Supporting Information

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

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Brief description of the assays used for checking promiscuous inhibitors

Asp protease assay

Inhibition of the β-secretase activity was measured by PanVera's BACE fluorescence resonance energy transfer assay kit as described in the protocol (www.invitrogen.com/content/sfs/panvera/L0724.pdf).

Glu receptor assay

Inhibition of an undisclosed metabotropic Glu receptor activity was measured in a radioligand binding assay using native rat cortical membranes. The principle of the assay is similar to that reported by Takeuchi et al. (*Z. Naturforsch.* **2001**, *57c*, 348-355).

Peptidergic GPCR assay

Inhibition of an undisclosed peptidergic GPCR activity was measured in an intracellular Ca^{2+} assay using CHO cells stably expressing the target. The principle of the assay is similar to that reported by Simpson et al. (*Eur. J. Pharmacol*. **2000**, *392*, 1-9).

Figure S1

The relationship between luminescent signal measured (RLU: relative light units) and the ATP concentration in the reaction buffer. The correlation coefficient (R^2) is 0.9997. Serial dilutions of ATP: 0.003; 0.01; 0.03; 0.1; 0.3; 1; 3 µM. Luminescence was recorded 10 minutes (■), 20 minutes (●) and 100 minutes (▲) after adding the Kinase-Glo™ reagent.

Figure S2

ATP-luminescence standard curve. Concentrations of ATP: 0.06; 0.1; 0.3; 0.6; 1 µM, in the excessof 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 presensce ● and in the abscence ■ GSK-3β.

Figure S3

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. $\Delta RLU=|RLU_{enzyme}-RLU_{blank}|$

Figure S4

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-3β. $\Delta RLU=|RLU_{enzyme}-RLU_{blank}|$

Table S1

Inhibition% of GSK-3β hits (as measured at concentration indicated in parenthesis) in three different assay systems

Table S2

Enrichment factors calculated at 1, 2, 5 and 10% of the ranked database for enrichment studies and virtual screening of the corporate sublibrary

Figure S1

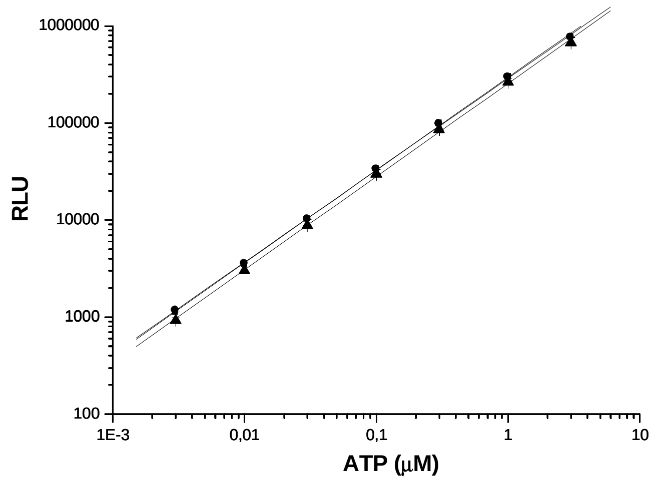

Figure S2

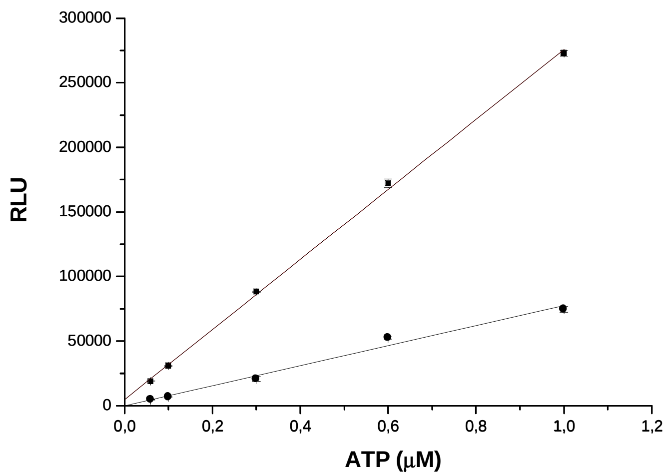

Figure S3

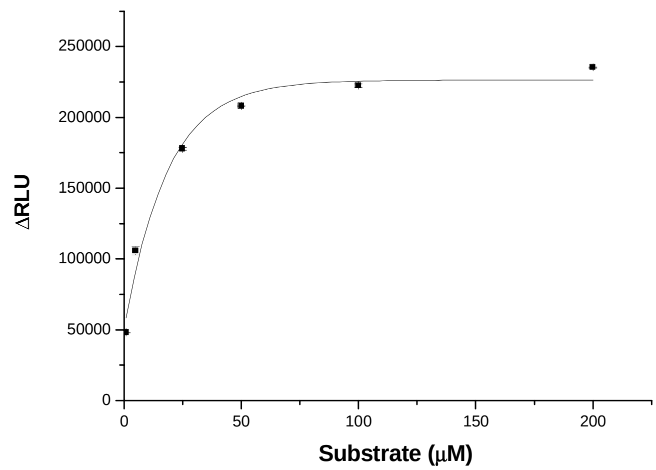

Figure S4

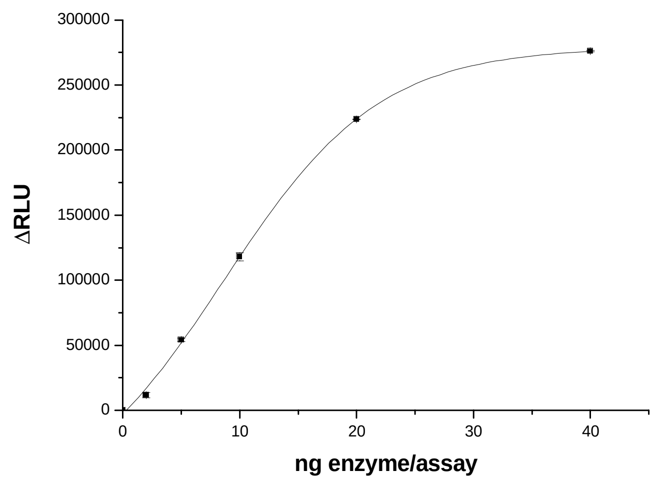

Table S1

	Inhibition %		
	Asp protease (30 μM)	Glu receptor (10 μM)	Peptidergic GPCR (5 μM)
Compound 1	33	< 30	< 30
Compound 2	< 30	56	< 30
Compound 3	< 30	< 30	< 30
Compound 4	< 30	< 30	< 30
Compound 5	< 30	< 30	< 30
Compound 6	< 30	< 30	< 30
Compound 7	< 30	< 30	< 30
Compound 8	< 30	< 30	< 30
Compound 9	< 30	< 30	< 30
Compound 10	< 30	< 30	< 30
Compound 11	< 30	< 30	< 30
Compound 12	< 30	< 30	< 30
Compound 13	< 30	< 30	< 30
Compound 14	< 30	< 30	< 30
Compound 15	< 30	< 30	< 30
Compound 16	< 30	< 30	< 30
Compound 17	< 30	< 30	< 30
Compound 18	< 30	< 30	< 30
Compound 19	< 30	< 30	70
Compound 20	< 30	34	< 30
Compound 21	< 30	< 30	< 30

Compound 22	< 30	47	< 30
Compound 23	< 30	44	< 30
Compound 24	< 30	< 30	< 30
Compound 25	< 30	< 30	< 30
Compound 26	104	44	< 30
Compound 27	< 30	< 30	< 30
Compound 28	< 30	< 30	< 30
Compound 29	< 30	< 30	< 30
Compound 30	< 30	< 30	< 30
Compound 31	< 30	< 30	< 30
Compound 32	< 30	< 30	< 30
Compound 33	< 30	< 30	< 30
Compound 34	< 30	< 30	< 30
Compound 35	57	< 30	< 30
Compound 36	< 30	< 30	< 30
Compound 37	< 30	42	< 30
Compound 38	< 30	< 30	< 30
Compound 39	< 30	< 30	< 30
Compound 40	< 30	42	< 30
Compound 41	< 30	< 30	< 30
Compound 42	< 30	< 30	< 30
Compound 43	< 30	< 30	< 30
Compound 44	< 30	< 30	< 30
Compound 45	< 30	< 30	< 30
Compound 46	< 30	< 30	< 30

Compound 47	< 30	< 30	< 30
Compound 48	32	< 30	< 30
Compound 49	< 30	32	< 30
Compound 50	< 30	33	< 30
Compound 51	< 30	25	< 30
Compound 52	< 30	< 30	< 30
Compound 53	< 30	< 30	< 30
Compound 54	< 30	< 30	< 30
Compound 55	< 30	< 30	< 30
Compound 56	< 30	< 30	< 30
Compound 57	58	< 30	< 30
Compound 58	< 30	60	< 30
Compound 59	< 30	< 30	< 30
Compound 60	< 30	< 30	< 30
Compound 61	< 30	< 30	< 30
Compound 62	N/A	< 30	< 30
Compound 63	N/A	< 30	< 30
Compound 64	< 30	< 30	< 30
Compound 65	< 30	< 30	< 30
Compound 66	< 30	< 30	< 30
Compound 67	< 30	< 30	< 30
Compound 68	< 30	< 30	77
Compound 69	< 30	< 30	76
Compound 70	< 30	< 30	< 30
Compound 71	< 30	< 30	< 30

Compound 72	< 30	< 30	< 30
Compound 73	N/A	< 30	< 30
Compound 74	< 30	< 30	< 30
Compound 75	< 30	< 30	< 30
Compound 76	< 30	< 30	< 30
Compound 77	< 30	< 30	< 30
Compound 78	< 30	< 30	< 30
Compound 79	< 30	< 30	< 30
Compound 80	< 30	< 30	< 30
Compound 81	< 30	< 30	< 30
Compound 82	< 30	< 30	< 30
Compound 83	< 30	< 30	< 30
Compound 84	< 30	< 30	< 30
Compound 85	< 30	< 30	< 30
Compound 86	< 30	< 30	< 30
Compound 87	< 30	< 30	< 30
Compound 88	< 30	< 30	< 30
Compound 89	< 30	< 30	< 30
Compound 90	< 30	< 30	< 30

Table S2

	1UV5-FlexX		1UV5-Phram		1Q4L-FlexX		1Q4L-Pharm	
	PMF/FlexX	FlexX/PMF	PMF/FlexX	FlexX/PMF	PMF/FlexX	FlexX/PMF	PMF/FlexX	FlexX/PMF
1 %	5	5	28	14	9	5	9	21
2 %	11	9	10.5	14	4.5	4.5	5	9.5
5 %	6	1.5	8	7.3	4	2.8	6	8
10 %	4	3	5	5	4.5	2	4.7	6.6

	1Q3D-FlexX		1Q3D-Phram		VS-Pharm
	PMF/FlexX	FlexX/PMF	PMF/FlexX	FlexX/PMF	PMF/FlexX
1 %	5	5	19	14	23
2 %	9	4.5	11.5	10	11
5 %	6	2.5	5	10	5
10 %	4	2.2	3.6	10	3