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Computational analysis of conformational changes in GSK3-β/CDK2 kinases for understanding inhibitor selectivity

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1. Introduction

Glycogen synthase kinase-3β (GSK3β) or tau-phosphorylating kinase is one of the serine/thereonine kinases which has been discovered firstly for its role in glycogen synthesis. Later GSK3β was itentified to play also a role in brain's neurons.[1] Cyclin-dependent kinases (Cdks) and their cyclin regulatory subunits control cell growth and division. Cdk2 kinase in complex with cyclin E is responsible for phosphorylating the retinoblastoma protein, which drives cells through the G1/S transition into the S phase of the cell cycle. [2] GSK3β and CDK2 are higly homolgous kinases showing high sequence identity especially in the kinase domain and the ATP's binding pocket. This similarity makes the task of developing GSK3β-selective inhibitor challgenging. Depending on inhibitors developed in in the group of Dr. Hilgeroth, computational chemistry methods are applied to get insight in the inhibitor binding, to predict the conformational changes, and to generate predictive models for estimating the binding affinity of novel compounds.

2. Derivatives of 1-Aza-9-Oxo-Fluorene

Novel 1-aza-9-oxafluorene derivatives have been synthesized in the group of Dr. Hilgeroth (Martin-Luther-University Halle-Wittenberg). Different substituents at position 3 and 6 produce inhibitors with varying potency for GSK3β and variable selectivity compared to CDK2.

Figure 1.

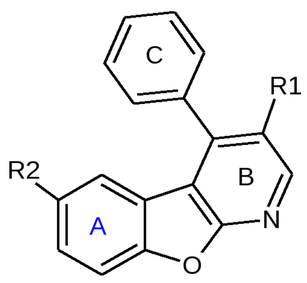


Figure 1. Chemical structure of 1-aza-9-oxo-fluorene derivatives

Cpd.	R1	R2	GSK3-β Ki µM	CDK2 Ki µM
1	OMe	OH	14.8	24.0
2	OMe	OMe	n.a (≥1000)	n.a (≥1000)
3	OBn	OH	5.8	6.4
4	OBn	OMe	0.02	147
5	OH	OH	0.02	82.3
6	OH	OMe	n.a (≥1000)	n.a (≥1000)

3. Suggested Binding Mode

Docking of the available compounds into three of the available crystal structures of GSK3-β (pdb id 1q3d.pdb, 1q5k.pdb, and 1j1b.pdb) suggests different binding modes. However, two binding mode were found to to explain the activities of these derivatives at GSK3-β and CDK2.

1. **Binding mode A:** the fluorene nitrogen makes a hydrogen bond with the hinge region residue Val135.
2. **Binding mode B:** the fluorene heteroatoms are located in the direction of the activation loop and make hydrogen bonds with Lys85 or Asp200, which is part of the DFG motif (charecteristic part of the activation loop).

a) Binding mode A **b) Binding mode B**

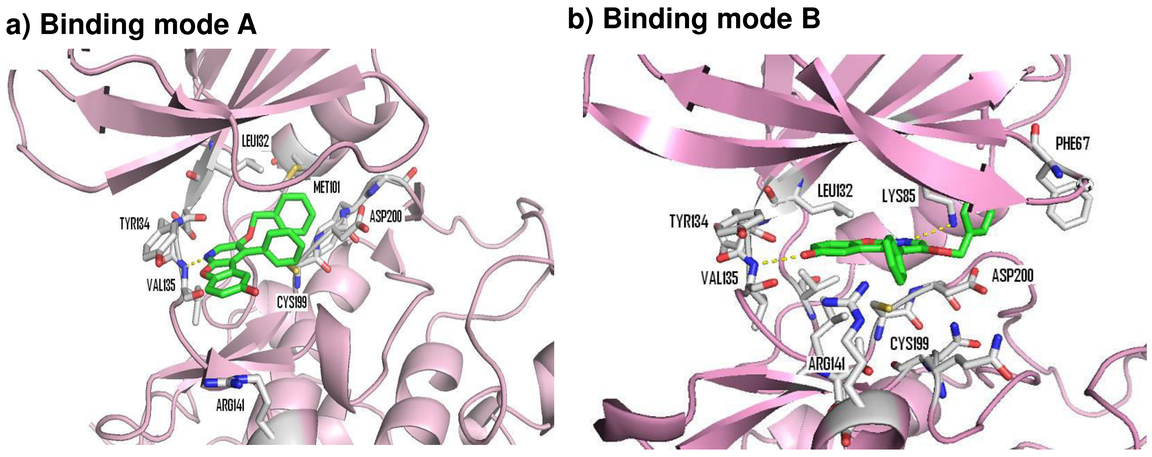


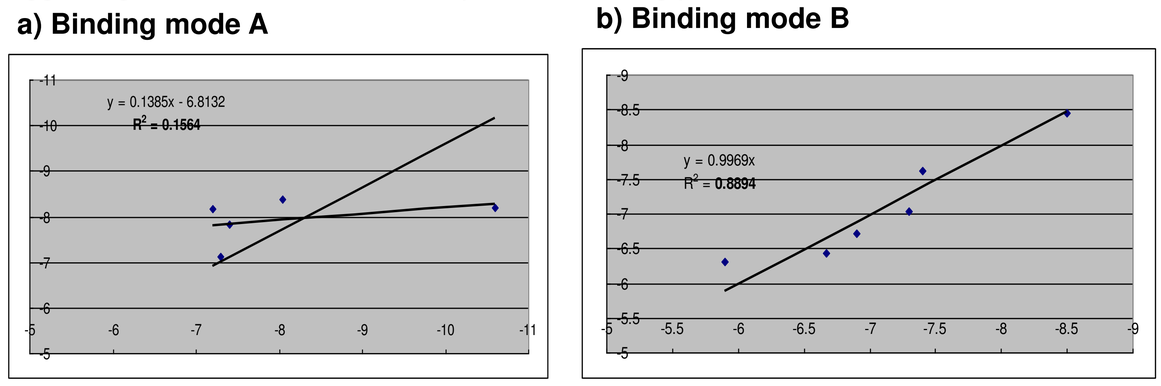
Figure 2: Possible binding modes of 1-aza-9-oxo-flourenes at GSK3β

The docking solutions of the derivatives with both kinases (3QWD and 1J1B for and 2W1H for CDK2) were dynamically simulated inside a box of water for 10 nanoseconds. Then, the MD trajectories were analysed for conformational changes in the kinase domain. Especially, the conformation of the P-loop and its distance from other residues of the ATP pocket and the bound inhibitor. Depending on these information the collection of kinase-inhibitor complexes were clustered. Further calculations were using the Linear Interaction Energy (LIE) mehtod developed by J. Aqvist [3].

4. Linear interaction energy models

Figure 3. LIE resulted in a predictive model for the six compounds in case of binding mode B (right). A statistically weaker model was obtained in the case of binding mode A, suggesting that model B is able to explain the binding process.

a) Binding mode A **b) Binding mode B**



5. Desolvation effect and role of water molecules

For both kinases, cpd. **6** shows weaker activity compared to cpd. **5**, obviously because the methylation of R1 hydroxyl (closest to the activation loop) results in a loss of the hydrogen bond with the activation loop's aspartate. However according to LIE calculations, the desolvation penalty becomes bigger for cpd. **1** in the case of GSK3-β whereas in the case of CDK2 the desolvation penalty doesn't change considerably.

Figure 4: Compound **5** bound with GSK3-β

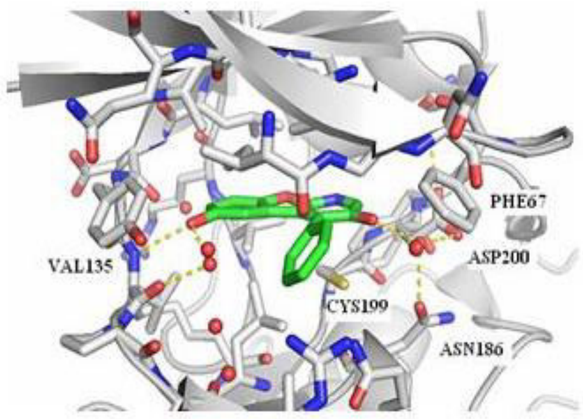
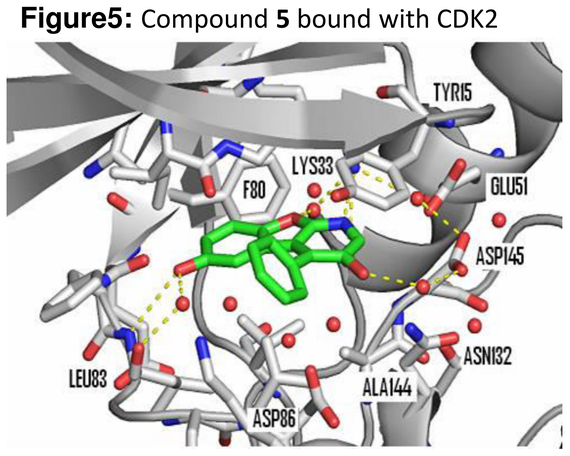


Figure5: Compound **5** bound with CDK2



6. Induced fit effect: the P-loop conformation

In case of the derivatives **3** and **4**, the substituent R1 (benzyl) optimizes its interactions using the flexibility of the glycine-rich P-loop, resulting in strong π-π interactions with the aromatic residue of the P-loop (Phe67 in GSK3-β and Tyr17 in CDK2). In case of CDK2 in complex with inhibitor **4**, the P-loop moves. As a consequence Tyr15 stays inside the binding pocket and makes the distance between P-loop and A-loop smaller This decreases the exposing of the hinge region to the bulk water.

Figure 6: Compound **3** bound to GSK3-β

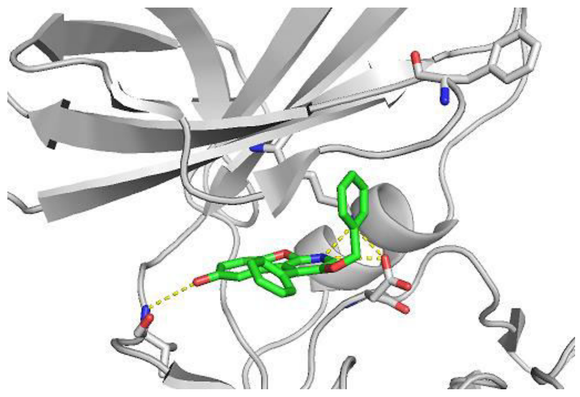
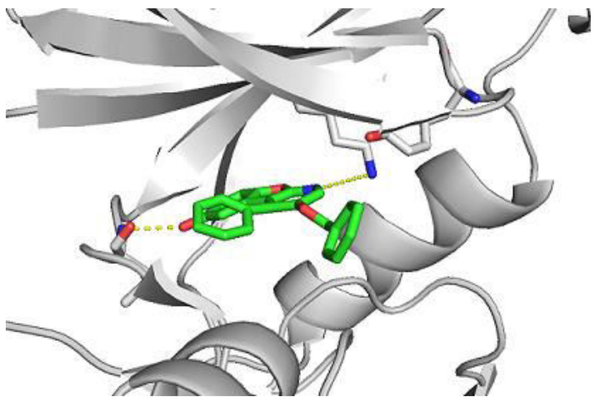


Figure 7: Compound **3** bound to CDK2



7. Conclusion

The application of the binding free energy method LIE taking into consideration the P-loop flexibiltiy was successful in generating predictive model to explain the varying activity of fluorene derivatives. Additionally MD simulation and the analysis of trajectories helped to understand the factors that affect the binding affinity. The models can now be applied to further optimize the inhibitor profile.

References

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