Comparative Virtual and Experimental High-Throughput Screening for Glycogen Synthase Kinase-3β Inhibitors

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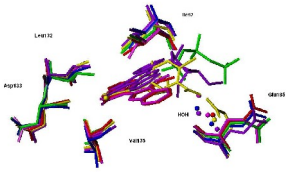
ABSTRACT

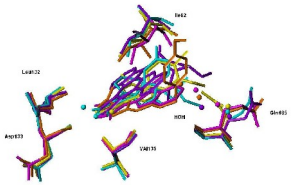
Glycogen synthase kinase-3β (GSK-3β) is a serine/threonine kinase that has recently emerged as a key target for neurodegenerative diseases and diabetes. As an initial step of our lead discovery program we developed a virtual screen to discriminate known GSK-3β inhibitors and inactive compounds using FlexX, FlexX-Pharm and FlexE. The maximal enrichment factor (EF=28) suggests that our protocol identifies potential GSK-3β inhibitors effectively from large compound collections. The effectiveness of our screening protocol was further investigated by a comparative experimental and virtual high-throughput screens performed for the same subset of our corporate library. Enrichment factors, the significantly higher hit rate of virtual screening (12.9%) than that of the HTS (0.55%) and also the comparison of active clusters suggest that our virtual screening protocol is an effective tool in GSK-3β-based library focusing. Head-to-head comparison of true/false positives and negatives, revealed the two approaches to be complementary rather than competitive.

DEVELOPING A VIRTUAL SCREENING PROTOCOL

Comparison of active sites of the publicly available X-ray structures



Active sites of six X-ray structures: 1Q3W: blue, 1PYX: green, 1UV5: magenta, 1Q41: red, 1Q3D: violet, 1Q4L: yellow. Only those residues are visible that are given in the pharmacophore constraints. Conserved water molecules are also signed (HOH).



Active sites of the X-ray structures used for virtual screening and the two recently released X-ray structures. 1Q5K: orange, 1R0E: cyan.

Enrichment study

Structure	FlexX	FlexX-Pharm	FP-Torsion
1Q3D	14	19	15
1UV5	14	**28**	25
1Q4L	18	21	18
Ensemble	14	-	-

$$EF(\%) = \frac{\left(N_{active(\%)} / N_{(\%)} \right)}{\left(N_{active} / N_{all} \right)}$$

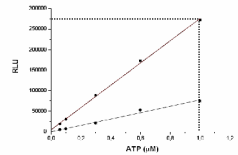
Best enrichment factors (EF) at 1% of ranked database for GSK-3β structures. FP-torsion: FlexX-Pharm with torsion energy constraints

DEVELOPING A HIGH-THROUGHPUT SCREENING PROTOCOL

The Kinase-Glo™ Luminescent Kinase Assay developed by Koresawa and Okabe is a homogeneous, high-throughput screening method of measuring kinase activity by quantifying the amount of ATP remaining in the solution following a kinase reaction. This assay can be performed with any kinase and substrate combination and does not require radiolabelled components. The Kinase-Glo™ assay was adapted and optimized for screening against GSK-3b. In order to get the best performance in selecting between active and inactive compounds the optimization of kinase reaction conditions was performed regarding both of the Promega's protocol and the results of Koresawa et al.

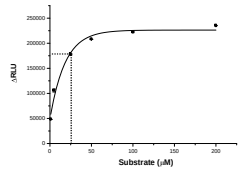
Determining optimal ATP concentration

ATP-luminescence standard curve. Concentrations of ATP: 0.06; 0.1; 0.3; 0.6; 1 μM, in the excess of substrate, 20 ng GSK-3β in final volume of 40 μl (30°C and 30 minutes). Control samples were measured in the same reaction mixture and under the same reaction conditions containing no GSK-3β. Measurements in the presence ● and in the abscence ■ GSK-3β.



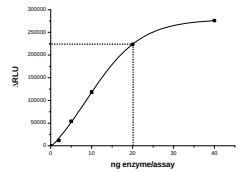
Determining optimal substrate concentration

Determining the optimal substrate concentration. Substrate concentrations: 1; 5; 25; 50; 100; 200 μM. The blank samples contained the same amount of substrate and ATP without GSK-3β. DRLU=|RLU$_{enzyme}$-RLU$_{blank}$



Determining optimal GSK-3β concentration

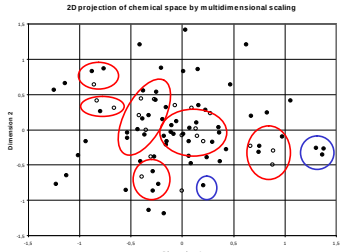
The optimal GSK-3β concentration was determined in the presence of 1 μM ATP and 25 μM substrate. The enzyme concentration was 2; 5; 10; 20; 40 ng. The blank values contain the same amount of ATP and substrate without GSK-3b. DRLU=|RLU$_{enzyme}$-RLU$_{blank}$|



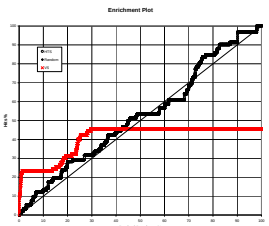
VIRTUAL AND HIGHT-THROUGHPUT SCREENING OF THE CORPORATE SUBLIBRARY (16299 COMPOUNDS)

RESULTS: CLUSTERS FOUND BY VS AND HTS

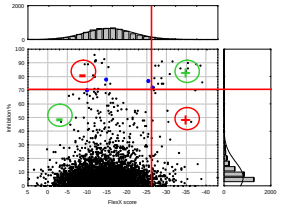
Chemical class	HTS	VS	VS+SS
Cluster 1	8 (83)	2 (81)	34 (19)
Cluster 2	6 (83)	2 (90)	4 (87)
Cluster 3	6 (75)	2 (83)	2 (83)
Cluster 4	3 (90)	1 (90)	2 (93)
Cluster 5	4 (90)	0	0
Cluster 6	4 (100)	0	0



2D projection of chemical scpece by multidimensional scaling based on Tanimoto distances calculated for hits obtained by virtual and experimental screening. Open circle: false negative molecules 69; black dot: true positive (21) compounds.



Enrichment plot of VS and HTS data. x – enrichment plot of virtual screening; the ratio of the hits found by the virtual screen vs. the ratio of the ranked database. The linear thick line indicates the random distribution of active molecules. O - enrichment plot of high-throughput screening; the ratio of the hits found by the high-throughput screen vs. the ratio of the ranked database, here the database is ranked by time.



Inhibition % vs FlexX score. Blue dots show HTS hits that were not validated. The 1 % of the ranked database is at FlexX score = -26,4. From the 90 validated HTS hits only 41 could be docked using FlexX-Pharm, 49 hits did not fulfill the pharmacophore constraints, therefore these points can not be plotted here.

CONCLUSIONS

The effectiveness of our screening protocol has been partially demonstrated by comparing the results of a virtual screen to those obtained by experimental screening. Virtual screening picked up 4 out of 6 series of compounds identified by HTS but the large number of false positives and the high rate of false negatives indicate significant limitations. Although FlexX-Pharm with the combined FlexX/PMF scoring functions was able to give reasonable enrichment factors in both artificial and real screening situations we showed that the inaccuracy of the docking scores, the application of pharmacophore constraints and the neglected flexibility of the active site might be responsible for these limitations.

On the basis of the success criteria used in the screening literature our virtual screening protocol was successful in producing remarkable enrichment and identifying the majority of active clusters at much lower cost and time relative to HTS. These results suggest that this protocol can be useful for pre-filtering our in-house library and can complement experimental screening when investigating large, commercially available virtual libraries for GSK-3β inhibitors.

We feel that this work – being the first truly comparative study – might be interesting for the broader community of lead discovery teams when planning experimental and virtual screening activities.