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New leads for selective GSK-3 inhibition: pharmacophore mapping and virtual screening studies

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Summary

Glycogen Synthase Kinase-3 is a regulatory serine/threonine kinase, which is being targeted for the treatment of a number of human diseases including type-2 diabetes mellitus, neurodegenerative diseases, cancer and chronic inflammation. Selective GSK-3 inhibition is an important requirement owing to the possibility of side effects arising from other kinases. A pharmacophore mapping strategy is employed in this work to identify new leads for selective GSK-3 inhibition. Ligands known to show selective GSK-3 inhibition were employed in generating a pharmacophore map using distance comparison method (DISCO). The derived pharmacophore map was validated using (i) important interactions involved in selective GSK-3 inhibitions, and (ii) an in-house database containing different classes of GSK-3 selective, non-selective and inactive molecules. New Lead identification was carried out by performing virtual screening using validated pharmacophoric query and three chemical databases namely NCI, Maybridge and Leadquest. Further data reduction was carried out by employing virtual filters based on (i) Lipinski's rule of 5 (ii) van der Waals bumps and (iii) restricting the number of rotatable bonds to seven. Final screening was carried out using FlexX based molecular docking study.

Introduction

Non-insulin dependent diabetes mellitus (NIDDM) is characterized by elevated blood glucose level that result from inadequate insulin action in insulin-sensitive tissues [1]. Various targets are being considered for drug development to treat NIDDM and insulin resistance. Of these, Glycogen Synthase Kinase-3 (GSK-3) is emerging as an important target owing to the available knowledge on this enzyme [2]. Glycogen Synthase is a terminal enzyme in the insulin-signaling pathway and is defined as the rate-limiting enzyme of glycogen biosynthesis. One of the major characteristics of diabetic muscles is severe inhibition of Glycogen Synthase (GS) and hence loss of glycogen synthesis [3, 4]. GSK-3 is a serine/threonine kinase, which phosphorylates and inactivates GS [5, 6]. In response to insulin stimulation in the phosphatidylinositide pathway, GSK-3 becomes inhibited by PKB mediated phosphorylation of N terminus [7, 8], facilitating the dephosphorylation and activation of glycogen synthase. GSK-3 was recently implicated in several human diseases such as cancer [9], chronic inflammatory processes [10], and neurological diseases such as bipolar [11] or Alzheimer's disease [12], etc. apart from type-2 diabetes [4, 13].

Presently, three distinct regions of GSK-3 are being targeted to suppress the enzyme activity: (1)

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Metal ion (Mg^{+2}) binding site, (2) Substrate interaction domain and (3) ATP binding pocket [14]. The majority of the known GSK-3 inhibitors are known to inhibit the enzyme in ATP competitive manner. A number of potent GSK-3 inhibitors (Figure 1) such as hymenialdisine [15], paullones [16], indirubins [17], etc. have been reported. These substrates inhibit other protein kinases also, more importantly, the Cyclin-Dependant Protein Kinases (CDKs), which are homologous to GSK-3, and lead to side effects. However, these side effects are not completely undesirable, lack of specificity is a cause of concern, and it is important to develop inhibitors specific to GSK-3. SmithKline Beecham reported SB 216763 (Figure 1) a member of maleimide series of compounds as a selective GSK-3 inhibitor [18]. A set of reported GSK-3 inhibitors which show greater degree of selectivity against CDKs include bis-7-azaindolylmaleimides [19, 20], 6-heteroaryl-pyrazolo[3,4-*b*]pyridines [21], CT-98023 [22], 1-(4-aminofurazan-3-yl)-5-dialkylaminomethyl-1-H-[1,2,3]triazole-4-carboxylic acid derivatives [23], etc. However, none of these found their way to practical application and hence modulation of these series of compounds is required to design new potent and selective GSK-3 inhibitors.

Vulpetti et al. have described structure-based approaches to improve selectivity between CDK2 and GSK-3β [24]. They employed Consensus Principal Component Analysis (CPCA) along with binding site analysis in rationally designing novel potent and selective CDK2 benzodipyrazole inhibitor. Dessalew and Bharatam are employing selective molecular field approach in designing new selective GSK-3 inhibitors [24]. In this article, a 3D pharmacophore mapping methodology based on distance comparison technique followed by virtual screening analysis is reported. Two criterions were adopted for the validation of developed

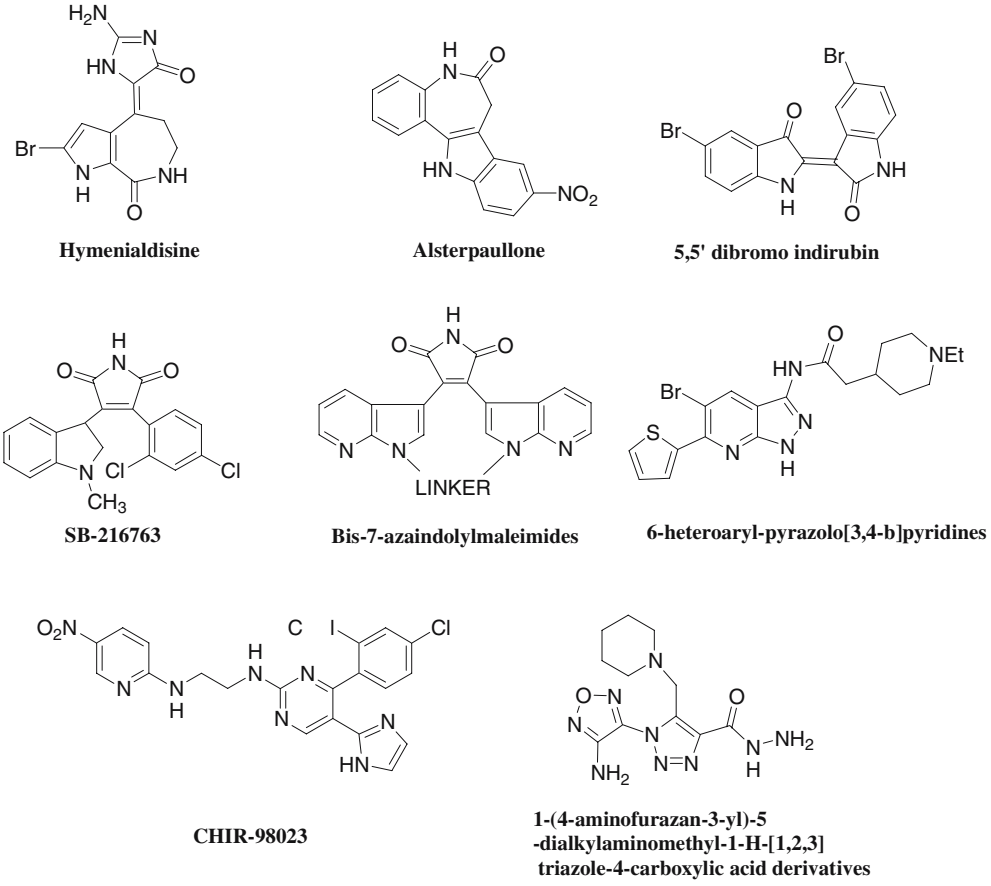

Figure 1. ATP-competitive Non-selective and Selective GSK-3 Inhibitors.

pharmacophoric queries – (i) based on the important interactions shown by the reference molecule and (ii) submitting the pharmacophoric queries to an in-house database of reported compounds on GSK-3 inhibition. The validated pharmacophoric query was then submitted to three different databases to identify new hits.

Computational details

Dataset selection and minimization

A dataset (Figure 2) of four potent and selective GSK-3 inhibitors from different classes, namely, 3-anilino-4-arylmaleimide (**1**) [18], oxadiazole derivative (**2**) [23], triazole derivative (**3**) [23], and macrocyclic polyoxygenated bis-7-azaindolylmaleimide derivative (**4**) [19] were selected for the generation of the pharmacophore model by DISCO module of Sybyl6.9 [25]. The bioactive conformation of **1** was extracted from the co-crystal of **1** and GSK-3β (PDB code: 1Q4L), minimized using the Tripos force field to obtain a local minimum. The initial structures of **2–4** were obtained by performing conformational search using the multi-search option of SYBYL6.9.

Pharmacophore model generation

Assignment of the initial pharmacophore features for the DISCO based pharmacophore mapping was done using the following features – aromatic and aliphatic ring centroids as hydrophobic centers, hydrogen bond donors and acceptors, and external site points representing receptor-associated hydrogen bond acceptor sites and donor sites. DISCO proposes a common pharmacophoric arrangement by choosing a template from amongst all structures and during each attempt the coordinates of the site points assigned to the template are compared to those of each of the molecules [26]. DISCO search algorithm uses a clique detection method to identify pharmacophoric map. Different DISCO runs were executed with varied number and types of features. When the specified elements were met within the set

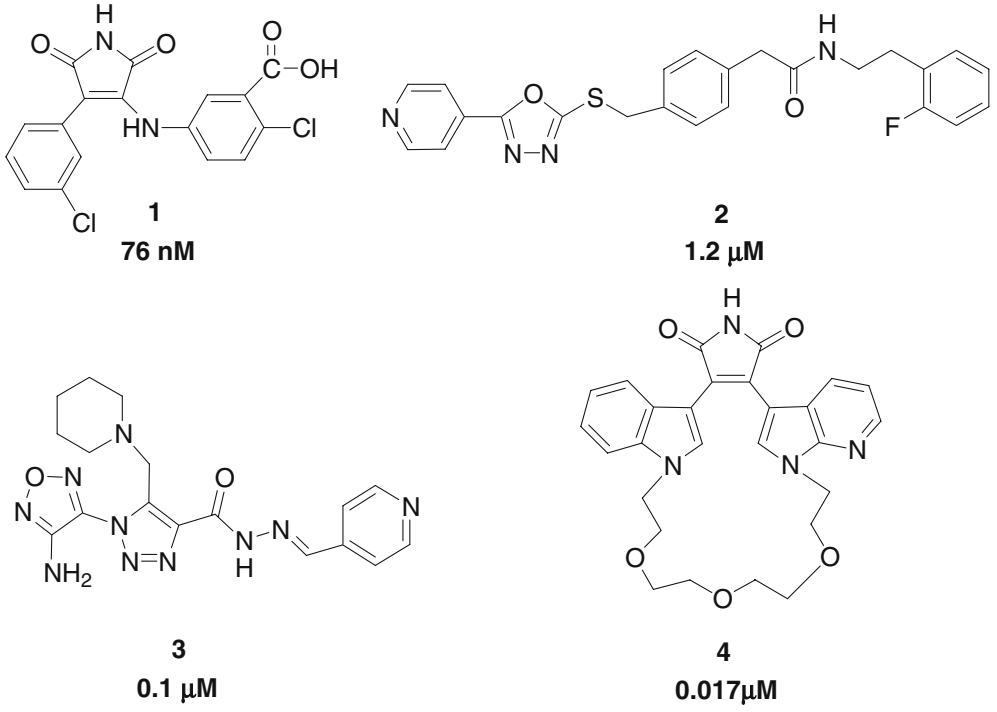

Figure 2. Set of molecules selected for generation of pharmacophore model. These molecules were reported to show GSK-3 selectivity. Also included are the reported IC_{50} values of these molecules against GSK3.

conditions, a valid pharmacophore map was established. On the other hand in case of no solution, the distance tolerance was increased and calculations were repeated.

Validation criterion for the pharmacophore models

Validation of the pharmacophoric models was carried out using two methods – (i) based on the important interactions and (ii) using in-house database search.

Validation based on the important interactions

The important interactions shown by the reference compound **1** (3-anilino-4-arylmaleimide derivative) (Figure 2) for which crystal structure information is available was taken as the validation criteria. Two most important interactions are (i) hydrogen bond acceptor atom showing H-bonding interaction with the donor site due to Val135 and (ii) hydrogen bond donor atom to the acceptor site due to Asp133. Molecular docking studies on the reported ATP competitive GSK-3 non-selective inhibitors like AMP-PNP, staurosporine, hymenialdisine, indirubin-3'-monoxime, alsterpaullone and GSK-3 selective inhibitors like 3-anilino-4-arylmalemide derivatives, 6-Heteroaryl- [3,4-*b*] pyridine derivatives show these two key H-bonding interactions [18, 21]. Other important interactions shown by reference compound **1** are towards amino acids Arg141, Gln185, and one conserved water molecule W1 near Thr138 (Figure 3) [27].

Validation using a database search

To check the validity of the developed pharmacophore maps, a 3D-database (GSK-3DDB) was built, with the reported active and inactive molecules (minimized by AM1 [28]) from all the categories of GSK-3 inhibitors. This database contained the total of 378 compounds including 130 selective GSK-3 inhibitors, 216 non-selective GSK-3 inhibitors, and 32 inactive molecules. An acceptable pharmacophoric query should be able to pick up most of the potent and selective GSK-3 inhibitors and should omit the non-selective GSK-3 inhibitors as well as inactive molecules from GSK-3DDB.

Virtual Screening

Virtual screening is one of the important strategies for lead identification. A schematic diagram showing the virtual screening strategy adopted in this work is given in Figure 4. Three different

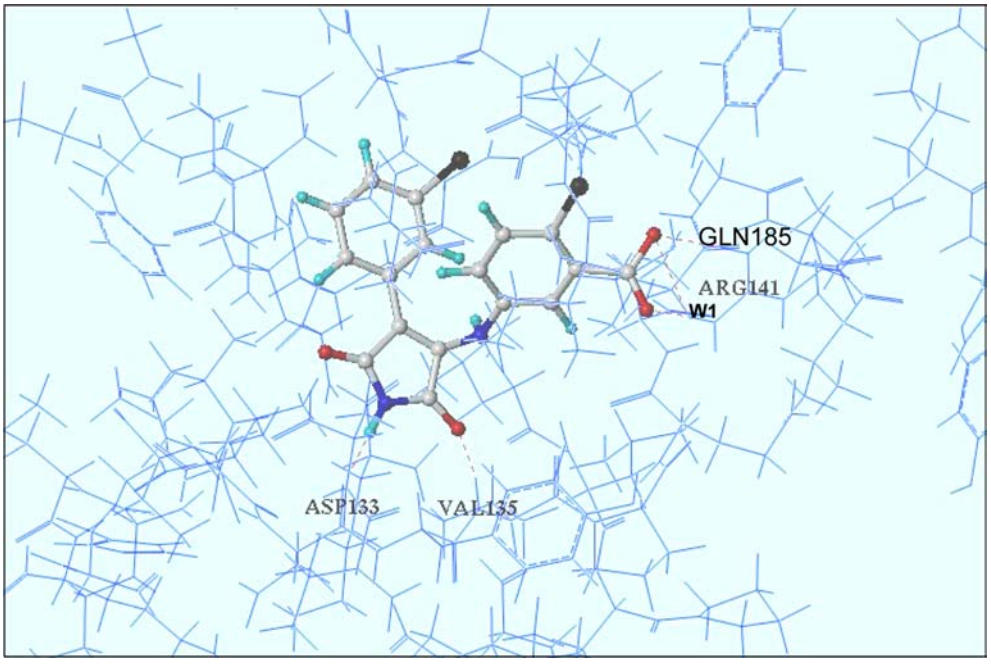

Figure 3. Interactions shown by the compound **1** in the active site of GSK-3β.

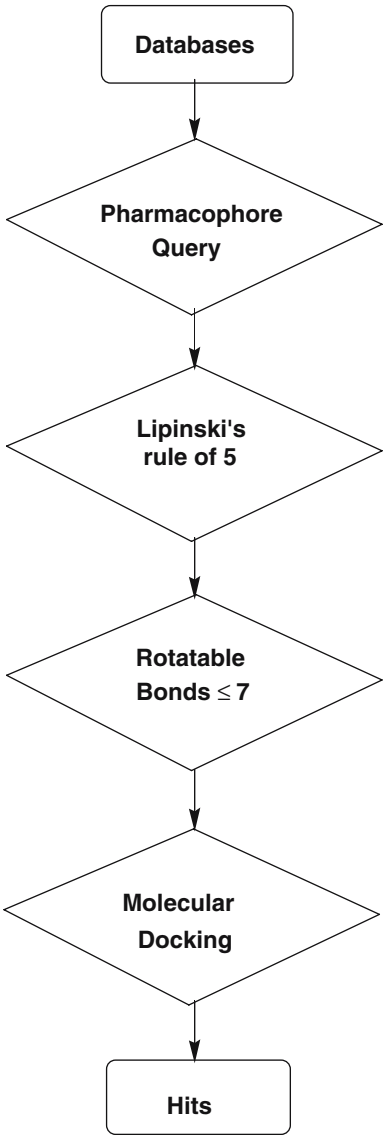

Figure 4. Schematic representation of the steps adopted in the virtual screening.

databases, NCI (number of molecules: 234,055), Maybridge (55,541) and Leadquest (41,393), were used for searching new lead compounds by employing the developed pharmacophoric queries [29]. Initially Lipinski's Rule of five (limit the range for Molecular Weight ≤ 500, calculated octanol–water partition coefficient ($C \log P \leq 5$), and hydrogen bond donors, and acceptors (OH's and NH's ≤ 5; N's and O's ≤ 10)) [30] and van der Waals bumps (polar surface area (PSA) < 120 Å^2) [31] were opted as the filtration criterion. Additional screening was carried out by restricting the number of rotatable bonds to a maximum of 7 [31, 32]. Several hits were obtained from each of the databases, which were further screened using molecular docking studies.

Molecular docking

FlexX [33] based molecular docking study was carried out to perform screening and validation of the hits obtained from database searching. FlexX method of molecular docking involves incremental construction of ligands from smaller fragments in the cavity of a receptor. All the hits obtained in database searching were docked into the ATP binding site in the co-crystal of 3-anilino-4-arylmalemide derivatives with human GSK-3β enzyme (PDB accession number 1Q4L), after extracting the ligand. Receptor description file (RDF) was created within the area of 6.5 Å around the co-crystallized ligand and the core interactions (Val135, Asp133, Arg141, Gln185 and bridging water molecule W1 near to Thr138) were defined in the RDF. For a comparative analysis of the hits obtained in database searching, FlexX score, G_score [34], PMF_score [35], D_sorce [36] and Chem_score [37] were estimated using the C-score module of the Sybyl6.9.

Results and discussion

Primary inspection of all types of molecules present in the dataset for generation of pharmacophoric model lead to a general conclusion that a pharmacophore for such molecules should have 2 acceptor atoms with the corresponding 2–3 donor sites, a donor atom along with the corresponding acceptor site, and two hydrophobic centers with variable distance tolerance range. Several initial DISCO runs were performed by varying the (i) tolerance, (ii) range of required features, (iii) detailing of some specific features by class. Table 1 shows the details of the initial efforts (Run-1–Run-5) where many models with limited number of pharmacophoric features were obtained. All these models turned out to be very non-specific because they have only about 4–5 features. DISCO-Run-6 resulted in 12 models with 9 pharmacophoric features each, however, in this case a price had to be paid in terms of large tolerance limit of 3.0 Å. Out of the 12 models generated in DISCO-Run-6,

Table 1. Number of models obtained along with the pharmacophoric features and tolerance values for each of the DISCO pharmacophoric run.

Run name	Features required	Energy value	Tolerance	Number of models	Number of pharmacophoric features
Run-1	3–8	0	0.5	2	4
Run-2	5–8	0	1.0	2	5
Run-3	DC: 1 A.A	0	0.5	1	4
Run-4	DC: 2 A.A	0	1.0	3	4
Run-5	DC: 2 A.A & 1D.A.	0	1.0	2	4
				1	5
Run-6	6–9	2	3.0	12	9

DC – Detail by Class, A.A – acceptor atom, D.A. – donor atom.

only three models showed the necessary key features for GSK-3 inhibition. These three models (labeled Mod-1, Mod-2 and Mod-3) were selected for further refinement.

The three chosen models were employed in performing virtual screening of the in-house database GSK-3DDB. Varying tolerances (3.00, 2.75, 2.50 Å) were allowed while performing this screening to map-out the effect of distance constraints, the results are listed in Tables 2–4. All three models Mod-1, Mod-2 and Mod-3 have same number of pharmacophoric features but differ in positions of donor sites in the models. Out of these, Mod-3 (Figure 5) was able to pick-up greater ratio of selective GSK-3 inhibitors from all classes of GSK-3 selective series (even at a

Table 2. Effect of the tolerance in model Mod-1 on the selectivity for GSK-3.

Model no.	Tolerance[a]	Total hits*	Selective GSK-3 hits	Non-selective hits	Inactive hits
Mod-1a	3.00	275	120 (0.92)	135 (0.62)	20 (0.62)
Mod-1b	2.75	230	115 (0.88)	97 (0.44)	18 (0.58)
Mod-1c	2.50	211	103 (0.79)	90 (0.42)	18 (0.56)

[a]units are in Angstroms, *Total hits picked by the model from the in-house database, values in parentheses are the hit rates for each category.

Table 3. Effect of the tolerance in model Mod-2 on the selectivity for GSK-3.

Model no.	Tolerance[a]	Total hits*	Selective GSK-3 hits	Non-selective hits	Inactive hits
Mod-2a	3.00	271	118 (0.91)	132 (0.61)	21 (0.66)
Mod-2b	2.75	226	113 (0.87)	96 (0.44)	17 (0.53)
Mod-2c	2.50	189	93 (0.72)	78 (0.36)	18 (0.56)

[a]units are in Angstroms, *Total hits picked by the model from the in-house database, values in parentheses are the hit rates for each category.

Table 4. Effect of the tolerance in model Mod-3 on the selectivity for GSK-3.

Model no.	Tolerance[a]	Total hits*	Selective GSK-3 hits	Non-selective hits	Inactive hits
Mod-3a	3.00	274	120 (0.92)	134 (0.62)	20 (0.62)
Mod-3b	2.75	227	116 (0.89)	96 (0.44)	18 (0.56)
Mod-3c	2.50	194	108 (0.83)	71 (0.33)	15 (0.46)

[a]units are in Angstroms, *Total hits picked by the model from the in-house database, values in parentheses are the hit rates for each category.

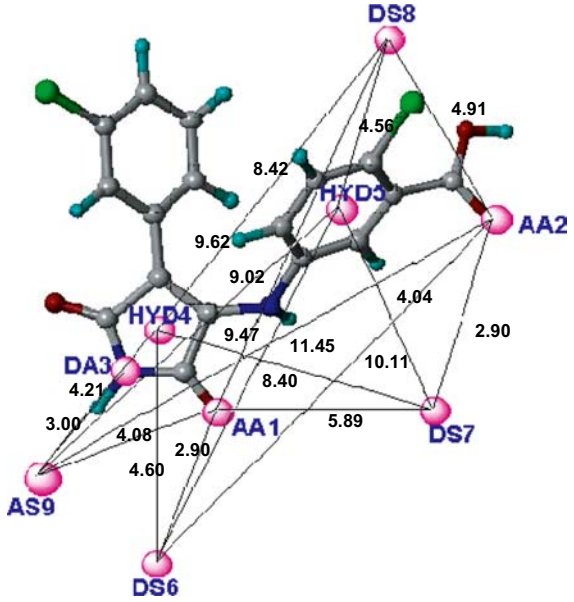

Figure 5. Pharmacophoric features and their distance relation (ligand point-site point) noticeable in Mod-3 along with a derivative of [3-anilino-4-arylmaleimide (**1**)].

tolerance of 2.5 Å) hence, it was considered for further refinement.

The effect of variation in the tolerance in Mod-3 between the features AA1–DA3 and DA3-HYD4 was carried out manually (AA – acceptor atom, DA – donor atom and HYD – hydrophobic center) and optimum value was assigned based on the performance of the model in identifying molecules from the database GSK-3DDB. Between the features AA1–DA3, the distance tolerance at 0.5 Å was found to be optimum. Similarly, between the features DA3–HYD4 a tolerance of 1.50 Å, was found to be optimum. Using this set of optimum values, the model performed quite well in identifying the GSK-3 selective inhibitors from the in-house database, while omitting majority of the non-selective GSK-3 inhibitors (Table 5).

Further improvement in model Mod-3c was introduced by removing the donor site DS8 and modification of other tolerances. The resultant query was found to be relatively satisfactory and labeled **DSP1** (Figure 6a). This query picked more number of GSK-3 selective inhibitors and only a few non-selective GSK-3 inhibitors. Table 6 lists the inter feature distances noticed in the query **DSP1**. The total number of hits identified by the query **DSP1** from the in-house database has been listed in Table 7. An attempt was made to improve the query **DSP1** by adding a hydrophobic feature HYD6, through UNITY interface. The resultant query **DSP2** (Figure 6b) with a tolerance of 2.5 Å has a total of 9 features (2 AA, 1 DA, 3 HYD, 2 DS and 1 AS). The query **DSP2** picked greater number of selective GSK-3 inhibitors from the in-house database but lacked diversity – excluded an entire class of GSK-3 selective molecules (Table 7), and hence not considered further.

The removal of hydrophobic feature HYD5 from the **DSP2** query resulted in new query **DSP3** (Figure 6c), which has 8 features in total. The query **DSP3** differs from query **DSP1** only in position of the hydrophobic center. The tolerances among the features were optimized to get higher selectivity of the pharmacophore for GSK-3. The optimized tolerances among the features and hits obtained from in-house database are shown in the Table 7. The query **DSP3** picked 96 selective

Table 5. Effect of internal tolerance in model Mod-3c between AA1–DA3 and DA3–HYD4 on selectivity of GSK-3 inhibitors.

Model No.	Features	Tolerance between features	Total hits*	Selective GSK-3 hits	Non-selective hits	Inactive hits
Mod-3c	AA1–DA3	0.5	91	74	12	5
	DA3–HYD4	1.5				

*Total hits picked by the model from the in-house database.

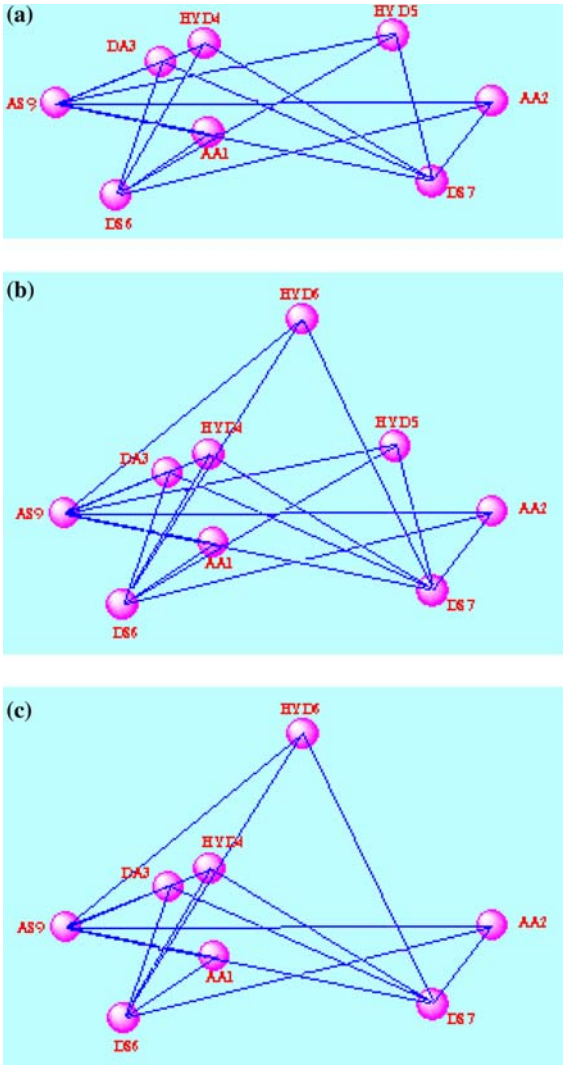

Figure 6. The pharmacophoric queries (a) **DSP1,** (b) **DSP2,** (c) **DSP3** generated from the pharmacophore model Mod-3c after removal of the donor site DS8. The inter feature distances related to these queries are given in Table 6. .

Table 6. Inter feature distances (in Å units) between various features in query **DSP1.** In the queries **DSP2** and **DSP3** also the same distance pattern is present.

Feature	AA1	AA2	DA3	HYD4	HYD5	DS6	DS7	AS9
AA1	–	7.48	2.27	2.36	5.53	2.90	5.89	4.08
AA2	7.48	–	8.77	7.75	3.67	10.11	2.90	11.45
DA3	2.27	8.77	–	1.21	6.11	3.73	7.66	3.00
HYD4	2.36	7.75	1.21	–	4.96	4.60	6.89	4.21
HYD5	5.53	3.67	6.11	4.96	–	8.40	4.04	9.02
DS6	2.90	10.11	3.73	4.60	8.40	–	8.15	2.89
DS7	5.89	2.90	7.66	6.89	4.04	8.15	–	9.96
AS9	4.08	11.45	3.00	4.21	9.02	2.89	9.96	–

AA – Acceptor atom, DA – Donor atom, HYD – Hydrophobic center, AS – Acceptor site, DS – Donor site.

Table 7. Hits picked by the queries **DSP1**, **DSP2**, **DSP3** from the database GSK-3DDB.

Query no.	Total hits*	Selective GSK-3 hits	Non-selective hits	Inactive hits
DSP1	111	96	11	4
DSP2	85	79	4	2
DSP3	103	96	5	2

*Total hits picked by the model from the in-house database

GSK-3 inhibitors out of 130, 2 inactive molecules from a total of 32 and 5 non-selective GSK-3 inhibitors out of 216 present in the in-house database. **DSP3** has the characteristic features required for an ideal pharmacophoric query, because it possessed the important interactions required for this series of selective GSK-3 inhibitors, (Bertrand et al. [27]) and is performing satisfactorily on the in-house database. Hence, **DSP3** was selected for virtual screening purpose.

Virtual screening with the help of **DSP3** and employing Lipinski rule of 5, resulted in the selection of 3640 hits from the NCI database, 1071 hits from the Maybridge database and 1167 hits from the Leadquest database. Further refinement of these hits was carried out by restricting the number of rotatable bonds to a maximum of 7, which reduced the number of hits to 466 from NCI, 276 from Maybridge and 225 from Leadquest, respectively (Figure 7).

The reference molecule, compound **1** was first docked into the ATP binding site of 1Q4L using FlexX method. The C_score module of SYBYL6.9 was employed to obtain various scores. The major interactions shown by **1** in GSK-3 binding site are the important H-bonding interactions with Asp133, Val135, Gln185 and Arg141 (Figure 3). Similarly, other molecules chosen (**2–4**) for the pharmacophore generation were also docked into the same active site, Table 8 lists the various docking scores. The 967 hits obtained from virtual screening were also docked into the same site, producing 61 hits having higher FlexX score than compound **1**. On comparing other scores (G_score, PMF_score, D_score and Chem_score) of these hits with that of compounds **1–4**, a set of 9

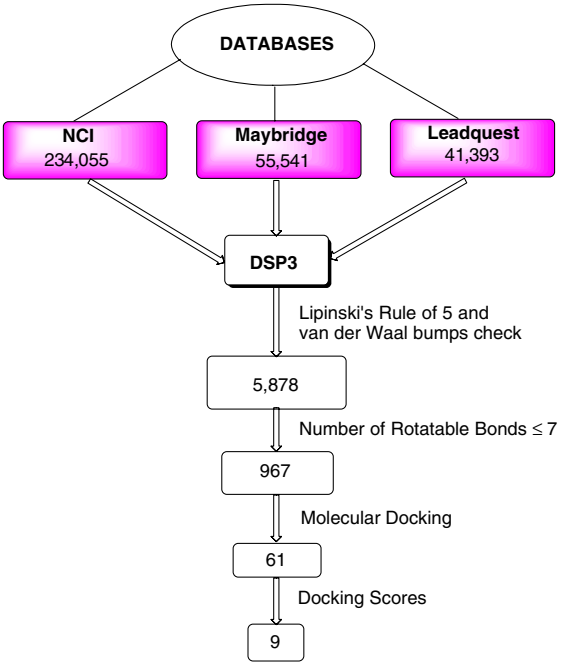

Figure 7. The results obtained from the virtual screening of the three databases (NCI, Maybridge, Leadquest). The numbers given the figure represent the number of molecules after employing the filters.

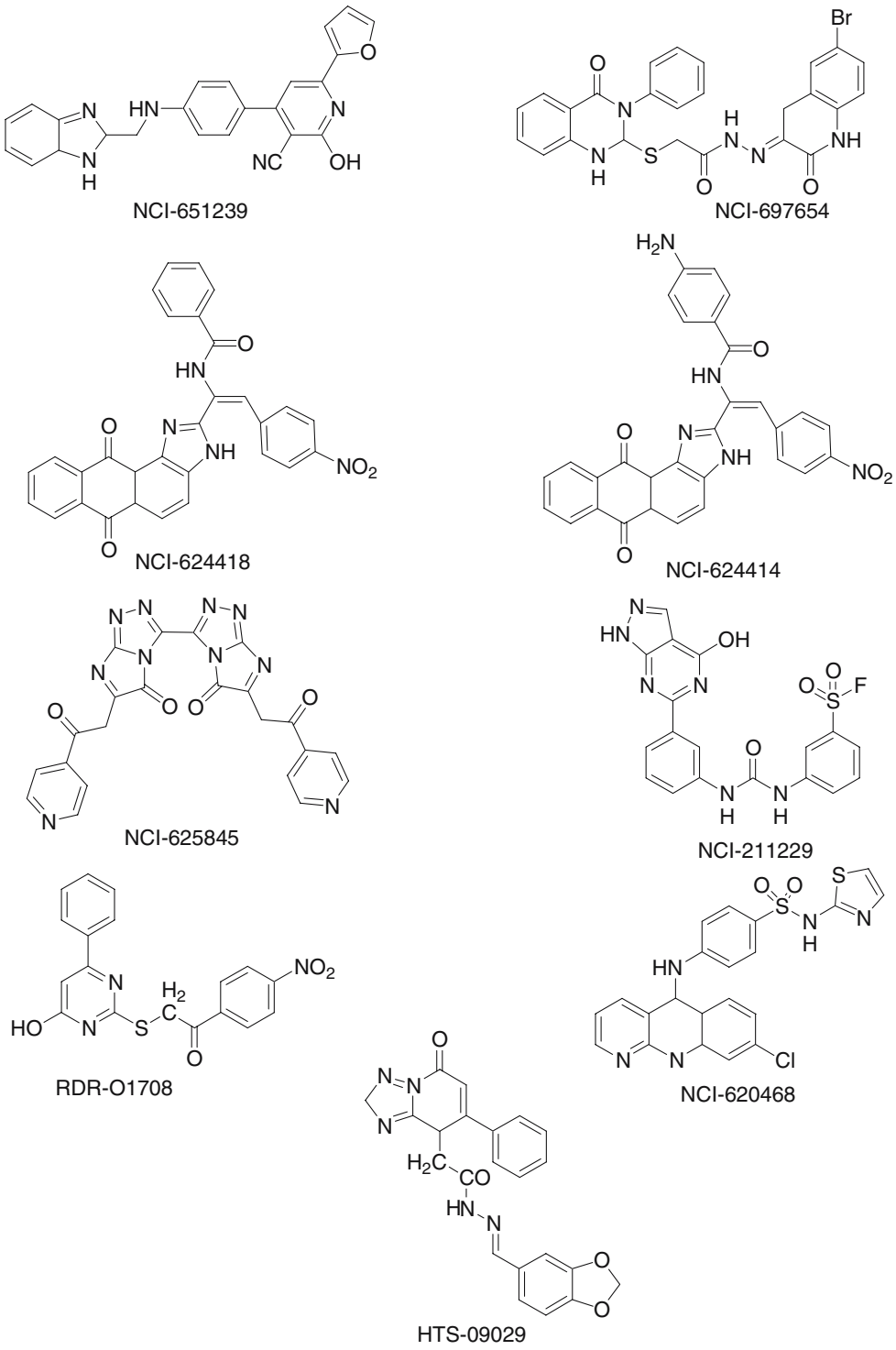

Figure 8. Structures of final nine hits selected from the virtual screening analysis.

Table 8. Docking scores of reference compounds (**1–4**) and the final hits obtained after docking study.

Compounds	FlexXscore	G_score	PMF_score	D_score	Chem score	CS score
1	−30.64	−189.71	−41.99	−114.96	−36.31	4
2	−25.50	−266.84	−19.09	−152.37	−34.41	4
3	−25.00	−35.93	−24.46	−137.13	−33.05	5
4	−15.00	−160.67	−76.62	−71.35	−22.10	5
NCI-651239	−33.22	−197.34	−73.22	−129.31	−40.47	5
NCI−624414	−37.59	111.32	-43.41	−120.02	−120.02	4
NCI-211229	−36.51	−210.54	−39.44	−133.45	−37.11	5
NCI-624418	−35.92	94.31	−58.39	−112.54	−36.02	4
NCI−697654	−32.63	−108.18	−53.97	−119.33	−36.30	4
NCI-625845	−31.85	−239.82	−90.42	−149.15	−29.80	4
NCI-620468	−30.79	−231.35	−38.22	−152.60	−42.74	5
HTS-09029	−32.17	−130.67	−62.94	−148.44	−38.52	5
RDR−01708	−30.96	−190.29	−45.19	−123.56	−34.84	5

new potential lead were identified (Figure 8, Table 8). All the 9 potential leads show core H-bonding interactions with Asp133 and Val135.

The better binding scores of the selected hits are due to the additional stabilizing interactions, for example, NCI-211229 shows interactions with Asn64 and Asp186 in addition to the core interactions (Figure 9). Thus these potential leads are expected to possess the critical pharmacophoric features required for selective GSK-3 inhibition

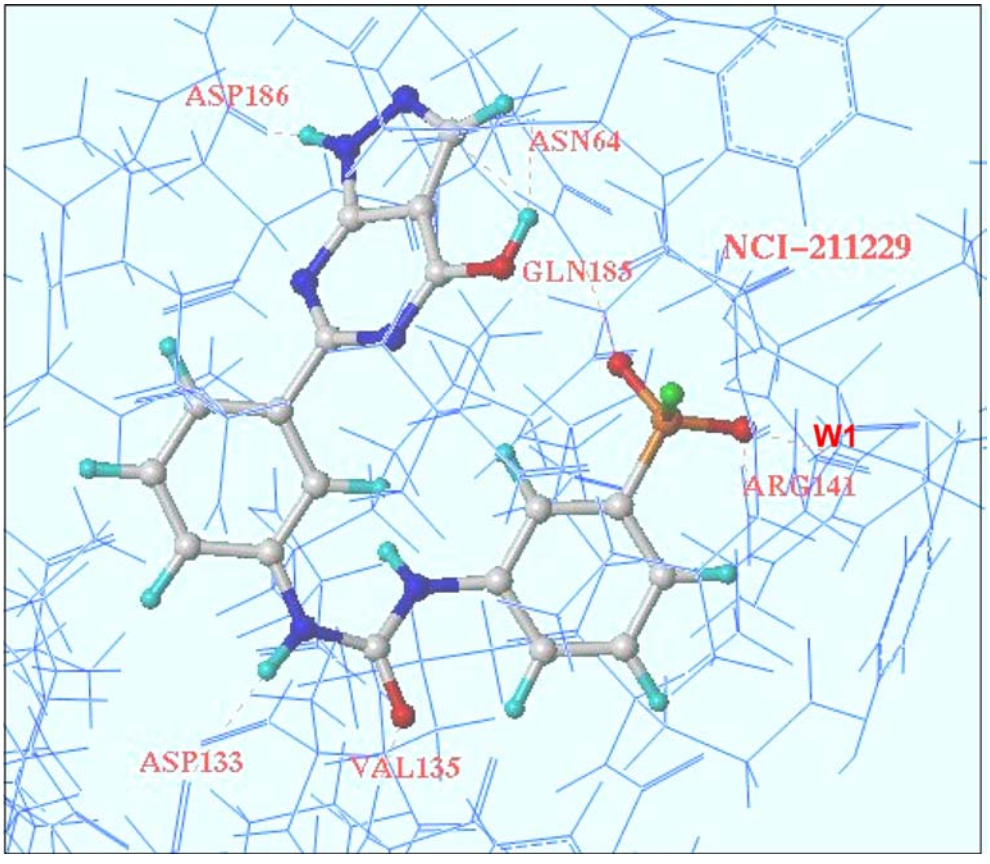

Figure 9. Interactions shown by the lead molecule (NCI-211229) obtained from virtual screening.

and are also expected to induce improved binding affinity with GSK-3.

Conclusions

A pharmacophoric map for selective GSK-3 inhibitors was generated from four diverse classes of compounds having specificity for GSK-3 over CDK-2 using DISCO module. Validity of the derived pharmacophoric map was ascertained (a) by analyzing the important interactions shown by the reference molecule and (b) by submitting the pharmacophoric query to a test database (which consists of selective, non-selective and inactive molecules from different classes). Out of the several maps generated, pharmacophoric query **DSP3** was found to be satisfactory because it identified greater number of active molecules from the test database and also the identified molecules are from all classes of GSK-3 selective inhibitors. The pharmacophoric query **DSP3** was employed to screen three well known chemical databases, namely, NCI, Maybridge and Leadquest to find new hits. Further data reduction was brought out by employing filters like, Lipinski's rule of 5 and van der Waals bumps check. These filters helped in selecting 5878 molecules from a total of 330,989 molecules. Further reduction in number of the hits was achieved by restricting the number of rotatable bonds to a maximum of 7, which gave 967 hits. Molecular docking based method was employed as the final filter to identify 61 hits. Finally, 9 molecules were identified as new potential leads for GSK-3 selective inhibition on the basis of five different docking scores in comparison to the already known best molecules. These potential leads are expected to possess selectivity towards GSK-3 because (i) they posses pharmacophoric features (ii) they all are drug like molecules according to Lipinski rules, (iii) they have limited flexibility with less than seven rotatable bonds, (iv) they all dock well and show greater binding affinity to GSK-3 than the reference molecule.

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