Available online at **www.joac.info**

ISSN: 2278-1862

Journal of Applicable Chemistry

2020, 9 (1): 102-115

(International Peer Reviewed Journal)

3D QSAR Pharmacophore based Virtual Screening and Molecular Docking for Identification of Potential IGF1R Inhibitors for Cancer Treatment

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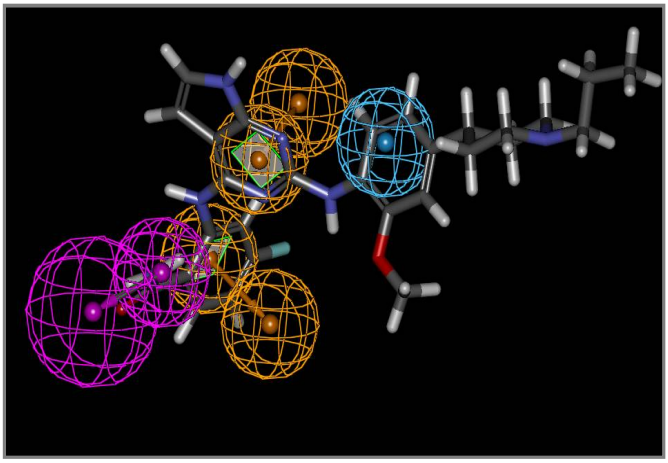
Accepted on 28th December, 2019

ABSTRACT

3D pharmacophore models were developed using chemical features for IGF1R based on the known inhibitors using Discovery Studio 2.0 and validated using external test set. The best pharmacophore model, Hypo1, includes hydrogen bond donor, hydrophobic and ring aromatic features, which has the highest correlation coefficient (0.90), cost difference (77.80), low RMS (1.55), as well as it shows a high goodness of fit and enrichment factor. Hypo1 was used as a 3D query for virtual screening to retrieve potential inhibitors from GOSTAR and ZINC databases. The hit compounds were subsequently subjected to molecular docking studies and finally, 44 compounds were obtained based on consensus scoring function for biological evaluation.

Graphical Abstract



Best pharmacophore model Hypo1 aligned to training set active Compound 1-1.

Keywords: IGF1R, Pharmacophore, HYPOGEN, Molecular docking, Ligand Fit, Cancer.

INTRODUCTION

IGF- 1R is a heterotetramer composed of two extracellular alpha-subunits that contain the ligand-binding domain and two beta-subunits that contain the cytoplasmic kinase domain. Binding of the ligands IGF- 1 and IGF-2 to the extra cellular domain of the receptor leads to autophosphorylation of the cytoplasmic beta-subunit and activation of the intrinsic kinase activity of the receptor. Activation results in the phosphorylation of insulin receptor substrates (IRS-1- 4) and Src-homology containing adapter protein (Shc). These in turn activate the PI-3K/Akt/m TOR survival pathway and the mitogenic RAS/Raf/MAPK pathway respectively [**1, 2**]. IGF-1R signaling has pleiotropic effects ranging from cell proliferation, differentiation, and migration to regulation of the apoptotic machinery. The crosstalk observed between epidermal growth factor receptor (EGFR) and IGF-1R signaling suggests wide potential for using IGF-1R inhibitors in combination therapy with other targeted agents, cytotoxic, and radiation therapy. IGF-1R activation has also been implicated in the development of resistance toward trastuzumab treatment in breast cancer [**3**] and lung cancer [**4**]. In the current study, we have generated series of 3D pharmacophore models based on the known IGF1R inhibitors, training set and validated using known test set compounds. Pharmacophore models were used as 3D queries for searching large databases to identify novel IGF1R inhibitors and were also used as predictive tool for estimating biological activity of IGF1R inhibitors through virtual screening or molecular designing on the basis of structure-activity analysis.

MATERIALS AND METHODS

Pharmacophore: Pharmacophore modelling is one of the most potent and rapid method to discover a novel scaffold and it can be generated either based on ligands or on the active site of proteins. Two different methods were applied for the ligand based pharmacophore model: HipHop and HYPOGEN. HipHop also known as common features hypothesis, which is generated based on the common features present in the training set molecules. HYPOGEN uses the activity values of the small compounds in the training set to generate the hypothesis. In this study, HYPOGEN algorithm [**5**] was applied to build the 3D QSAR pharmacophore models using DS V2.0 software (Accelrys Inc., San Diego, CA, USA).

Test and training set preparation: For generating hypotheses, training set molecules have to satisfy certain set of laws like it must be broadly colonized (minimum 18 compounds) by structurally diverse representatives and wrap an activity range of at least four order magnitude. All the biologically relevant data must be obtained by homogeneous processes along with the most active compounds have to be necessarily be integrated in training set. For this study, 18 diverse compounds with activity value (IC50) between 0.0003 µM and 489 µM were selected as training set from various literatures, which span over four orders of magnitude, was used to engender the hypotheses which span over four orders of magnitude. To validate the hypothesis the test set was prepared using the same protocol as training set. Test set contains 34 structurally different compounds from the training set with wide range of activity values. MDL-ISIS Draw 2.5 was used to sketch the two-dimensional (2D) chemical structures of all compounds which were converted into 3D structures using DS. Maximum numbers of 250 conformations were generated for each compound using the Best Conformation model generation method based on CHARMm force field and Poling algorithm to assure the energy-minimized conformation. The conformations with energy higher than 20 kcal mol^{-1} from the global minimum were rejected. Compounds with their conformational models were then submitted to DS for generating hypothesis.

Pharmacophore generation using HYPOGEN: An automated 3D QSAR pharmacophore was created by comparing the activity values of compounds in the training set, which could be used for quantitative assessment of activities while screening large databases [**6**]. Selecting the chemical feature is one of the most important steps in generating pharmacophore. Feature mapping module from DS was used to select the chemical features for hypothesis generation. While generating

hypotheses hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), hydrophobic aromatic and ring aromatic (RA) features were selected based on the training set compounds with the minimum of 0 to maximum of 5 features were selected. The 'Uncertainty' values for the 18 compounds in the training set were set to 2, and the default values for other parameters were kept constant. Subsequently, pharmacophore models were computed using 3D QSAR Pharmacophore module and the top 10 scoring hypotheses were collected. The qualities of the hypotheses were dependent on the fixed cost, null cost, and total cost values [7]. Assessment of pharmacophore quality and database screening Evaluation of the quality of the pharmacophore using test set showed a correlation value of 0.92 between the experimental and predicted activities. All queries were performed using the Ligand Pharmacophore Mapping protocol. In order to further validate the Hypo1, GF and EF were calculated [8] using the formula ((Ha/4HtA)(3A+Ht)) x (1-((Ht-Ha)/(D-A))) and (Ha/Ht)/(A/D)b respectively. Finally, Hypo1 was selected as the best hypothesis and used as a 3D query to retrieve a novel scaffold for IGF1R inhibitors from various databases like GOSTAR and ZINC.

Structure based molecular docking: Molecular docking and pharmacophore model are the two potent methods in drug discovery process. Virtual screening followed by docking has become one of the reputable methods for drug discovery and enhancing the efficiency in lead optimization. The main advantage of pharmacophore based docking was to focus on specific key interaction for protein-ligand binding. Ameliorate the selection of active compounds it is optimal to use both methods like molecular docking and pharmacophore [9–15].

Molecular docking was executed for accurate docking of ligands into protein active sites using Ligand Fit module in DS. There are three stages in Ligand Fit protocol: (i) Docking: attempt is made to dock a ligand into a user defined binding site (ii) In-Situ Ligand Minimization and (ii) Scoring: various scoring functions were calculated for each pose of the ligands. The protein complexes were selected from protein databank (PDB, www.rcsb.org). Till date, there are many IGF1R complexes were reported, among them PDB ID: 3O23 was selected based on the resolution of the complex and the size of the co-crystal. For docking study, initially protein was prepared by removing all water compounds and CHARMm force field was applied using Receptor-Ligand Interactions tool in DS. After the protein preparation, the active site of the protein has to be identified. The active site of the protein was represented as binding site; it's a set of points on a grid that lie in a cavity. Two methods were applied to define the binding site for a protein: (i) Firstly, binding sites were identified based on the shape of the receptor using ''eraser'' algorithm and (ii) secondly, volume occupied by the known ligand pose already in an active site. In this study, binding site was defined using second method and the critical amino acids were identified by analyzing the protein–ligand interactions from IGF1R co-crystal structures which were deposited in PDB. During the docking process top 10 conformations was generated for each ligand based on dock score value after the energy minimization using the smart minimizer method, which begins with steepest descent method and followed by the conjugate gradient method. Ligand binding affinity was computed based on the dock score and other scoring functions like LigScore1, LigScore2, Jain, Potential of Mean Force (PMF), Piecewise Liner Potential (PLP) ligand.

RESULTS AND DISCUSSION

HYPOGEN model for IGF1R inhibitors: A training set of 18 compounds with diverse scaffold were collected from various literature and used in this study. Structures of the training set compounds were shown in figure 1. 3D QSAR Pharmacophore Generation module/Discovery Studio (DS) was used to construct pharmacophore model using hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), hydrophobic aromatic and ring aromatic (RA) chemical features. It produced ten top-scored hypotheses based on the activity values of the training set molecules. Best Hypo1 establishes the highest cost difference (77.80), best correlation coefficient (0.90), maximum fit value (10.81) and lowest root mean square (RMS) of 1.5. The fixed and the null cost values are 72.1806 and 174.255, respectively. Fixed total cost was dependent on summation of the cost components: weight cost, error

cost and configuration cost. Two key values were used for cost analysis: one is the difference between the fixed and null costs and another one is the difference between null and total cost (cost difference). The fixed cost represents a cost of the theoretical ideal hypothesis, which could absolutely predict the activity of compounds in the training set with lowest deviation, while null cost represented the cost of hypothesis with no features that estimates every activity to be the average activity. The difference between these two costs should be 70 bits to show the over 90% statistical significance of the model. The cost difference should be greater than 60 bits to represent a true correlation data.

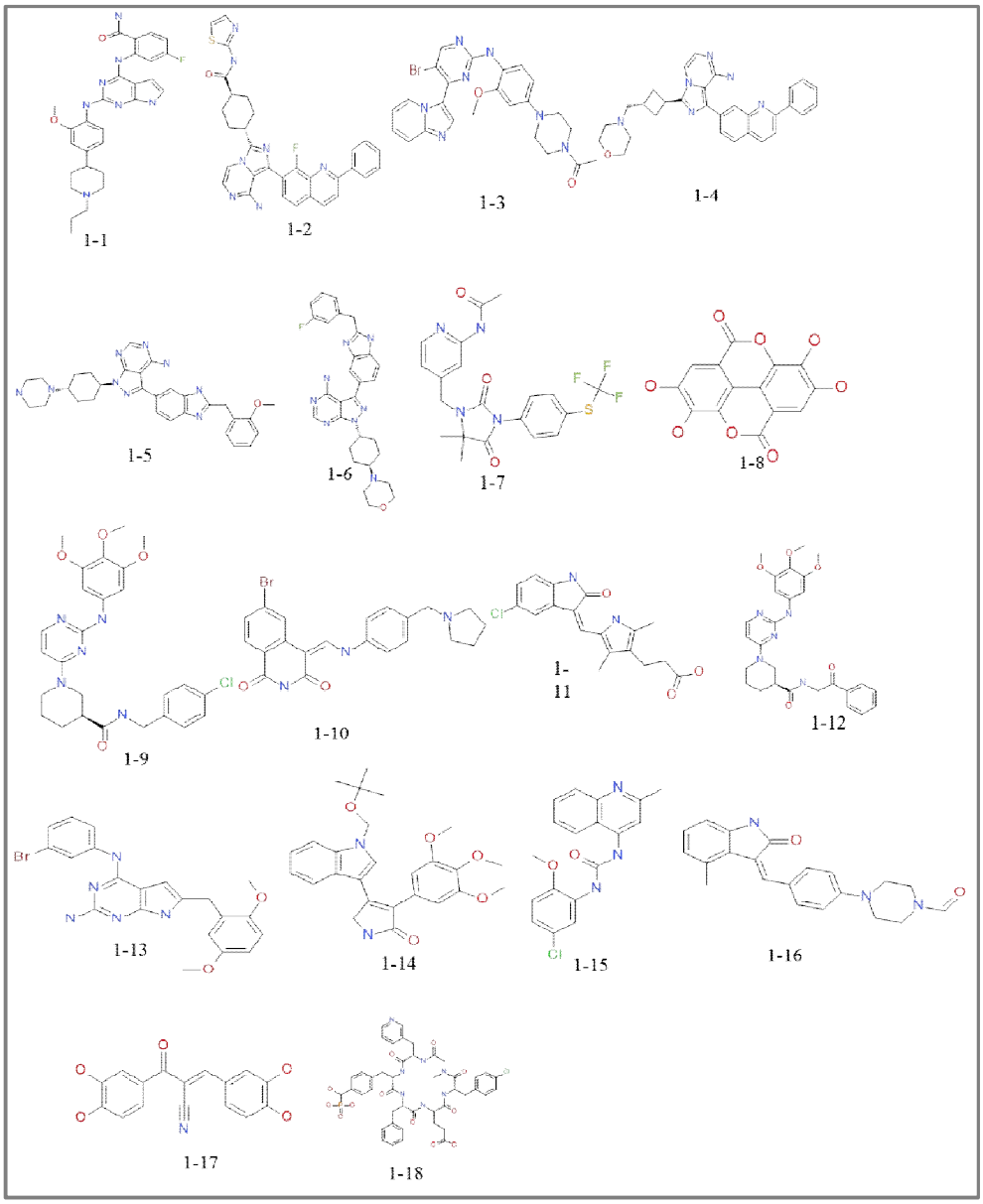


Figure 1. Chemically diverse 18 compounds used as training set in 3D QSAR Discovery Studio/Pharmacophore generation.

The cost difference between null and fixed cost was found to be 77.80 and it was more than 70 bits. All hypotheses had a correlation coefficient of higher than 0.80, but Hypo1 showed the highest correlation coefficient values of 0.90, demonstrating good prediction ability of Hypo1. Higher cost difference and correlation value with low RMS and error values have been observed for Hypo1 when compared with other hypotheses as in (Table 1). Hence, Hypo1 was selected as a best hypothesis and

employed for further analyses and the most active and inactive compounds in the training set were aligned in Hypo1 was shown in figure 2, 3 and 4.

Table 1. Results of top 10 hypotheses as a result of automated 3D QSAR pharmacophore generation

Hypo no.	Total cost	Cost difference	RMSD	Correlation Coefficient	Max fit
Hypo1	96.45	77.804	1.55	0.901849	10.813
Hypo2	97.79	76.4672	1.6	0.894331	10.721
Hypo3	110.9	63.402	2	0.828048	10.731
Hypo4	118.3	55.942	2.25	0.77564	9.4718
Hypo5	118.4	55.862	2.23	0.78089	10.168
Hypo6	119.9	54.313	2.24	0.777569	10.649
Hypo7	121.7	52.516	2.32	0.757503	9.679
Hypo8	122.4	51.897	2.33	0.755567	9.8787
Hypo9	123	51.213	2.2	0.79727	9.4865
Hypo10	123.2	51.022	2.27	0.775756	8.7828

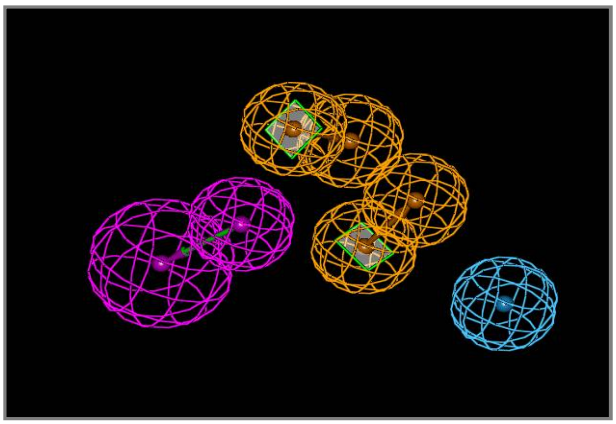


Figure 2. HYPOGEN pharmacophore model.

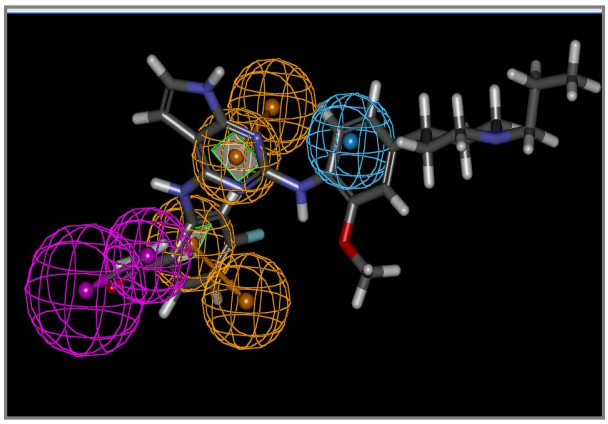


Figure 3. Best pharmacophore model Hypo1 aligned to training set active Compound 1-1.

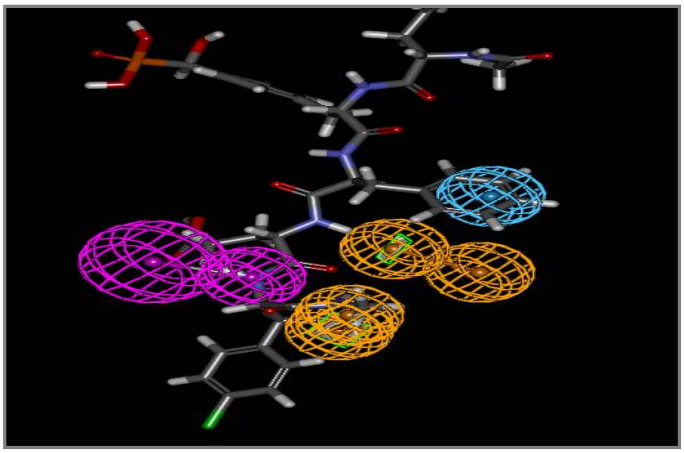


Figure 4. Best pharmacophore model Hypo1 aligned to training set inactive Compound 1-18.

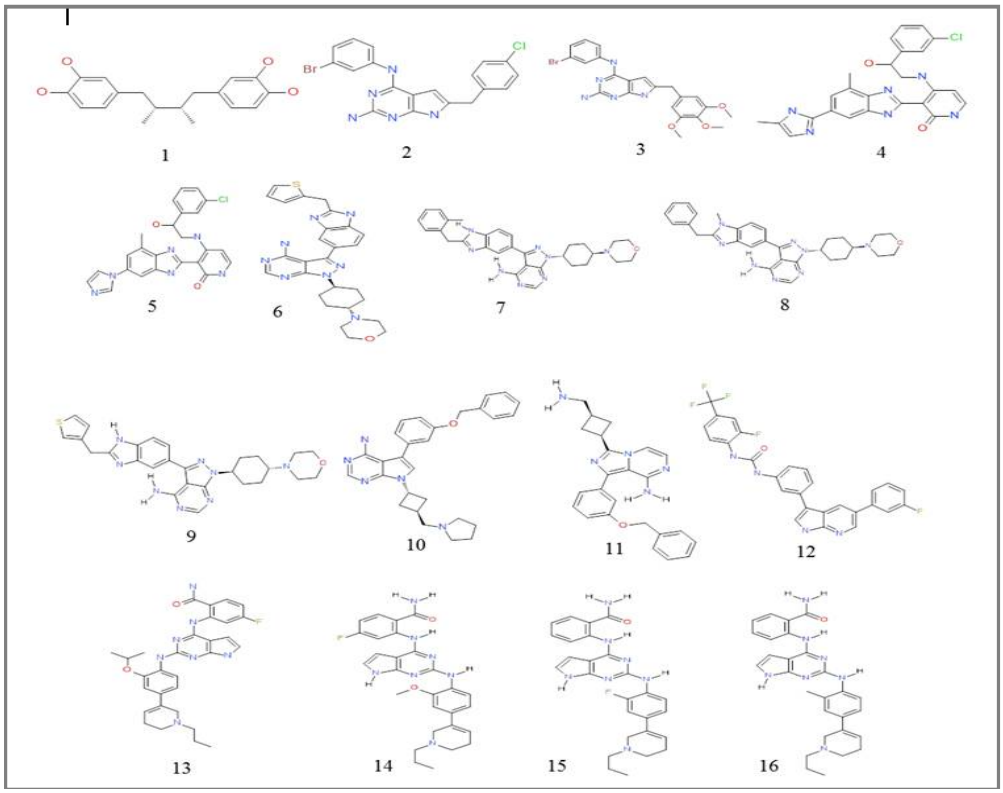
Training set compounds were classified relatively into three sets based on their activity values: highly active IC50 < 0.3 µM =+++; moderately active 0.3 <= IC50 < 3 µM = ++ and inactive IC50 < 500 µM = +. The experimental and estimated activities by Hypo1 for 18 training set compounds are shown in table 2.

Table 2. Actual and predicted activity of the training set molecules based on pharmacophore model

Compound	Fit value	Exp.IC50 µM	Predicted IC50 µM	Exp.IC50 nM	Predicted IC50 nM	Experimental scale	Predicted scale
1-1	9.71211	0.0003	0.000212	0.3	0.212318	(+++)	(+++)
1-2	8.15366	0.002	0.007681	2	7.68142	(+++)	(+++)
1-3	7.79072	0.003	0.017717	3	17.7166	(+++)	(+++)
1-4	7.79847	0.017	0.017403	17	17.4034	(+++)	(+++)
1-5	7.75566	0.026	0.019206	26	19.2063	(+++)	(+++)
1-6	7.6422	0.058	0.024941	58	24.9405	(+++)	(+++)
1-7	6.53012	0.098	0.322832	98	322.832	(+++)	(++)
1-8	5.37831	0.25	4.57922	250	4579.22	(+++)	(+)
1-9	5.38351	1.87	4.52475	1870	4524.75	(++)	(+)
1-10	5.29531	2.42	5.54355	2420	5543.55	(++)	(+)
1-11	4.7371	2.7	20.0446	2700	20044.6	(++)	(+)
1-12	5.35738	4.1	4.80529	4100	4805.29	(+)	(+)
1-13	5.54499	5.1	3.11969	5100	3119.69	(+)	(+)
1-14	5.36254	7.7	4.74859	7700	4748.59	(+)	(+)
1-15	4.84587	12	15.6039	12000	15603.9	(+)	(+)
1-16	5.13079	67.6	8.09673	67600	8096.73	(+)	(+)
1-17	4.95646	114	12.096	114000	12096	(+)	(+)
1-18	5.32488	489	5.17869	489000	5178.69	(+)	(+)

Validation of hypo1: Validating the hypothesis is one of the significant methods in pharmacophore generation. There are several methods to confirm the quality of pharmacophore like preparing test set, etc. The generated hypotheses were mainly validated to check whether the best hypothesis selected the active compounds during the screening process such as the percentage of active compounds picked from dataset, correlation between the predicted and estimated values of test set along with its efficiency in reducing true negatives and false positives.

Test set: Test set was prepared using the same protocol as training set prepared and used to determine whether the hypothesis was able to predict the active compounds (Figure 5) other than the training



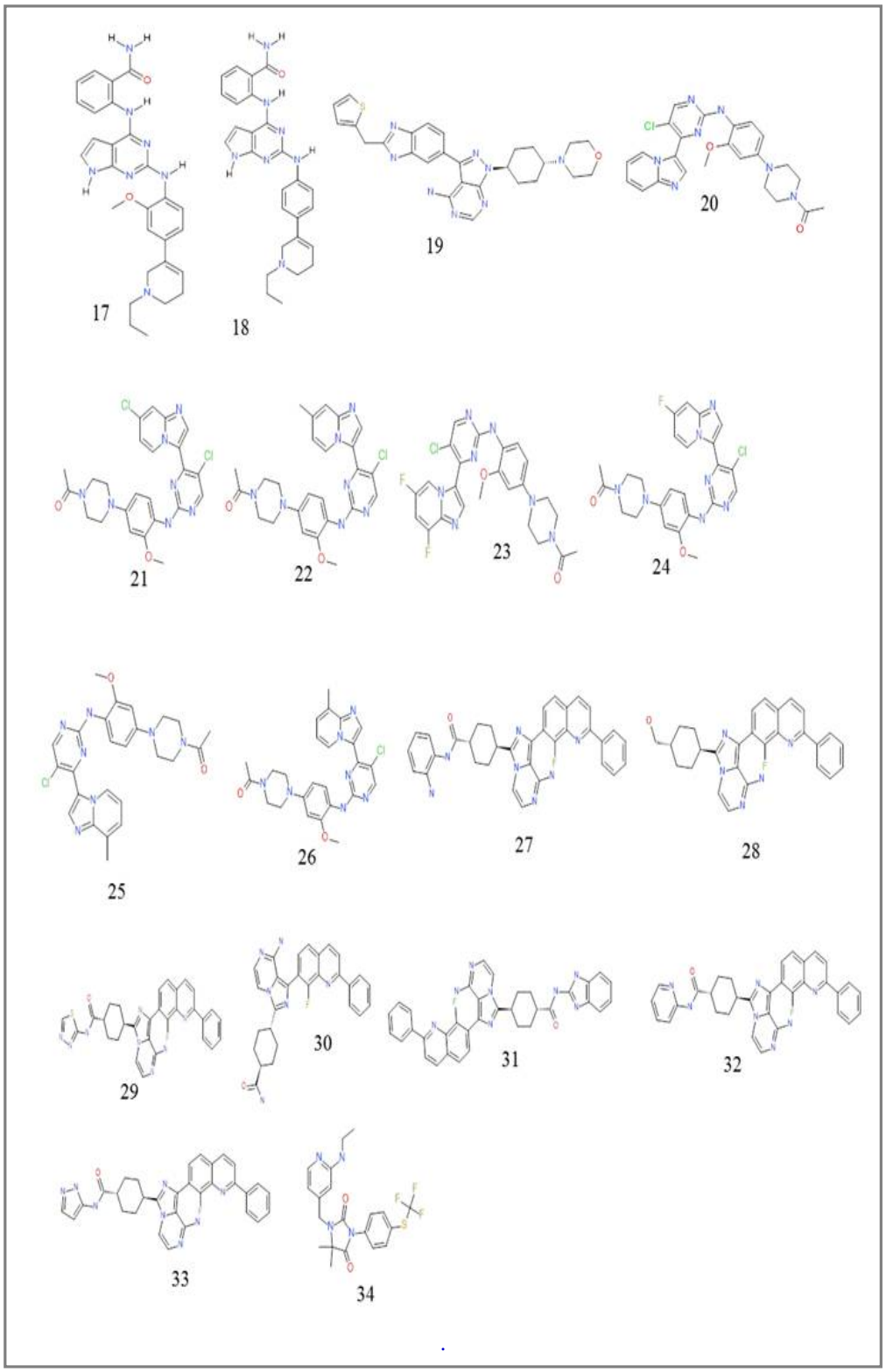


Figure 5. Chemically diverse 34 compounds used as test set in 3D QSAR Discovery Studio.

set molecules. The correlation coefficient (r) for the test set given by Hypo1 was 0.94. The experimental and predicted activities of Hypo1 as applied to test set are shown in table 3. This result was used for further legalization of Hypo1 and we also suggest that the Hypo1 not only fit for training set compounds but also for the external compounds.

Table 3. Experimental and predicted IC50 data values of 34 test set molecules against IGF1R

Compound No.	Fit value	Exp.IC50 µM	Predicted IC50 µM	Exp.IC50 nM	Predicted IC50 nM	Experimental scale	Predicted scale
1	5.03396	24.6	10.1191	24600	10119.1	(+)	(+)
2	5.23554	10.1	6.36148	10100	6361.48	(+)	(+)
3	5.24009	12.6	6.29519	12600	6295.19	(+)	(+)
4	5.87349	0.14	1.46422	140	1464.22	(+++)	(+)
5	6.62296	0.18	0.2607	180	260.697	(+++)	(+++)
6	7.6134	0.023	0.02665	23	26.6501	(+++)	(+++)
7	7.3568	0.038	0.04812	38	48.1172	(+++)	(+++)
8	7.34134	0.035	0.04986	35	49.8614	(+++)	(+++)
9	7.50796	0.032	0.03397	32	33.9733	(+++)	(+++)
10	7.04922	0.078	0.0977	78	97.6962	(+++)	(+++)
11	7.24895	0.06	0.06168	60	61.6815	(+++)	(+++)
12	6.25662	0.605	0.60601	605	606.009	(++)	(++)
13	7.73461	0.0032	0.02016	3.2	20.1599	(+++)	(+++)
14	7.73855	0.0008	0.01998	0.8	19.9778	(+++)	(+++)
15	7.70506	0.0016	0.02158	1.6	21.5794	(+++)	(+++)
16	8.31776	0.005	0.00526	5	5.26427	(+++)	(+++)
17	8.10311	0.0006	0.00863	0.6	8.62959	(+++)	(+++)
18	8.27368	0.0016	0.00583	1.6	5.82673	(+++)	(+++)
19	7.56501	0.025	0.02979	25	29.7915	(+++)	(+++)
20	7.67501	0.01	0.02313	10	23.1256	(+++)	(+++)
21	7.69875	0.01	0.0219	10	21.8954	(+++)	(+++)
22	7.70531	0.009	0.02157	9	21.567	(+++)	(+++)
23	7.51683	0.022	0.03329	22	33.287	(+++)	(+++)
24	7.69901	0.011	0.02188	11	21.8823	(+++)	(+++)
25	7.65393	0.013	0.02428	13	24.2758	(+++)	(+++)
26	7.63104	0.01	0.02559	10	25.5892	(+++)	(+++)
27	7.79926	0.004	0.01737	4	17.3716	(+++)	(+++)
28	7.81335	0.008	0.01682	8	16.8169	(+++)	(+++)
29	7.80899	0.005	0.01699	5	16.9868	(+++)	(+++)
30	8.09006	0.006	0.00889	6	8.89292	(+++)	(+++)
31	7.80796	0.006	0.01703	6	17.027	(+++)	(+++)
32	7.78798	0.005	0.01783	5	17.8289	(+++)	(+++)
33	7.81575	0.005	0.01672	5	16.7242	(+++)	(+++)
34	6.8415	0.154	0.15762	154	157.615	(+++)	(+++)

Databases screening: Virtual screening of small-molecule libraries forms one aspect of a sophisticated approach to drug discovery [16]. Hypo1 was used to screen the various databases like GVK and ZINC database, which consists of million compounds. In drug discovery process virtual screening of databases is an effective alternative to high throughput screening (HTS). Totally, 30000 compounds satisfied all the critical features in Hypo1 and 2000 compounds were considered for further analyses based on the maximum fit value. Drug-likeness properties are an important indicator for selecting the compounds for in vitro studies, which includes molecular or physicochemical properties that contribute to favorable Lipinski's rule of five. So, we further sorted these 2000 compounds using the Lipinski's rule of five and finally 1000 compounds were further considered for docking studies. To evaluate the fit of the pharmacophore to the binding site of the crystal structure of IGF1R (PDB ID:3O23), the pharmacophore model Hypo1 was compared with the bound conformation of the compound IGF1R ligand ((5S)-5-methyl-1-(quinolin-4-ylmethyl)-3-{4-[(trifluoromethyl)sulfonyl]phenyl}imidazolidine-2,4-dione). complexed with IGF1R. We compared the pharmacophore model with the active site of IGF1R crystal structure and it clearly shows a good agreement with the target based pharmacophore.

Molecular docking studies of IGF1R: Molecular docking is a computational technique that samples conformations of small compounds in protein binding sites; scoring functions are used to assess which of these conformations were best complements to the protein binding site. There are two main aspects to assess the quality of docking methods: (i) docking accuracy, which recognizes the true binding

mode of the ligands to the target protein and (ii) screening enrichment which measures the relative improvement in the identification of true binding ligands using a docking method versus random screening [**17**]. Training set of 18 compounds as well as 1000 hit compounds retrieved from the databases which satisfied drug like properties were docked in the active site of IGF1R using Ligand Fit (Figure 6). Totally, 60 compounds show showed the hydrogen bond interactions with Lys 1033A, Met1082A and Asp1153A.

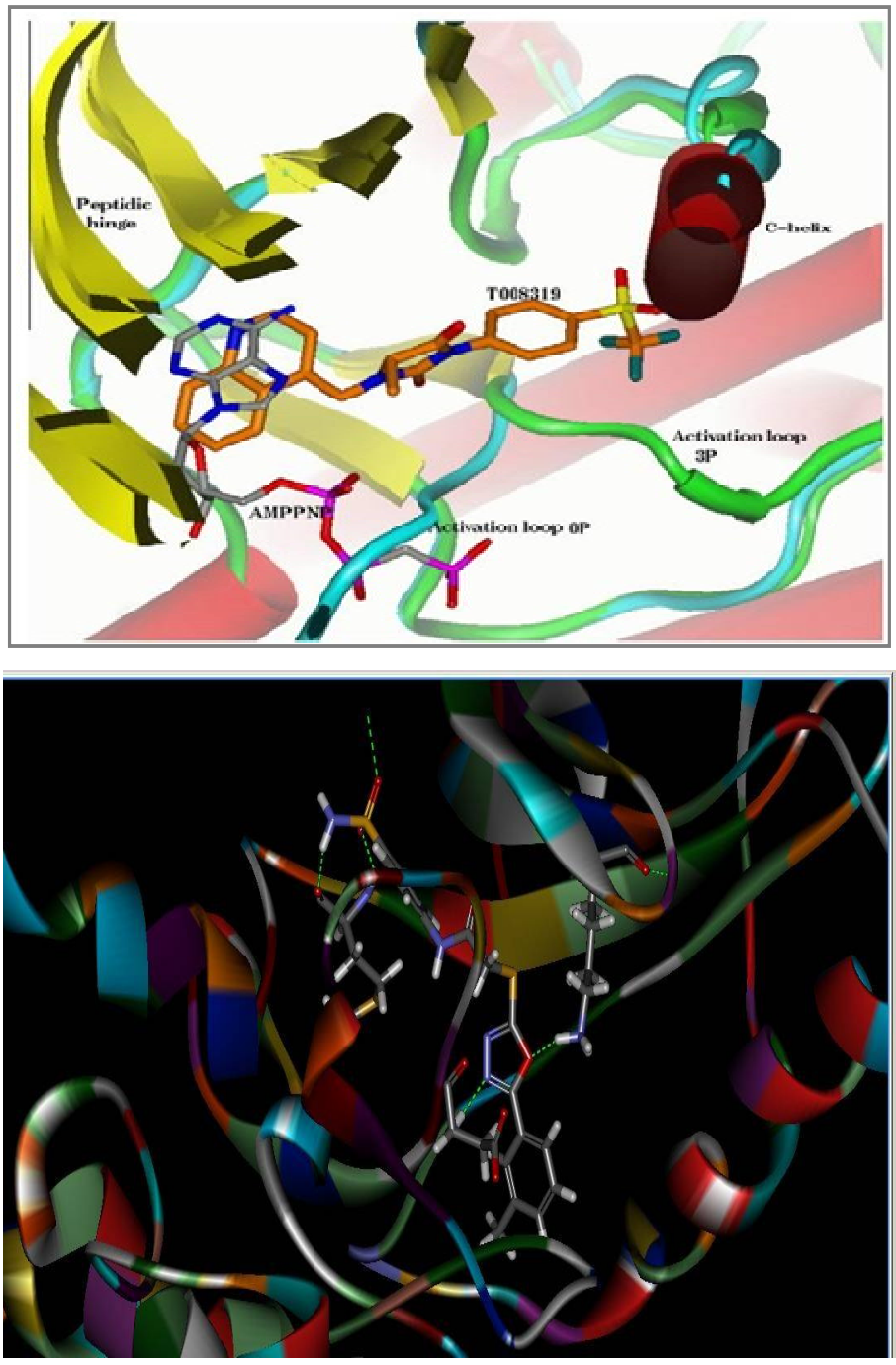
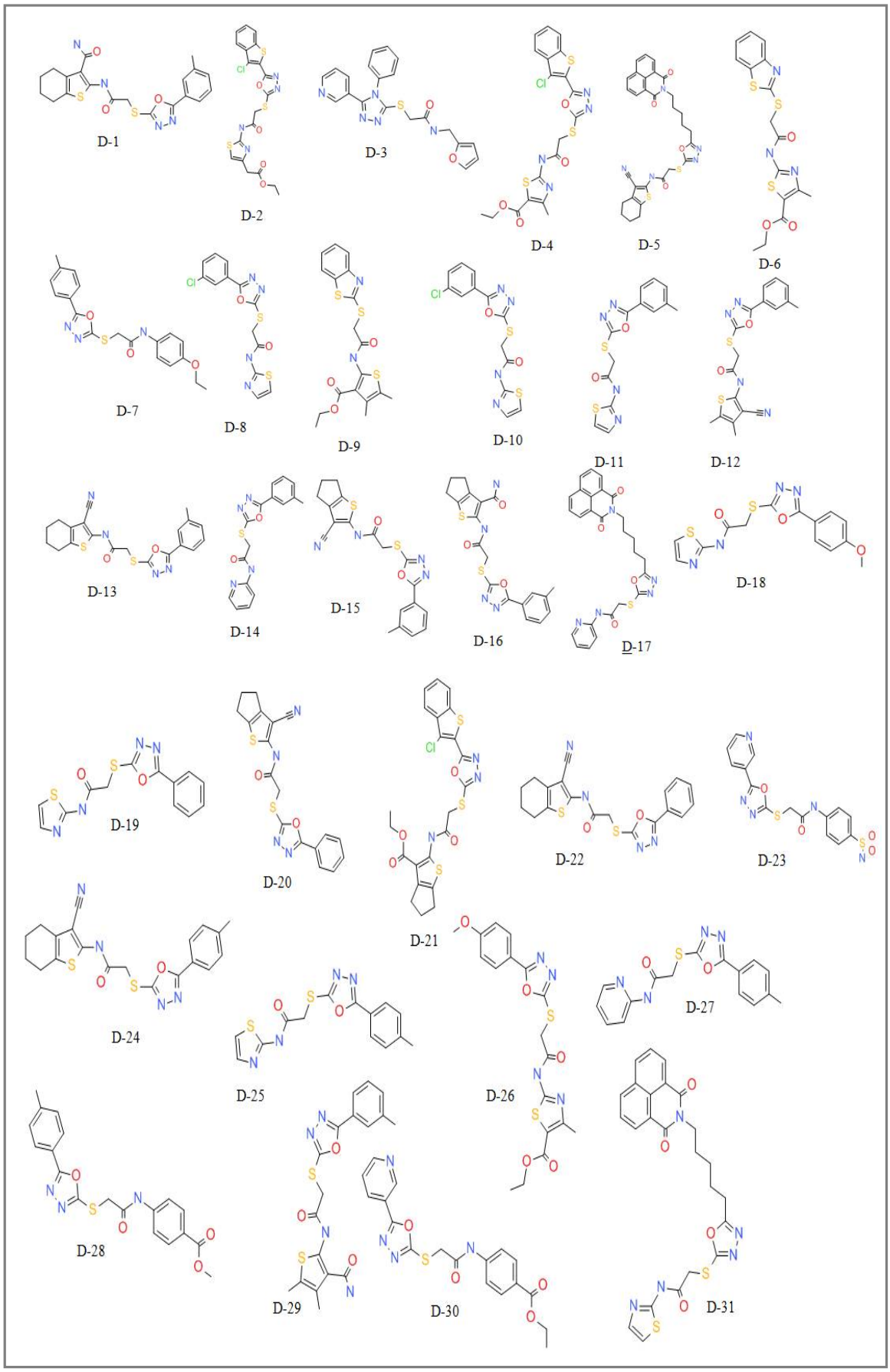


Figure 6. Comparison of D-44 and 3O23 ligand in active site of IGF1R (PDB ID: 3O23).

The training set compounds showed the dock score greater than 80 and the maximum fit value. All of the hit compounds possessed the good fit value and the dock score. Based on the consensus scoring function finally 44 compounds were sorted for further vitro studies (Figure 7).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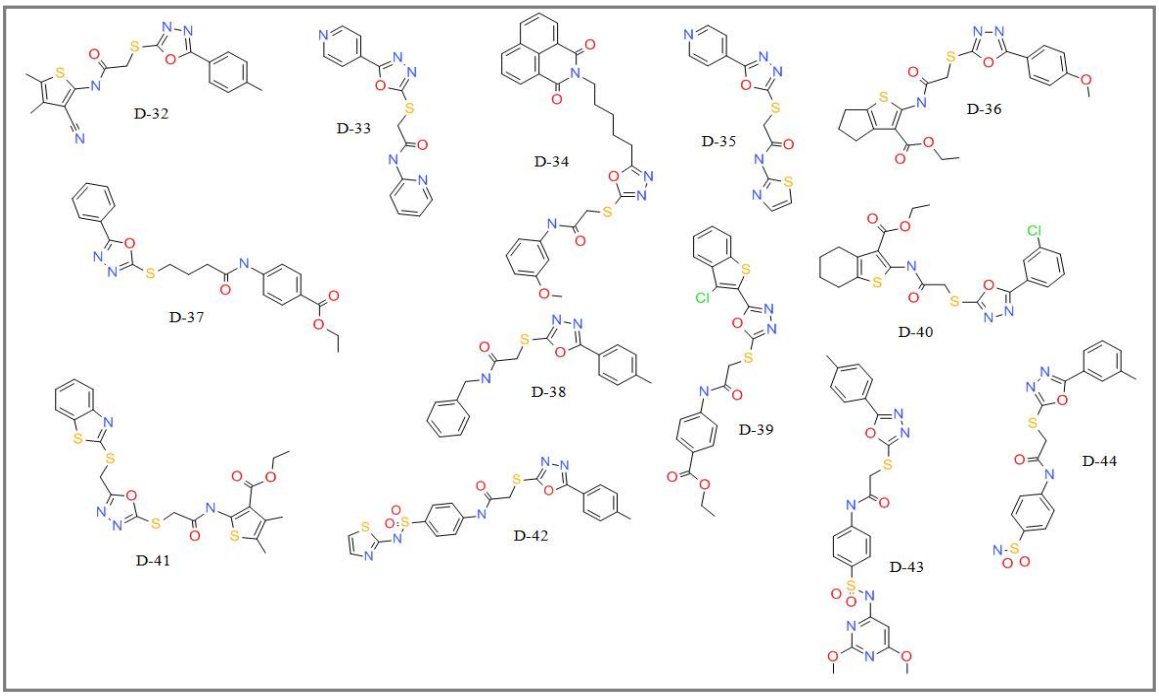

Figure 7. Compounds retrieved based on pharmacophore and docking.

All the ligands in the complex structures showed the hydrogen bond interactions with Lys 1033A, Met1082A and Asp1153A. This clearly indicates that these hydrogen bonded amino acids play a crucial role in IGF1R inhibitions and the result was shown in table 4 [**18-31**].

Table 4. Analyses of critical amino acids for IGF1R inhibition 16 crystal structures deposited in PDB

S.No.	PDB-1D	Resolution A	Ligand	Hydrogen bond integrations
1	3LW0	1.79	CCX	Vol1063(A)
2	3O23	2.10	MQY	Lys 1033, Asp 1153, Met 1082
3	3NW5	2.14	LGX	Glu 1050, Met 1052
4	3NW6	2.2	LGW	Glu 1050, Met 1052
5	3NW7	2.11	LGV	Asp 1123, Met 1052
6	3I81	2.08	EBI	Asp 1123, Met 1052
7	3D94	2.3	D94	Glu 1050, Lys 1003 Met 1052
8	2OJ9	2	BMI	Glu 1050, Met 1052
9	1JQH	2.1	ANP	MG 304, Glu 1080, Lys 1033, Met 1082, Ser1009
10	1K3A	2.1	ACP	Glu 1050, Met 1052, Ser 979
11	3QQU	2.9	01P	Asp 1150, Met 1079
12	3ETA	2.6	315	Asp 1150, Glu 1047, Glu 1077, Met 1079
13	3F5P	2.9	741	Lys 1033, Met 1082
14	2ZM3	2.5	575	Glu 1080, Met 1082
15	1IGR	2.6	FUC	Asn 105, Asp 132, His 223
16	3LVP	3	PDR, EPE	Met 1082

APPLICATION

The purpose of this study was not only to construct the pharmacophore model to predict the estimated activity of the compounds, but also to employ the hypothesis on virtual screening to search novel scaffolds.

CONCLUSION

In this work, it have built 3D pharmacophore models from 18 IGF1R inhibitors and the best quantitative pharmacophore model, Hypo1, consisted of five features like two HBA and three H. The

features were perfectly complementary to the IGF1R active site and directed to relative protein residues, showing that Hypo1 represents the characteristic of IGF1R active site. For predicting activity, the correlation coefficient of Hypo1 with training and test sets were 0.93 and 0.91 respectively, suggesting a good predictive power of the hypothesis for the majority of IGF1R inhibitors. Hypo1 was used as a 3D query for screening large databases like GOSTAR and ZINC. Finally, 44 compounds were selected as potent IGF1R inhibitors based on the consensus scoring function. From the overall analyses, we conclude that the Hypo1 pharmacophore truly reflects the features of IGF1R inhibitors and this pharmacophore could be used as fast and accurate tool to assist discovery of novel IGF1R inhibitors.

ACKNOWLEDGEMENT

Thanks to Jawaharlal Nehru Technology University, Hyderabad for their support, thanks to GITAM Deemed to be University for their continuous help in process development, thanks to GVKBIO for Analytical support.

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