Fragment-based drug design of small molecule EPHA4 kinase inhibitors

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Aim and scope of the thesis

Chapter 2

1. Aim and scope of the thesis

Due to the large interest in protein kinases as drug targets, the high structural similarity between kinase domains, the focus on the catalytic cleft for inhibitor development and the confined characteristics of interacting ligands (e.g. flat, heteroatom containing, aromatic hinge binding scaffolds), the diversity in kinase-ligand space is limited. The identification of novel, IP free, hinge binding fragments and novel chemistry is therefore required. With the approval of Zelboraf® (Vemurafenib, PLX4032)[1] the power of FBDD, in making the drug development process more efficient, has been demonstrated. The better coverage of chemical space and typically higher hit rates, when compared to HTS, allows the screening of smaller libraries. These conditions make FBDD an excellent method for drug discovery efforts within an academic setting. Fragment-based biophysical- and *in silico*-screening methods have proven to be good research tools for the identification of scaffolds that interact with a particular target.

1.1 Research Aims

In this project we set out to use a combination of these techniques for the identification of novel EPHA4 interacting fragments. For this purpose, a consortium of three academic research groups and three companies was created. The inhibitory properties of the identified compounds are assessed by biochemical assays. Computer aided drug design and X-ray crystallography serve as key tools in the structure based optimization of EPHA4 inhibiting fragments into more potent analogs. The synthesis of novel compounds should lead to high affinity, IP-free, EPHA4 inhibiting lead compounds with good physicochemical properties. *In silico* efforts are undertaken to better understand the kinase-inhibitor-selectivity problem.

2. Outline of the thesis

From an analysis of 409 kinase-inhibitor complexes in the Protein Data Bank (PDB),[2] it became evident that the majority of kinase inhibitors target the hinge region by forming one or two hydrogen-bond interactions.[3] Inhibitors that make three hydrogen-bonds with the hinge are less common. The gatekeeper residue in the EPHA4 receptor is a threonine residue, which is capable of forming hydrogen-bonds with ligands. This offers an opportunity to design inhibitors that make four consecutive hydrogen-bonds in the ATP binding site. Since ~20% of the kinases contain such a gatekeeper amino acid, specifically targeting this *4 in a row* structural element may therefore be favorable in terms of potency and selectivity over other kinases. In **chapter 3**, the *in silico* identification and subsequent optimization of a fragment that makes these *4 in a row* hydrogen-bonds is described. A combination of pharmacophore screening and docking was used to identify this hinge binding fragment and steer the structure based optimization process. The optimization efforts led to the identification of a sub-micromolar inhibitor of the EPHA4 receptor. Soaking of a close analog into a crystal of the EPHA4 kinase domain confirmed the putative *in silico* generated binding mode of the starting fragment, including the utilization of the *4 in a row* binding motif. The inhibitor was found to be moderately selective when screened against a panel of 124 kinases. Interestingly, the identified scaffold was a novel kinase inhibitor at the time of discovery.

Hit optimization was therefore considered particularly attractive, even though the synthetic chemistry associated with this scaffold was challenging. More specifically, the synthesis of a key intermediate compound produced two regioisomers, of which the product ratio seemed depending on the electronic properties of the *para*-substituent on a phenyl ring. To open up the chemistry space that is represented by the hit scaffold, the reaction was investigated in detail. As the isomers were difficult to separate from the reaction mixtures in a quantitative manner, a ^{13}C-incorporation strategy was designed. This method enabled direct determination of the product ratio in the reaction mixtures and is described in

chapter 4. By using this approach new insight in the reaction mechanism of these types of condensation reactions is gained.

To better understand the factors that steer kinase inhibitor selectivity, the complete Protein Data Bank was data mined for co-crystal structures of all human kinase domains in complex with small molecule inhibitors. This has resulted in the creation of a **K**inase-**L**igand **I**nteraction **F**ingerprints and **S**tructure (KLIFS) database. In **chapter 5**, an extensive overview of the binding modes of >1200 inhibitor – kinase complexes are described. The analysis helps to identify structural features that are important for affinity and selectivity of inhibitors. The database contains a consistent alignment of the kinase catalytic cleft and can be searched for specific interactions or conformational changes (like DFG-status). This setup enables the identification of family- or group-specific interaction features. The database can serve as a guide for future kinase inhibitor design.

References

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