RESEARCH ARTICLE

Identification of Potent Angiotensin Converting Enzyme 2 Inhibitors through Virtual Screening and Structure-Based Pharmacophore Design

C. Zozimus Divya Lobo[1], A. Syed Mohamed[1]*, C.Vedhi[2], S.V. Rajesh[3], V. Aroulmoji[4] and Gnanendra Shanmugam[5]*

[1]Department of Chemistry, Sadakathullah Appa College (Autonomous), Tirunelveli, Tamilnadu, India.
[2]Department of Chemistry, VOC College, Tuticorin, Tamilnadu, India
[3]Department of Botany, Vivekanandha college of Arts and Sciences (Autonomous), Tiruchengode, Namakkal, Tamilnadu.
[4]Center for Research & Development, Mahendra Educational Institutions, Namakkal-637503,Tamilnadu, India
[5]Department of Biotechnology,College of Life and Applied Sciences, Yeungnam University, Gyeongsan, South Korea.

ABSTRACT: Angiotensin Converting Enzyme (ACE), a metallo-peptidase is the best known important drug target in the treatment of hypertension and responds to broad range ACE inhibitors such as Captopril. Whilst, many phytochemical compounds including alkaloids and flavonoids were also reported with anti-hypertensive activity. On the other hand, ACE2 is considered as an interesting new cardio-renal disease target as it is close and unique ACE homologue. In this scenario, the anti-hypertensive activities of 17 phytochemical compounds were analyzed through docking studies with ACE2. Also, the other ACE inhibitors with reported IC_{50} values were considered for docking interactions and used as training set. Further, the best docked phytochemical compound Rosemarinic acid and the training set compounds with ACE inhibitor activity were used to design the pharmacophore and validated. The generated 3D pharmacophore is subjected to screen the compounds with the significant chemical features against May bridged database consisting of more than one lakh compounds and subsequently, the hit compounds were screened using various filters such as estimated activity, Lipinski's rule of five, and ADMET properties and resulted Eight compounds. The anti-hypertensive activities of these 5 compounds with good fit values were selected for further docking studies with ACE2. The five compounds PD 00533, CD 01374, CD 04888, CD 01278 and BTB 04932 exhibited the best docking scores and also favors the necessary hydrogen bond interactions with in the activity site of ACE and thus identified as novel leads with anti-hypertensive activity.

KEYWORDS*:* Pharmacophore, Angiotensin Converting Enzyme, ACE inhibitors, ADMET, docking studies.

1. INTRODUCTION

Hypertension and congestive heart failures are becoming epidemic throughout the world [1]. In recent years, the drastic increase in the number in the adult population of the world with hypertension was recorded and more than 20 million people were affected with heart failure. Angiotensin-converting enzyme 2 (ACE2) is a newly discovered membrane-bound aminopeptidase [2]. This enzyme has been proven to be critical in impacting cardiovascular and immune systems by 2 distinct physiologically important mechanisms. ACE2 catalyzes the production of vasodilatory peptides, including angiotensin 1 to 7 and thus is responsible in counterbalancing the potent vasoconstrictor effects of angiotensin II. This counterbalancing property of ACE2 is proposed to be important for the development of novel pharmacotherapy against hypertension and related cardiovascular diseases [3-4]. In the process of hypertension, ACE plays an important role in regulating blood pressure, and ACE inhibitors are considered to be one of the therapeutic methods for treating anti-hypertension. Angiotensin-converting enzyme is secreted in the lungs and kidneys by cells in the endothelium of blood vessels, and it is the part of the renin-angiotensin system (RAS). It indirectly increases blood pressure by causing blood vessels to constrict by converting angiotensin-I to angiotensin-II [5-6]. Thus, the ACE considered as an ideal target for controlling blood pressures and heart failures and synthetic compounds are being used as ACE inhibitors to treat heart problems. These inhibitors inhibit the conversion (angiotensin-I to angiotensin-II), dilate the blood vessels and control the blood pressures. Several ACE inhibitors, including captopril, lisinopril, fosinopril and enalapril, are synthetic molecules which are clinically used as anti-hypertension agents [7].

In 1990 Paul Ehrlich [8] introduced pharmacophore as 'a molecular framework that carries (phoros) the essential features responsible for a drug's (pharmacon) biological activity'. The design of pharmacophore are necessary to reveal specific functional group that are optimal for the interactions which can trigger the potential targets either by inhibiting or enhancing the biological function of those receptors [9]. The generation of pharmacophore plays a crucial role in the drug discovery pipeline in term of time and cost. The crucial step in the design of pharmacophore involves the alignment of multiple ligands (training set) which can determine the essential chemical features that are essential for their bioactivity. The alignment of these multiple ligand can be achieved by superposing a set of active molecules [10]. In general, the pharmacophore

***Corresponding Author:** *science@gmail.com & asm2032@gmail.com*
Received: 12.05.2017 **Accepted:** 10.06.2017 **Published on**: 27.07.2017

modelling involves the knowledge of two or more known active compounds. In this method, the known compounds (training set) are aligned and the commonly shared chemical entities are established common pharmacophore features [11]. These shared features of the training set compound are considered as the essential chemical entities. More commonly, the pharmacophore models are generated with the combination of known active and inactive compounds and been used to validate the models. Utmost care is taken while choosing the known active and inactive compounds for the training set, as they significantly influence the quality of the model [12]. Most preferably, the selection of inactive compounds in the training set is based on the activity low binding affinities and high IC_{50} values to avoid the generation of inappropriate pharmacophore model. Ultimately, to achieve the good pharmacophore models, the training set should contain the structurally diverse set of compounds. Thus the present study is designed to explore the chemical features that ascertain the ACE inhibitors activity was put-forth through pharmachophore designing.

2. METHODOLOGY

Virtual Screening of AHL Analogue Library

The target ACE2 and selected ACE inhibitors were converted in to PDBQT files by using the PyRx software [13] for virtual screening studies. For docking purpose the grid was set to the predicted binding pocket of ACE2 as that inhibitors would have flexibility in binding. The ACE inhibitors were docked with ACE2 using AutoDock Vina option of PyRx. The docking was carried out using Lamarckian Genetic Algorithm and with parameters as follows: 10 docking trials, population size of 150, maximum number of energy evaluation ranges of 250000, maximum number of generations of 27,000, mutation rate of 0.02, cross-over rate of 0.8 and an elitism value of 1 [14]. The ACE inhibitors with the best docking score (binding energy) were used for the Pharamacophore modelling.

Pharmacophore modeling and 3D database Screening

The pharmacophore model was generated by using the Pharamacophore option of discovery studio software [15]. The best docked ACE inhibitor was used as training compound for the generation of a Pharamacophore by using option Auto Pharmacophore Generation which considers the Hydrogen bond acceptor (HB_ACCEPTOR), Hydrogen bond donor (HB_DONOR), Hydrophobic feature (HYDROPHOBIC), Negative ionizable feature (NEG_IONIZABLE), Positive ionizable feature (POS_IONIZABLE) and Aromatic ring (RING_AROMATIC) feature types to generate a selective pharmacophore model from a single ligand. The Principal value of 2 and the Maxis set to the Training ligand which ensures that all of the chemical features in the compound should be considered in building the pharmacophore space. The Auto Pharmacophore Generation option enumerates a set of candidate pharmacophore models from the features and chooses the pharmacophore with the highest selectivity as predicted by a Genetic Function Approximation (GFA) model. Using this generated pharmacophore hypothesis, compound screening was performed against, Maybridge database [16] consisting of one lakh compounds and assessed the compounds matching the pharamacophore by considering the Fit Values.

Molecular Docking

The Top 5 obtained hits from Maybridge database with the highest Fit Value were docked with in the active site of ACE2 by using FlexX [17] with following parameters i) default general docking informations, ii) base placement using triangle matching, iii) scoring of full score contribution and threshold of 0,30 and No score contribution and threshold of 0,70. iv) chemical parameters of clash handling values for protein ligand clashes with maximum allowed overlap volume of 2.9 A03 and intra-ligand clashes with clash factor of 0.6 and considering the hydrogen in internal clash tests. v) default docking details values of 200 for both the maximum number of solutions per iteration and maximum number of solutions per fragmentation. Further, the interactions of database molecules with SdiA in the docked complex were analyzed by the pose-view of LeadIT.

3. RESULT AND DISCUSSION

Virtual Screening

The 3D structures of 24 ACE inhibitors as ligand compounds in SDF (structure data file) format are virtually screened to reveal their binding efficiencies through docking in the binding pockets of ACE2 receptor using FlexX module of LeadIT suite. Among these 24 compounds, 17 compounds (Figure.1) were the phytochemical with anti-hypertensive activities and 7 were the compounds with IC50 values. The docking parameters such as triangle matching base placements, zero full score and No score contributions and threshold for full score and no score contributions of 30 and 70, respectively, Clash handling values of 2.9 A^{03} and 0.6 for protein ligand clashes with maximum allowed overlap volume and intra-ligand clash factors while considering the hydrogen in internal clash tests and 200 as the default docking values for maximum number of solutions per iteration and also per fragmentation [18].

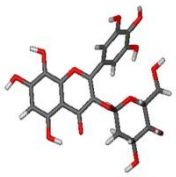
Docking Interactions

The docking interactions that envisage the binding affinities of the lead compounds with the binding pockets amino acids in the modelled structures are analyzed by using pose-view module of LeadIT suite [19] which clearly picturizes the Hbond and non-bond interactions. Among these compounds the best docked phytochemical compound Rosemarinic acid (Figure.2) was selected for the further pharamacophore modelling studies.

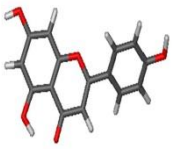
Pharmacophore Modelling and Validation

The pharmacophore model is generated by using the pharmacophore module of Discovery Studio. The Pharmacophore hypothesis generation is achieved by using auto pharmacophore generation option in Discovery Studio which considers the chemical feature types such as the hydrogen bond acceptor (HB_ACCEPTOR), hydrogen bond donor (HB_DONOR),

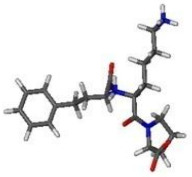
hydrophobic feature (HYDROPHOBIC), negative ionizable feature (NEG_IONIZABLE), positive ionizable feature (POS_IONIZABLE) and aromatic ring (RING_AROMATIC) for the selected ligand. The ten pharmacophore models are generated by using Common Feature pharmacophore Model Generation protocol in Discovery studio. For a statistically significant pharmacophore model, correlation coefficient and root mean square deviation (RMSD) are calculated [20]. The best pharmacophore model was selected based on the high correlation coefficient and lower RMSD. The generated pharmacophoric features based on the Rosemarinic acid is shown in figure.3.



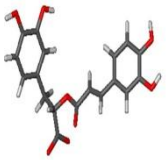
CID:44259992
(Hibiscetin-3-glucoside)



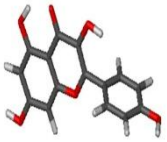
CID:12358401
(Apigenin)



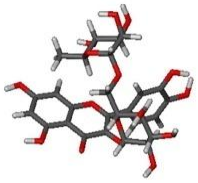
CID:5362119
(Lisinopril)



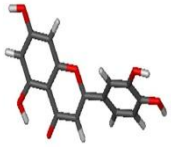
CID:5315615
(Rosemarinic acid)



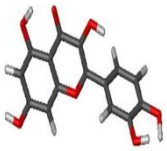
CID:5280863
(Kaempferol)



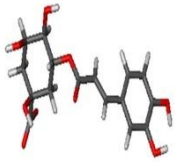
CID:5280805
(Rutin)



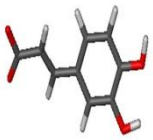
CID:5280445
(Luteolin)



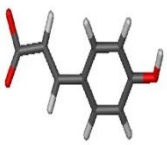
CID:5280343
(Quercetin)



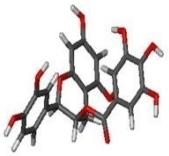
CID:1794427
(Chlorogenic acid)



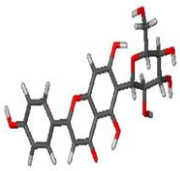
CID:689043
(Caffeic acid)



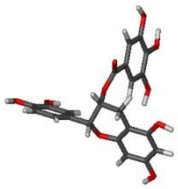
CID:637542
(Coumaric acid)



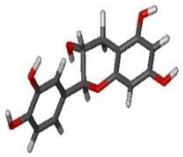
CID:367141
(Epicatechingallate)



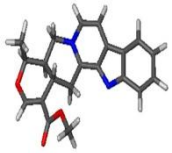
CID:162350
(Isovitexin)



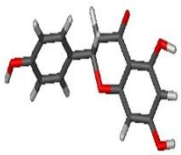
CID:107905
(Epicatechin)



CID:73160
(Catechin)



CID:73073
(Serpentine)



CID:932
(Naringenein)

Figure.1: The 17 phytochemical compounds used in the study for the phamacophore generation

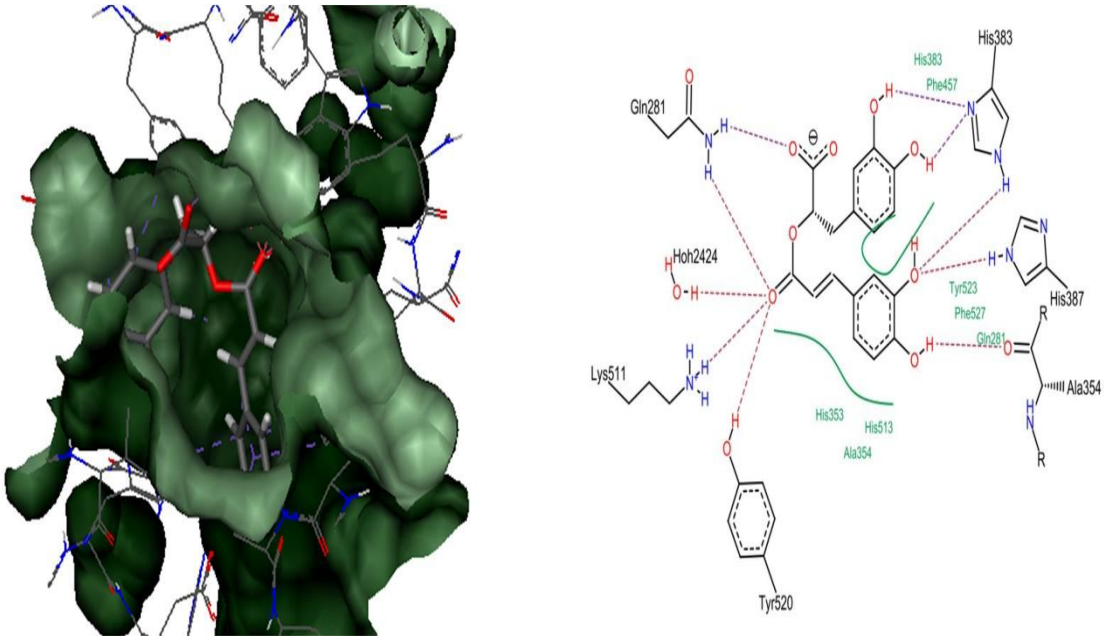


Figure.2 Docking complex and interactions of Rosemarinic acid (CID: 5315615) (-34.6473 kJ/mol)

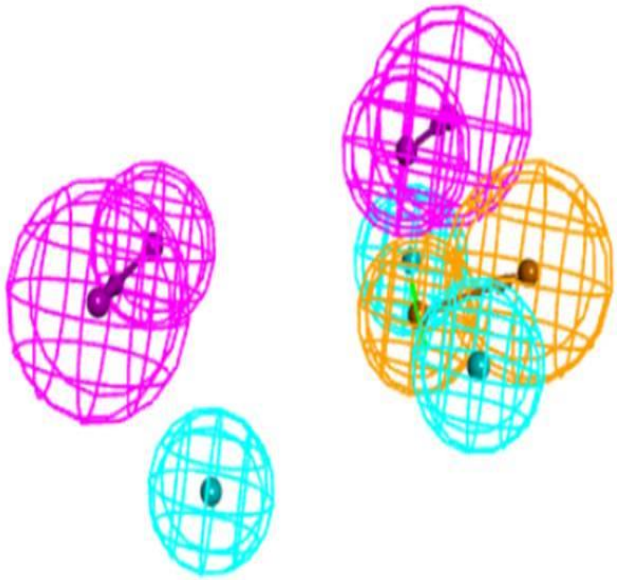


Figure.3: Generated Pharmacophore based on the Rosemarinic acid Hydrogen bond acceptor (green); Hydrogen bond donor (magenta) Hydrophobic (cyan) ; Ring aromatic (orange)

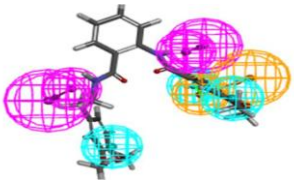
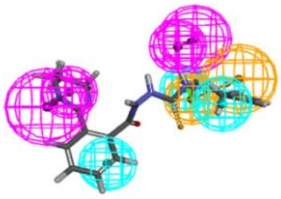
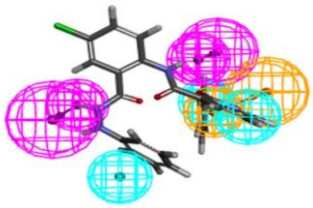
Compound	Mapped Pharmacophore	Fit value
PD 00533		4.66957
CD 01374		3.86604
CD 04888		2.56234
CD 01278		2.54782
BTB 04932		1.89542

Figure.4: Maybridge compounds mapped against the generated pharmacophoric and their fit values

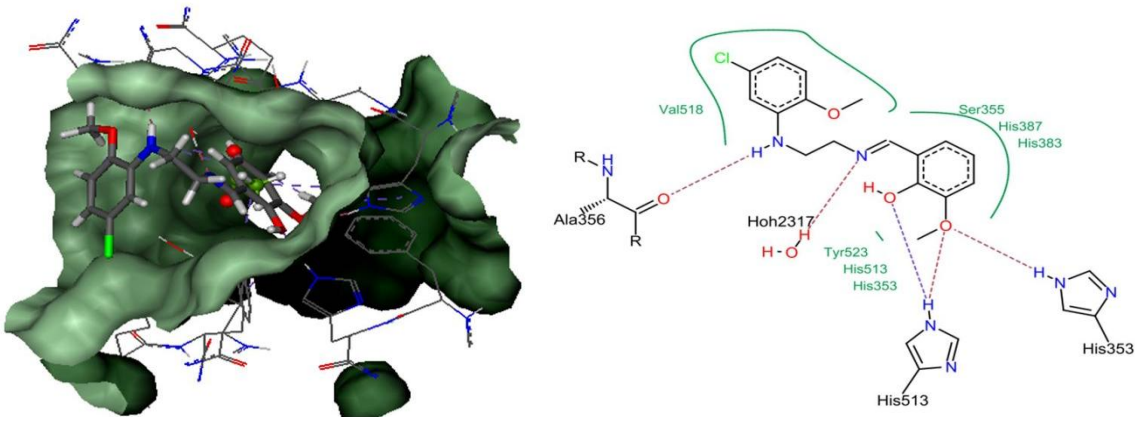


Figure.5: Docking complex and interactions of PD 00533 (-38.4372 kJ/mol)

Table.1: Maybridge compounds with the fit values and docking scores

Compound	Fit value	Docking score
PD 00533	4.66957	-38. 4372
CD 01374	3.86604	-34.5687
CD 04888	2.56234	-32.5624
CD 01278	2.54782	-30.4587
BTB 04932	1.89542	-28.25687

3D Database Screening

Search 3D Database protocol with best search option implemented in DS is used for database screening against Maybridge database consisting of more than one lakh compounds. The obtained database hits is screened using various filters such as estimated activity, Lipinski's rule of five [21], and ADMET properties [22]. The final hit compounds after filtering are known as hit list and ranked according to the fit value, which is the degree of consistency with the pharmacophore model. To decrease the number of hits, a minimum fit value of >3, which is the lowest limit to qualify as a hit compound, is applied. This lower limit of fit value is chosen according to the fit value obtained from the active molecule. The molecules with good fit scores are selected for further docking studies. The generated 3D pharmacophore is subjected to screen the compounds with the significant chemical features against May bridged database, exhibited 5 potential compounds that matches the generated pharmacophore. The five MayBridge Database compound, PD 00533, CD 01374, CD 04888, CD 01278 and BTB 04932 exhibited the best docking scores and also favors the necessary hydrogen bond interactions with in the activity site of ACE and thus identified as novel leads with anti-hypertensive activity. These Hits are defined as those compounds that possess chemical functionalities that spatially overlap with corresponding features within the pharmacophoric model. The hits were subsequently fitted against the pharmacophore and assessed by Fit Value (Figure.4).

Molecular Docking

The Top 5 obtained compounds PD 00533, CD 01374, CD 04888, CD 01278 and BTB 04932 were docked with in the active site of ACE2, and their docking interactions with their binding energies along with their pharmacophoric fit values were tabulated (Table.1). Among the obtained 5 hits from maybridge database, the compound PD 00533 exhibited the highest docking score of -38.4372 kJ/mol (Figure.5). The docking studies implies that the amino acids Alanine (Ala356), Histidine (His 513 and 353) and Water molecule (Hoh 2317) in the binding pockets of ACE are vital in posing the better binding interaction with the maybridge screened compound (PD 00533). While the non bonded interactions are favoured by Valine (Val518), Serine (Ser355), Histidine (His 353,387,383 and 513). These docking interactions also envisages that the =O (keto group) present in the compounds and NH (amino group) on the amino acids favors the Hbond interactions. Thus the pharmacophoric design and 3D database search along with the docking studies revealed that the May Bridge compound PD00533 having the better binding energy of -38.4372 kJ/mol might have a better inhibition activity against the ACE2 receptor.

4. CONCLUSION

The ACE2, a metallo-peptidase is considered as a interesting cardio-renal disease target and is the best known important drug target in the treatment of hypertension that responds to Captopril. Alternatively many phytochemical compounds with their anti-hypertensive activities were also reported. In this study, the 17 phytochemical compounds and 7 other ACE inhibitors with reported IC_{50} values were considered for docking interactions and used as training set to design the pharmacophore and validated. The best docked phytochemical compound Rosemarinic acid has resulted in the generation of 3D pharmacophore is used to screen against May bridged database. The hit compounds were screened using various filters such as estimated activity, Lipinski's rule of five, and ADMET properties and resulted five compounds PD 00533, CD 01374, CD 04888, CD 01278 and BTB 04932 with best fit values and also the best docking scores. This compound also favors the necessary hydrogen bond interactions with in the activity site of ACE and thus identified as novel leads with anti-hypertensive activity.

REFERENCES

[1]. McMurray JJ, Petrie MC, Murdoch DR, Davie AP 1998. Clinical epidemiology of heart failure: public and private health burden. Eur Heart J. 19 (Suppl P):P9–P16.

[2]. Turner AJ, Hooper NM 2002. The angiotensin-converting enzyme gene family: genomics and pharmacology. *Trends Pharmacol Sci*. 23:177–183.

[3]. Ishiyama Y, Gallagher PE, Averill DB, Tallant EA, Brosnihan KB, Ferrario CM 2004. Upregulation of angiotensin-converting enzyme 2 after myocardial infarction by blockade of angiotensin II receptors. *Hypertension*. 43:970 –976.

[4]. Donoghue M, Wakimoto H, Maguire CT, Acton S, Hales P, Stagliano N, Fairchild-Huntress V, Xu J, Lorenz JN, Kadambi V, Berul CI, Breitbart RE 2003. Heart block, ventricular tachycardia, and sudden death in ACE2 transgenic mice with down-

regulated connexins. *J Mol Cell Cardiol*. 35:1043–1053.

[5]. Skeggs LT, Jr, Kahn JR, Lentz K, Shumway NP 1957. The existence of two forms of hypertensin. J Exp Med. 99: 275–282.

[6]. Skeggs LT, Dorer FE, Kahn JR, Lentz KE, Levine M 1976. The biochemistry of the renin–angiotensin system and its role in hypertension. Am J Med. 60: 737–748

[7]. Vázquez-Valadez VH, Abrego VH, Martínez PA, Torres G, Zúñiga O, Escutia D, Vilchis R, Velazquez AM, Martinez L, Ruiz M, Camacho B, Lopez-Castanares R, Angeles E 2013. Docking Studies of Methylthiomorpholin Phenols (LQM300 Series) with Angiotensin-Converting Enzyme (ACE). *The Open Medicinal Chemistry Journal*, 7: 30–38.

[8]. Ehrlich P 1909. Ueber den jetzigen Stand der Chemotherapie. Ber. Dtsch. Chem. Ges. 42, 17–47.

[9]. Sheng-Yong Yang 2010 Pharmacophore modeling and applications in drug discovery : challenges and recent advancesDrug discovery Today, 15:444-450.

[10]. Van Drie JH 2004. Pharmacophore discovery: a critical review. In Computational Medicinal Chemistry for Drug Discovery (Bultinck, P., ed.), pp. 437–460, Marcel Dekker.

[11]. Poptodorov K 2006. Pharmacophore model generation software tools. In Pharmacophores and Pharmacophore Searches (Langer, T. andHoffmann, R.D., eds): 17–47, Wiley–VCH

[12]. Mutasem O Taha, Amal G Al-Bakri and Waleed A Zalloum 2006. Discovery of potent inhibitors of pseudomonal quorum sensing via pharmacophore modeling and in silico screening, Bioorganic & Medicinal Chemistry Letters, 16 : 5902–5906.

[13]. Wolf LK. Digital briefs: New software and websites for the chemical enterprise. C&EN. 2009;87:32

[14]. Gnanendra Shanmugam, Syed Mohamed, Jeyakumar Natarajan 2013. Identification of potent inhibitors for Salmonella typhimurium quorum sensing via virtual screening and pharmacophore modeling, Combinatorial Chemistry & High Throughput Screening, 16 (10): 826 – 839.

[15]. Accelrys Software Inc., 2011. *Discovery Studio Modeling Environment, Release 2.5*, San Diego: Accelrys Software Inc.,.

[16]. Maybridge Database www.maybridge.com/

[17]. Rarey M, Kramer B, Lengauer T, Klebe G 1996. A fast flexible docking method using an incremental construction algorithm. J Mol Biol. 261:470-89.

[18]. Gnanendra Shanmugam, Anusuya Shanmugam, Natarajan Jeyakumar 2012. Molecular modeling and active site analysis of SdiA homolog, a putative quorum sensor for Salmonella typhimurium pathogenecity reveals specific binding patterns of AHL transcriptional regulators, J Mol. Model., 18 (10): 4709 – 4719.

[19]. Stierand K, Maab P, Rarey M 2006. Molecular Complexes at a Glance: Automated Generation of two-dimensional Complex Diagrams. Bioinformatics. 22: 1710-1716.

[20]. Sanam R, Vadivelan S, Tajne S, Narasu L, Rambabu G, Jagarlapudi SA 2009. Discovery of potential ZAP-70 kinase inhibitors: Pharmacophore design, database screening and docking studies. Eur. J. Med. Chem., 44, 4793-4800.

[21]. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ 2001. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Del. Rev., 46, 3-26.

[22]. Walters WP, Murcko MA 2002. Prediction of 'drug-likeness'. Adv. Drug. Deliv. Rev, 54: 255-271.