VU VRIJE UNIVERSITEIT AMSTERDAM

VU Research Portal

Fragment-based drug design of small molecule EPHA4 kinase inhibitors

van Linden, O.P.J.

2013

document version
Publisher's PDF, also known as Version of record

Link to publication in VU Research Portal

citation for published version (APA)
van Linden, O. P. J. (2013). *Fragment-based drug design of small molecule EPHA4 kinase inhibitors*.

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

E-mail address:
vuresearchportal.ub@vu.nl

Download date: 17. Jan. 2022

Summary and conclusions

Chapter 6

Summary and conclusions

This STW funded project was designed to develop new leads for the EPHA4 receptor tyrosine kinase with clinical potential to treat skin and prostate cancer. A typical Fragment-Based Drug Discovery (FBDD) approach was foreseen in which a combination of biophysical (in particular NMR), biochemical and *in silico* approaches would be used to identify and confirm hit finding and guide subsequent hit optimization efforts towards potent EPHA4 inhibitors. With these ligands, *in vitro* efficacy and activity in EPHA4 expressing tumor cells (*e.g.* PC3 cells) was to be assessed. The research has been performed by a consortium of three groups with expertise in their respective fields, i.e., biophysical approaches and target expertise at Leiden Institute of Chemistry (LIC) of Leiden University (the group led by Dr. Gregg Siegal) and kinase activity studies at the Leiden University Medical Center (LUMC) (the group led by Dr. Kees Tensen). Design and Synthesis (amongst others, the work described in this thesis) was performed at VU University Amsterdam. Additional support was provided by several small biotech companies that participated in the user committee of this project.

The described consortium faced several challenges that are typical for FBDD studies that target kinases. An intrinsic aspect of fragment-based drug discovery is that a high concentration of fragments is needed during the screening. Typically, however, kinase dir0ected scaffolds suffer from poor solubility characteristics. This is due to the fact that the catalytic cleft is mostly hydrophobic and typically accommodates flat, aromatic, heteroatom containing scaffolds. This poses a challenge to the design of the library to be screened. Despite these physicochemical challenges, hit compounds were identified using biophysical approaches by the LIC group. Biochemical validation of these hits was not always straightforward for several reasons, including the limited potency and compromised solubility of the hits and the fact that the protein proved difficult to handle during protein production, purification and assay development. In efforts to design more potent and soluble EphA4 binders, a first round of fragment hit exploration was done by considering commercially available structurally related analogs. Subsequently, three scaffolds were further optimized by synthesizing series of derivatives.

These studies were aimed to identify the pharmacophores that are important for EphA4 binding and to explore available vectors for fragment growing. These initial hit optimization studies are not described in this thesis, since the hits that were obtained using *in silico* approaches, as described below, were prioritized. This was done after considering the SAR, IP position, synthetic feasibility and the complicated correlation between biochemical and biophysical assays that was associated with the hits that were found by the biophysical approaches.

Thus, complementary to biophysical screening techniques, *in silico* screening was used to identify novel EPHA4 binding fragments and these studies have been described in **chapter 3**. Using the structural information derived from crystal structures of two ligands bound to related kinases, a mixed pharmacophore model for the binding site of EPHA4 was constructed, which describes a *4 in a row* hydrogen-bond structural element of the EPHA4 receptor. These efforts led to the fragment-based discovery of a 6,7,8,9-tetrahydro-3*H*-pyrazolo[3,4-*c*]-isoquinolin-1-amine fragment, a novel EPHA4 hinge binding molecule. The putative binding mode of the fragment was investigated by synthesizing analogs and growing of the fragment towards the hydrophobic back cleft of EPHA4. More specifically, probing of the selectivity pocket BP-I, increased the affinity for EPHA4 and yielded an inhibitor with 2 μM (IC_{50}) activity and good Ligand Efficiency (see below). Soaking of this compound into a crystal of the EPHA4 kinase domain confirmed the predicted binding mode of the scaffold. The structure of this inhibitor-kinase complex has been deposited in the protein data bank (pdb:2xyu). Interestingly, profiling of the scaffold against a panel of 124 protein kinases revealed that this compound is a moderately selective kinase inhibitor. The *in silico* identification and optimization of this scaffold illustrates the efficiency of computer-aided drug design in FBDD.

Having performed initial hit optimization, the next step would have been to address properties like toxicity, bioavailability and solubility. For the latter, the lipophilicity of the hits would have to be reduced during further growing and optimization efforts. In recent months, the FBDD community is discussing the importance of keeping the lipophilicity as low as possible, *e.g.*, by introducing terminology as molecular obesity[1-2] to raise awareness of the problems of "fatty"

molecules. Also, metrics to guide fragment hit optimization like Ligand Efficiency have been further developed to include lipophilicity information. As an attractive metric, scientists from Astex introduced LLE_{AT}.[3] It would have been interesting to use these insights for hit and lead optimization. However, the hit and lead optimization work on this scaffold was stopped when Merck disclosed a patent that covered identical EphA4 inhibitors. The reported IC_{50} values of the disclosed structures are consistent with the data presented in this work. Although a major disappointment for our project, the competing work of Merck underlines the efficiency of our approach and also illustrates the potential of the scaffold as a possible new drug for the treatment of cancer and neuronal injuries by inhibition of the EPHA4 receptor tyrosine kinase.

At the time of our hit finding of the scaffolds described above, efficient synthesis routes to obtain these types of compounds were scarcely available in literature. While exploring the synthesis route, uncertainty about the regioisomeric outcome of key intermediates also arose. More specifically, the condensation of substituted 2-aroyl-cyclohexanones with 2-cyanoacetamide yielded tetrahydroisoquinolines and tetrahydroquinolines as products. The extent to which either isomer was formed seemed to be depending on the electronic properties of the *para*-substituent on the phenyl ring. To make the chemistry space that is represented by the hit scaffold more accessible, the reaction was investigated in detail, as described in **chapter 4**. Toward this end, we analyzed the effect of a strategically chosen set of *para*-substituents on the formations of tetrahydroisoquinoline and tetrahydroquinoline products. Since there are issues associated with separating and accurately quantifying such product ratios with conventional methods, we strived to determine the product ratio directly from the crude reaction mixtures. A ^{13}C-incorporation approach was designed, which allowed the determination of product ratios *in situ*, enabled by advanced 1D and 2D NMR studies and our in house 500 MHz Cryoprobe NMR. Semi-empirical MO calculations on the starting materials support the notion that a combination of activation strain and of the energy gap between the LUMO and LUMO+ is responsible for the observed regioisomeric ratios. The results of this Hammett study have given insights in the mechanisms associated with the reaction, which is of importance to fundamental organic

chemistry. The exploration of the synthesis route enabled us to efficiently synthesize a key intermediate compound that is useful for probing kinases

Although our efforts focus on EphA4 receptors, the inhibitors evidently bind to more kinases. This is a typical aspect of kinase drug discovery, with molecules that target the hinge region of these proteins binding to a panel of kinases, rather than being selective.[4] Identification of kinase-, group- or family-specific structural features can therefore significantly attribute to the development process of more selective inhibitors. Toward this end, we conducted a thorough analysis of kinase-ligand interaction patterns on all regions of the catalytic site of all currently (publically) available human kinase crystal structures. By mapping this kinase-ligand interaction space, cross family ligand binding features were studied. Molecular Interaction Finger Prints (IFP) were used to describe the binding modes of >1200 kinase-ligand co-crystal structures present in the Protein Data Bank (PDB) and is presented in **chapter 5**. The resulting, freely available **K**inase **L**igand **I**nteraction **F**ingerprints and **S**tructure Database (KLIFS), contains the aligned 85 amino acid pockets and ligands of these complexes, including their IFP analysis string. Systematic mining of this kinase-ligand interaction space has gained insights into how conserved and selective kinase interaction hot spots can accommodate the diversity of chemical scaffolds observed in kinase ligands. The growing number of crystal structures has led to an increased identification of novel sub pockets and binding modes in kinase-ligand interaction space. These studies lead to an improved understanding of the structural requirements of kinase binding that will be useful in future ligand discovery and design studies.

In all, we have described a wide variety of aspects that are important for the development of kinase inhibitors, ranging from making relevant chemistry space accessible, mapping kinase biology space and explore efficient methods to connect biology with chemistry. A variety of technologies were used, *e.g.*, by using available crystallographic structural data, computational chemistry approaches like pharmacophore placement and *in silico* docking were used to identify novel hinge binding fragments. More potent analogs of the hits were obtained using synthetic organic chemistry. Fundamental insights into the mechanisms of a key reaction were obtained using a combination of synthetic organic chemistry, advanced NMR

techniques and MO calculations. Explorations in the human kinome, by systematic *in silico* mining of crystallographic data, led to an extensive overview of kinase-ligand interaction space, which attributes to the development of novel kinase inhibitors and facilitates in obtaining more selective ligands. Overall, in this medicinal chemistry project we have gained more understanding on efficiently targeting EphA4 and other kinases.

References

1. Hann, M. M. Molecular obesity, potency and other addictions in drug discovery. *MedChemComm* **2011,** 2, 349-355.
2. Keserü, G. M.; Makara, G. M. The influence of lead discovery strategies on the properties of drug candidates. *Nat. Rev. Drug Discov.* **2009,** 8, 203-212.
3. Mortenson, P.; Murray, C. Assessing the lipophilicity of fragments and early hits. *J. Comput. Aided Mol. Des.* **2011,** 25, 663-667.
4. Bamborough, P.; Brown, M. J.; Christopher, J. A.; Chung, C.-w.; Mellor, G. W. Selectivity of Kinase Inhibitor Fragments. *J. Med. Chem.* **2011,** 54, 5131-5143.