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Design of fructose-2,6-bisphosphatase inhibitors: A novel virtual screening approach

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Abstract

Fructose-2,6-bisphosphatase (FBPase-2) is a switch between gluconeogenesis and glycolysis in the hepatic cells. The structural features required for inhibitory activity of FBPase-2 were unidentified; no leads are available for inhibiting this important enzyme. In this paper pharmacophore mapping, molecular docking methods were employed in a virtual screening strategy to identify leads for FBPase-2. A receptor based pharmacophore map was modeled which comprised of important interactions as observed in co-crystal of rat liver isozyme with the product inhibitor fructose-6-phosphate. The pharmacophore model was validated against two databases of best docked structural analogues of fructose-2,6-bisphosphate and fructose-6-phosphate. The query generated was submitted for flexible search of ligands in chemical databases, namely LeadQuest, Maybridge and NCI. The hits obtained were further screened by molecular docking using FlexX.

Keywords: Diabetes Mellitus; Pharmacophore mapping; Virtual screening; Molecular docking; Fructose-2,6-bisphosphatase

1. Introduction

One of the major contributing factors to fasting hyperglycemia in Type 2 Diabetes Mellitus (T2DM) is increased Hepatic Glucose Output (HGO) in liver. HGO is a consequence of two distinct and highly regulated processes: gluconeogenesis and glycogenolysis. The balance of these pathways is severely altered in patients with T2DM contributing to chronically elevated plasma glucose concentrations. Therefore, an understanding of the regulation of these fluxes can provide important insights into the mechanisms and potential treatment of diabetes [1]. Hepatic glucose production plays an important role in maintaining blood glucose homeostasis, and can be modulated by manipulating hepatic fructose-2,6-bisphosphate (F-2,6-P_2) content. Thus the enzyme involved in this process, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK-2/FBPase-2) is a point of intervention for therapies aimed at lowering blood glucose in diabetes [1,2]. The PFK-2/FBPase-2 is known as ‘‘tandem enzyme’’ because it possesses bifunctional enzyme activity, thus it is a responsible for the synthesis and breakdown of F-2,6-P_2 under the control of adrenaline and glucagon via adenyl cyclase, 3′,5′-cyclic AMP and protein kinase A [3–6]. This enables adrenaline and glucagon to switch on gluconeogenesis in liver tissue and stabilize the blood glucose concentration. High concentrations of ATP favor the binding of ATP to the bisphosphatase domain, which might induce a global structural change of that domain and this in turn affects the catalytic core of the kinase domain, resulting in the activation of PFK-2 [7]. The advantage of having two activities on a single protein makes it possible to co-ordinate regulation (synthesis/degradation) of F-2,6-P_2 by a single signal [8]. The F-2,6-P_2 has been found to be involved in maintaining blood glucose level by two mechanisms, i.e. (i) by inhibiting the activity of enzyme FBPase-1 and thus discontinuing the process of gluconeogenesis and (ii) by activating the enzyme PFK-1 involved in glycolysis [9]. Recent studies revealed that increased F-2,6-P_2 overcomes insulin resistance and also reduces obesity [10]. The importance of F-2,6-P_2 to the regulation of carbohydrate metabolism has made this bifunctional enzyme a target for therapeutic intervention [11].

Since FBPase-2 is responsible for degradation of F-2,6-P_2, its inhibition by xenobiotics may represent a important

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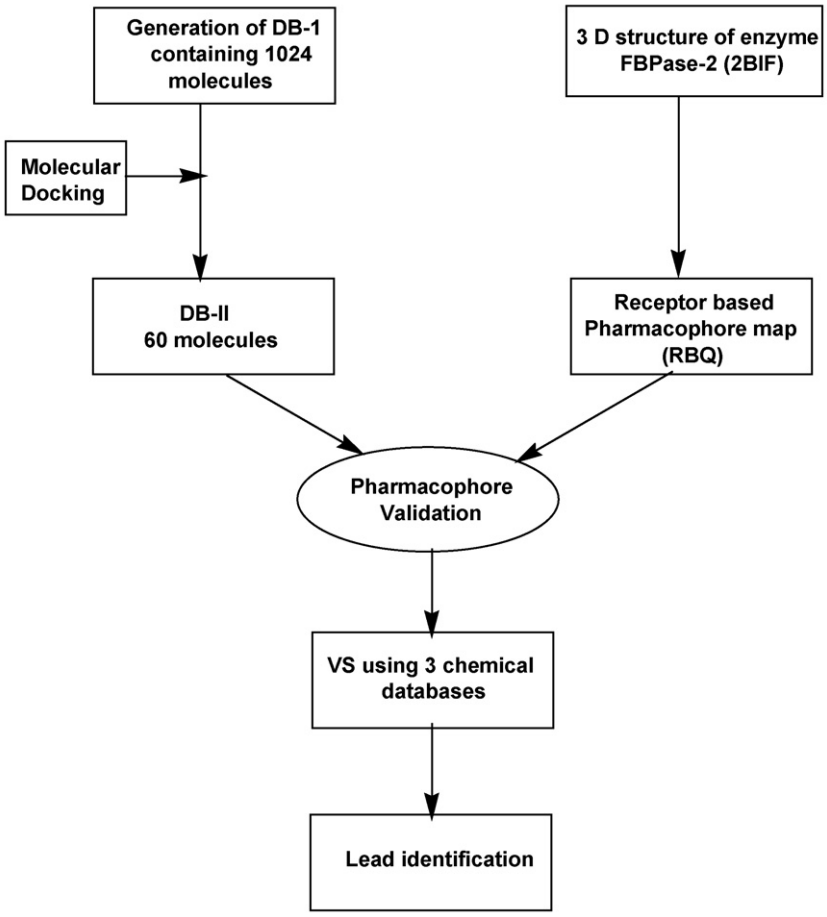

Fig. 1. Flowchart for the work carried (DB = databank).

therapeutic strategy for the treatment of diabetes. It is well known that FBPase-2 is competitively inhibited by inorganic phosphate, substrate itself at its saturating concentration, noncompetitively by ATP and product of dephosphorylation, i.e. F6P [12]. In case of enzyme FBPase-2 few other compounds were also known to bind which includes natural substrates, ligands and inhibitors, viz.; F-1,6-P_2, F-2,6-P_2 [13] and many others [14–17] but those could not be used for drug discovery or therapeutic application because of specificity problems. Since this target is important for insulin resistance, there is an urgent need to identify leads to inhibit this enzymatic action. Computational approaches are quite suitable to tackle this problem. In this work, we report a virtual screening strategy adopted to identify new leads for FBPase-2 inhibition. Virtual screening (VS) is a chemoinformatic technique for the computational screening of compounds. It can be defined as the use of high performance computing to analyze large database of compounds for finding possible leads [18]. Virtual screening is an important component of the computer-based search for novel lead compounds. There are two approaches (i) 'VS by docking', which requires knowledge of the 3D structure of the target protein binding site to prioritize compounds by their likelihood to bind to the protein and (ii) 'similarity-based VS', where no information on the protein is necessary instead, one or more compounds that are known to bind to the protein are used as a structural query. The screening procedure extracts compounds from the database according to an appropriate similarity criterion. In order for the screening procedure to be effective, this criterion should regard molecules that bind tightly to the same proteins as similar [19]. Since, as stated earlier, none of the known ligands could be used for such similarity-based search, in these circumstances a receptor/structure based pharmacophore mapping technique was employed [20–22]. Such pharmacophore are generally generated, based on the important interactions taking place in the active site on ligand binding. In this paper, we report the generation of a pharmacophore map which constitutes features identified from F6P–FBPase complex. The pharmacophore map was validated using a database of well-docked structures. This model was subjected to 3D search in three different databases containing approximately 0.35 million molecules for identification of lead. A schematic representation of the virtual screening strategy adopted in this work has been described in Fig. 1.

2. Computational details

2.1. Generation of virtual library

The catalytic hydrolysis reaction with F-2,6-P has been shown to be inhibited by the product F6P. This mechanism for the inhibition of the enzyme is similar to *mechanism based inhibitors* (Fig. 2) where the product inhibits the catalysis [23].

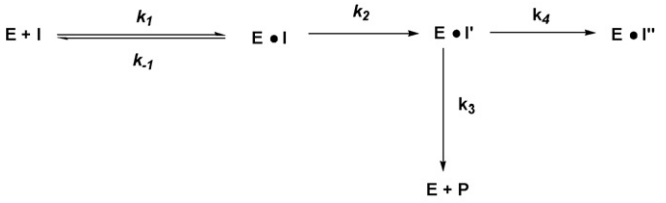

Fig. 2. Mode of inhibition by mechanism based inhibitors.

Since the hydrolysis of F-2,6-P by FBPase-2 involves the dephosphorylation at position 2 and the product is reported to be an inhibitor, a design strategy involving development of F-2,6-P and F6P mimics has been developed. The replacements at position 6-OH group were made with carboxylic acid, carboxylic esters, amides and sulfonamides groups. Following this strategy and making various substitutions at R, R_1, R_2 and R_3 a virtual library of about 1024 molecules were generated of which 512 belong to series A (F-2,6-P_2 mimics), with structural backbone as shown in Fig. 3a and another set of 512 molecules, series B (F6P mimics) had a backbone structure as shown in Fig. 3b. A virtual databank of these 1024 molecules (DB-I) was created after performing energy minimization using conjugate gradient method and Tripos force field [24,25].

2.2. Molecular docking

Docking studies were performed using FlexX [26] module of SYBYL 6.9 [27] which involves incremental construction algorithm. The crystal structure 2BIF (resolution 2.4 Å) is a dimer of FBPase-2 with two F6P molecules in two different chains [28]. Since the temperature factor (B factor) of the chain B containing F6P-550 was better, for docking studies residues in a sphere of radius 6.5 Å around F6P in chain B was defined as active site pocket. The customization involved inclusion of some vital residues in the receptor, viz.; Glu-325, Tyr-336, Arg-350, Lys-354, Tyr-365, and Gln-391 [13,28–30]. The torsional angles were properly rotated to have perfect ligand–receptor interactions. Since the active site of FBPase-2 is hydrophilic, water molecules must be considered for docking. Hence two water molecules, namely H_2O-844 and H_2O-909 were retained while others were dropped from the receptor description file. The docking studies were used for two purposes, i.e. (i) to identify a set of compounds which can be further used for validation of receptor based pharmacophore and (ii) to identify potential lead compounds from hit list of the UNITY 3D search during virtual screening. The validation of the docking was carried out by docking the original ligand itself, i.e. F6P, in to the active site. Different scoring functions were used for scoring the docked conformations, viz.; FlexX [31,32], C-Score [33], DrugScore [34], PMF_Score [34–36], D_Score [37], G_Score [37], and ChemScore [38].

2.3. Generation of pharmacophore map

A receptor-based pharmacophore was generated using UNITY–SITE-ID interface within SYBYL 6.9 package. The SITE-ID was used to define which cavity must be used for the query generation while the module UNITY 4.4 [39] generated structure-based queries from the crystal structure with the PDB Code 2BIF. Initially the UNITY 4.4 randomly identified several pharmacophoric features from the receptor site in the substrate binding domain of the enzyme. By comparing the pharmacophoric features from the literature reports and the crystallographic data, selection of important pharmacophoric features were made. The UNITY 4.4 then automatically generated pharmacophoric query corresponding to substrates. A 3D map of these pharmacophoric features was then generated by defining the distances and distance tolerance between the pharmacophoric features. This 3D map was validated with the help of databank (DB-II) generated by molecular docking strategy. Slight modifications were made into the 3D map so that the pharmacophore should identify maximum number of molecules from DB-II which include an *excluded volume feature*. These modifications included changes in the number and types of features as well as in the distances and distance tolerances between the features. After several rounds of modifications RBQ was chosen as a validated pharmacophore which contained six pharmacophoric features from the receptor site along with projected complimentary ligand features.

3. Results and discussions

The combinatorial approach on the F-2,6-P and F6P generated a database DB-I containing 1024 molecules. This database was submitted to molecular docking and a set of 60 (30 pairs, series A and series B) best docked molecules was prepared as database DB-II. Multiple scoring functions were used to score the docked ligands, viz.; FlexX, C-Score,

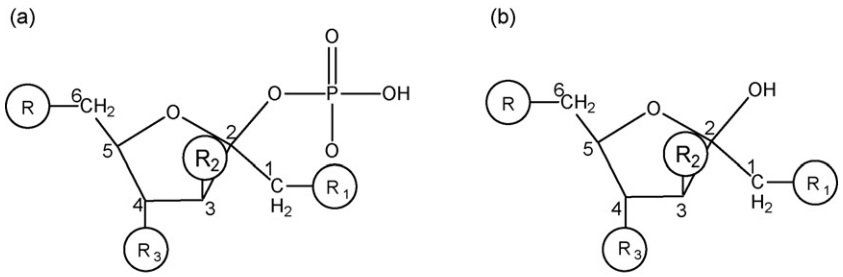

Fig. 3. (a) Backbone structure for series A and (b) backbone structure for series B.

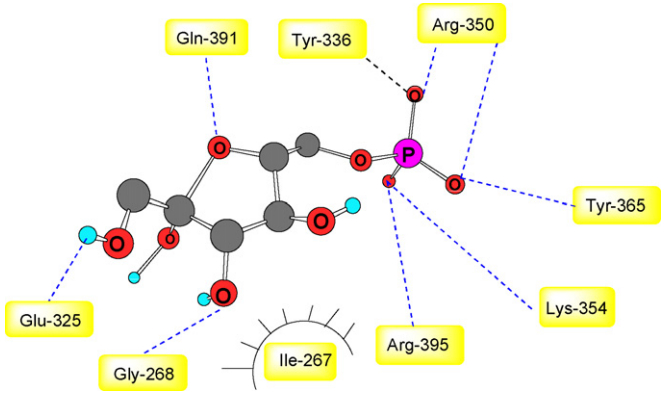

Fig. 4. A diagram of the receptor site showing some important interactions with fructose-6-phosphate.

DrugScore, PMF_Score, D_Score, G_Score, and ChemScore. While studying, it was observed that most of the best docked series A ligands are having sulfonamide side chain which was singly substituted with aromatic ring hence it is evident that aromatic sulfonamides may appear as leads for this target in future. Similarly from series B some esters were observed to have interaction at Gln-391 instead of ring oxygen of furan.

3.1. Details of generation and validation of pharmacophore map

The active site of FBPase-2 is very hydrophilic [28,40,41] thus H-bonding interactions are mainly responsible for binding the inhibitors. Hence a model was build depending on hydrogen bonding interactions at UNITY–SITE-ID interface. The cavity size of the substrate binding domain is 111 $Å^3$ as measured by SITE-ID module of SYBYL. It was found that almost all the hydrogen bonding interactions are due to hydrogen donors in the receptor (Fig. 4). F6P is held in the active site by a very strong hydrogen bond with Gln-391. The other important interactions are hydrogen bonds with Tyr-336, Lys-354 and Glu-268. It was known from literature that F6P also forms a hydrophobic interaction with Ile-267 in the rat testis isozyme cavity but not the rat liver isozyme [28]. Hence this interaction was not considered while building a pharmacophore model.

For the validation of the pharmacophore a set of 60 best docked compounds in the database DB-II was used. The initial query picked all the 60 compounds from DB-II. The query was then subjected to the other data set of 200 best docked molecules. This caused a selection of almost all the molecules and hence it was considered a very general pharmacophoric query and unsuitable for virtual screening exercise. One of the ways to improve specificity associated with the query is to fix the distance tolerance values systematically such that it must yield the hits which would have best complementarity to the receptor and hence best binding. Such attempts lead to the query which had a distance tolerance value of ±0.5 between each receptor point and the expected ligand point and ±1.3 between two receptor points. The tolerance values between ligand points varied between 0.3, 0.5, 1.5 and 2.0. However such attempts did not yield a query with improved specificity.

From the available crystal structure information it is known that catalytic site in FBPase-2 lies very close to the allosteric site (the site for the product inhibitor F6P binding) [12,28] also His-256 is known to be the most important amino acid in the enzyme [28,40,41]. Consequently ligands binding to this amino acid or residues in its vicinity must be labeled as competitive inhibitors. Since the interactions of F6P were used, which is noncompetitive inhibitor [42] of the enzyme it was essential to define some excluded volume *feature* to avoid the search of ligands which may occupy that particular volume. The excluded volume features in UNITY permits the user to specify the radius of the excluded volume sphere around each atom. This feature is actually a constraint which avoids the possibility of finding structure having substitutions in that particular region. A sphere of radius 3 Å was defined as

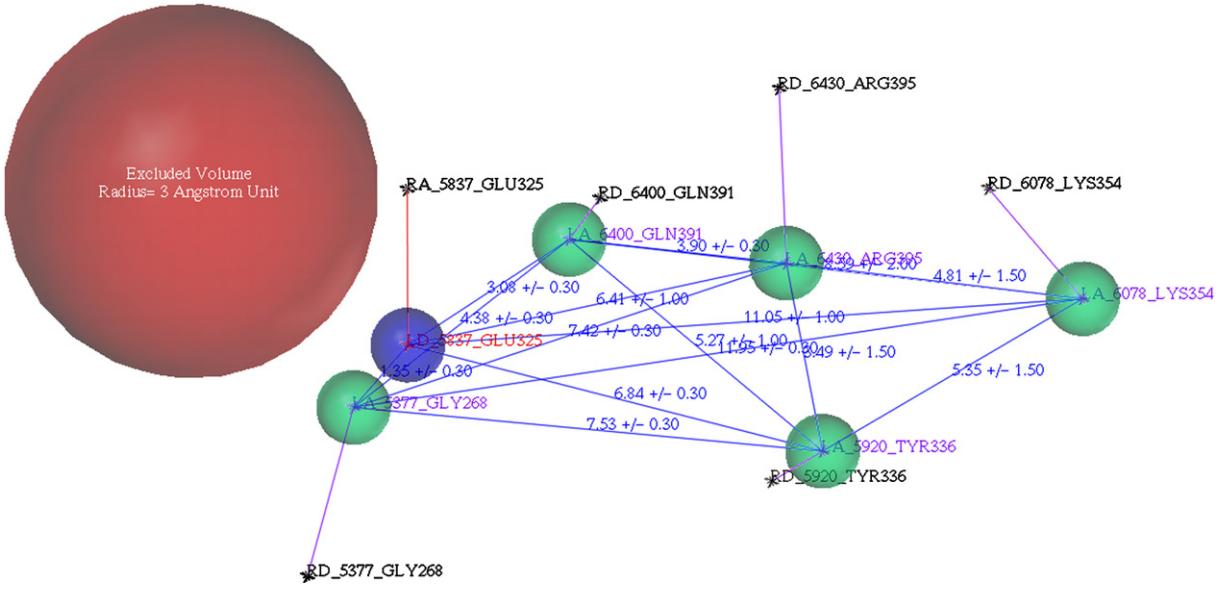

Fig. 5. Query RBQ.

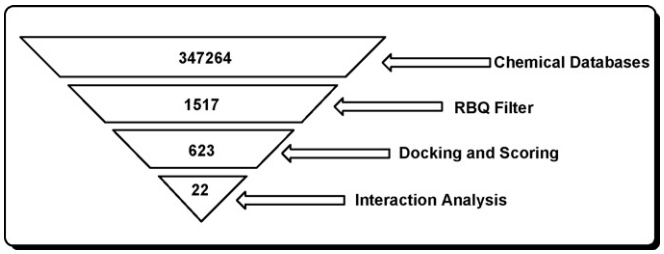

Fig. 6. Summary of virtual screening process.

excluded volume around detached PO_4^- unit. Thus, the generated query RBQ included the vital hydrogen bonding interactions (donors and acceptors) and excluded volume (Fig. 5). RBQ picked 30 structures from the DB-II database (16 molecules from series A and 14 molecules series B, respectively). These 30 molecules contained most of the best docked ligands. This validated query was considered for virtual screening.

3.2. Virtual screening

UNITY 4.4 was used for 3D database searching of the pharmacophore model generated by UNITY–SITE-ID interface. The databases into which query was searched include LeadQuest database (41,395 molecules) [43], Maybridge database (55,618 molecules) [44] and NCI database (250,251 molecules) [45] (Fig. 6). UNITY's conformationally flexible 3D searching was executed on the aforementioned databases which was restricted with modified Lipinski's rule and for further screening the number of rotatable bonds was set to 8. The modified Lipinski's rule was based on the concept of Lead Likeness of hits. For better intestinal absorption and bioavailability leads must follow Lipinski's ''Rule of Five''. In general this rule is for description of molecules having drug-like properties. Drug-likeness is a property that is most often used to characterize compound libraries such as combinatorial or screening libraries that are screened to find novel lead chemical compounds [46]. According to this rule no good absorption is to be expected if MW > 500, log $P > 5$, H-bond donors > 5, and H-bond acceptors > 10 [47]. In their optimization to clinical candidates, leads most often become large and lipophilic. Thus, leads should have some different values for the screening purpose [48–50]. Hence to account for this, parameters were defined in flexible search for the pharmacophore model RBQ. Total 1517 molecules found after flexible search were 188, 255 and 1074 each from LeadQuest, Maybridge and NCI databases, respectively.

3.3. Screening by molecular docking

The set of 1517 molecules obtained after employing RBQ filter were selected for molecular docking analysis using FlexX software. On the basis of docking poses and scores, 59, 104 and 460 molecules were selected from LeadQuest, Maybridge and NCI databases, respectively making a total of 623. These 623 molecules exhibit higher scores than F6P and hence they were considered to be the potential leads. To screen further, the binding interactions of the ligands were keenly observed. As the residues Arg-395, Gln-391, Lys-354, Tyr-365, Tyr-336, Gly-268 and Glu-325 were considered to be potential binding

Table 1
The docking scores of the reference molecules along with the molecules obtained from pharmacophoric search

Molecules from	Molecules	FlexX	G_Score	PMF_Score	D_Score	ChemScore	C-Score
Reference set	F6P	−28.3	−191.60	−65.92	−131.83	−15.75	3
	F-2,6-P_2	−37.6	−232.46	−121.87	−202.49	−15.9	5
LeadQuest	1525-03054	−34.06	−225.38	−74.69	−122.19	−38.88	5
	1525-1947	−29.99	−197.91	−105.12	−128.06	−34.2	3
Maybridge	BTB-05499	−47.01	−111.26	−110.94	−128.74	−36.45	3
	HTS-04937	−40.88	−82.92	−75.70	−96.53	−40.14	2
	HTS-02332	−39.55	−225.69	−98.65	−117.93	−38.01	4
	KM-06874	−32.49	−219.63	−81.69	−127.17	−35.72	5
	HTS-08532	−29.42	−231.76	−45.91	−116.24	−31.96	4
	RDR-02080	−34.14	−151.18	−69.56	−99.83	−32.29	4
NCI	626629	−34.71	−129.83	−102.94	−77.60	−28.92	3
	53958	−32.09	−157.79	−74.05	−180.49	−32.93	5
	74197	−34.02	−123.01	−77.79	−100.01	−39.56	3
	288730	−42.73	−169.88	−79.39	−119.25	−41.37	4
	87701	−47.46	−120.49	−82.85	−128.23	−39.95	2
	630904	−34.95	−211.64	−77.91	−117.02	−37.25	4
	61054	−33.15	−156.13	−101.62	−108.73	−36.42	5
	338516	−34.43	−78.58	−63.30	−86.63	36.12	3
	10953	−29.03	−141.82	−102.24	−116.97	−43.91	4
	631852	−46.34	−134.0	−96.71	−89.92	−32.69	3
	262639	−34.19	−84.83	−79.98	−86.41	−31.2	3
	366115	−36.28	−108.27	−88.03	−80.41	−37.55	3
	334199	−34.43	−78.58	−63.30	−86.63	−36.12	3
	11693	−30.60	−60.15	−57.25	−89.23	−24.06	2

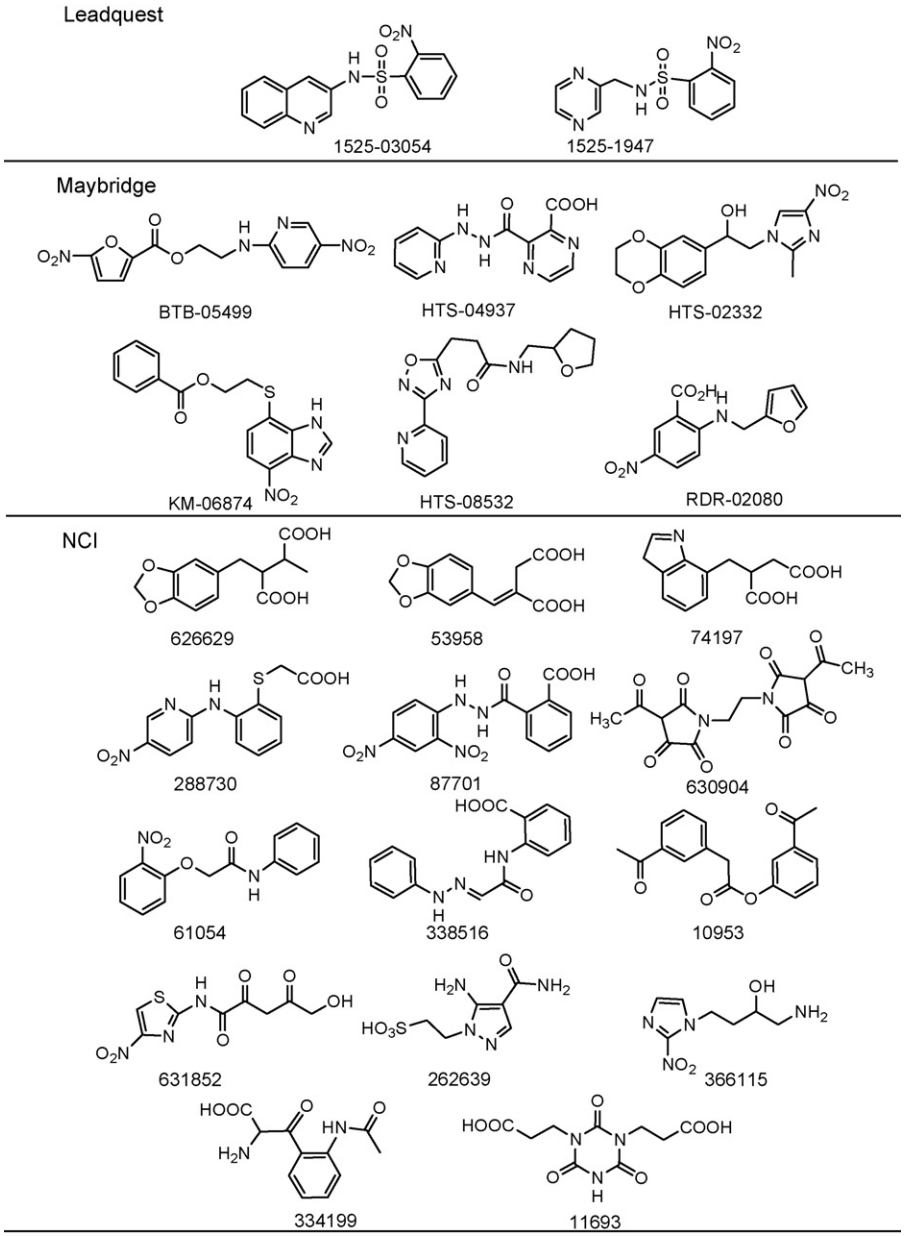

Fig. 7. Hits from LeadQuest, Maybridge and NCI database which mimic binding pattern of F6P.

sites in the cavity for F6P, the interactions of different high scoring ligands were observed, those molecules showing larger number of interactions were taken as the leads from UNITY hits. A total of 22 molecules are chosen after employing this filter. Table 1 lists the docking scores of 22 hits obtained from virtual screening. It was observed that most of the compounds are nitro aromatics. It is due to the need for a negative center that can bind with basic residues in the receptor. The hits obtained from such screening are shown in Fig. 7.

4. Conclusions

A receptor based pharmacophore map RBQ was designed which comprised of important interactions from co-crystal of rat liver FBPase-2 with the product inhibitor fructose-6-phosphate, using UNITY–SITE-ID interface. The important interactions considered for pharmacophore generation were with the amino acid residues Glu-325, Tyr-336, Arg-350, Lys-354, Tyr-365, Arg-395 and Gln-391. Glu-325 was considered as hydrogen bond acceptor while all others were hydrogen bond donors. This query had a distance tolerance value of ± 0.5 between each receptor point and the expected ligand point and ± 1.3 between two receptor points. The tolerance values between ligand points varied between 0.3, 0.5, 1.5 and 2.0. An excluded volume feature was introduced to improve selectivity in the pharmacophore model. The model was validated against a small database of best docked structural analogues of fructose-2,6-bisphosphate and fructose-6-phosphate. The query generated, RBQ was submitted for flexible 3D search of ligands from three databases, namely LeadQuest, Maybridge and NCI (347,264 molecules). Initial pharmacophore based screening identifies 1517 compounds. These hits were further screened

using docking studies. The criterion for selection of hits as lead molecules was based on predicted binding affinities, i.e. scores, as well as binding interactions of the ligands exposed by FlexX. Different scoring functions were used for scoring the docked conformations, viz.; FlexX, C-Score, PMF_Score, D_Score, G_Score, and ChemScore. Finally, 22 molecules were considered to be the potential leads. This study should provide further insights to support structure based design of antidiabetic drugs to develop novel FBPase-2 antagonists with improved activity profiles.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jmgm.2007.06.004.

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