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3-D QSAR modeling of benzoxazole benzenesulfonamides substituted derivatives and feature based inhibitor identification as antidiabetic agent

Jagbir Singh, Rani Mansuri, Ganesh Chandra Sahoo and Mahesh Kumar

Abstract
Since fructose-1,6- bisphosphatase regulates the gluconeogenesis pathway and it is a rate limiting enzyme, makes itcrucial enzyme of gluconeogenetic pathway and a important drug target. Since the compounds used for study were already synthesized as anti-diabetics by Chunqiu *et al*. So the *3-D* QSAR model of benzoxazole benzenesulfonamide derivatives was developed and analyzed using PHASE3.4 (Schrodinger9.0). A statistically valid and reliable *3-D* QSAR (AAADRR.27) model with R^2= 0.9686 and validation with correlation coefficients (q^2 = 0.72) was built, that indicate good statistical predictive ability. Effect of hydrophobic/nonpolar, withdrawing/ H-bond acceptor, H-bond donor groups of the compounds were correlated with the activity. *3*-D QSAR model was used for virtual screening. Five compounds (CoCoCo.ID 456,043, 419,095, 456,554, 445,527, 406,692) showed better docking score than most potent analog41 of benzoxazole benzenesulfonamide. Therefore these compounds can also be used as lead for lead modification and optimization for antidiabetic drug discovery and development.

Keywords: *Fructose-1, 6-biphosphate*, benzoxazole benzenesulfonamide, PHASE3.4, *3-D QSAR*, ADME, Toxicity

1. Introduction
The gluconeogenic pathway produces glucose from three-carbon precursors (lactate, alanine and glycerol) in the liver and kidney to fulfill the supply of glucose to brain and red blood cells depend for functioning [1].
According the national diabetes statistics (NDS) 2016, WHO projected diabetes as the seventh leading cause of death in the United States upto 2030? The age-adjusted rate of diagnosed diabetes among Asian and American adults was found 4.4% for Chinese, 11.3% for Filipinos, 13.0% for Asian Indians, and 8.8% for other Asians. New cases of diabetes are risen upto 422 million[2]. Diabetes is becoming the reason of blindness, kidney failure and heart strokes etc. So there is need of development of new drug or identification of leads for structure based drug desingning [2].
Human enzyme fructose -1,6-bisphosphatasecatalyses rate limiting step in the gluconeogenesis pathway by converting fructose-1,6-bisphosphate into fructose-6-phosphate. So it has long been worked upon this enzyme as potential therapeutic target for the treatment of type 2 diabetes [3-6]. The vital role of gluconeogenesis was confirmed by inhibition of Fructose 1,6-Bisphosphatase as inhibition of this enzyme reduces excessive glucose production in the body [7]. Since fructose -1,6-bisphosphatase can be a good target against diabetes and no potent inhibitors of this enzyme as antidiabetic reported over last few decades [8-9]. *3-D* pharmacophores is one of the most significant contributions of computational chemistry to drug discovery. QSAR (Quantitative structure activity relationship) applied to develop correlation between physicochemical properties and biological activities of chemical substances [10-12].
In present research study QSAR model was developed for the inhibitors of fructose -1,6-bisphosphatase, congeneric derivatives of series of benzoxazole benzenesulfonamides [13-14] using PHASE3.4 module of Schrodinger9.0 suite [15]. Best QSAR model was selected as query for virtual screening of CoCoCo. asinex compound library as Virtual screening is the very potent method to identify new fittest and new scaffold for any protein. Fifteen compounds

were found non carcinogenic, non-mutagenic, nontoxic and also found to good ADME properties where five out fifteen compounds found to have better docking score than most potent analog41 among benzoxazole benzenesulfonamide derivaives. Hence these compounds can further be tested for antidiabetic activity and can be used as lead molecules for lead modification and optimization to design potent antidiabetic drug against fructose -1,6-bisphosphatase.

2. Computational Methodology

PHASE3.4 module of Schodinger9.0 was used for *3-D* QSAR modeling. Phase is a flexible product for common pharmacophore development, activity prediction of external as well as internal compounds, and 3*D* database searching. Phase identifies pharmacophores found common among a set of aligned ligand conformations and each common pharmacophore hypotheses using a range of scoring techniques in the relative manner in which the molecules are likely to bind with target receptor. Developed hypothesis may be then combined with active compound dataset to make a 3-D *QSAR* model.

Phase module of Schrodinger9.0 can be used for lead discovery, structural activity relationship; lead optimization and. Phase may also be used screening large compound databases and libraries [15].

2.1 QSAR model development

2.1.1 Compound preparation and Dividation of compound dataset into actives and inactives

Compounds were prepared by using Ligprep2.5. Compounds were divided into active and inactive compounds on the basis of pLogIC50 value by setting threshold value. The compounds having pLogIC50 value above and below the threshold value were considered as actives and inactives respectively.

Conformations for each ligand were generated with a maximum number of 1000 conformations for each ligand using ConfGen module of Schrodinger9.0 applying OPLS_2005 force field and distance-dependent dielectric solvation.

2.1.2 Find common pharmacophore for actives and Score hypothesis

Aligned actives were used for common pharmacophoric features hypothesis responsible for interacting with the receptor. Hence all the actives were aligned to find common pharmacophoric features.

Then pharmacophore sites were created based on six pharmacophore features, including hydrogen bond acceptor (A), hydrogen bond donor (D), hydrophobic group (H), negatively charged group (N), positively charged group (P) and aromatic ring (R). The rules of feature definitions were applied to plot the positions of each pharmacophore site. Pharmacophore features were characterized based on set of chemical structure patterns of the studied compounds [15].

Scoring of the entire hypotheses step was done. Scoring hypothesis procedure provided a ranking order to the different hypothesis generated on the basis of survival and survival-inactive scores. The appropriate pharmacophoric hypothesis generated should differentiate between actives and inactives. So the hypothesis which could not differentiate between actives and inactives were not a valid hypothesis and rejected

2.2.3 Built QSAR model

Training set and test set were made randomly. Training set 70% of the total compounds set and test set of 30% of total dataset were prepared. *3D*-QSAR approaches involve the production of a common pharmacophore hypothesis on the basis of alignment of pharmacophoric features of the training set of actives. Parameters (*Random seed, grid spacing*) were kept default except *PLS factor* was kept 4. Eliminate variables with *[t-value]* < 2.00 was selected to eliminate uninformative variables that increase the model generality. Applying Partial Least Square (PLS) statistical analysis, all Pharmacophore hypotheseswere further used to develop *3D*-QSAR models using. The favoring and disfavoring bioactivity volume occluded maps were generated for the pharmacophore hypothesis for observing and explaining of variation in activity by the variation in the structural features.

2.2 Pharmacophore based virtual screening

Virtual screening is the very potent method to identify new fittest and new scaffold for any protein. Pharmacophore based virtual screening has been succeed in finding the novel scaffold against many targets. Virtual leads can be identified using Virtual screening approach; this makes the importance of virtual screening in drug discovery. The best 3D pharmacophore will be used for screening CoCoCo.asinex compound database to find novel scaffold for target protein. The hit molecules selected only if it maps at least three out of six pharmacophoric features of the hypothesis used and scored Phase Match value one that means molecules passes the criteria using Phase module of Schrodinger9.0. The screened compounds were then further sorted by using Lipinski's rule of five, ADME and toxicity as screening filters using Prepare and filter ligands and calculate molecular properties tools of Discovery studio 3.5 [16-18]. The molecules which passed the filters were the subjected to docking studies.

2.3 Molecular docking

LibDock module of DS3.5 was used for docking. Protein Fructose-1,6- bisphosphatasewas prepared using DS3.5.Binding site was created by selecting Analog 41 using Discovery studio3.5 having binding site dimensions X (-15.14), Y (13.25), and Z directions (-18.08) for docking. The compound preparation and minimization was done prior docking. The conformations of compounds were generated with maximum 225 numbers of conformations to be generated and conformations of separate isomers were also created within the threshold of 20.0 kcal/mol relative energy. The CHARMM force field was applied to minimize the compounds with 1000 steps of Steepest Descent (SD) algorithm. The compounds poses having RMSD less than 1.0 Å were considered as duplicates as the higher specified RMSD value will reduce the number of ligand poses returned and a RMS gradient 0.001 [19].

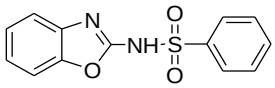
LibDock is a high-throughput algorithm developed by Diller and Merz, which consist of the polar and apolar features as "Hotspots". The molecules which passed the applied filters were docked with the crystal structure of Fructose-1,6-bisphosphatase(PDB id.2FIX). Most potent analog 41 (derivative of benzoxazole benzenesulfonamide)[14] was also docked to analyze and compare interacting amino acids of analog 41 and proposed screened compounds. CHARMM force field was applied for docking and scoring and other docking parameters were kept default. Ligand-receptor minimization *in situ* during dockingwas performed on the complexes to remove any ligand van der Waals clashes prior scoring and calculating binding energy. The 5,000 steps of SD with free movement of atoms within the binding site sphere were minimized [20]. The binding energy of protein–ligand

complexes was calculated from the free energies of the complex, and free energies of individual protein and the ligand using CHARMM force field and implicit salvation method [21].

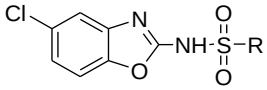
3. Compound Dataset

Dataset of 41 derivatives of benzoxazole benzenesulfonamide as given in Table.1 used to for developing *3D-QSAR* model in the present study and validation. IC50 values >50 were used as IC50 = 50 to eliminate calculation error. The pIC50 values were used instead of IC50. Data set consist of some highly active, few moderately and inactive molecules out of which 28 molecules were randomly chosen for training set and 13 molecules were selected for test sets [14].

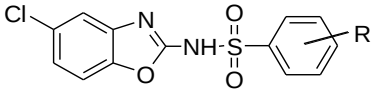
Congeneric derivatives (Table1) of following three topological class of benzoxazole benzenesulfonamide (Compound- A) i.e. A.1, A.2, A.3 are used in present *3-D QSAR* model building as input dataset.



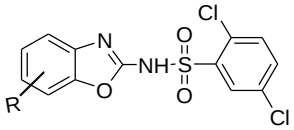
Compound-A:- *N*-(1,3-Benzoxazol-2-yl)benzene sulfonamide



A.1. 5- Chloro-*N*-(dioxide- substituted-sufanyl)-1, 3benzoxazol-2-amine



A.2.*N*-(5-Chloro-1,3-benzoxazol-2-yl) substituted-benzene sulfonamide



A.3.*N*-(1,3- (Substituted-benzen-oxazol-2-yl) -2,5-dichloro benzene sulfonamide

4. Results and Discussion

Benzoxazole benzenesulfonamide derivatives having efficacy potential towards *fructose-1,6-bisphosphatase* were used to make *3D QSAR* model. Following Common pharmacophore *AAADRR.27*** with Survival score = 3.741, Site score = 0.97, Vector score = 0.998, Volume = 0.775, Activity = 5.720, SD = 0.1167,* R^2 *= 0.9686,* Q^2 *= 0.7203, Pearson R = 0.791* selected as a best 3-D QSAR model.

The compound dataset (Table 1) was divided into actives and inactive sets by setting threshold in pIC_{50} (pIC_{50} more than 5.2 as active and pIC_{50} less than 5.0 as inactive) values of molecules and between this ranges are moderately active molecules. Pharmacophoric sites were created. Common pharmacophores matching at least 17 out of 18 active compounds were identified and scored for hypothesis (shown in dark black color in Table 2). The dataset was divided randomly into training set (70% of dataset) and test set (30% of dataset). The common pharmacophores were aligned and trained with training set and validated with test set. Out of found QSAR models AAADRR.27*** found best shown in dark black color in Table 3. The variant AAADRR.27**(Pharmacophoric features as shown in Fig. 1) mentioned in Table 2 found as best common pharmacophore to make *3-D* QSAR model and a best Square of Regression (R^2 = 0.9686 at PLS factor 4) line for AAADRR.27*** (Three H-Acceptor, one H-Donor and Two aromatic) was produced as shown in Table 3. *3-D* QSAR model AAADRR.27*** gave regression line a best fit line for all compounds that showed good predictability of selected QSAR model as shown in Fig. 2. *3-D* QSAR model was validated by correlating the test dataset to the selected *3-D* QSAR model AAADRR.27***. Distances between the all common pharmacophoric features of selected *3-D* QSAR model were measured that may help in screening compounds databases as shown in Fig. 3. Alignment of most active ligands (5, 41), all active ligands and all (active+inactive) ligand with the bestselected *3-D* QSAR model shown respectively shown in Fig. 4(A),(B),(C) indicated that most active molecules align perfectly on the common pharmacophoric features of final best *3-D* QSAR model (AAADRR.27***).

3-D QSAR model.AAADRR.27*** was visualized from QSAR model where Fig5 showed volume occlusion maps for hydrophobic/nonpolar, electron withdrawing, H-bond donor group effect in activity and inactivity of ligands. Volume occlusion map for hydrophobicity of most active ligand (ligand 41) in QSAR model as shown in Fig. 5(A) confirmed thatlarge volume occupied by dark blue cubes contours in QSAR model indicated that increase in hydrophobicity in that region on particular positionsof benzoxazole benzenesulfonamide may increase the overall bioactivity. The hydrophobic volume occupied by red contour may disfavor the overall activity. That can be analyzed by comparing the hydrophobic occlusion map of inactive ligand shown in Fig. 5(B) and the most active ligand shown in Fig. 5(C) which described that lack of hydrophobic/nonpolar groups in the region occupied by red contour cubes may be disfavoring the bioactivity of most inactive compound no. 27 like ligands as shown in Fig. 5. (B). Effects of electron-withdrawing groups viewed by QSAR model as shown in Fig. 5.(D) where yellow contour region defined the presence of electron-withdrawing groups in that region favours the bioactivity. The effect of H-bond donor viewed from QSAR model as shown in Fig. 5.(E) where green contor defined that the H- bond donor groups in that region favours the activity and red contor defines the disfavoring region of H – bond donor.

Favoring and Red cubes contors are inactivity favoring. (C)- Most active ligand-41. (D)- Electron withdrawing effects from QSAR model of most active ligand. (E)- H-bond donor effect from QSAR model of most active ligand.

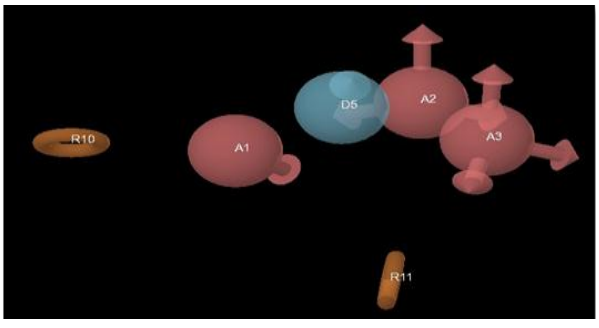


Fig 1: Common Pharmacophore hypothesis AAADRR from actives. Best common pharmacophore for predictive atom based QSAR model.

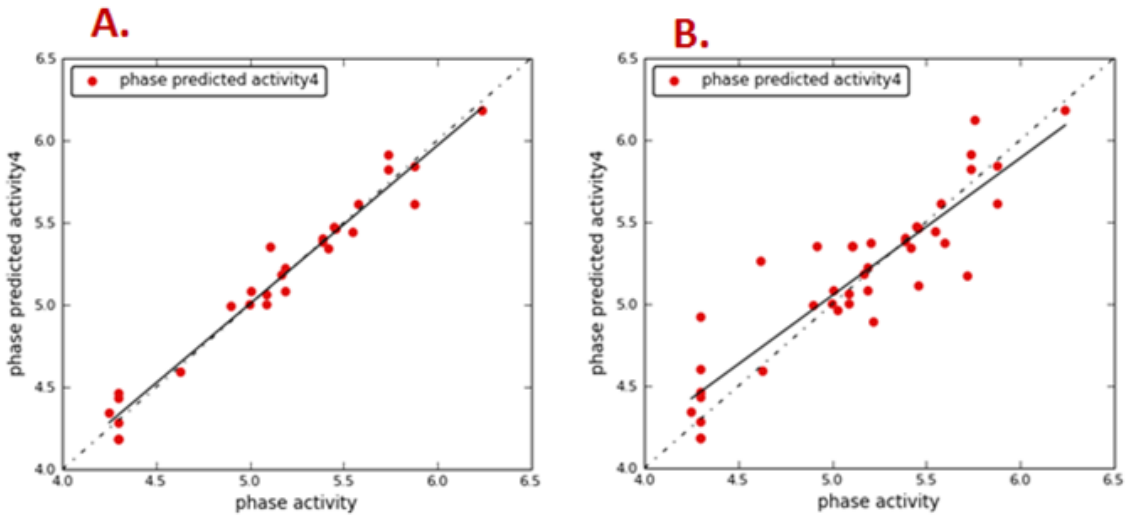


Fig 2: Graph between Predicted PIC50 and Actual PIC50 of test set for 3-D QSAR approaches. (A). Graph for phase predicted and phase activity of training set molecules. (B). Graph for phase predicted and phase activity of all molecules used for 3-D QSAR.

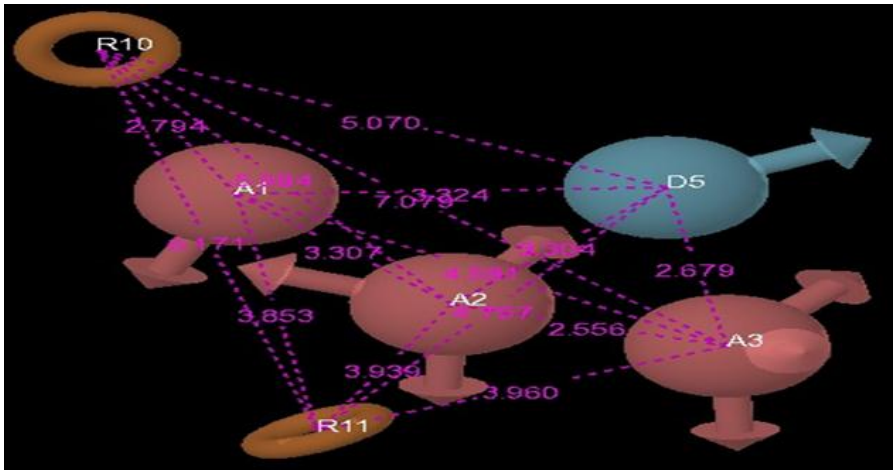


Fig 3: Distance map of pharmacophore features of best QSAR model AAADRR.27.

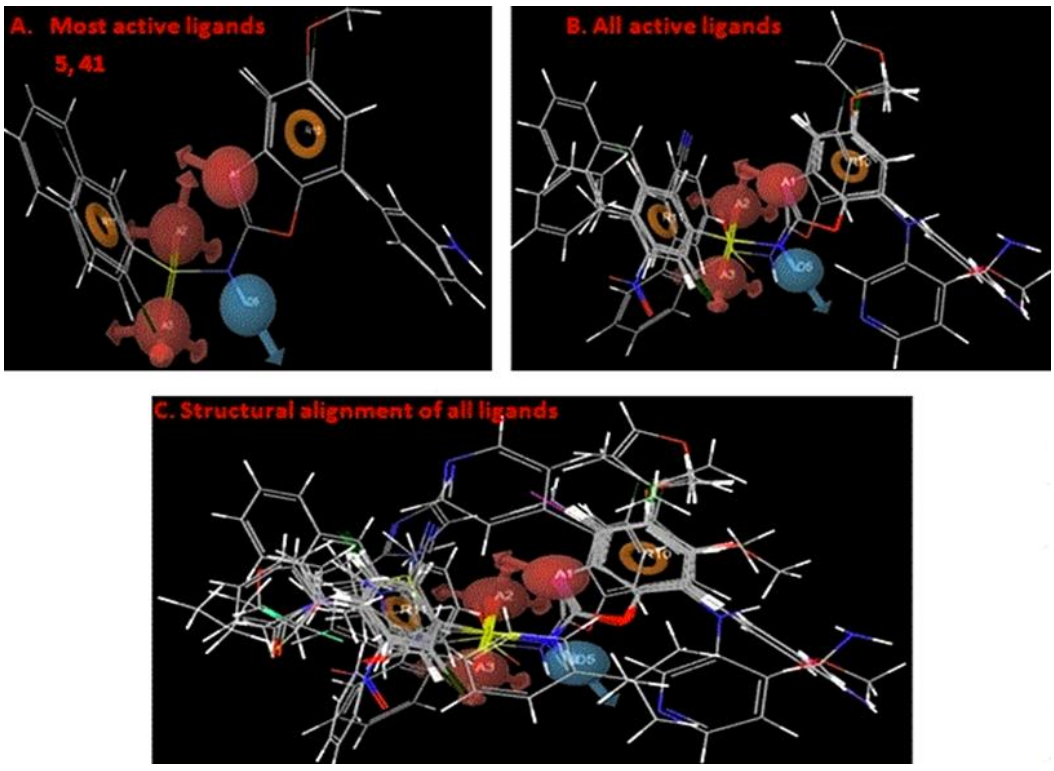


Fig 4: (A) Alignment of most active ligand 5, 41 on common pharmacophor. **(B)** Alignment of all active compounds on common pharmacophore features **(C)** Alignment of all ligands on common pharmacophore of best QSAR model AAADRR.27.

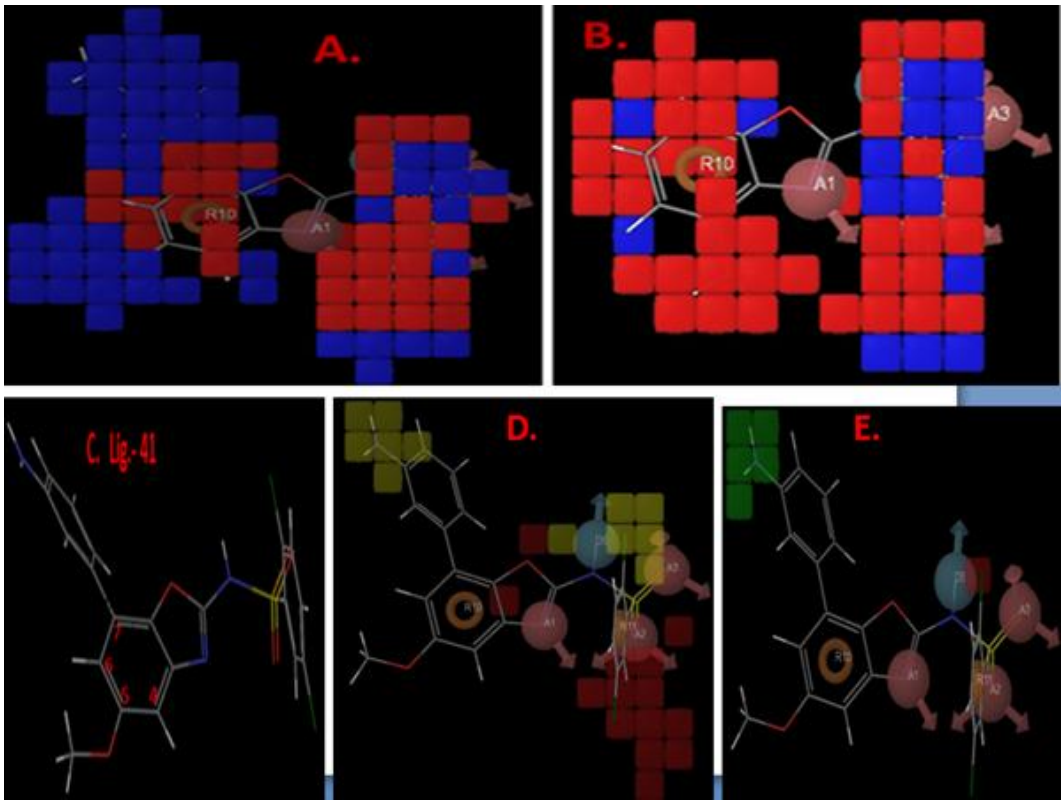
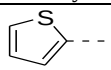
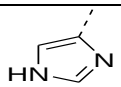


Fig 5: Volume occlusion map of most active ligand in QSAR model (A)- Hydrophobic effect of active ligand (41 ligand). (B)- Hydrophobic effect of inactive ligand (27 ligand). Blue cubes contors are activity.

Table 1: Compound dataset used for 3d-qsar model building with their pic50, actual and predicted activities.

S. No.	Compound	R	Actual PIC50	Predicted PIC50	Residuals	LibDock	Binding Energy Kcal/mol
1.	A.1.a	1,4-Dichlorobenzene	5.46	5.11	-0.35	79.0324	-108.6
2.	A.1.b	Phenyl	5.11	5.35	0.24	75.30	-150.5
3.	A.1.c		4.9	4.99	0.09	75.23	-82.7
4.	A.1.d		5.03	4.96	-0.07	78.8835	-86.8
5.	A.1.e	Naphthalene	5.6	5.37	-0.23	78.6641	-115.1
6.	A.1.f	Anthracene	5.46	5.46	0	78.3442	-59.4
7.	A.1.g	Me	4.3	4.92	0.62	77.072	-121.6
8.	A.1.h	n-Bu	4.3	4.18	-.012	73.512	-132.7
9.	A.2.a	2-Br	5.19	5.08	-0.11	73.2439	-117.5
10.	A.2.b	2-CN	5.22	4.89	-0.33	77.8163	-107.1
11.	A.2.c	2-Ph	5.42	5.34	-0.08	75.3235	-114.0
12.	A.2.d	2-(Imidazol-1-yl)	4.63	4.59	-0.04	75.2882	-130.8
13.	A.2.e	3-Cl	5.88	5.61	-0.27	75.0588	-109.6
14.	A.2.f	3-NO2	5.74	5.91	0.17	74.9741	-132.9
15.	A.2.g	3-Ph	5.008	5.08	0.072	73.3067	-87.4
16.	A.2.h	3-(4-t-Bu-benzyloxy)	4.3	4.43	0.13	72.1309	-143.8
17.	A.2.i	4-F	5.55	5.44	-0.11	75.3235	-97.7
18.	A.2.j	4-OCF3	5.107	5.35	0.243	75.2882	-77.6
19.	A.2.k	4-Me	5.207	5.3	0.163	75.0588	-123.1
20.	A.2.l	4-t-Bu	4.62	5.26	0.64	74.5646	-97.7
21.	A.2.m	4-Ph	5.39	5.38	-0.01	76.2414	-80.5
22.	A.2.n	4-(3-Furanyl)	5.17	5.18	0.01	76.1525	-69.7
23.	A.2.o	4- NHCOCH2CH2CH3	4.92	5.35	0.43	73.3067	-133.4
24.	A.2.p	2,6-Di-Cl	4.3	4.60	0.3	73.1049	-108.9
25.	A.2.q	2-Cl, 6-Me	4.3	4.46	0.16	72.8595	-113.2
26.	A.3.a	4-I	4.3	4.18	-0.12	72.5654	-96.8
27.	A.3.b	4-Me	4.25	4.34	0.09	72.3774	-110.5
28.	A.3.c	4-(4-Pyridyl)	4.3	4.28	-0.02	74.7555	-137.0
29.	A.3.d	5-Br	5.72	5.17	-0.55	76.0146	-98.6
30.	A.3.e	5-Me	5.19	5.22	0.03	75.6986	-118.3
31.	A.3.f	5-t-Bu	4.49	4.41	-0.08	72.3654	-123.1
32.	A.3.g	5-(3-Furanyl)	5.39	5.40	0.01	77.8163	-109.7

33.	A.3.h	6-Cl	5.09	5.00	-0.09	78.20	-101.8
34.	A.3.i	6-Me	5.0	5.00	0	78.40	-118.8
35.	A.3.j	6-MeO	5.09	5.06	-0.03	75.80	-99.3
36.	A.3.k	6-MeO, 7-(4-MeO-3-Pyridyl)	5.45	5..47	0.02	75.30	-131.4
37.	A.3.l	5-MeO, 7-(3-HO-Ph)	5.76	6.12	0.36	72.70	-133.2
38.	A.3.m	5-MeO, 7-(4-HO-Ph)	5.74	5.82	0.08	76.80	-143.2
39.	A.3.n	5-MeO,7-(3-NH2CH2-Ph)	5.58	5.61	0.03	78.54	-62.2
40.	A.3.o	5-MeO, 7-(4-NH2-Ph)	5.88	5.84	-0.04	78.20	-154.4
41.	A.3.p	5-MeO, 7-(3-NH2-Ph)	6.24	6.18	-0.06	80.0	-155.8

* Molecules were selected as test set randomly

Table 2: QSAR hypothesis scoring of common pharmacophores**.**

ID	Survival	Survival-inactive	Posthoc	Site	Vector	Volume	Selectivity	# Martches	Activity	Inactives
AAADHR.216	3.677	1.258	3.677	0.94	0.997	0.743	1.878	16	5.207	2.418
AAADHR.126	3.675	1.258	3.675	0.94	0.996	0.741	1.87	16	5.207	2.417
AADHRR.111	3.685	1.232	3.685	0.92	0.997	0.769	2.182	16	5.72	2.453
AAAHRR.201	3.669	1.255	3.669	0.93	0.997	0.74	2.23	16	5.207	2.414
AAADRR.27**	3.741	1.147	3.741	0.97	0.998	0.775	1.645	17	5.72	2.594

** Selected common pharmacophore hypothesis for QSAR Model**.**

Table 3: QSAR models for common pharmacophores of benzoxazole benzenesulfonamide derivatives.

ID	Factors	SD	R^2	F	P	RMSE	Q^2	Pearson-R
AAADHR.216	1	0.3642	0.5931	37.9	1.65e-006		0.2334	0.4966
	2	0.2562	0.8064	52.1	1.221e-009	0.4209 0.4769	0.016	0.3703
	3	0.1723	0.9159	87.2	4.811e-013	0.4064 0.4067	0.2853	0.5568
	4	0.1383	0.9481	105	2.014e-014		0.3841	0.5812
AAADHR.126	1	0.3709	0.5779	35.6	2.683e-006		0.2343 -	0.4927
	2	0.2616	0.7982	49.4	2.051e-009	0.4206 0.4958	0.0635	0.3078
	3	0.1844	0.9037	75.1	2.453e-012	0.4375 0.4264	0.1719	0.466
	4	0.1397	0.9471	102.8	2.508e-014		0.4134	0.5158
AADHRR.111	1	0.378	0.5617	33.3	4.433e-006		0.2619	0.516
	2	0.2713	0.7829	45.1	5.12e-009	0.413 0.4731	0.0315	0.3795
	3	0.1961	0.8911	65.5	1.061e-011	0.4275 0.4099	0.2092	0.4995
	4	0.1398	0.947	102.7	2.55e-014		0.4728	0.5636
AAAHRR.201	1	0.3636	0.5944	38.1	1.58e-006		0.2416	0.5002
	2	0.2569	0.8053	51.7	1.313e-009	0.4186 0.4861	-0.0224	0.3461
	3	0.1727	0.9155	86.7	5.112e-013	0.4252 0.4165	0.2177	0.5033
	4	0.1291	0.9548	121.4	4.113e-015		0.4492	0.55
AAADRR.27***	1	0.357	0.609	40.5	9.707e-007		0.2958	0.5468
	2	0.2629	0.7962	48.8	2.323e-009	0.4034 0.4554	0.3025	0.4484
	3	0.1629	0.9249	98.5	1.258e-013	0.3774 0.3963	0.5836	0.6482
	4	0.1167	0.9686	149.8	4.097e-016		0.7203	0.791

*** selected as common pharmacophore model for QSAR.

4.1 Visualization of Atom Based Phase *3D-QSAR* Model Volume Map

4.1.1 Effect of Hydrophobic/Nonpolar groups on bioactivity

Hydrophobicity favours activity to the much extent. Hydrophobic volume by workspace most active (compound-41) of QSAR model was mapped as shown in Fig. 6(A) where dark blue color contours occupying the big volume suggested that hydrophobic groups placement on 7thpostion [7-(3amino-phenyl)] and 5th position [5-(methoxy)] of benzoxazole heterocyclic ring may favour the bioactivity. Red color contour on or between the 6th and 4thposition also suggested that the hydrophobic/nonpolar in that region or position may disfavor bioactivity. Big volume occupied by dark blue color contours on 3rd, 4th and 5th of the 2,5- dichlorobenzene ring of benzenesulfonamide suggested that hydrophobic/nonpolar group replacement may favour the bioactivity at these positions. Hence hydrophobic groups at given positions may be favour bioactivity.

4.1.2 Effect of Hydrogen donor groups on bioactivity

Volume occupied by dark blue contour on 3-amino group of 7-phenyl ring of benzoxazole heterocyclic ring and on the nitrogen as amino group in benzenesulfonamide ofbenzoxazole benzenesulfonamide suggested that presence of hydrogen donor group on these positions in the benzoxazole benzenesulfonamide like compound may favour the bioactive interaction toward the *Fructose-1,6-bisphosphatase as shown in* Fig. 6(B) where yellow color contour region confirmed about the position or region where hydrogen donor group replacement on that particular position or region may lead to decrease in activity.

Effect of electron-Withdrawing group on bioactivity

Dark blue color contour region present on nitrogen [7-(3-amino) phenyl ring and oxygen (5-methoxyphenyl) atoms of benzenesulfonamide heterocyclic ring as shown in Fig. 6(C) suggested that the presence of electron withdrawing groups on these positions may lead to enhancement of bioactivity of the molecule. Where red contour region defined the electron withdrawing group replacement in that region disfavored the bioactivity of the molecule.

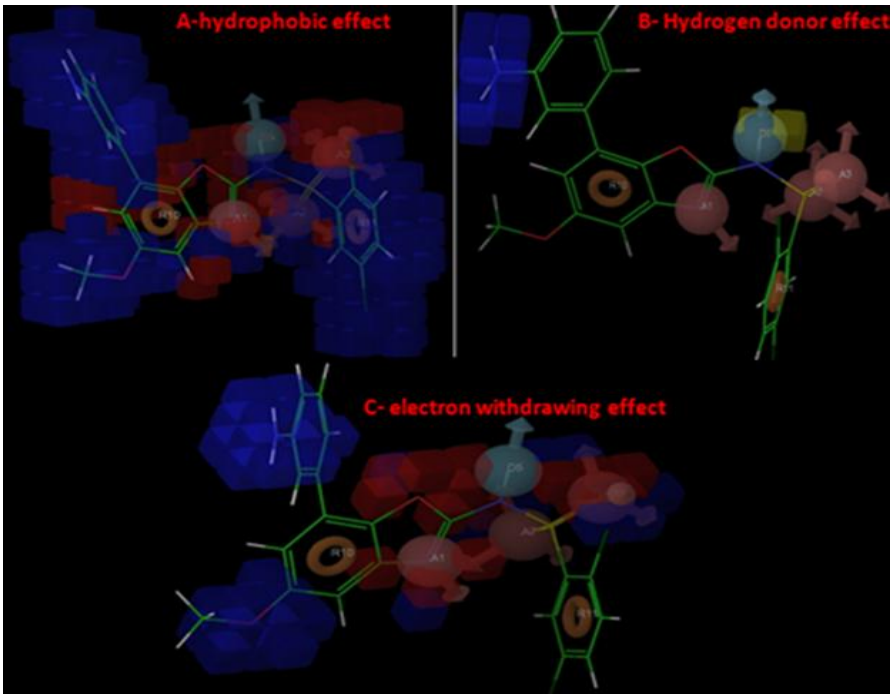


Fig 6: Volume occlusion map of most active ligand in workspace (blue color favors activity and red/yellow disfavors activity). (A). Volume occupied by favoring and disfavoring regions of hydrophobic group effects. (B). Volume occupied by favoring and disfavoring regions of hydrogen donor group effects. (C). Volume occupied by favoring and disfavoring regions of electron-withdrawing group effects.

4.2. Pharmacophore Based Virtual Screening

The best *3-D* QSAR hypothesis was selected as query for pharmacophore based virtual screening for identification of new scaffolds as inhibitor of *Fructose-1,6- bisphosphatase*. CoCoCo.Asinex compound database was selected and screened using Phase module of Schrodinger9.0. Only thousand compounds (PhaseMatch score =1) matched the hypothesis. Using Prepare and filter ligands module of Discovery Studio3.5 these compounds were first screened on the basis of Lipinski rule (LogP ≤ 5, molecular weight ≤ 500, number of hydrogen bond donor ≤5 and acceptor are ≤ 10). The molecules which passed the rule of five were further screened for ADMET (human intestinal absorption LogP, aqueous solubility, blood brain barrier (BBB), heptotxicityand CYPP450 2D6 interaction) and toxicity (carcinogenicity, mutagenicity, toxicity etc.) using Calculate molecular properties tool of Discovery studio3.5.Compounds with no lipinski violation, good ADMET and free of toxic effect were used for molecular docking. As absorption, solubility, BBB, and toxicity prediction plays important role in selection of compounds to move next stages in drug discovery. Level of 3, 3or 4, 0 for BBB, solubility and absorption respectively were considered best for good ADMET of compounds. Only fifteen compounds (Fig. 7) could satisfied these filters and were found non carcinogenic, non mutagenic, nontoxix, no interaction with CYPP450 2D6 along with good ADME properties as shown in Table.4.

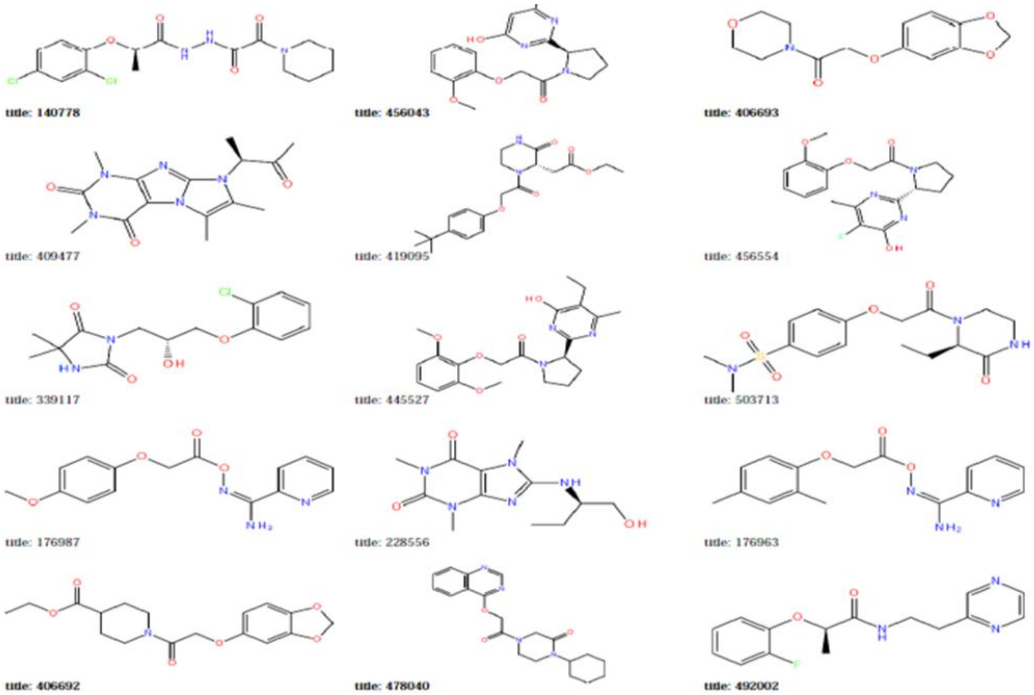


Fig 7: Molecular structure of screened molecules on the basis of 3*D* QSAR model.

Table 4: Molecules screened on the basis of pharmacophore based virtual screening.

S. no.	CoCoCo. ID	Phase score	Rule'5 violation	BBB level	Solubility level	Absorption level	Carcinogenic	mutagenic	Toxic	Cyp2D6 Interaction	Lib Dock Score	Binding Energy Kcal/mol
1	140778	1	0	3	3	0	No	No	No	No	68.1	-122.7
2	176963	1	0	3	3	0	No	No	No	No	67.5	-120.5
3	176987	1	0	3	3	0	No	No	No	No	64.6	-140.2
4	228556	1	0	3	3	0	No	No	No	No	74.3	-86.3
5	339117	1	0	3	3	0	No	No	No	No	72.8	-115.8
6	406692	1	0	3	3	0	No	No	No	No	86.8	-155.2
7	406693	1	0	3	4	0	No	No	No	No	70.3	-109.2
8	409477	1	0	3	3	0	No	No	No	No	70.6	-81.6
9	419095	1	0	3	3	0	No	No	No	No	86.9	-169.4
10	445527	1	0	3	3	0	No	No	No	No	82.2	-137.1
11	456043	1	0	3	3	0	No	No	No	No	81.2	-147.4
12	456554	1	0	3	3	0	No	No	No	No	83.4	-122.9
13	478040	1	0	3	3	0	No	No	No	No	64.3	-119.1
14	492002	1	0	3	3	0	No	No	No	No	62.5	-120.6
15	503713	1	0	3	4	0	No	No	No	No	62.8	-124.2

4.3. Molecular Docking

Fifteen compounds sorted using different screening filters were docked into the binding pocket of*Fructose-1,6-bisphosphatase* where benzoxazole benzenesulfonamide analog 41 binds using Lib Dock module of Discovery Studio3.5. Binding pocket of analog 41 was selected as binding site for docking of compounds. Analog 41 interacts with C=O of amino acids Gly26 and Thr27 of a single monomer [14]. Screened compounds and protein were prepared using Prepare ligand and Prepare protein module respectively of Discovery Studio3.5. The most potent analog 41 (from Table.1) of benzoxazole benzenesulfonamide found to have LibDock score 80.6. The compounds with CoCoCo.ID 419095, 406692, 456043, 456554 and 445527 found to have LibDock score 86.9, 86.8, 81.2, 83.4 and 82.2 respectively than most potent benzoxazole benzenesulfonamide analog 41. The binding energies of top five indentified compounds were approx. similar to the potent internal compounds but two compounds CoCoCo.ID 419095 and 406692 scored lowest binding energies among all five indentified compounds as well as lower binding energy than internal compounds as given in Table.4. Compound 419095 also formed hydrogen bonds with Gly28, Thr31 and Gly21. That compound also interacted, alike most potent analog.41 within the binding pocket. Compound 419095 used two Gly and one Thr residues for binding with target protein as shown in Fig.8.

Compound 406692 also interacted with Gly28, Thr31, Leu30, Gly21, Tyr113 amino acids. Compound used two Gly and one Thr31 for binding which were also used by compound 419095 for interacting with target protein. Besides these amino acids compound 406692 also used Leu30 as hydrogen donor and Tyr113 as hydrogen acceptor for interaction as shown in Fig.9. Hydrogen bond interactions formed by these two top scoring compounds 419095 and 406692 was found similar to that of most potent internal compound 41.

Five indentified compounds and top potent compounds 40 and 41 were superimposed within the binding pocket and were found properly aligned and superimposed. The compounds indentified based on 3-D QSAR model were fitting in an orientation similar to potent internal compounds 40 and 41 within the same binding pocket as shown in Fig10. So these molecules can further be investigated as lead and may directly be used as template for drug designing for antidiabetic development.

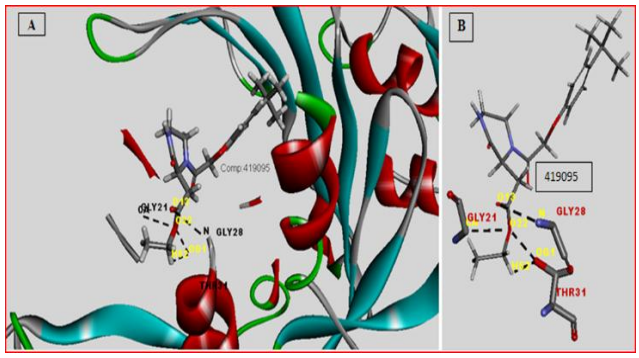


Fig 8: Interacting orientation of identified compound 419095 within target protein (A). Binding orientation of compound into the binding site. (B). Interacting amino acids and donor and acceptor group atoms are shown. Donor/acceptor group atoms are in Yellow color.

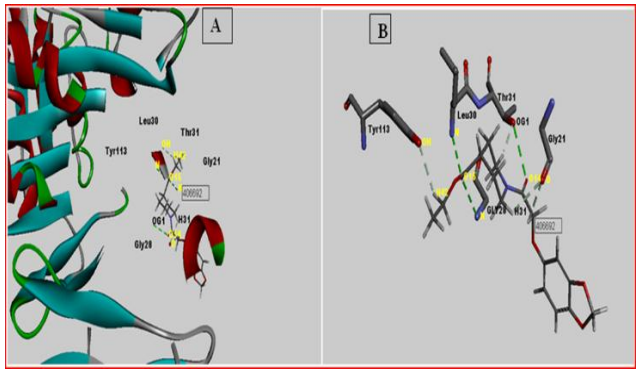


Fig 9: Interacting orientation of identified compound 406692 within target protein (A). Binding orientation of compound into the binding site was shown. (B). Interacting amino acids and donor and acceptor group atoms are shown. Donor/acceptor group atoms are in Yellow color.

5. Conclusion

Antidiabetic congeneric series of N -(1,3- (substituted alkyl/aryl) benzen-oxazol-2-yl) -2,5-dichloro benzene sulfonamide, N-(5-chloro-1,3-benzoxazol-2-yl) (substituted alky/aryl) benzene sulfonamide, 5- chloro-N-(dioxide-aryl/alkyl substituted sufanyl)-1,3 benzoxazol-2-amine derivatives of benzoxazole benzenesulfonamide were used to make *3-D QSAR* model using PHASE 3.4 of Schodinger9.0. Bioactivity favoring and disfavoring volume occlusion maps for donor, electron withdrawing and hydrophobic effects from QSAR model as well as most active ligand were analyzed.

Volume occlusion maps showed that increasing in the hydrophobic, donor and electron withdrawing group effect in their particular region at particular positions on benzoxazole heterocyclic ring and benzenesulfonamide part of benzoxazole benzenesulfonamide may increase the bioactivity of benzoxazole benzenesulfonamide molecules against Fructose-1,6- bisphosphatase. *3-D* QSAR model.AAADRR.27*** selected as best model that may meet the pharmacophoric features of binding mode of benzoxazole benzenesulfonamide within the active site of Fructose-1,6-bisphosphatase. Newer derivatives can further be designed and synthesized on the basis of *3-D* QSAR model AAADRR.27***. Compounds with CoCoCo.ID 419,095, 406,692, 456,043, 456,554, 445527 were identified as best scoring compounds. The five identified compounds showed good LibDock score than most potent analog 41 of benzoxazole benzenesulfonamide and also have good ADMET profile. But on the basis of LibDock score and binding energy two compounds CoCoCo.ID 419095 and 406692 found better interaction with target protein. So these can further be tested for antidiabetic activity and used as lead for antidiabetic drug development.

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