Structure-based tailoring of compound libraries for high-throughput screening: Discovery of novel EphB4 kinase inhibitors

Supplementary Material

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Keywords: EphB4, tyrosine kinases, angiogenesis, cancer, docking, high-throughput screening

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October 25, 2007

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Compound ranking. It is necessary to discard poses with unlikely binding modes through the application of filters before calculating the binding energies with LIECE.[1] The quotient of the van der Waals energy and the molecular weight (the "van der Waals efficiency") is a very useful filter. Compounds **1-3** were identified in rankings using it as a filter. The three applied filter/ranking combinations were: (i) ranking according to the vdW-efficiency; (ii) vdW-efficiency $\leq -0.116\,\mathrm{kcal{\cdot}g^{-1}}$, then ranking according to ΔG^L (free energy of binding predicted by the LIECE model); (iii) vdW-efficiency $\leq -0.1\,\mathrm{kcal{\cdot}g^{-1}}$, number of rotatable bonds ≤ 5, and van der Waals interaction energy $\leq -35\,\mathrm{kcal{\cdot}mol^{-1}}$, then ranking according to ΔG^L. The cutoffs were obtained from the distributions of the respective values. Compounds **1-3** and the five weak EphB4 inhibitors with different anchors were among the best 300 poses in at least one of these rankings. Compounds **4-8** were not present in the original library of 728202 molecules.

FRET-based enzymatic assay. The EphB4 FRET enzymatic assay (Z'Lyte Tyr kinase assay, Invitrogen, USA) was performed according to the manufacturer's protocol. Briefly, EphB4 kinase, at a concentration of 3.5 ng per reaction well, was incubated for one hour with FRET-peptide substrate in the presence of ATP at a concentration of 30 μM, which is near its K_m. Reactions were developed and stopped and fluorescence was measured at 440 nm and 520 nm emission with excitation at 400 nm. IC_{50} values were calculated with GraphPad Prism 5.0 (GraphPad Software).

FRET-based enzymatic assay at Cerep. The compounds showing the strongest inhibition effects were verified in the FRET-based assay[2] offered by Cerep (France). Briefly, compounds were measured in duplicates at eight different concentrations ranging from 10 nM to 20 μM. The concentrations of EphB4 and ATP in the assay were 0.2 μg/ml and 0.75 μM (which corresponds to its K_m), respectively.

References

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