reported against α-chymotrypsin uptill now, to obtain the best shared-feature pharmacophore base VS results. Pharmacophore models are computationally efficient due to simplicity, and suitable for virtual screening, during pharmacophore generation some steps require such as [a] identification of ligands [b] interpretation of ligands [c] creation of pharmacophore [d] visualization and export for virtual screening. For the generation of a good and effective / high quality pharmacophore model several criteria need to be considered, such as structural similarity, structural flexibility, biological activity and chemical properties. Ligand Scout generates the following common feature Pharmacophore model [Fig.1].

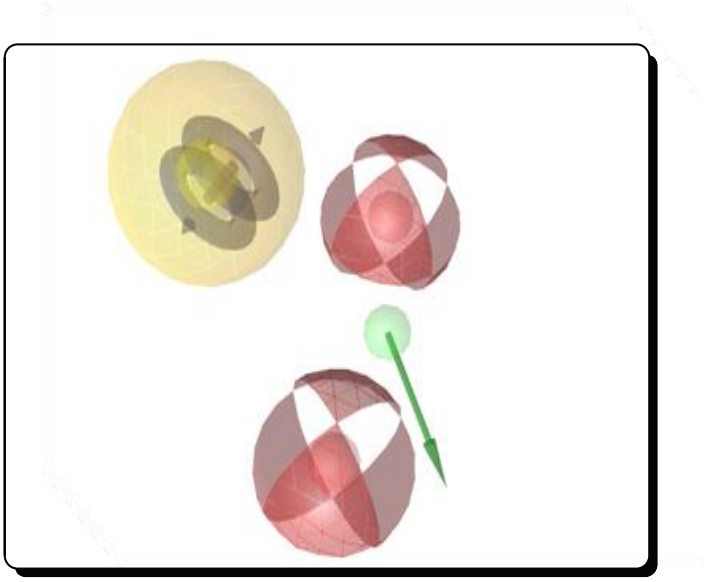


Fig 1: Illustrating a four point shared-feature pharmacophore model derived by using Ligand Scout software, depicting the shared features of two drug molecules Chymostatin and Biotin, the observed features include one H-donor of amide Nitrogen atom , two H-acceptor features of Carbonyl oxygen atom and a hydrophobic aromatic ring centroid .

Ligand-based Pharmacophore model can be used as an important tool for virtual screening, when the 3D structure of receptor / protein is unknown or the docking techniques are not successfully applicable. Ligand-based VS depends on the pharmacophore based information of known active compounds. These molecules are used to derive a pharmacophore model which high lights the important basic structural features of molecules that are necessary for biological activity, such as hydrogen-bonding, hydrophobic region, proton donor, proton acceptor which a molecule should possess to bind with target receptor / protein, appropriately [26]. This pharmacophore model can be used as a template to search and identify the most promising candidates from the chemical library. In this regard simple selection and considerations of a set of active molecules may greatly reduce the search space. The strength of pharmacphore-based screening in comparison with other similarity search screening approaches is more successful

to treat a diverse data set of putative active compounds with totally different chemical scaffolds. The advantage of this approach lies in the fact that in the worst case, when there is no clues on the binding conformation of any of the ligand is available, for a set of conformation a pivot ligand can be enough to computed [27-29], although a pharmacophore is an abstract concept of functional groups assemble at a particular distance [r] in space, that accounts for the compounds towards biological activity against their target receptor / protein. Pharmacophore-based Virtual screening of *in-house data-base* was carried out by using MOE software. Ligand-based virtual screening is based on similarity search.

To conduct the ligand-based virtual screening, at first a shared-feature four point Pharmacophore model was derived by using chymostatin and biotin, the model Pharmacophore fit score value was 86.0, it was the training set, and we used this training set to find out the Pharmacophore fit score of our virtually screened hits of test sets. More near fit score value to the training set means best fit with the test model therefore, more potent inhibitor. In our case all the hits showed less than 86.0 Pharmacophore fit score value of training set [Table 3].

***In-Vitro* Screening of Compounds:-**

Identified hits of thirty three 33 drug-like molecules were selected for bioassay screening. However, nineteen compounds 19 were subjected for *in-vitro* screening and eight showed some extent of inhibitory potential while three were found to be as moderately actives [Table 2].

Bio- assay Screening Protocol:-

The inhibitory activity IC_{50} of selected ligands were checked against α-chymotrypsin enzyme by using 50 mM Tris-HCl buffer of pH 7.6 with 10 mM $CaCl_2$. while α-chymotrypsin [12 units / mL was prepared in the same above mentioned buffer] along with different concentration of test compounds prepared in DMSO, then it was allowed to incubate at 30 °C for 25 min. The reaction was started by the substrate, N-succinyl-L-phenylalanine-p-nitroanilide] [SPpNA] addition with 0.4 mM prepared buffer mentioned as above]. As p-nitro aniline was released, variation in absorbance was observed which was continuously monitored at 410 nm. All the reactions were carried out in triplicate with the final volume of 200 L, and using a micro plate reader [Spectra Max M2, Molecular Devices, CA, and USA].

Structure Activity Relationship [SAR]:-

The three active compounds [Table 2 (**6-8)]** were found to be moderately actives against α-chymotrypsin, and it is clearly observed that these all three molecules contain polyhydroxy phenols, while two belongs to flavonoid class of natural product, comprises of benzopyrane ring, docked poses shows [Fig. **2- 4**] that this inhibitory potential comes due to the presence of H-bonding b/w flavonoid with the active site amino acid residues. Before that some compounds have already been reported as active serine protease inhibitors from flavonoid class of natural product.

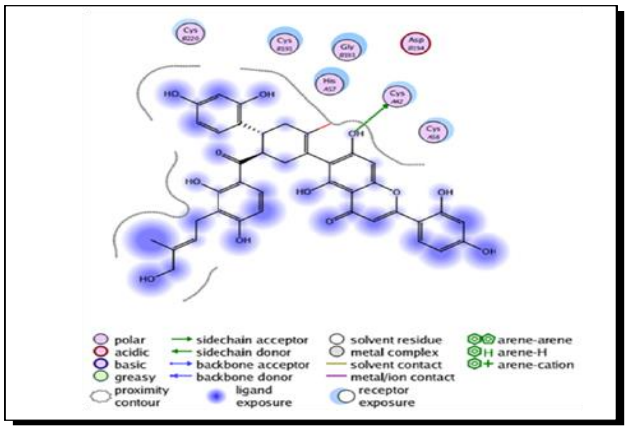


Figure 2: In the above docked pose of the ligand with receptor binding in proximity contour within 5 Å region. It is clear that the proximity contour is surrounded by some other amino acids apart from catalytic traid, this includes Aspartic acid194, Cysteine191 and Serine214.

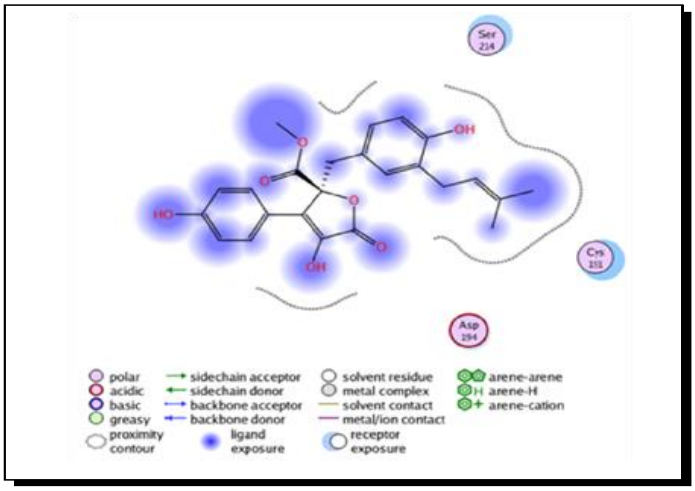


Figure 3: In the above docked pose of ligand with receptor binding within 5 Å regions. Its clear that the lone pair of oxygen atom of hydroxyl group of flavonoid ring making H-bonding with SH group of the cysteine amino acid resides at position 42 which furthermore in contacts with water molecules. The keto group of ring is also in solvent contact as well as with water molecule.

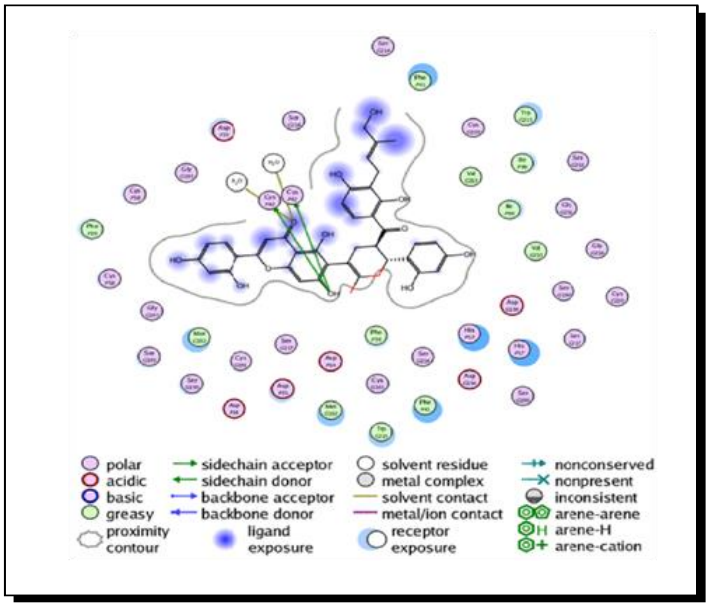


Figure 4: In the above docked pose of the ligand with receptor binding in proximity contour within 5 Å regions. It's clear that the lone pair of oxygen atom of hydroxyl group of flavonoid ring making Hydrogen bonds with the backbone SH group of cysteine amino acid residue resides at position 42.

Results and Discussion:-

Computational techniques have great scope in rational drug designing and discovery process, therefore successfully applying to increase research and development [R&D] productivity of pharmaceutical industry, specifically for speeding up the identification of targets hits, and reduce the cost and time. *α*-chymotrypsin is an important serine protease enzyme. To evaluate the therapeutic potential of *in-house data-base* for treating GERD, IBS and pancreatitis, we conducted structure-based virtual screening of *in-house data-base* against *α*-chymotrypsin by using two docking softwares *GOLD* [genetic algorithm for ligand-protein docking] and *FRED* [fast rigid exhaustive

docking], *GOLD* has already been reported to use for the docking against the same target enzyme and its scoring functions are not supportive for it [*GOLD* software manual] while, *FRED* was used due to its large scoring functions range and fastest speed, *FRED* docked results against *α*-chymotrypsin of PDB I.D 2P8O was used to calculate the enrichment factor of 5%, 10%, and 20% of decoy set of *in-house data-base*, for 5% of *data-base* **shape gauss** scoring function was dominant 1.26, which deals with the shape of a molecule, while for rest of the, 10%, 15% and 20 %. PLP scoring function was dominant one, which deals with the information about, H-donor, H-acceptor, hydrophobic region and metal ion presence if it is present in the molecule. The value of an enrichment factor for 5%, 10%, 15% and 20% was found to be, 1.26, 0.07, 1.05 and 0.89 respectively [Table 1a-1d]. The maximum value obtained 1.26, which is not sufficient to validate the docked results, because docking gives the best fit pose of a ligand to its binding receptor / protein, which is estimated by various scoring functions of a particular docking software, and calculated by an enrichment factor, if the enrichment factor value is low, it clearly indicates that ligands are not appropriately bound with the provided active site of receptor during docking, however, it is binding with some other sites due to allosteric interactions. Docking is usually used to locate or explain the binding affinity pattern of ligand molecules to the provided protein-ligand binding site [active-site], and used to explain the binding pattern of interactions of ligand with amino acid residues of receptor active site, and other non-covalent allosteric interactions. The calculated low [decrease] enrichment factor value for individual scoring function of *FRED* docking software raised a question that why does molecular-docking can't always give or predict good and successful enriched docking results ?. It could be explained through the Molecular docking result's description which are not successful for all kinds of receptor-ligand interactions, the reason behind is, it could be due to non-efficient selection and performance of docking software. Non-availability of well resolved ligand-protein co-crystallizes structure. Role of H_2O molecule, in ligand-protein binding, presence of allosterric interactions in which ligands are not efficiently and tightly bind with the provided active site, instead of that it binds with some other amino acid residues apart from active site residues. Molecular dynamics effect of ligand also plays a very important role, which is not present in rigid docking, in which the interaction of ligand with protein active site is restricted up to certain conformers. However, in the real system [*in-vitro*] molecules are continuously in dynamics mode and try to attain best fit-binding pose with receptor active site, because of a low enrichment factor, we adopted a new approach to hit the leads by ligand-based Pharmacophore modeling and virtual screening.

A Ligand-base pharmacophore model is a very important tool in the absence of 3D protein-ligand co-crystallize structure or in case of non-successful docking with low enrichment factor result, it can be helpful towards *drug-like* discovery by virtual screening, In this case study we developed a four point [tetrahedral] shared-feature ligand-based pharmacophore model from two potent drug molecules, *chymostatin* and *biotin* which includes four pharmacophore features, such as one hydrophobic aromatic centroid feature, one [H-donor] and two [H-acceptors]. Pharmacophore fit score value of the model was obtained as 86.00, it was the training set, and we used this training set to find out the Pharmacophore fit score of our virtually screened hits of test sets. More near to the training set ligand Pharmacophore fit values means best fit with the test model therefore, more potent inhibitor. The generated or developed pharmacphore model was used to search the 3D [SYBYL Mol_2 format] of the *in-house data-base* [ICCBS-2012 *data-base*], which comprises of synthetic, semi-synthetic, naturally isolated and biotransformed compounds.

This model was used to similarity search in the decoy set of filtered molecules by using MOE which search and identified 33 molecules. Out of the 33 virtually screened hits, nineteen compounds were made available to subject for *in-vitro* screening, eight molecules showed inhibitory potential, while three showed moderate activity, with IC_{50} 319.8 ± 1.80 µ M, 474.8 ± 5.08 µ M and 481.3 ± 9.10 µ M respectively. Out of the eight molecules five belongs to benzopyran derivatives, in which two are good actives with % inhibition 54.1%, 65% and IC_{50}= 319.89±1.8 µ M, 474.88±5.08 µ M respectively [Table 2]. Eight compounds showed inhibitory activity against the enzyme *α*-chymotrypsin [Table 3].

We used Molecular Operating Environment [MOE] software to observe the binding interaction pattern of the active ligands with the receptor. In our case study all the hits showed less than 86.0 Pharmacophore fit score values which has proved that these are not closely related with the drug Pharmacophore models. And this has been further evaluated by *in-vitro* bio-assay screening results which didn't show any remarkable activity.

Enrichment factor of FRED scoring functions [for 5% of *data-base*]:-

Table 1a: Showing the scoring function shape gauss is dominant in 5% EF of *data-base*

Scoring functions	No of actives in 5%	Enrichment factor	% E.F value
Chemgauss-2	2	2/415*87.36	0.42
Chemgauss-3	1	1/415*87.36	0.20
Chem-score	1	1/415*87.36	0.20
Oechmscore	1	1/415*87.36	0.20
Shape gauss	**6**	**6/415*87.36**	**1.26**
PLP	2	2/415*87.36	0.42
Screen score	0	0	0.00
Consensus score	0	0	0.00

Enrichment factor for 10%:-

Table 1b: Showing that the scoring function PLP is dominant for 10% EF of *data-base*.

Scoring functions	No of actives in 10%	Enrichment factor	% E.F value
Chemgauss-2	5	5/8300*87.36	0.05
Chemgauss-3	5	5/8300*87.36	0.05
Chem-score	5	5/8300*87.36	0.05
Oechmscore	4	4/8300*87.36	0.04
Shape gauss	6	6/8300*87.36	0.06
PLP	**7**	**7/8300*87.36**	**0.07**
Screen score	4	4/8300*87.36	0.04
Consensus score	4	4/8300*87.36	0.04

Enrichment factor for 15%:-

Table 1c: Showing that the scoring function PLP is dominant for 15% EF of *data-base*

Scoring functions	No of actives in 15%	Enrichment factor	% E.F value
Chemgauss-2	6	6/1245*87.36	0.42
Chemgauss-3	10	10/1245*87.36	0.70
Chem-score	7	7/1245*87.36	0.49
Oechmscore	3	3/1245*87.36	0.20
Shape gauss	9	9/1245*87.36	0.63
PLP	**15**	**15/1245*87.36**	**1.05**
Screen score	8	8/1245*87.36	0.56
Consensus score	4	4/1245*87.36	0.28

Enrichment factor for 20%:-

Table 1d: Showing that the scoring function PLP is dominant for 20% EF of *data-base*

Scoring functions	No of actives in 20%	Enrichment factor	% E.F value
Chemgauss-2	9	9/1660*87.36	0.47
Chemgauss-3	15	15/1660*87.36	0.78
Chem-score	11	11/1660*87.36	0.57
Oechmscore	4	4/1660*87.36	0.20
Shapegauss	10	10/1660*87.36	0.52
PLP	**17**	**17/1660*87.36**	**0.89**
Screen score	9	9/1660*87.36	0.47
Consensus score	8	8/1660* 87.36	0.42

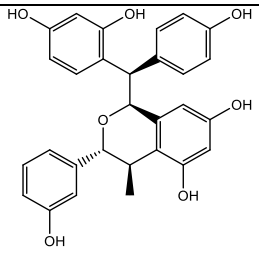
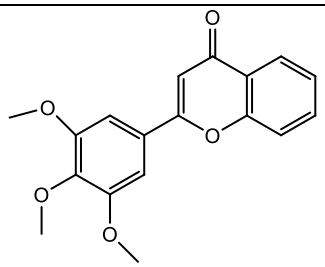
Table 2: Bio-assay screening results of identified hits against enzyme α-chymotrypsin:

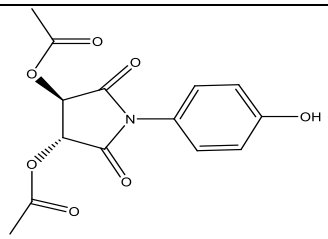
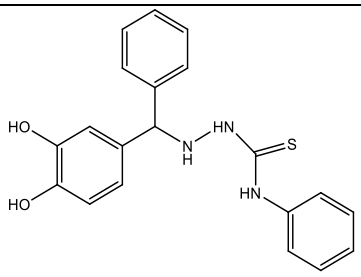
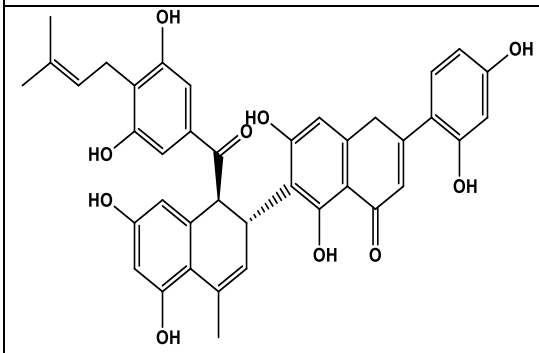
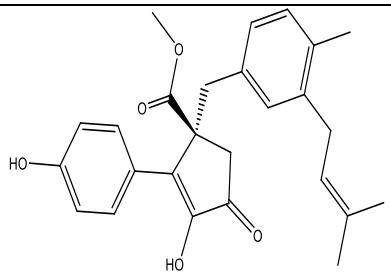
Compounds no	% inhibition	IC_{50} [µ M]
1	19.6	-
2	20.1	-
3	21	-
4	22.6	-
5	36	-
6	52.1	**481.3 ± 9.10**
7	54.6	**474.8 ± 5.08**
8	65	**319.8 ± 1.80**
Standard inhibitor	**Chymostatin**	**IC_{50} 7.5 µM**

Table 2: Showing the bio-assay screening results against α-chymotrypsin enzyme, compound [6-8] were identified and evaluated as new actives.

Table 3: Showing the Pharmacophore fit score values of actives:

Table 3: Showing the Pharmacophore fit score values of actives:

Compounds	Pharmacophore Fit Score	%Inhibition	IC_{50} value µM
 **(1S,3R,4R)-1-((R)-(2,4-dihydroxyphenyl)(4-hydroxyphenyl)methyl)-3-(3-hydroxyphenyl)-4-methylisochroman-5,7-diol**	**29.6500**	**19.6**	**-**
 **2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one**	**46.4900**	**20.1**	**-**

 **(3R,4R)-1-(4-hydroxyphenyl)-2,5-dioxopyrrolidine-3,4-diyl diacetate**	**36.4500**	**21**	**-**
 **2-((3,4-dihydroxyphenyl)(phenyl)methyl)-N-phenylhydrazinecarbothioamide**	**35.9200**	**22.6**	**-**
 **(1'S,2'R)-1'-(3,5-dihydroxy-4-(3-methylbut-2-enyl)benzoyl)-6-(2,4-dihydroxyphenyl)-1,3,5',7' -tetrahydroxy-4'-methyl-1',2'-dihydro-2,2'-binaphthyl-8(5H)-one**	**60.4400**	**36**	
 **(S)-methyl 3-hydroxy-2-(4-hydroxyphenyl)-1-(4-methyl-3-(3-methylbut-2-enyl)benzyl)-4-oxocyclopent-2-enecarboxylate**	**66.0100**	**52.1**	**481.3 ± 9.10**

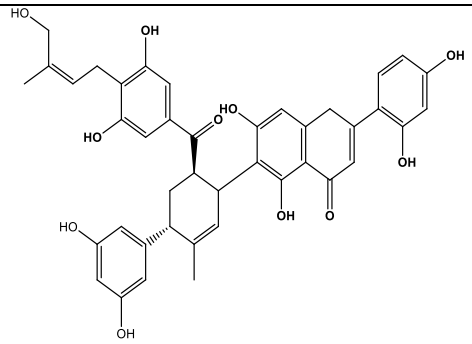
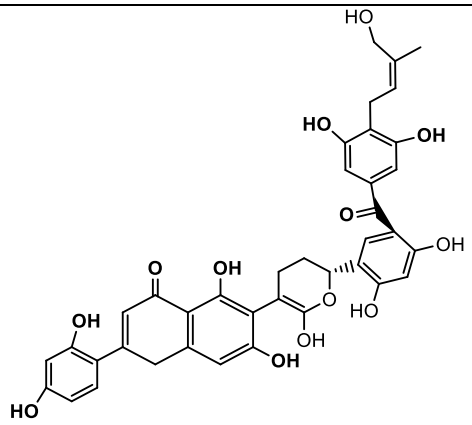
 **7-((4R,6R)-6-(3,5-dihydroxy-4-((Z)-4-hydroxy-3-methylbut-2-enyl)benzoyl)-4-(3,5-dihydroxyphenyl)-3-methylcyclohex-2-enyl)-3-(2,4-dihydroxyphenyl)-6,8-dihydroxynaphthalen-1(4H)-one**	**65.4400**	**54.6**	**474.8 ± 5.08**
 **(R,Z)-7-(2-(5-(3,5-dihydroxy-4-(4-hydroxyvmethylbut-2 enyl)benzoyl)- 2,4-dihydroxyphenyl)-6-hydroxy-3,4-dihydro-2H-pyran-5-yl)-v3-(2,4-dihydroxyphenyl)-6,8-dihydroxynaphthalen-1(4H)-one**	**66.4400**	**65**	**319.8 ± 1.80**
Standard inhibitor chymostatin			**7.5 µM**

Table 3: Showing the Pharmacophore fit score values of active inhibitors.

Conclusion:-

A ligand-base pharmacophore model is a very important tool in the absence of 3D protein-ligand co-crystallize structure or in case of non-successful docking with low enrichment factor result, it can be helpful towards drug-like discovery by virtual screening, In our study the bio-assay screening results revealed that eight molecules showed inhibitory potential, while three showed moderate activity, with IC_{50} 319.8 ± 1.80 µ M, 474.8 ± 5.08 µ M and 481.3 ± 9.10 µ M respectively. Out of the eight molecules five belongs to benzopyran derivatives, in which two are good actives with % inhibition 54.1%, 65% and IC_{50}= 319.89±1.8 µ M, 474.88±5.08 µ M respectively.

These compounds can be furthermore used for lead optimization to improve the therapeutic potential of these compounds for further drug designing steps of the inhibitors.

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Conflict of Interest:-

The authors declare that they have no competing interests.

References:-

1. Polgar. L. the Catalytic traid of serine peptidases CMLS Cell Mol Life Sci **2005**;62:2161–217 222-25.
2. Wouters J1., Huygens M., Pochet L., Pirotte B., Durant F MB., Structural approach of the mechanism of inhibition of α-chymotrypsin by coumarins. Bio org Med Chem Lett. **2002**;12: 1109–12.
3. Lionel P., Caroline D.,Georges D., Johan W., Bernard M., Miche. Reboud-Ravaux Bernard P. Coumarinic Derivatives as Mechanism-Based Inhibitor Chymotrypsin and Human Leukocyte Elastase. Bioorg Med Chem. **2000:**8: 1489–1501.
4. Bonnie M., Ashe RLC., Selective Inhibition of Human Leukocyte Elastase and Bovine α-Chymotrypsin by Novel Heterocycles. J Biol chem. **1981**; 256[22]:11633–11606.
5. Baek DJ, Reed PE DSbk. Alternate substrate inhibitors of an α-chymotrypsin: enantioselective interaction of aryl-substituted enol lactones. J.A Biochem. **1990**; 29 4305–11.

6. Lipinski, C.A F. Lombard, B.W.Dominy, and P.J.Feeney. "Experimental and Computational Approaches to Estimate Solubility and permeability in Drug Discovery and Development Settings, ". Adv Drug Deliv Res. **1997**; 25: 3–25.
7. David J. Huggins Ashok R. Venkitaraman and DRS Rational Methods for the selection of Diverse Screening Compounds. ACS Chem.biol . **2011**;6: 208–217
8. Lyne PD. Structure-based virtual screening: an overview. Drug Discov Today. **2002**; 7:1047–1055.
9. Oprea, T.I. and Matter H. Integrating virtual screening in lead discovery. Curr Opin Chem Biol. **2004;**3:49–358.
10. Willet P. Similarity-based virtual screening using 2 fingerprints. Drug Discov Today. **2006**; 11:1046–1053.
11. Murray CW1., Clark DE., Auton TR., Firth MA., Li J., Sykes RA, Waszkowycz B, Westhead DR YS. Combining structure-based drug design and combinatorial chemistry for rapid lead discovery. J. Comput Aided Mol Des. **1997**; 11:1997.
12. Ingo Muegge SO off. Advances in virtual screening. Drug Discov today Technol. **2006**; 3:405–411
13. . Sakkiah S1, Thangapandian S, John S LK. Pharmacophore-Based Virtual Screening, molecular docking studies to design potent heat shock protein 90 inhibitors. Eur J Med Chem. **2011**; 46:2937–47.
14 www.pdb.org
15 Pochet L.; Frederick R.; Masereel B., Coumarin and Isocoumarin as Serine Protease Inhibitors Curr Pharm Design, November **2004**, 10: 3781-3796.
16 Khalid M K, Uzma R M, et al Synthesis and Chymotrypsin Inhibitory Activity of Substituted Oxazolones Let Drug Des Discov, **2008;** 5:52-56.
17 Raphael Frederick, Severine Robert, et al, 3,6-Disubstituted Coumarins as Mechanism-Based Inhibitors of Thrombin and Factor Xa J. Med. Chem., **2005**; 48: 7592–7603.
18 FRED docking software manual user guide.
19 McGann. M., J. FRED Pose Prediction and Virtual Screening Accuracy, Chem. Inf. Model., **2011;** 51:578-596.
20 Jérôme H., Peter W., and David J., Wilton., Western B., U.K. Pierre Acklin., Kamal Azzaoui, Edgar Jacoby and AS. New Methods for Ligand-Based Virtual Screening: Use of Data Fusion and Machine Learning to Enhance the Effectiveness of Similarity Searching J. Chem Inf Model . **2006**; 462–470.
21 Aziz-ur-Rehman, 1A. Siddiqa, 1M. A. Abbasi, 1S. Rasool, 1S. Z. Siddiqui, 1S. Gul, 2M. Ashraf and 3R. Nasar Enzyme Inhibition Studies on N-Substituted Sulfonamides Derived from m-phenetidine Pak. J. Chem. **2013**;3[3]: 100-106.
22 Breu F, Guggenbichler S, Wollmann J. Ligand-Based Pharmacophore modeling of anticancer histone deacetylase inhibitors. African, J Biotechnol. **2008**; 9:3923–3931.
23 Khan HN, Kulsoom S, Rashid H. Ligand-based pharmacophore model development for the identification of novel antiepileptic compound. Epilepsy Res. **2012**; 98:62–71.
24 Yuhong Xiang, Zhaoyan Hou ZZ. Pharmacophore and QSAR Studies to Design Novel Histone Deacetylase 2 Inhibitors. ChemBiol Drug Des. **2012**; 79:760–770.
25 William L. Jorgensen. The Many Roles of Computation in Drug Discovery Science. **2004**; 303March:1813–1818.
26 Dror O, Schneidman-Duhovny D, Inbar Y, Nussinov R, Wolfson HJ. Novel approach for efficient pharmacophore-based virtual screening: method and applications. J Chem Inf Model. **2009**; 49:2333–43.
27 Gerhard Wolber, and Thierry Langer, LigandScout: 3-D Pharmacophores Derived from Protein-Bound Ligands and Their Use as Virtual Screening Filters J. Chem. Inf. Model. **2005**, 45, 160-169.
28 Thangapandian, S.; John, S.; Sakkiah, S.; Lee KW. Potential virtual lead identification in the Discovery of pharma inhibitors Application of ligand and structure-based pharmacophore modeling approaches. Eur J. Med Chem. **2011**; 46:2469 – 2476.