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Identification of potential glycogen kinase-3 inhibitors by structure based virtual screening

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Abstract

Glycogen synthase kinase-3 (GSK3) is a serine/threonine kinase that has attracted much drug discovery attention in recent years. Structural crystallography of the kinase has produced several high resolution inhibitor–GSK3 complexes and this is offering valuable information about the important pharmacophoric features present in the inhibitor, the protein target and the bioactive conformation. The availability of several GSK3–inhibitor co-crystals was successfully exploited to derive a pharmacophore query which retains the all important inhibitor–GSK3 interaction chemistry. A hypothesis containing three features: two hydrogen bond donors and one hydrogen acceptor was found to explain much of the inhibitor–GSK3 interaction. Subsequently, the query has been submitted to three databases for electronic screening. The hits obtained were docked into glycogen synthase kinase-3β active site. A total of 21 novel potential leads were proposed after thorough examination by a combination of methods: (i) visual examination of how well they dock into the glycogen synthase kinase-3β binding site, (ii) detailed analysis of their FlexX, G_Score, PMF_Score, ChemScore and D_Score values, (iii) comparative investigation of the docking scores of the hits with that of the thus far reported inhibitors (iv) determination of the binding mode and examination of how the hits retain interactions with the important amino acid residues of the kinase binding site. The hydrophobic heterocycles identified in this investigation are expected to be important additions to the armamentarium of GSK3 hyperactivity antagonism. Further more, the present work may further our current knowledge of the molecular basis of activation, inhibition and regulation of this pharmaceutically important kinase.
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Keywords: GSK3; Pharmacophore mapping; Virtual screening; Docking; FlexX

1. Introduction

Protein biophosphorylation is an important reaction in the regulation of protein functions. Biological phosphorylation mostly occurs on hydroxyl containing amino acids such as serine, threonine and tyrosine and is catalyzed by protein kinases whose number is well over 800 in the human genome. Given the importance of protein phosphorylation as a major post-translational mechanism used by cells to regulate enzymes and other proteins and the fact that many human disorders are either caused by or are consequences of aberrations of protein phosphorylation [1], protein kinases have become one of the most promising targets for drug discovery [2–7]. Glycogen synthase kinase-3 (GSK3) was originally identified and studied for its function in the regulation of glycogen synthase [8–10], the rate-limiting enzyme in glycogen biosynthesis [11]. It is a serine/threonine kinase that is composed of two isoforms (α and β) in mammals. These isoforms share high homology (>90%) at the catalytic domain and are expressed ubiquitously in cellular systems and have similar biochemical properties [12]. GSK3 has multiple substrates [13,14] and plays a critical role in glucose homeostasis [15], CNS function and cancer [13], circadian rhythm, cell death, cell survival and others. Given the importance of phosphorylation in physiological events and the diverse substrates this kinase has, GSK3 inhibitors have got a

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wide spectrum of therapeutic potential which includes major disorders such as diabetes, neurodegenerative diseases [16], bipolar disorders [17], stroke, cancer and chronic inflammatory diseases [18].

Fig. 1 shows some classes of GSK3 inhibitors. Although a number of diverse classes of GSK3 inhibitors have been reported including paullones [19], indirubins [20], maleimides (bisarylmaleimides [21], anilinomaleimides [22], bisindolylmaleimides [23], azaindolyl maleimides [24]) and despite the high therapeutic potential the kinase has as a target, currently there is no drug that targets GSK3. Studies have produced a wealth of information regarding the molecular basis of GSK3 activation and inhibition [25]. Currently, there are a number of complexes of GSK3 with different inhibitors reported in the Brookhaven

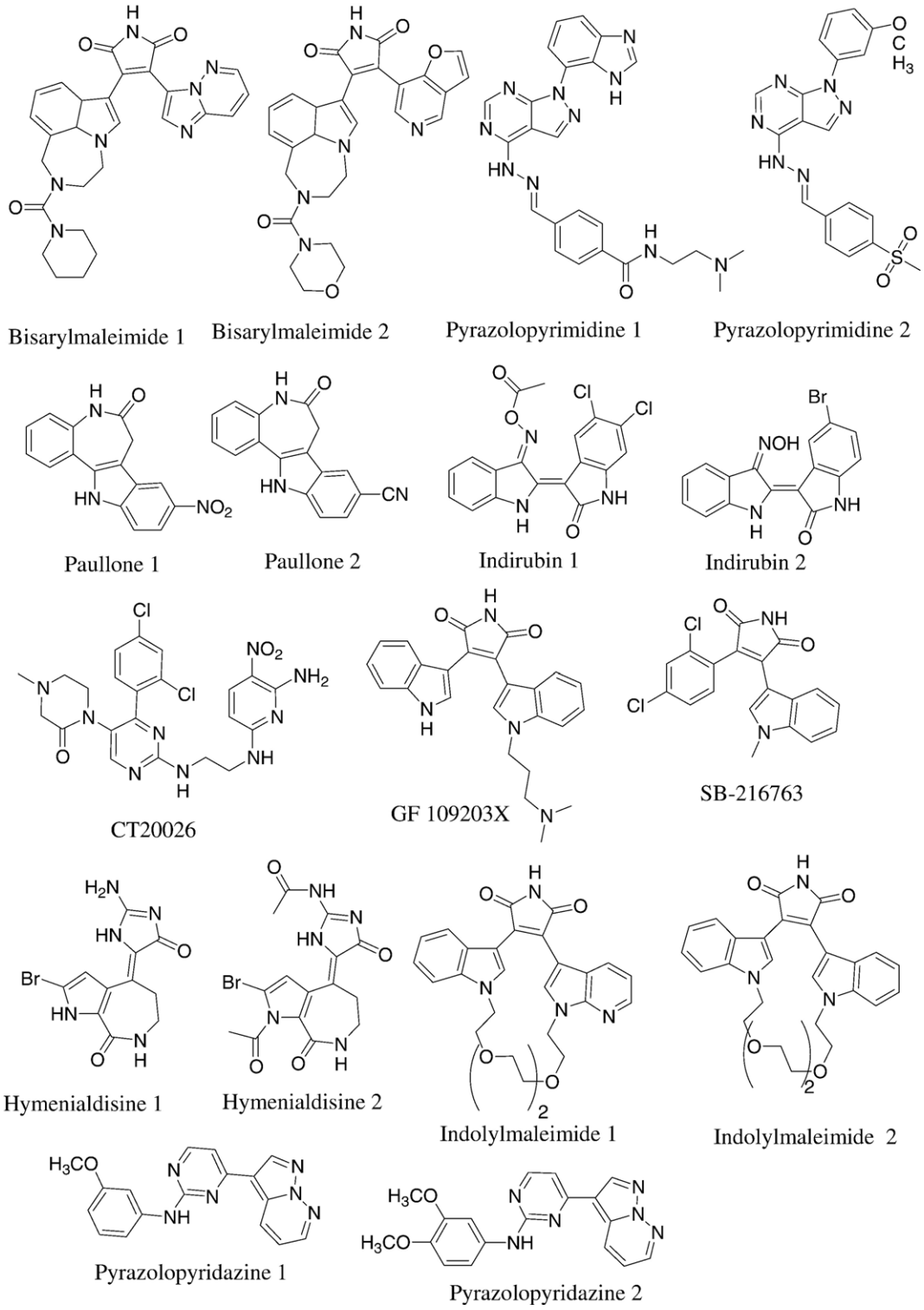

Fig. 1. Examples of GSK3 inhibitors that are also used as reference compounds used for calibration of the docking methodology.

protein databank. These complexes of GSK3 have unfolded how the structurally diverse chemical scaffolds interact with the same binding site of the kinase. Such a scenario represents an ideal condition for medicinal chemistry to exploit the vast information and translate it into a clinically useful agent. As there is a wealth of crystallographic information, a methodology that can take this structural information into account to aid in designing novel scaffolds is worthwhile. Structure based pharmacophore mapping [26,27] and its use in structure based virtual screening appears to be well suited here. A pharmacophore model postulates that there is an essential three-dimensional (3D) arrangement of functional groups that a molecule must possess to be recognized by the binding pocket. It collects common features distributed in 3D space that is intended to represent groups in a molecule that participates in important interactions between drugs and their active sites. Hence, a pharmacophore model provides crucial information about how well the common features of a subject molecule overlap the hypothesis model. Pharmacophore identification can be done either manually [28,29] or it can be automated [30–33]. When there is enough structure activity data and when the molecules are relatively rigid, a manual pharmacophore mapping can be initiated particularly when these are coupled by the presence and thorough investigation of high resolution inhibitor–receptor complexes. In a previous application, we have attempted to construct a pharmacophore hypothesis using 21 diverse GSK3 inhibitors, using HipHop [34] module of CATALYST [35]. The results of the study point out a hypothesis containing four features: two hydrophobic, one hydrogen bond donor and another hydrogen bond acceptor was found to be the best from the 10 common feature hypotheses produced by HipHop [34]. However, the previous report employed only inhibitors, i.e., it is solely ligand based with no attention given to the chemical characteristics of the receptor. Hence, there is a need to derive a pharmacophore that exploits the vast crystallographic information of the numerous GSK3–GSK3–inhibitor complexes so as to come up with a conclusive result that incorporates the essential features of both the available inhibitors and that of the kinase binding site. In the present study we have exploited the available GSK3–inhibitor complexes [36] to derive a pharmacophore and the resulting pharmacophore was submitted to electronically screen three databases: NCI, LeadQuest and Maybridge. The hits obtained along with known inhibitors were docked into the GSK3 binding site and the binding mode have been determined and detailed comparative and structural analysis have been made to validate the hits using a series of standard hit validatory measures.

2. Materials and methods

2.1. Determination of the pharmacophore geometry

Crystallographic data has been employed to derive a pharmacophore model for inhibiting GSK3. Five structurally different structures whose complex with GSK3β is available from the Brookhaven protein data bank were used in this study. Fig. 2 shows these complexes and their protein data bank code. The important features that interact to the common amino acids of GSK3β (Val135, Asp133 and Gln185) were identified from previous crystallographic study [36]. A careful investigation shows that all the five structurally different ligands form three common hydrogen bonds with these amino acids. Three of the ligands (alsterpaullone, staurosporine and indirubin-3-monoxime) were noted to interact with a common water molecule while the remaining two ligands (AMP–PNP and anilinomaleimides) are interacting without intermediate water molecule with Gln185. AMP–PNP and anilinomaleimide have an –OH group that interacts with Gln185 via hydrogen bonding while the OH of the intermediary water molecule of the rest three ligands is interacting with the same Gln185. Hence this common water molecule was taken to serve as a common hydrogen bond donor feature for the inhibitors which interact with the kinase through it while the –OH on AMP–PNP and anilinomaleimide served as the equivalent hydrogen bond feature. The carbonyl group present in anilinomaleimide, staurosporine, alsterpaullone and indirubin-3′-monoxime is acting as a hydrogen bond acceptor feature that forms hydrogen bonding with the –NH of Val135 of the binding site. Moreover, the nitrogen atom on the pyrimidine ring of AMP–PNP is serving as a hydrogen acceptor feature for AMP–PNP to form the same hydrogen bonding with the NH of Val135. Since all interact with hydrogen bonding with the same amino acid, the carbonyl group in anilinomaleimide, staurosporine, alsterpaullone and indirubin-3′-monoxime and the N on the pyrimidine ring of AMP–PNP were taken to represent equivalent hydrogen bond acceptor features. The NH_2 group on the adenine ring of AMP–PNP, the NH adjacent to the carbonyl group in indirubin-3′-monoxime and staurosporine, and the NH on the maleimide ring of anilinomaleimide are seen to make hydrogen bonding interaction with the carbonyl group of Asp133. Furthermore, alsterpaullone makes hydrogen binding interaction with the carbonyl of Asp133 through a bridging water molecule. Hence the NH centers on indribunin-3′-monoxime, staurosporine, AMP–PNP, anilinomaleimide and the OH of the water that helps alsterpaullone to interact with the same CO of Asp133 are considered equivalent hydrogen bond donor features. The over all common features were found to be two hydrogen bond donor features that interact with the NH of both Val135 and Gln185; one hydrogen bond acceptor feature that interacts with the carbonyl of Asp133. These amino acids were also found to be important with other inhibitors of GSK3 [36]. Following the identification of the important common bonding interaction, the ligands were extracted from their complexes in the protein data bank and brought to SYBYL [37]. As conformations of the inhibitors represent the bioactive ones, there was no need to go for conformational analysis in order to establish the bioactive conformation. Hence the inter feature distances were determined from the extracted bioactive conformation. The inter feature distances were determined using the manage feature options of UNITY implemented in SYBYL6.9 for all of the five inhibitors. For the inhibitors which interact via bridging water molecule, both the water molecule and the corresponding feature on the inhibitor were used to determine the distance

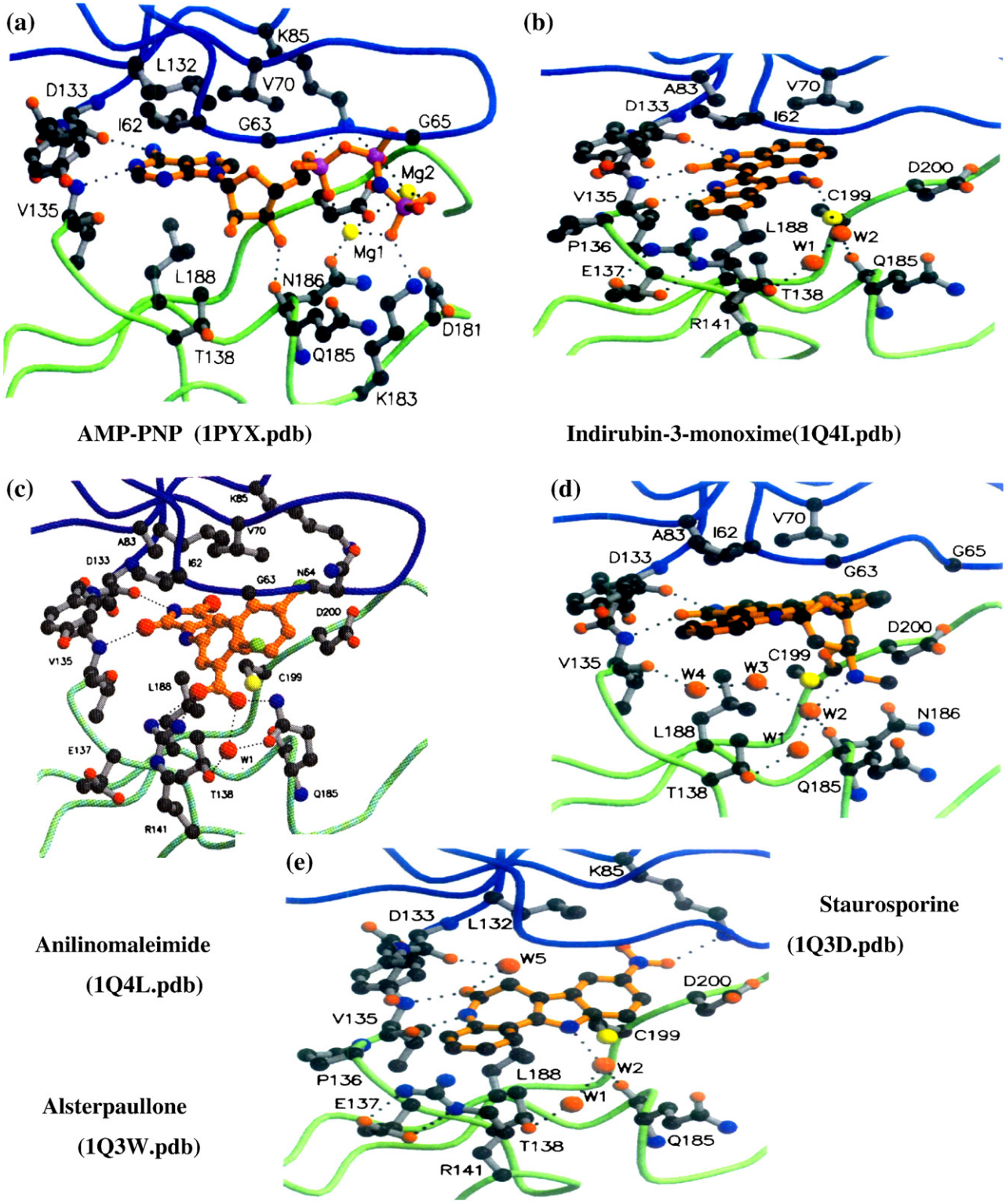

Fig. 2. Crystal structures of GSK3β–inhibitor complexes.

ranges. The corresponding equivalent features and the distance ranges of the five inhibitors is displayed in Fig. 3. Examination of the inter feature distances shows that the values for the corresponding equivalent features do not exactly overlap, which is not surprising given that some of the inhibitors are interacting via another bridging molecule (water). So, in order not to miss potential inhibitors that may interact with the receptor directly or indirectly via a bridging water molecule, the pharmacophore triangle was determined by taking the union of distance ranges between equivalent features. This is important as the query is going to be used in the preliminary screening and the hits obtained from this preliminary study will be subjected to more

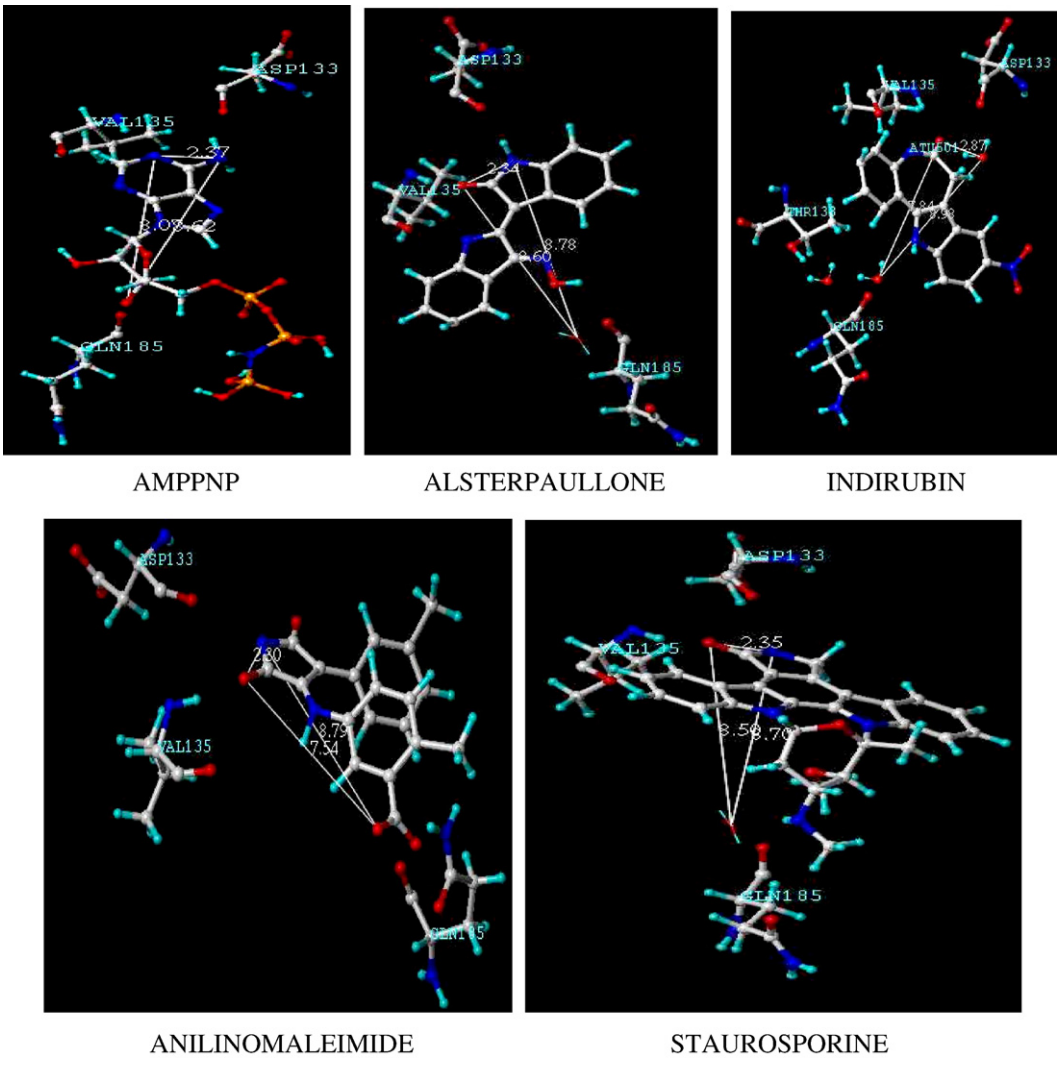

Fig. 3. The corresponding equivalent features and the distance ranges of the inhibitors used to determine the pharmacophore geometry.

exhaustive and sequential structural verification strategies. The pharmacophore triangle thus obtained is shown in Fig. 4. Finally, in order not to miss other hydrogen bond donor and acceptor features, the –OH and –NH positions were assigned general hydrogen bond donor feature sites while the CO feature was generalized into a hydrogen bond acceptor feature by using the FEATURE option of UNITY.

2.2. Virtual screening

The developed query was submitted to three database; NCI, Maybridge and LeadQuest databases. In order to omit unreasonable chemistries, virtual screening filters such as Lipinsk's rules [38,39] were applied. The maximum molecular weight was set to 500; hydrogen bond donors (OH's and NH's) were set to <5; hydrogen bond acceptors (N's and O's) to <9; MLogP <4.15. Apart from this, the number of rotatable bonds was limited to 7. The flexible search methodology was employed as it takes into account the conformational flexibility of molecules. Unless otherwise stated, all other parameters were kept at their default values.

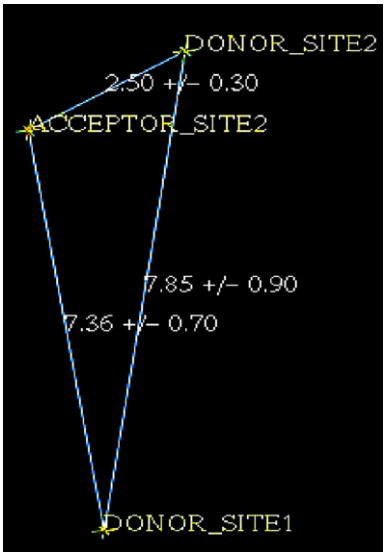

Fig. 4. The pharmacophore triangle.

2.3. Molecular docking

In order to aid in the prioritization and comparative investigation of the hits with the so far reported inhibitors, the hits obtained from the search were docked into the binding pocket of GSK3 using the FlexX program [40] interfaced with SYBYL6.9. FlexX employs a fast algorithm for flexible docking of small ligands into a fixed protein binding site using an incremental construction process. Standard parameters of the FlexX program as implemented in SYBYL6.9 were used during docking. To further evaluate the docking experiment, the G_Score [41], PMF_Score [42], D_Score [43] and ChemScore [44] values were estimated using the CScore module of SYBYL. As CScore is a consensus scoring function, the different scoring functions in it provide multiple approaches to evaluate ligand–receptor interactions and such different scores are expected to better aid in prioritization.

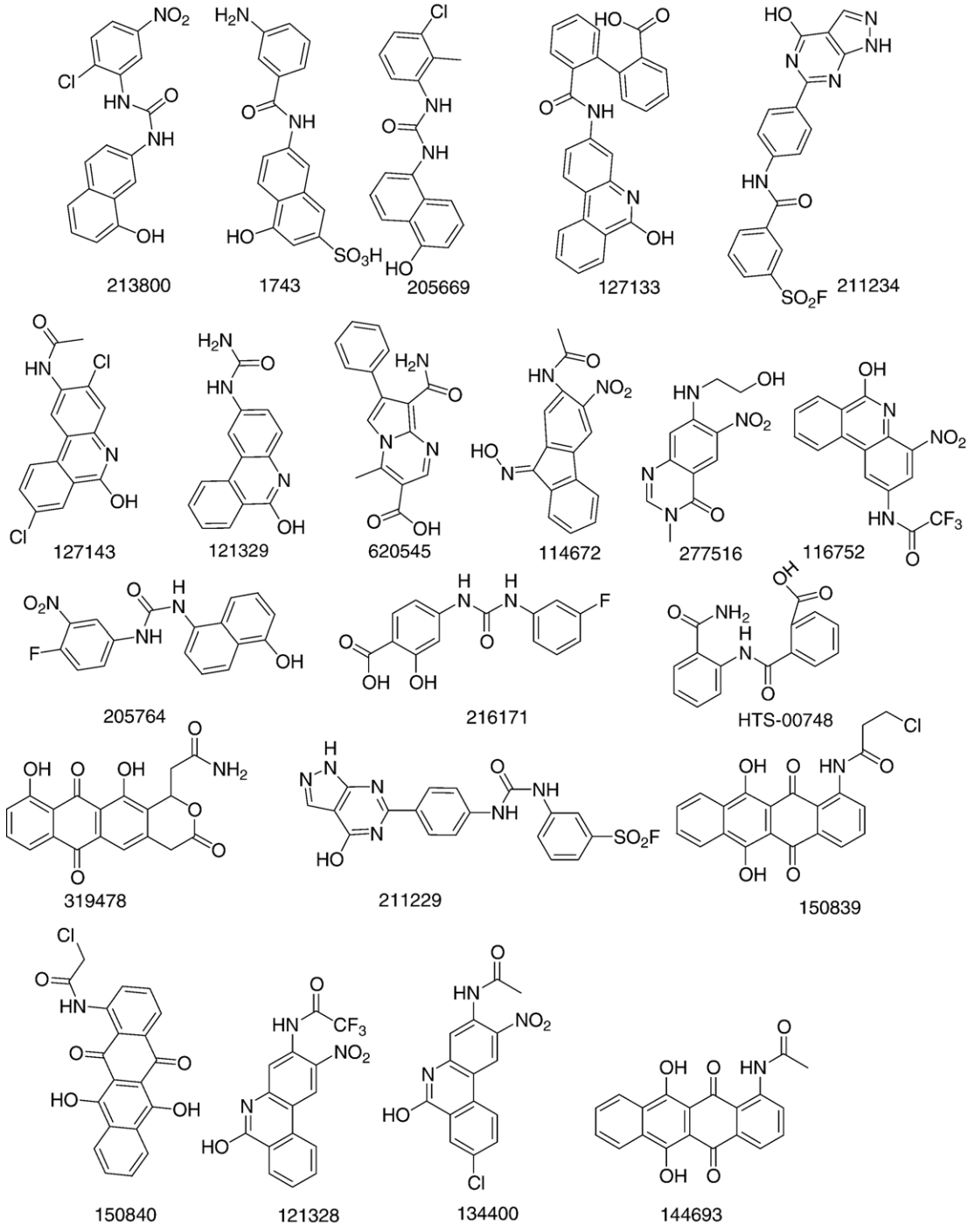

Fig. 5. Structures of the hits identified from the NCI database. HTS-00748 is obtained from Maybridge database. The numbers for each structure indicate the code number of the molecules in the databases.

3. Results and discussion

Crystallography assisted pharmacophore modeling is a well established method for lead identification particularly in the early phases of drug discovery projects. That the pharmacophore in the present study contains just three features can be explained on the basis of the fact that pharmacophores generally contain three features and rarely four and almost never more than four [27]. Our previous work on ligand based pharmacophore mapping also substantiates this point [34]. Moreover, hydrogen bonding is found to be the major contributor to binding of inhibitors with GSK3 [45,46] and indeed drug receptor interaction [47] in general. The derived pharmacophore query was submitted to three UNITY databases: LeadQuest, Maybridge and NCI. Searching LeadQuest yielded 62 hits while 192 hits were obtained from the Maybridge database search (Fig. 5). The NCI database on the other hand returned 1299 hits. The high number of hits returned from the search has to do with the relatively large distance ranges in the query and with the relatively large number and classes of structures in the chemical databases. But this may not be a disadvantage as this is just a preliminary step and the hits will be subjected to more robust filtering strategies subsequently. The relatively high number of hits perhaps improves the probability of not missing very promising hits. The difference in number from the difference chemical databases was not unexpected given the differences in the number and diversity of the chemical composition of the three databases. NCI contains 234,055 drug like compounds while LeadQuest and Maybridge respectively contain 41,393 and 55,541 chemical compounds. The hits obtained were then docked into the binding site of GSK3β using the FlexX docking algorithm. Docking is generally employed to estimate the binding affinities of ligands to a given receptor. This was very important to rank-order and hence to prioritize the hits for further investigation given the large number of hits obtained from the search. In order to be assured of the docking experiment, it is desirable to assess how the FlexX and other docking scores used predict the affinities of known inhibitors which have an experimentally determined IC_{50} values. For this purpose, 11 different experimentally known ligands representing the most active molecules from their respective classes were taken from the available literature and were docked into the same GSK3β binding site the hits were docked using the same methodology. The docking scores of the reference molecules together with their IC_{50} are shown in Table 1. As this table shows the FlexX score varies from −14.37 to −35.13 for the previously reported most active inhibitors. Since the inhibitors are reported by different groups in different labs direct comparison of IC_{50} with the docking scores is obviously difficult. Nevertheless, irrespective of this direct correlation comparison a hit with FlexX score higher than the reported inhibitors should ideally present a potential lead. This is because the procedures taken for all reference molecules were identical—the same protein, the same active site, the same software and hardware and as the different docking scores are measures of the free energy of inhibitor–GSK3 binding. It is suspected that everything that contributes to the differences in docking scores should be inherent to the nature of ligand structure. As a further means of validating the FlexX score the second most active molecules were taken into the docking experiment in order to see how the FlexX and other scores correlate with the inhibitory potencies of the ligands.

Eight molecules were taken from a series and the next most active molecules were taken for the study. The results are displayed together with most active 11 molecules in Table 1 where the suffixes 1 and 2 to the names of the inhibitors indicate the most active and the second most active inhibitors in their respective series. The result shows that FlexX score appears to parallel with experimental IC_{50} quite accurately. FlexX was found to differentiate even a very small difference between the IC_{50} values. The only exception was seen in case of anilinomaleimides where the second most active molecule

Table 1
IC_{50} and docking scores of the reference inhibitors

Series no.	Name	IC_{50} (nM) (ref)	Docking scores					
			FlexX	G_Score	PMFScore	D_Score	ChemScore	CScore
1	Bisarylmaleimde1	0.7 [21]	−35.13	−256.53	−27.62	−138.91	−38.24	3
2	Bisarylmaleimde2	0.8 [21]	−30.71	−282.25	−30.56	−142.95	−36.67	2
3	Indirubin1	5 [20]	−29.84	−175.06	−47.89	−99.10	−33.57	5
4	Indirubin2	4 [20]	−29.76	−174.59	−45.06	−105.89	−31.64	5
5	Paullone1	4 [19]	−26.91	−126.69	−39.12	−84.14	−35.62	4
6	Paullone2	10 [19]	−21.32	−150.40	−27..31	−83.56	−31.62	4
7	Hymenaldisine1	10 [48]	−23.44	−141.48	−41.45	−79.70	−23.73	4
8	Hymenaldisine2	130 [48]	−21.39	108.56	−26.31	−43.18	−20.52	2
9	Pyrazolopyridazine1	10 [49]	−21.54	−60.29	−41.20	−58.87	−30.62	5
10	Pyrazolopyridazine2	12 [49]	−17.14	−58.84	−43.75	−66.44	−27.53	5
11	Indolylylmaleimde1	22 [23]	−14.87	−151.31	−38.67	−68.03	−24.16	5
12	Indolylylmaleimde2	26 [23]	−14.87	−149.98	−38.09	−67.59	−24.22	5
13	SB-216763	75 [50]	−23.93	−187.76	−34.41	−202.87	−33.35	5
14	CT2006	4 [51]	−28.53	−101.69	−62.30	−96.41	−28.60	4
15	Pyrazolopyrimidine1	1 [52]	−27.84	−84.64	−35.35	−120.26	−29.94	4
16	Pyrazolopyrimidine2	2 [52]	−23.10	−114.36	−49.55	−123.94	−30.37	5
17	GF109203	190 [53]	−29.39	−253.72	−42.55	−316.77	−35.61	4

was predicted higher than the most active compound. However, examination of the bioassay protocol of anilinomaleimides shows that the IC_{50} was done on the α-subtype of GSK3 while the docking study was carried out on GSK3β as this is the only crystal structure available in the Brookhaven protein data bank at the time of this work. This all shows that FlexX score might be reliable enough to make a decision as to which hits to take for further study. And hence FlexX score was used as a basis to select compounds that would be expected to bind with a higher affinity. The highest FlexX score that could be obtained from the reference molecules is −35.13. Since there is only one molecule that has above −35.00 in the reference structures, hits with a FlexX score better than −35.00 were taken to represent a novel potential leads for docking scores are measures of the free energy of binding. Using this cut off, the NCI database search yielded 20 hits while only one was obtained from the Maybridge search. The LeadQuest database did not give any hit with FlexX score better than −35.00. Apart from this, the scores from other scoring functions were also requested in the hit verification. In most of the cases, the PMF, Chem_, G_ and D_Scores do correlate with the experimental IC_{50} and hence these too were used to aid in prioritization. As can be seen these docking scores for the hits are mostly better than the reference molecules. And indeed one reference molecule was found to have a positive G_Score value. Furthermore, the final hits remained were visually observed how they dock into the GSK3β binding site. Hits that dock well were finally chosen and are anticipated to be new potential hits. Last but importantly, the binding modes of the hits were determined and visually analyzed. The hits were found to dock well into the GSK3 active site. The interaction of the two highest FlexX scoring hits displayed in Figs. 6 and 7 attests that the hits do retain the important common binding mode that is known for the so far reported inhibitors. Chemically, the hits are flat, hydrophobic nitrogen heterocycles which is seen as a general feature in the previously reported inhibitors. The structures of these 21 validated hits identified are shown in Fig. 4 while the docking scores are shown in Table 2.

3.1. Interactions of Hit-134400 with GSK3β

The interaction of Hit-134400 with GSK3β active site is shown in Fig. 6. As the figure shows the nitro group is seen to make two hydrogen bonding interactions with the two NH_2 of Arg141. The carbonyl of Pro136 is also seen to make hydrogen bonding interactions with the two NH_2 of the amide group. Besides, the carbonyl group of the amide moiety is noticed to make hydrogen bonding interactions with the hydroxyl of Tyr134. Ile62 and Val70 are observed close to the phenyl rings of the hit which indicates a potential hydrophobic interactions. The NH of Val135 is also making hydrogen bonding interactions with the pyrimidine nitrogen while the hydroxyl on the pyrimidine nitrogen is seen to make hydrogen bonding interactions with the carbonyl of Asp133.

3.2. Interactions of Hit-144693 with GSK3β

The interactions of Hit-144693 with GSK3β binding site is shown in Fig. 7. The side chain NH of Gln185 is seen to make hydrogen bonding interactions with the carbonyl oxygen of the amide bond. Moreover, the amide NH is also seen to make hydrogen bonding interactions with the carbonyl oxygen of Ile62. Possible hydrophobic interactions exist between the hydrocarbon groups of Val70, Ile62, Leu188, Val110, Val83,

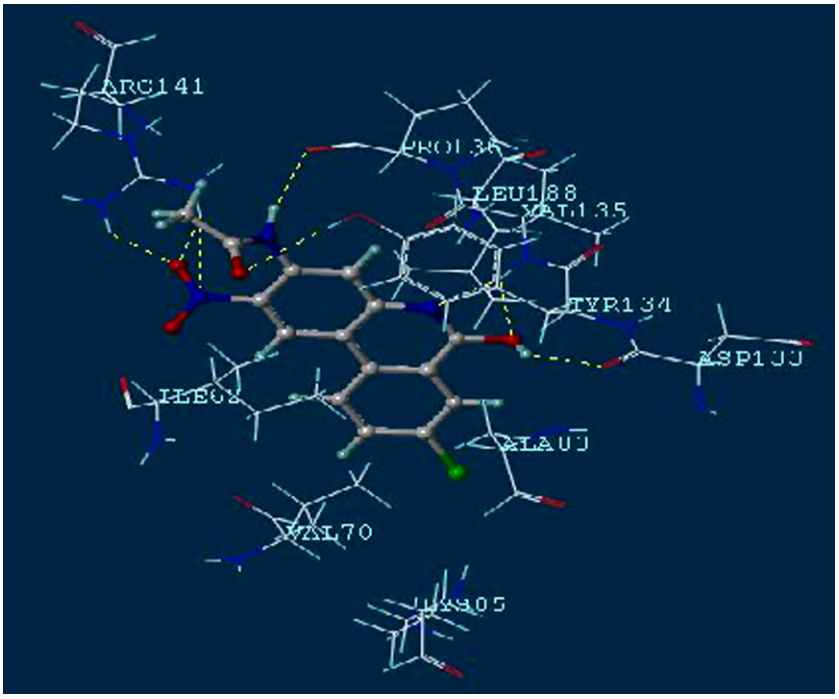

Fig. 6. Interactions of Hit-134400 with GSK3β.

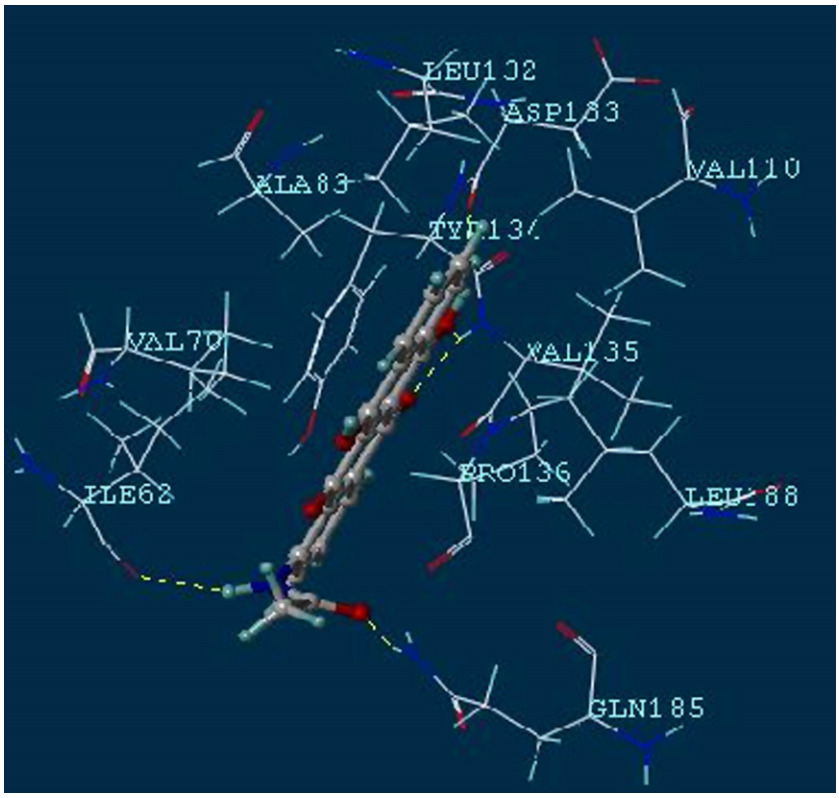

Fig. 7. Interactions of Hit-144693 with GSK3β.

Ile132 and the tetracyclic ring system of the hit. Asp133 and Val135 are also interacting via hydrogen bonding with the OH of the hit. Finally, the NH of Val135 is observed to make favorable hydrogen bonding interactions with one of the carbonyl of the quinone ring. These hydrogen bonding interactions are acknowledged to be common to all of the previously experimentally known GSK3 inhibitors [36]. In both cases the hits were able to reproduce this common binding mode seen so far.

Table 2
Docking scores of the hits identified

Series no.	ID no.	Docking scores					
		FlexX	G_Score	PMF_Score	D_Score	ChemScore	CScore
1	134400	−42.24	−100.01	−68.16	−97.46	−35.55	4
2	121328	−41.25	−93.10	−53.31	−88.28	−33.74	3
3	144693	−41.09	−158.49	−48.68	−111.69	−42.13	5
4	150840	−41.13	−174.68	−49.97	−119.82	−42.36	4
5	150839	−40.05	−159.87	−46.76	−120.61	−41.73	2
6	114672	−39.63	−95.50	−47.21	−87.96	−36.86	4
7	211229	−38.26	−180.96	−28.85	−132.34	−40.16	4
8	319478	−37.88	−153.61	−51.10	−107.96	−38.37	3
9	277516	−37.64	−74.25	−47.81	−79.64	−27.71	5
10	620545	−37.59	−107.88	−70.44	−83.34	−39.42	3
11	127133	−37.47	−102.86	−49.27	−117.73	−41.42	5
12	205764	−37.32	−141.63	−61.80	−104.66	−33.88	5
13	216171	−36.60	−92.09	−46.71	−74.31	−36.77	4
14	211234	−36.44	−130.52	−40.68	−96.90	−30.36	5
15	121329	−35.92	−103.14	−46.98	−75.18	−34.80	3
16	127143	−35.68	−124.25	−41.78	−92.66	−35.84	4
17	205669	−35.45	−151.37	−48.61	−98.31	−36.17	5
18	1743	−35.30	−194.21	−58.28	−123.12	−43.22	5
19	213800	−35.33	−117.41	−18.35	−87.07	−29.28	3
20	116752	−35.11	−93.39	−21.82	−77.86	−29.37	1
21*	HTS−00748	−35.29	−16.42	−37.32	−63.34	−30.32	1

Note: A number with * refers to hits from Maybridge database while the rest are from NCI database.

4. Conclusions

Pharmacophore modeling is best applied in the initial phase of drug discovery programs. Crystallography is now a well recognized tool in drug design. It offers valuable information about important features present in ligands or protein targets, about the bioactive ligand conformation and its geometry. The availability of several co-crystals of GSK3β with several varied inhibitors has been exploited to successfully derive a pharmacophoric query. The so developed query which retains the all important inhibitor–kinase interaction chemistry has been submitted to three databases for electronic screening. The hits have been further investigated through a combination of several standard hit validation measures: visual examination of how well they dock into the GSK3β binding groove, detailed analysis of their docking scores using five different scoring functions, comparative investigation of the docking scores of the hits with that of the thus far known 17 inhibitors of the kinase, and, last but importantly, how the hits retain interactions with the important amino acid residues of GSK3β binding site with which the known GSK3 inhibitors are known to interact. The hydrophobic heterocycles identified in this investigation are expected to expand the available GSK3 inhibitors, albeit future structure–activity studies will be critically important to validate the predictive analysis of the present study. Further more, the present work may further our knowledge of the molecular basis of GSK3 activation and inhibition and regulation of the phosphorylating capability of this pharmaceutically important kinase.

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