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An increasing number of kinase inhibitor candidates is entering clinical development, representing an important change in the pharmaceutical industry, notably the development of small molecule kinase inhibitors for signal transduction therapies. Six distinct small-molecule kinase drugs, imatinib, gefitinib, erlotinib, sorafenib, sunitinib and dasatinib act on a distinct subset of dysregulated, and often cancer-relevant kinases. The entire research field gains tremendous insights through the ongoing research and clinical trials with these six drugs and with the fast following second generation kinase inhibitors, many of which are in different phases of clinical development. In addition, novel chemogenomic and chemoproteomic technologies are emanating from the current kinase research area, focussing efforts on the generation of spectrum-selective inhibitors for anticancer therapies as opposed to the mono-specific inhibitors for the remaining therapeutic areas.

Initially, the family of protein kinases has been regarded as a family of non-drugable enzymes, mainly due to the fact that protein kinases share high sequence homology in their catalytic site and a conserved mechanism of action. Therefore, it has been assumed that small molecules, which are competing with ATP for binding, are most likely lacking the necessary level of selectivity in order to be pharmacologically tolerated and safe. Despite these prejudices and the fact that some of the known broad spectrum kinase inhibitors are indeed toxic to cells and animals, researchers at Ciba-Geigy and subsequently at Novartis managed to rationally design a selective kinase inhibitor, imatinib. Imatinib has been optimized to inhibit the Bcr-Abl kinase in order to treat chronic myelogenous leukaemia. It is regarded as the first rationally designed kinase inhibitor on the market. Intensive research and design of kinase inhibitors like imatinib, gefitinib and others has demonstrated that the ATP binding site of a particular kinase target is not a static entity. Indeed, the catalytic sites of different kinase targets are highly conserved. Next to the ATP binding site, X-ray structures of different kinases (in combination with various inhibitors) revealed the presence of a hydrophobic back-pocket, a surface-exposed front pocket and the ribose binding site as substructures, which are accessible to inhibitor design. Despite being evolutionary conserved, especially the front- and the back-pockets are not necessarily conserved in terms of their amino acid sequence and shapes. This insight led to the first important strategy in kinase inhibitor design, which was to address the hinge domain, which connects the two lobes of a typical kinase domain, by competing with the binding of ATP. Starting from there, such an ATP competitive core can be functionally extended into the hydrophobic back-

pocket. Inhibitors, like gefitinib, erlotinib and dasatinib represent good examples of such a strategy. In fact they achieve a remarkable level of selectivity although they are far from being mono-specific. By serendipity, imatinib has another important feature. Co-crystallization of imatinib with Abl revealed that it binds to the inactive form of the kinase. It is a so-called DFG-out binder. Somehow, the drug manages to rearrange the active site, extending the hydrophobic pocket into a deep pocket and exploiting additional unique interaction sites. These structural findings for imatinib have been obtained mostly in hindsight. A close look into second generation kinase inhibitors, like lapatinib, BIRB-796 and AZD-1152 reveals that these inhibitors are making use of similar structure features and together with the marketed angiogenesis inhibitor sorafenib, constitute a constantly growing group of DFG-out binders. In the meantime, it has been well recognized that DFG-out binding might offer an additional opportunity to generate selectivity for a particular kinase inhibitor. In essence, today we know three different design principles for the generation of ATP competitive kinase inhibitors: 1, addressing the hydrophobic back-pocket, 2, DFG-out binders and 3, spectrum-selective compounds, which are competing with ATP, but are not necessarily highly selective (e.g. sunitinib).

Despite the initial resentments against the generation of protein kinase drugs, we can now apply these three different design principles using structural and rationale approaches. We still do not completely understand the network of signal transduction and the distinct roles of the ~530 protein kinases of the human kinome, which are sometimes overlapping and even opposing in function. Therefore inhibitor profiling is an important, but also a huge task. The determination of a selectivity profile for a particular kinase inhibitor is therefore always a function of the available technologies. Selectivity profiling in the various kinase selectivity panels (enzymatic profiling) is limited to the number of kinases in the respective panel and does not take into account that kinase inhibitors might also bind to off-targets different than kinases. In order to better control the specificity and selectivity of kinase inhibitors in drug discovery, novel chemoproteomics tools are becoming an integral part of inhibitor design. The applications of these tools, like the immobilization of kinase inhibitors and subsequent determination of its comprehensive target and off-target profile, are not just limited to selectivity determination. Furthermore, they are supportive in using the full capacity of a particular kinase inhibitor core structure.

For example, we have previously identified members of the benzothiophene class of compounds as potent inhibitors of the mycobacterial protein kinase, PknG. AX20017 has been described as a particular selective inhibitor of PknG with an  $IC_{50}$  of ~0,5 $\mu$ M. The original hit, AX20017, has been further optimized to a lead. Repeatedly, we experimentally determined the selectivity using the kinase inhibitor immobilization technology. Aurora A has been identified as one of the few kinase off-targets of a particular subclass of the

benzothiophenes. Aurora A is well known as an oncogene and it controls centrosome maturation and bipolar spindle assembly during mitosis. Therefore, it is a highly rated cancer target. With a limited effort, we could apply an optimization strategy for the benzothiophenes in order to generate nanomolar and pharmacologically active Aurora inhibitors, which are acting on both cancer relevant Aurora family members, Aurora A and Aurora B. Although these leads do not even extend into the hydrophobic back-pocket, they show a surprising level of selectivity against a panel of selected kinases. In addition, they induce classical Aurora (A & B) dependent phenotypes in various cellular models. In addition, we see activity in mouse xenograft models. Co-crystallization experiments have revealed the binding mode of the benzothiophenes to Aurora A. Subsequent optimization efforts are now directed to add functional groups at the 5'-position of the benzothiophene core in order to fill the hydrophobic pocket and potentially induce deep pocket binding. In essence, we try to integrate the knowledge from other kinase inhibitor optimization programs, to quickly come up with a drug-like Aurora inhibitor, which might be even well tolerated, due to its superior selectivity.