

Signal Transduction Therapy with Rationally Designed Kinase Inhibitors

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Abstract: Signal transduction therapy has become one of the most important areas of drug research. Signaling disorders represent a major cause for the pathological states and many of the recently identified validated target molecules of drug research are signal transduction related macromolecules, mostly kinases. Rational drug design is aimed to achieve the selective inhibition of distinct pathologically relevant signaling enzymes or receptors. In the previous years, the concept of rational drug design has been expanded for a complex process including pathomechanism-based target selection, target validation, structural biology, molecular modeling, structure-activity relationships, pharmacophore-based compound selection and pharmacological optimization. The two main branches of the chemical rational drug design are structure-based design and ligand-based design. Some important examples for the application of 3D structure-based rational drug design in the development of clinically relevant kinase inhibitors are presented. The Nested Chemical Library™ (NCL) technology is a ligand-based design approach and relies on a knowledge-based approach, where focused libraries around published leads and selected cores are used to generate extended pharmacophore models (Prediction Oriented QSAR). NCL was designed on the platform of a diverse kinase inhibitor library, consisting of small molecule heterocycles, which are organized around 108 core structures. Some examples for testing the library on various targets and Prediction Oriented QSAR models will also be presented. The core elements of the kinase family-biased masterkey concept are the so-called privileged structures that emerge from a sophisticated molecular design and optimization process that encodes for a target family-wide structural commonality in ligand binding. The combination of a kinase family-wide imprinted commonality with additional structural fragments in the molecular periphery of a once established privileged structure allows to synthesize highly active and selective kinase inhibitors. In addition, several kinase inhibitors in preclinical or clinical development and application of 3D structure based rational drug design in the development of clinically relevant kinase inhibitors are reviewed.

Key Words: Signal transduction therapy, rational drug design, kinase inhibitor, masterkey, cancer, QSAR.

THE CONCEPT OF SIGNAL TRANSDUCTION THERAPY

It has become evident that intra- or intercellular communication disorders represent the basis of a majority of complex pathomechanisms. Thus, modern drug research has become increasingly focused on signal transduction therapy.

The normal cell has multiple independent mechanisms that regulate its growth and differentiated function, and several separate events are needed to override these control mechanisms, as well as to induce a pathological state. The processes of cellular growth and differentiation, as well as the maintenance of specialized functions show a remarkable degree of coordination. According to convincing evidence, the latter involves intercellular communication, rather than relying entirely on intracellular programming. The pathological state per se is a multi-step process. It requires that interdependent systems, which control proliferation,

differentiate functions and cellular homeostasis in certain malfunctioning cell populations, are uncoupled. Moreover, certain cellular functions or malfunctions (including proliferation of infected, transformed or inflammatory cells) are stimulated in such a way as to result in the generation further damage inflicting [1].

Proliferation of infected, damaged or malfunctioning cells is very often a key factor for the generation of the pathological state, in not only cancer and infectious diseases, but also in inflammation or autoimmune related diseases like arteriosclerosis, arthritis or certain inflammation related neurodegenerative diseases. Inflammation has been found the ultimate cause of many chronic diseases, where the false signaling-related proliferation of immune cells turned out to be a critical factor in the pathogenesis of the disease.

The majority of molecular pathomechanisms result from intra- or intercellular communication disorders, whereas a series of genomic changes are potential causes and consequences of this breakdown of communication. In a healthy organism, normal cells fulfill their duties, do not send or receive false messages and are firmly controlled by the external messages of the communication network. By

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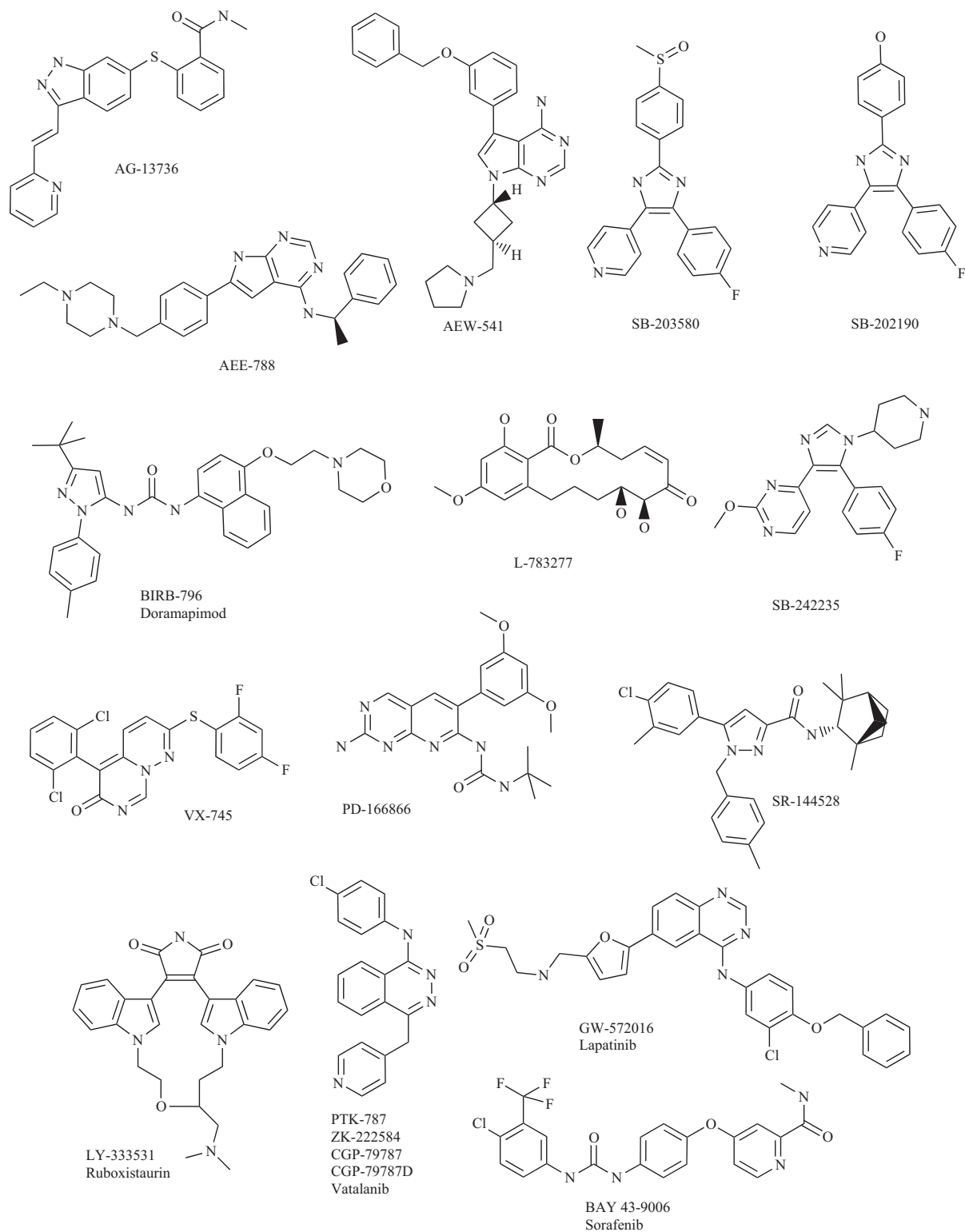


Fig. (1). Structures of the selected listed kinase inhibitors I.

contrast, cancer cells, for example, generate a false, mimicked proliferation signal for themselves *via* the oncogenes and other genomic changes. Whether this communication failure is the result of environmental factors and/or external messages (with an influence exerted at the genomic level) or whether it is encoded in the genetic program that is a question, which can be answered only on a case-by-case basis. However, it needs to be considered that cells like human beings live in a well-organized society, and in a given ecosystem. To a certain extent, the latter determines their receptivity and responsiveness, as well as defines the properties of the systemic response to various carcinogenic agents and effects. In other words, carcinogenic compounds can be carcinogenic in a given *in vitro* system or in a given organism but the same agent can have different effects in different systems, of course depending on the extent of the effect. Evidently, changes at the genomic level are critical steps of carcinogenesis, whereas the manifestations of these genomic changes and of the systemic response are dependent on the communication state and responsibility of the system to the microenvironment or personal ecosystem.

In normal cells, the regulation of cellular functions and the communication with the external world, the ecosystem, is controlled by a complex relationship between pieces of genetic information and by a large series of external factors and mediators, which provide the fine-tuning of coordinating the homeostasis of the cells. Since signaling disorders represent a major cause for the pathological states and most of the recently identified validated target molecules of drug research are signal transduction related macromolecules (mostly kinases), signal transduction therapy has become one of the most important area of drug research [2-5].

KINASE INHIBITORS IN SIGNAL TRANSDUCTION THERAPY

Approximately 20-25 percent of the druggable genome consists of kinases involved in signal transduction. Currently, however, only four kinase inhibitors are used in clinical practice, which means wide perspectives for drug discovery. The first proof of concept drug for signal transduction therapy was Gleevec - an inhibitor of BCR-ABL kinase - and has been launched in May 2002 [6,7]. Gleevec is indicated for the treatment of chronic myeloid leukemia (CML) and has a success rate of 90% in affected patients. According to our present knowledge, almost 200 compounds with kinase inhibitory activity against more than 50 kinase targets are in the various stages of preclinical and clinical development in signal transduction therapy. This definitely underlines the significant influence of this area on drug research [8,9]. Of the launched and late stage clinical development compounds for cancers, the most important small molecule synthetic tyrosine kinase inhibitors are Gleevec, the epidermal growth factor receptor tyrosine kinase inhibitors: ZD1839/gefitinib/Iressa [10] and OSI-774/erlotinib/Tarceva [10], and the vascular endothelial growth factor receptor (VEGF-R) kinase inhibitor SU11248/Sunitinib [11].

1. VEGF-R

Targeting VEGF-R might lead to the discovery of new anticancer agents. Manley and co-workers developed potent and selective VEGF-R inhibitors [224]. One of the most

important processes in tumor growth is angiogenesis, by which new blood capillaries sprout from pre-existing blood vessels [13-15]. Since VEGF stimulates angiogenesis, inhibition of its receptor will inhibit angiogenesis, a viable principle to inhibit tumor formation. The authors selected vatalanib, a first-generation VEGF-R inhibitor as a starting point in the development of the second-generation VEGF-R inhibitors. Based upon the binding model and the pharmacophore elements of vatalanib, an anthranil amide scaffold was selected for further examination (scaffold morphing). Database mining identified a compound, which inhibited VEGF-R with IC₅₀ value of 3.7 μ M as predicted. Lead optimization of this compound resulted in the identification of AAL993, a highly potent and selective inhibitor of VEGF-R with good biopharmaceutical properties. The authors determined the crystal structure of the complex of AAL993 and VEGF-R, and showed that the ligand binds to the inactive conformation of the enzyme, similarly to the binding mode of Gleevec to BCR-ABL kinase.

Other potent kinase inhibitors inhibiting VEGF signaling pathways are SU5416 [10], PTK787/ZK-222584/CGP-79787/CGP-79787D/vatalanib [16], SU6668 [17,18], CP-547632 [19] and AZD2171 [20]. The multiple target inhibitor SU11248/sunitinib (Flt3, c-Kit, VEGF-R2 and PDGF-R β) is now in Phase III clinical development.

ZD6474/vandetanib is a potent, orally active, low-molecular-weight inhibitor of KDR/VEGF-R2 tyrosine kinase activity and also displays inhibitory activity towards EGF-R and oncogenic RET kinase. It is currently in Phase II clinical development for a range of solid tumors, both as mono-therapy and in combination with certain anticancer agents [21-23].

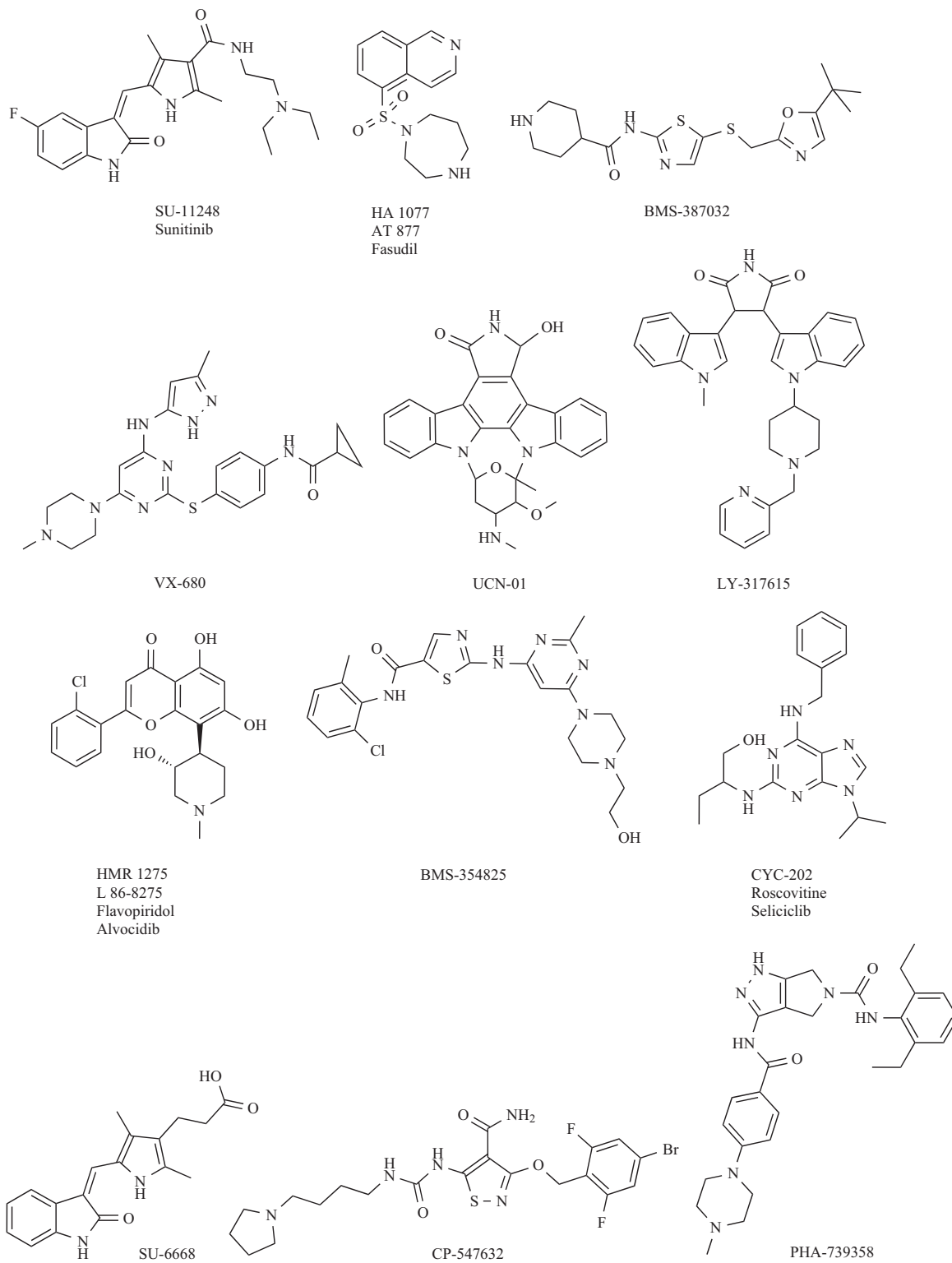
2. Flt-3

Flt-3 inhibitors, like CEP-701 showed clinical (Phase I) activity in patients with acute myeloid leukemia [24]. Tandutinib (MLN-518) is in clinical Phase III for the treatment of various cancers [25]. CHIR-258 is an orally active small molecule that exhibits potent inhibitory activity against multiple RTKs involved in tumor growth and angiogenesis (IC₅₀ \leq 10 nM for VEGF, FGF, PDGF and c-KIT receptor kinases; 37 nM for CSF-R1). CHIR-258 is in clinical Phase I [26].

3. BCR-ABL

The BCR-ABL tyrosine kinase inhibitor, imatinib (Gleevec) has greatly improved the outcome for patients with chronic myeloid leukemia (CML). Unfortunately, induced mutations, which cause imatinib-resistance are leading to relapses in some patients [27]. Unlike imatinib, BMS-354825 inhibits the active BCR-ABL enzyme. It binds differently to the ATP-binding site of BCR-ABL. As a result of that, it does not only inhibit wild-type BCR-ABL, but also 14 of the 15 reported BCR-ABL mutants, leaving only the gatekeeper mutant BCR-ABL^{T315I} unaffected. The safety and efficacy of BMS-354825 is presently being evaluated in clinical Phase I and II trials in Gleevec-resistant CML patients [28].

In addition to BMS-354825, the Aurora kinase inhibitor VX-680 and the p38 inhibitor BIRB-796 also inhibit the imatinib-resistant ABL^{T315I} kinase [29].

**Fig. (2).** Structures of the selected listed kinase inhibitors II.

Two other receptor tyrosine kinases, c-kit and the platelet derived growth factor receptor (PDGF-R) are two serendipitous, but important imatinib off-targets. Overexpression of c-kit, a tyrosine kinase, is the underlying cause for the formation of gastrointestinal stromal tumors (GISTs). Some of the Gleevec treated patients, in analogy to Gleevec-induced resistance in CML, develop Gleevec-resistant GISTs, expressing the imatinib-resistant KIT^{V559D/T670I} kinase. The KIT/FLT3 inhibitor SU-11248/sunitinib shows potent activity against the imatinib-resistant KIT^{V559D/T670I} kinase, consistent with the clinical efficacy of SU-11248 against imatinib-resistant GISTs. Again, very similar to that the epidermal growth factor receptor mutant (EGF-R) inhibitors EKB-569 and CI-1033, but not GW-572016/lapatinib and ZD-6474, potentially inhibit the gefitinib- and erlotinib-resistant EGF-R mutant, EGF-R^{L858R,T790M} [29].

4. EGF-R

The EGF-R inhibitors Iressa and Tarceva are effective in the treatment of various tumor types. Another quinazoline derivative, which inhibits EGF-R, is lapatinib. Despite their structural similarity, the molecules bind in different modes to the hinge domain. It was shown that lapatinib binds to the ATP binding site in a conformation that resembles an inactive kinase structure; it is just opening up the hydrophobic pocket and inducing the inactive conformation, while Tarceva binds to the active conformation [30]. The difference between the binding mode of the two molecules results in the fact that lapatinib has a slower off-rate from the active site.

The reactive cysteine situated in the ATP binding site of EGF-R permits the design of selective, irreversible EGF-R inhibitors, which form a covalent bond with the reactive cysteine. These types of compounds are being investigated in clinical trials [31,32]. Wissner and co-workers [32] identified 4-anilinoquinazoline inhibitors based on modeling studies and after lead optimization, and they started the clinical trials of inhibitor EKB-569. This compound inhibits EGF-R with an IC₅₀ of 0.083 μM and it inhibited the growth of different carcinoma cell lines.

Successful examples of drug design at Novartis described PKI166 as a dual inhibitor of both the EGF-R and the HER-2 kinases [10,33,34].

GW-572016/Lapatinib is a dual EGF-R/HER-2 inhibitor in Phase III [35].

BMS-599626 is an orally bioavailable inhibitor of the HER1, HER2 and HER4 tyrosine kinases (IC₅₀=22, 32 and 190 nM, respectively), it is currently in clinical Phase I [36].

The Her2/ErbB2 inhibitor of Pfizer, CP-724,714 is currently in Phase II trials [37].

AEE-788 is an anticancer agent of Novartis with various effects: it shows VEGF antagonist, EGF-R family tyrosine kinase receptor inhibitory, ErbB2 tyrosine kinase receptor inhibitory and AKT protein kinase modulatory activity. It is in clinical Phase I [38].

The successful kinase inhibitors (e.g. Gleevec and Iressa) are ineffective on certain mutated kinases [39]. It has been

reported recently that the T790M mutations in EGF-R as secondary mutations that arise in previously sensitive NSCLCs harboring an activating mutation, is associated with the emergence of acquired resistance. (Similar resistance and mutation has been described in the case of Gleevec in CML.) However, it has been shown now that in the case of EGF-R, this mutation is present only in a subset of cases, and even tumors that harbor the T790M mutation may contain only a small fraction of cells with this mutation. These observations imply that multiple resistance mechanisms can coexist in recurrent tumors after an initial response to Gefitinib or Gleevec. Because most of the kinase inhibitors have to reach an intracellular target, specific membrane transporters may significantly modulate their effectiveness. In addition, the hydrophobic kinase inhibitors may interact with so-called multidrug transporters and thus alter the cellular distribution of unrelated pharmacological agents. It has been demonstrated recently that certain TKIs, already in the clinical phase of drug development, directly interact with the ABCG2 multidrug transporter protein with a high affinity. Low concentrations of the TKIs examined selectively modulated ABCG2-ATPase activity, inhibited ABCG2-dependent active drug extrusion, and significantly affected drug resistance patterns in cells expressing ABCG2. The results indicated that multidrug resistance protein modulation by TKIs may be an important factor in the clinical treatment of cancer patients. These data also raised the possibility that an extrusion of TKIs by multidrug transporters, e.g., ABCG2, may be involved in tumor cell TKI resistance [40,41].

This multiple resistance problem also shows the importance of personalized therapy [42,43], where the drug is applied after gene expression and mutation analysis.

5. IGF-R

Insulin-like Growth Factor-1 (IGF-1) and its receptor play a pivotal role in many cancers; it is an attractive target for the design of inhibitors. A new family of catechol mimics has been reported to be a substrate-competitive inhibitor of IGF-1 receptor (IGF-1-R) [44]. Inhibition of IGF-1-R autophosphorylation by novel 6-5 ring-fused compounds (pyrrolopyrimidines, furopyrimidines) are reported [45]. A compound from Novartis (AEW-541) inhibiting IGF-1-R is in clinical Phase I. It is being developed against cancer and multiple myeloma [46].

6. FGF-R

Mohammadi and co-workers [47] determined the X-ray structure of FGF-R co-crystallized with their oxindole (indolinone) derivatives. It has been shown that overexpression of FGF-R can be detected in a population of breast cancers [48], human pancreatic cancers [49], astrocytomas [50], salivary gland adenocarcinomas [51], Kaposi's sarcomas [52], ovarian cancers [53], and prostate cancers [54]. They have shown that the oxindole group binds to the adenine region of the ATP binding site of FGF-R and the other groups interact with the residues of the hinge region. The structural information provided by this article can be used for designing novel, more potent and selective FGF-R inhibitors. For example, Hyun and co-workers developed reliable CoMFA and CoMSIA models for FGF-R inhibitors based on

the crystal structure of the oxindole derivatives [55]. They selected their best CoMSIA model to screen a virtual library to find new inhibitors.

7. Met

Met is commonly overexpressed in tumors and point mutations have been identified in hereditary and sporadic papillary renal carcinomas, gastric, hepatocellular and head and neck carcinomas. Several potent and selective small-molecule inhibitors of anti-HGF/c-Met (PHA665752, SU11274, SU11271, SU11606 and Kirin (Kirin has not selectivity data)) [56,57] are reported.

8. Src

The Src family of non-receptor tyrosine kinases has been in the center of interest ever since kinases have been identified as potential therapeutic targets. The widely expressed (and closely related) Src and Yes kinases are particularly attractive targets for therapeutic intervention in cancer, having being implicated in the growth and dissemination of breast and colon cancer. A major difficulty in the development of Src inhibitors for clinical development was the problem of selectivity, in particular the need to avoid targeting the Src family kinase Lck, a critical mediator of T-cell development and function. Advances have been achieved by SUGEN/Pharmacia and Novartis in developing ATP-mimetics with approximately tenfold selectivity for Src over Lck. SKI-606, a 4-Anilino-3-quinolinecarbonitrile dual inhibitor of Src and Abl Kinases, is a potent antiproliferative agent against Chronic Myelogenous Leukemia. SKI-606 is in clinical Phase I [58]. AZD0530 is a highly selective, dual-specific, orally available, small molecule inhibitor of Src kinase and BCR-ABL. AZD0530 is in clinical Phase I for the treatment of cancer [59].

9. p38

The p38 mitogen-activated protein kinase (MAPK) plays a crucial role in regulating the production of proinflammatory cytokines. Inhibiting these kinases would have an important role in treating various inflammatory diseases like rheumatoid arthritis or asthma. Several compounds have been reported to inhibit p38 kinase in nanomolar range: SB202190 [60], SB203580 [61], SB-242235 [62], BIRB-796/Doramapimod [63], VX-702 and VX-745 [64]. VX-745 and doramapimod are in clinical development phase. The p38 MAP kinase inhibitor, SCIO-323 is in clinical Phase II. It is being developed against rheumatoid arthritis, cerebrovascular ischemia and diabetes mellitus [65]. SCIO-469 is another small-molecule p38 MAP kinase inhibitor under development by Scios Inc as a potential oral therapy for inflammatory disorders. PS540446 is a p38 inhibitor of Pharmacopeia and currently in clinical Phase I for the treatment of rheumatoid arthritis [66]. GlaxoSmithKline's p38 inhibitor, 8565533 is also in clinical Phase I for the treatment of rheumatoid arthritis and chronic obstructive pulmonary disease [67]. TAK-715 is a p38 inhibitor of Takeda, which demonstrated significant *in vitro* and *in vivo* activities and was advanced into clinical Phase II trials [68]. Ro3201195, a p38 inhibitor of Roche is in clinical Phase I for the treatment of rheumatoid arthritis [37].

Gill and co-workers discovered new p38 α MAP kinase inhibitors by using fragment-based high-throughput X-ray crystallography [69]. This kinase regulates the biosynthesis of cytokines TNF- α and IL-1 β . Increased production of these cytokines can lead to various inflammatory diseases such as rheumatoid arthritis, Crohn's disease and inflammatory bowel disease. Therefore, an orally active p38 α MAP kinase inhibitor would be of great therapeutic value. They used their fragment library to identify two molecular fragments as hits by using fragment-based high-throughput X-ray crystallography. These molecules were optimized, using structure-guided chemistry approaches to obtain novel, potent and selective p38 α MAPK inhibitors.

10. ERK1/2

The MAPK pathway controls the growth and survival of a broad spectrum of human tumors. The MAPK pathways are located downstream of many growth-factor receptors. There is an important link between the MAPK kinase (MEK)-ERK pathway and cell-cycle machinery. Overexpression and activation of this receptor are commonly detected in various cancers, and several lines of evidence indicate that overexpression and activation of ERK play an important part in progression of cancer. The central role of RAF and MEK in transmitting signals through the growth factors related Ras-MAPK pathway make these kinases promising targets of anticancer drugs [70]. PD166866 [71], SR-144528 [73] and conivaptan [74] inhibit MAPK in low nanomolar concentration. PD089828 [72] inhibits MAPK in micromolar concentration. MEK inhibitors are the first highly selective inhibitors of the MAPK pathway to enter the clinic. Several potent MEK inhibitors have reached preclinical development, like U0126 [75], PD98059 [76], L-783277 [77], L-167307 [78], and RWJ-68354 [79]. CI-1040 (PD184352) is an orally administered, selective small-molecule inhibitor of MEK. This agent significantly inhibited growth of the colon carcinoma cell lines both *in vitro* and *in vivo* models. In addition to impairing tumor cell proliferation, CI-1040 blocked cell motility, disrupted the cell-cell contact inhibition that is required for invasion, and induced dose-dependent arrest of G1. Importantly, antitumor activity was achieved without evidence of toxicity, and was correlated with a reduction in the levels of activated MAPK in excised tumors. CI-1040 went through Phase I evaluation in cancer patients [80]. This agent seems to be well tolerated. PD 0325901 is a follow up project of CI-1040 in Phase I clinical trial [8]. It has been shown that ARRY-142886 (AZD6244), a potent, selective MEK1/2 inhibitor currently in Phase I trials, has demonstrated sustained inhibition of ERK1/2 phosphorylation in tumor models [81].

11. JNK

It has been demonstrated that the c-Jun N-terminal kinase (JNK) pathway plays a role in regulating the cellular processes that are involved in Parkinson's disease; the inhibition of JNK with SP-600125, a specific inhibitor of JNK, is effective in MPTP Parkinson's disease model [12,82,83].

12. PKC

Protein kinase C (PKC) is a family of closely related serine and threonine kinases. Overactivation of some PKC

isozymes has been postulated to occur in several disease states, including diabetic complications. Selective inhibition of overactivated PKC isozymes may offer a unique therapeutic approach to disease states such as diabetic retinopathy. LY-333531/Ruboxistaurin is a staurosporine derivative in clinical Phase III [84-86]. LY317615 is a PKC- β 2 inhibitor with a potent antiangiogenic activity. Fine and co-workers presented the preliminary result of Phase II LY317615 clinical trial on 32 patients with recurrent malignant gliomas in 2004 that demonstrated the antitumoral activity of LY 317615 [87]. PKC-412 is a PKC inhibitor being in clinical Phase II for the treatment of cancer [88].

13. CDKs

Since years, cell cycle kinases, like the cyclin-dependent kinases (CDKs) have been regarded as attractive cancer targets. Cellular and xenograft models lead to the assumption that inhibition of some cell cycle and transcriptional CDKs might lead to an arrest of proliferating cells and even into apoptosis [89]. As a result, almost every large pharmaceutical company has been looking for ATP-competitors of the CDKs. UCN-01 of Kyowa Hakko Kogyo Co. Ltd. is an anticancer compound, which inhibits a couple of kinases such as CDKs, PKC and Checkpoint kinase 1. This compound is in clinical Phase II [90,91]. Flavopiridol is the first potent inhibitor of cyclin-dependent kinases (cdks) to reach clinical trial. The sequential combination of docetaxel and flavopiridol has been investigated in a Phase I trial in patients with advanced non-small cell lung cancer, and a randomized Phase II study is under way [92]. BMS-387032 is a novel cyclin-dependent kinase 2 inhibitor, currently in Phase I clinical trials for anticancer therapy [93]. Seliciclib (CYC-202 or R-roscovitine) is a potent CDK inhibitor currently undergoing phase II clinical testing in lung and B-cell malignancies [94].

Honma and co-workers applied rational drug design methods to develop selective CDK4 inhibitors [95]. CDKs play important roles in the regulation of the cell cycle [96]. Irregular activity of CDKs can often be detected in a variety of cancer cells. Several non-selective CDK inhibitors were known at that time, but the authors tried to design selective CDK4 inhibitors in order to cause cell cycle arrest in the G₁ phase, because G₁ arrest was thought to reduce the stress for healthy cells more than in other phases. This way, an inhibitor with an enlarged therapeutic window could be developed. The X-ray structure of CDK4 was not available in the PDB, therefore they had to build a homology model of the kinase. Since CDK2 shows high sequence homology with CDK4, they used the 3D X-ray structure of CDK2 as a template to build the homology model. Then they applied the LEGEND de novo design program [97] supplemented with their in-house developed program, SEEDS. SEEDS was used to shepherd the structure generation process to get drug-like and commercially available structures. As a result, they selected, purchased and screened a set of 382 compounds in a CDK4/cyclin D assay. From this molecule pool, they could obtain 18 hits (hit rate: 4.7 %) of four molecular classes. They selected the diarylurea scaffold for further investigations, because there were five potent inhibitors in this group. Applying preliminary SAR, they identified a compound with IC₅₀ of 0.10 μ M, which was selected as the lead compound

for further modifications. The lead compound was docked into the homology model of CDK4, and based on the proposed binding mode, structural modifications were carried out. By doing this, they were able to identify an even more potent inhibitor with IC₅₀ value of 0.042 μ M. Ultimately, they validated the proposed binding mode of the lead compound by comparing it to the X-ray structure of the complex of the obtained compound and CDK2.

14. Auroras

Another attractive cell cycle target is represented by the members of the Aurora family of kinases. Aurora kinase family was discovered in 1997 and closely linked to tumor genesis [98]. The Aurora kinases are overexpressed in a wide range of human tumors. Elevated expression of Aurora-A has been detected in over 50% of colorectal [99,100], ovarian [101] and gastric tumors [102], and in 94% of invasive duct adenocarcinomas of the breast [103]. Researchers from Vertex published the three-dimensional atomic structure of Aurora 2 kinase in 2002 [104], a key scientific advance that enabled the design and optimization of multiple classes of small molecule Aurora kinase inhibitors. VX-680 was advanced to preclinical development, following evaluation of the compound's activity in tumor cell lines and in animal models of tumor growth. In June 2005, Vertex and Merck initiated a Phase I study of VX-680 in hematological cancers. VX-680 might have activity in these malignancies not because it inhibits the Aurora kinases, but also because Flt-3 is an important off-target, which is potently inhibited with VX-680 [29,105]. Two clinical studies are also underway in patients with recurrent or non-responsive solid tumors, or cancers for which standard therapy does not currently exist [106]. A compound of Nerviano Medical Sciences, PHA-739358, that inhibits Aurora A, B and C kinases and causes significant tumor growth delay and regression in various xenograft animal models has been selected for further development and is currently being evaluated in a multicenter Phase I dose escalation studies [107]. Co-crystallization of one of the compounds of Nerviano with Aurora-A has provided useful additional information about the binding mode [108].

15. Akt/Protein Kinase B (PKB)

Breitenlechner and co-workers designed selective Akt/protein kinase B (PKB) inhibitors based on the crystal structure of PKA [109]. Overexpression of PKB can be found in prostate, breast and ovarian carcinomas [110-112], therefore it is an attractive target molecule. The authors used PKA as a surrogate kinase for PKB, because these kinases show 47% sequence homology in the catalytic kinase domain. The ATP binding sites of PKA and PKB differ only in three to seven residues (depending on the definitions). Azepine derivatives of the kinase inhibitor (-)-balanol were used as starting point of the design. Based upon the analysis of the crystal structures, the authors managed to develop three nanomolar inhibitors of PKB, which were selective versus PKA.

16. Syk

Syk kinase is an important target in inflammation and asthma. Mast cells play important roles in both early and late

phase allergic reactions. Syk kinase is involved in IgE signaling in mast cells, and it is a transducer of signaling through the Fcε receptor of mast cells. Syk inhibitors could prevent both early and late phase allergic reactions blocking mast cell responses to allergic stimuli. Syk tyrosine kinase is commonly expressed by normal breast epithelial cells, that loss of Syk expression is associated with the acquisition of a malignant breast tumor phenotype, and that Syk may directly act as a tumor suppressor, presumably by controlling cell division [113]. Rigel's clinical candidate, an intranasal inhibitor of Syk kinase R-112, completed a Phase II clinical trial in 2004 [114-116]. In December 2004, Rigel initiated a Phase I clinical trial of R-406 [117].

17. JAK

JAK kinases are important targets in immunological disorders and in leukemia. AG490 family of tyrosine kinases has been shown to be a potent inhibitor of JAK2 and to a lesser extent, JAK3 [118,119]. WHI-P97 is a Jak3 tyrosine kinase inhibitor of Wayne Hughes Institute against asthma [120]. The novel JAK3 inhibitor CP-690550 is a potent immunosuppressive agent, which is in Phase II clinical trials by Pfizer [121].

TARGET SELECTION AND VALIDATION

With a drug discovery focus on the target gene family of protein kinases, it is tried to raise synergies by the fact that these highly homologous enzymes comprise the largest family of the human genome. The individual family members, approximately 530 kinases [13,122] constitute a functional basis for basically every physiological process. Nearly all aspects of life involve cellular responses to extracellular stimulation. All cellular events are governed by signal transduction events that rely on highly coupled intracellular networks of specific protein-protein interactions, which are in turn functionally controlled by reversible phosphorylation reactions catalyzed by protein kinases. Consequently, kinases play a central role in propagation of signal transduction in every type of cell [123]. Not surprisingly, kinases are reported to be involved in a plethora of diseases. They have been found dysregulated in terms of expression levels or catalytic activity and also mutated leading to hyperactive or inactive mutants. One could argue that there is not a single therapeutic indication where protein kinases *per se* could be excluded as targets.

The common feature conserved throughout the entire protein kinase family is the catalytic domain with its associated catalytic center. Almost all protein kinases employ ATP as a co-substrate in order to transfer the γ -phosphate of ATP onto an acceptor-protein, -peptide or -lipid substrate [13]. The identical catalytic mechanism together with a high degree of sequence homology, identical protein folding topologies, and the common co-substrate ATP initially led to the assumption that protein kinases constitute a non-druggable family of protein targets. This belief dominated the majority of the pharmaceutical industry until the middle of the 1990's. This attitude drastically changed when Novartis began work on Bcr-Abl and successfully carried the corresponding inhibitor STI571 (CGP57148B, imatinib, since 2002 marketed as Gleevec™) through clinical

trials [33,124]. Simultaneously, SmithKline Beecham initiated work on p38 inhibitors, yielding the lead compound SB203580 [125]. Since then, both academic research and pharmaceutical industry laboratories started a real conquest into the world of protein kinases and their small-molecule inhibitors. Currently, R&D spending for inhibitor development is highest for protein kinase based research within the pharmaceutical and biotechnology industries [126].

Against all odds, the pharmaceutical industry began an intensive search for kinase inhibitors [126] and achieved the result of currently 4 launched kinase inhibitors and 53 compounds in the various stages of clinical trials [127]. The cumulated experience in kinase inhibitor drug discovery projects collected over the last 10 years teaches us that fairly selective ATP-site specific protein kinase inhibitors can be generated, despite all the functional, sequential, and structural similarities that dominate the catalytic domains of the protein kinase family [128]. Results from clinical use of kinase inhibitors of the first generation demonstrate that protein kinases are druggable enzymes. Accessibility of the ATP-binding pocket for small molecule binding amenable by subsequent chemical optimization has been a major concern due to the high level of sequential, structural, and functional similarity amongst the kinases [122]. In contrast, it turns out that the highly conserved ATP-binding site is an ideal playground for inhibitor design given the new technologies, which have become available to researchers, e.g. molecular inhibitor design supported by co-crystallization efforts, molecular modeling, in combination with broad panels of protein kinases available for selectivity profiling and fragment-based high-throughput x-ray crystallography [69]. These and similar technologies were available only late into the programs for the first generation inhibitors and could not contribute significantly. However, the success of kinase inhibitors such as imatinib, fasudil, gefitinib, and erlotinib demonstrates that even without the sophisticated technologies, a fair level of selectivity for (a) particular kinase(s) has been achieved, sufficient to avoid general toxicity after application to cell culture systems, animals, and humans.

Given the degree of homology of the individual kinase family members on one hand and the amenability of the ATP-binding site to drug design on the other hand, synergies can be defined for the design of next-generation kinase inhibitors. Selected derivatives of an identical chemical core structure frequently recognize distinct kinases with different associated therapeutic relevance. This has been shown with the imatinib-like aminopyrimidines, in which the addition of a single methyl group to the aminopyrimidine core led to an unexpected change in selectivity from PKC to Abl [124]. It has become clear that the specificity and selectivity for a target is a function of the derivatization pattern of an underlying core structure. Once this core structure and its derivatives meet the characteristics of drug-likeness, it might represent the starting point for more than just one development candidate in various therapeutic areas. Subsequent optimization chemistries use previously made compounds and benefits from the medicinal chemistry strategies in the first therapeutic program [129,130]. This strategy might be also described as target hopping and represents an intelligent way of recycling pharmacologically beneficial medicinal

chemistry concepts, the critical parameter being the available series of molecules with associated profiles, as opposed to a proper genetically validated target [131].

In summary, large efforts are being made in the field of kinase inhibitor research and development [126]. The past ten years of effort in this field have altered our perception of the importance of kinase inhibitors to the pharmaceutical industries. This field is now vigorously populated and the availability of new technologies, especially from the field of chemogenomics and chemoproteomics augur a tremendous potential for the next generations of kinase inhibitors.

In trying to learn from the field of kinase inhibitor drug discovery, we try to follow an opportunistic approach and repeatedly exploit the common features of all protein kinases for the target selection and validation processes. Just around the new millennium, the -omes and -omics hype started to sweep over industrial research providing companies endless lists of putative targets, unfortunately devoid of any solid validation and without associated drug discovery approaches. The expected benefits from the -omes and -omics initiatives for drug discovery and development have not yet emerged within the pharmaceutical and biotech industry [132]. Genomics typically provided a list of dysregulated genes. From the dysregulated message to a drug that represents a process, which takes typically 12 to 15 years [122,124]. The validation of a target through biological means requires approximately 8 years in average and is regarded as a key activity in targeted drug discovery. During the target validation period, a suite of technologies is typically exploited to gain confidence in a particular target. These technologies include gene knockout studies in mice, mode-of-action investigations in cellular and animal models, siRNA technologies, etc. All of these technologies focus on the entire kinase gene or gene product and none really focuses on the kinase activity. Nevertheless, inhibition of the kinase activity is the best that can be achieved through the application of kinase inhibitors to cellular and animal models as well as to patients. There is evidence available on the independent roles of regulatory domains of protein kinases and on the impact of protein kinase molecules on binding partners in the formation of larger complexes, as it has been recently described for the Aurora kinases [133]. Given the discrepancy of employing tools for target validation, which affect an entire protein rather than just its activity, and the subsequent generation of pharmacological agents, which modulate the activity of kinase rather than affecting the whole kinase protein, a strategy has been chosen to validate potentially novel kinase targets with tools which are as close as possible to the final product, a pharmacologically active agent (see also above). This strategy is called chemical validation and relies on a representative collection of proven kinase inhibitors, i.e. a kinase-biased compound library [3,122]. In that setting, a novel kinase target of interest is screened against this collection and the hits from one or more chemical series are determined. Subsequently, these hits are employed in a cellular model combined with a direct cellular kinase assay. Provided that the hits show activity in the cell-free and in both cellular assays, the kinase of interest is considered validated and the tool inhibitors have already proven its druggability [122].

During the subsequent compound optimization efforts, the major drivers are to conserve the properties of a privileged structure and to ensure a sufficient level of selectivity of the optimized inhibitors in a panel of more than 50 different human protein kinases. Two important technologies were established to support this process:

1. Inhibitor-insensitive protein kinase mutants represent a tool for the validation of protein kinase targets in cellular disease models.
2. A kinase inhibitor-based affinity chromatography method (KinaTor™) is an ultimate selectivity-profiling tool, which provides a snapshot of the pharmacologically relevant environment for a particular kinase inhibitor of interest.

These two technologies are being described in the following two sections.

1. Inhibitor-insensitive kinase mutants

Chemistry-based validation technologies rely on combining orthogonal chemistry and genetics (chemical genetics) [122]. The analog-sensitive kinase alleles (ASKAs) use the fundamental approach of modifying first a small-molecule ligand (to render it “orthogonal”), followed by changing protein structure in a complementary way to accept finally the orthogonal ligand. It can be understood as a classical “lock and key” approach. Importantly, the mutation to the protein must only affect the binding to the orthogonal compound and not otherwise modify protein function [134]. The technology relies on the discovery of ASKAs (the “lock”) and corresponding small molecule analog compounds (the “key”) that specifically modulate ASKA activity [134]. All kinases have a bulky amino acid residue at a conserved position in the ATP-binding pocket, the so-called “gatekeeper”. Mutation of the gatekeeper to an alanine or glycine creates access to the deep hydrophobic pocket. Importantly, this mutation is silent in terms of kinase function and activity. Subsequently, potent inhibitors such as naphthyl-substituted PP1 analogs have been designed to fit only into the engineered kinases. Orthogonal kinase inhibitors like appropriately derivatized PP1-analogs are highly potent, cell permeable and have excellent bioavailability and low toxicity in mice. Thus, a specific kinase can be validated as a drug target by treating ASKAs in cells or whole animals with the orthogonal inhibitor and studying the genomic, proteomic, cellular, physiological and/or phenotypic consequences of such inhibition [134].

In addition, ASKA transgenic mice should provide crucial information regarding the therapeutic index. ASKA-based *in vivo* studies will be able to elaborate on mechanism and target-based efficacy and toxicity for most protein kinases [134].

Inhibitor-insensitive kinase mutants (IHKMs) are a conceptual reverse of the ASKAs. The first inhibitor-insensitive mutant was p38 α ^{T106M,H107P,L108F}, a mutant of p38 α , resistant against the pyridyl imidazole inhibitor, SB203580 [135], an ATP-competitive inhibitor of p38 with known anti-inflammatory properties [125,136]. Co-crystallization of p38 α with pyridyl imidazoles [137] identified Thr-106, the “gatekeeper” as critical for interaction. As predicted,

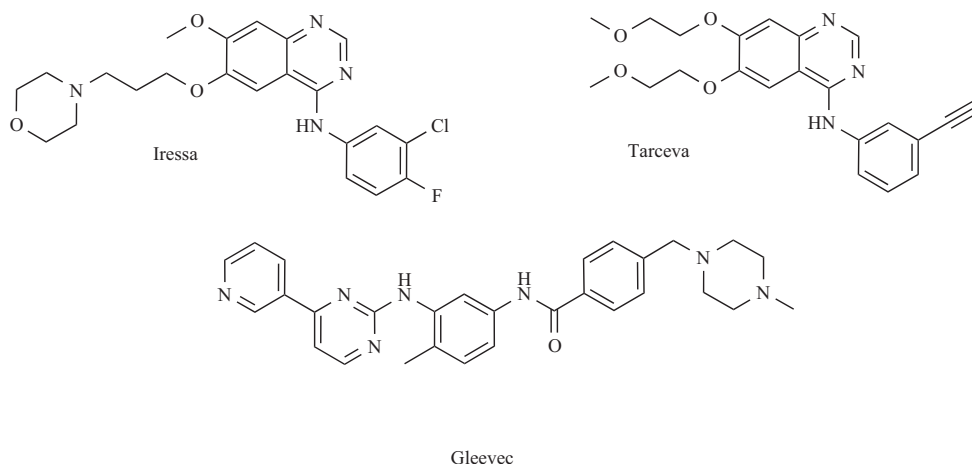


Fig. (7). Structure of approved kinase inhibitors.

Understanding the binding mode of kinase inhibitors can help researchers to develop additional selective inhibitors for other kinases as well.

LIGAND-BASED DRUG DESIGN

When the 3D structure of the target molecule is not known e.g. for many membrane bound proteins, which are extremely difficult to crystallize, one can mobilize the tools of ligand-based design.

Active Analog Approach (AAA)

The assumption of AAA is that all compounds that display similar activity profiles are able to adopt similar conformation [175]. The first step is searching the conformational space of a highly active compound and map its interatomic distances. The next step is searching the conformational space of other compounds to find their bioactive conformations based on the mapped interatomic distances of the first molecule. Then calculate molecular volumes of the “bioactive conformations”, superpose them and use regression analysis of the volumes to derive relationship to biological activity [176].

3D QSAR (QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS)

3D QSAR models relate the biological activity of ligands to three-dimensional fields surrounding the compounds.

One of the most popular methods is Comparative Molecular Field Analysis [177] (CoMFA). The basic idea of CoMFA is that the intermolecular interaction energy between the receptor and the ligand correlates with the steric, electrostatic, hydrogen bonding and hydrophobic fields of the ligands. These interaction energies are calculated between the ligand and the so-called hypothetic probe atoms, which are placed on certain number of grid points. The results of the calculations can be arranged in a matrix, in which every row represents a molecule and every column represents a calculated interaction energy value in a certain grid point; one additional column contains the biological activity data. These calculations generally generate thousands

of columns (descriptors), which strongly exceed the number of rows (molecules). The QSAR model is generated by a PLS (Partial Least Squares) analysis of the data matrix. Validated QSAR equations derived from CoMFA models can be used to predict the biological activity of new molecules.

The most difficult task in a CoMFA analysis is the superimposition of the molecules before the actual calculations. Small differences in the starting superimposed structures can lead to dramatic changes in the final QSAR equation. Finding the bioactive conformation of the molecules is another challenging issue. Several other 3D-QSAR methods were developed after introducing CoMFA. Examples of 3D-QSAR techniques are:

Comparative Molecular Similarity Analysis [178] (CoMSIA) can be viewed as an extension of the CoMFA methodology. It is based on the same assumption as CoMFA: changes in binding affinities of ligands are related to changes in descriptors, represented by various fields. In CoMSIA, both steric and electrostatic features, hydrogen bond donor, hydrogen bond acceptor and hydrophobic fields are considered. Potential energy functions are smoothed with Gaussian function. One of the major advantages of CoMSIA over CoMFA is that of less sensitivity to different ligand alignment.

Comparative Molecular Moment Analysis [179] (CoMMA) enables 3D-QSAR analysis without the requirement of molecular superimposition using descriptors such as moments of inertia, dipole and quadrupole moments. One drawback can be in CoMMA is that the value of these descriptors equals infinity for symmetric molecules whose dipole moment is zero [180].

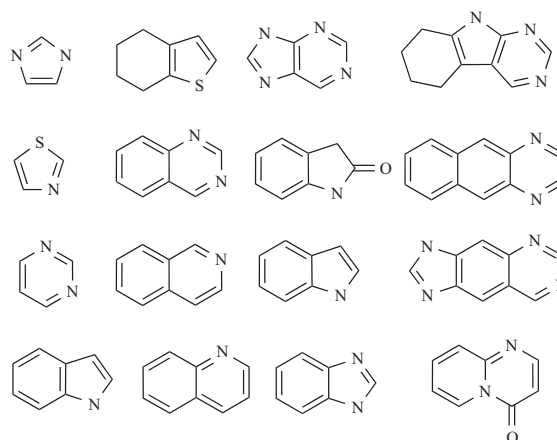
4D-QSAR [181] allows to use multiple conformation, orientation and protonation state of the ligands as well as simulation of local induced-fit phenomena. This technique can choose the bioactive conformation more precisely as each ligand molecule can be represented by an ensemble of conformations, orientations, and protonation states [182].

Table 1. The IC₅₀s for Not Depicted Individual Compounds Coming from the Respective Core Structures from the NCL on Selected Target Kinases

Target	IC ₅₀ (μmol)	Core Nr
AKT	0.13606	104
Aurora A	0.028978	4
Aurora B	1.30216	102
CDK1/CycB	0.01258	4
CDK2/CycA	0.003074	4
CDK3/CycE	0.1671	4
CDK4/CycD1	0.017501	105
CDK5/p35	0.008514	32
CDK6/CycD3	0.03838	105
CDK7/CycH	0.23074	4
CDK9/CycT1	0.00151	4
CK1-alpha	0.092072	103
c-Kit	11.754944	86
EGFR	0.000199	13
GSK-3beta	0.615749	4
Kit human	0.825977	108
p38a	0.03964	30
PAK1	9.612499	4
PKA	0.39313	1
PknG	0.005011	103
RICK	0.004199	30
RIP	0.280426	33
ROCK2	0.042713	1
SRPK1	0.03626	9
SRPK2	0.00211	9
UL97	0.012428	106
v-Abl	0.087099	4

[191,192] provided quite many inhibitory core structures. Unfortunately, not many peptide inhibitors have been found. On the other hand, these peptides had problems with bioavailability/ADMET properties. A possible solution was to develop more drug-like peptidomimetic compounds. Several such compounds including some tyrphostins, tyrosine derivatives, or acyl-hydrazones reached early phase preclinical development but were dropped due to “lack of selectivity” i.e. acted on more kinases at the same time [193].

Development of ATP like or ATP binding site directed inhibitors was opposed for a long time owing to the concern about possible lack of selectivity and side effects *via* various

**Fig. (9).** Selected core structures from the NCL.

ATP binding proteins. When 4-phenylamino-quinazolines were found to have excellent and quite selective EGF-R inhibitory activity [194], it boosted the research in this field and almost one hundred small heterocyclic cores were developed against various kinases. Although the known 530 kinases have highly conserved ATP binding site, several high affinity and selective inhibitors have been discovered and are under clinical evaluation [195].

Classifying the known kinase inhibitors by chemical structures, there are several exempts from the ATP-like structures: e.g. the very first inhibitors, oxanyl-hydrazones [193,196] showed only negligible similarity with ATP and that time the binding mode could not be explained. These compounds showed some common steric and electronic properties with some small peptide substrates, therefore these compounds were treated like substrate binding site inhibitors. Recently, X-ray diffraction experiments with co-crystallized inhibitors like BCR-ABL inhibitory Gleevec [154], p38 MAPK inhibitory BIRB-796 [63] proved allosteric type binding mode for these structures. Applying special “theoretical interactive surface” pharmacophore modeling on pre-filtered “non-ATP-like” compounds, the main structural features needed for allosteric binding could be identified. Appropriate molecular shape, size and electronic properties in suitable positions can afford selective and high affinity binding at allosteric binding sites of kinases. For ATP-like binding, size is a principal property: the molecule must fit into the ATP binding pocket. The most potent ATP inhibitors known can be fitted into a cube of approximately 9-10 angstroms. In the case of several allosteric inhibitory structures, the longest distance has been found that in the molecule between two suitable electronegative (or partially positively charged) groups is around 13.6-14.5 angstroms. A few hundreds of allosteric inhibitor analogs had been developed around proven allosteric-type core structures to find that in certain families of allosteric inhibitors, shortening or lengthening of this characteristic distance significantly decreases kinase inhibitory activity.

Therefore, it is probable that in these cases, two-point binding mode has to be considered, and often the structure of the “middle part” of the molecule does not significantly

solubility, permeability and interactions with transporter proteins. Three main methods can be used to measure the human intestinal absorption: bioavailability, percentage of urinary excretion of drug-related material following oral administration, and the ratio of cumulative urinary excretion of drug-related material following oral and intravenous administration [214].

Human Oral Bioavailability

Oral bioavailability is the percentage of a compound that reaches systematic circulation in unaltered form after oral administration. It consists of two processes: absorption and liver first-pass metabolism and can be viewed as the superposition of these two processes.

Blood-Brain Barrier (logBB)

Penetration through this barrier is important not only for drugs, which act on central nervous system (CNS), but also for drugs with peripheral target, because of the possible CNS side effects. BBB penetration is often quantified by logBB, the logarithm of the ratio of steady-state concentration of drug in brain to that in blood [215].

MDR

Multi drug resistance is one of the key issues in antitumor therapy. The P-glycoprotein (Pgp, MDR1) mediated multi drug resistance was the first discovered mechanism in multi drug resistance. There are two other ATP-binding cassette transporters, which participate in the multi drug resistance of tumors: the multi drug resistance protein 1 (MRP1), and the mitoxantrone resistance protein (MXR/BCRP) MDR1 transporter recognizes various hydrophobic and anionic drugs and drug conjugates [216].

Mutagenicity

The Ames test is widely used test to detect possible chemical carcinogens. It is based on mutagenicity in *Salmonella typhimurium* bacteria. In fact, some substances that cause cancer in laboratory animals (dioxin, for example) do not give a positive Ames test and vice-versa.

Qualitative and quantitative filters were used for early ADMET filtering. Lipinski's rule of five is an example of qualitative filter [217]. Quantitative filters are based on in-house developed QSPR (Quantitative Structure Property

Relationship) models (Table 2) e.g., LogP, LogS_w, human intestinal absorption [218], human oral bioavailability [218], blood brain barrier [218], and MDR1 Calcein assay model [219]. These in silico methods are used as predictors for the ADMET properties and they influence compound selection and design.

MASTERKEY CONCEPT APPLIED TO KINASE INHIBITORS

Apart from screening efforts of either diverse chemical libraries or target family-biased compound collections within the context of lead finding, the protein family of kinases with its more than 500 distinct members qualifies for the application of target family-biased masterkey concept, which is based on the utilisation of tailor-made privileged structures [129]. Rather than following the classical approach in which a single target protein, e.g. a protein kinase is tackled at a time within a distinct disease area, the masterkey concept offers the opportunity to process multiple related members from a target family simultaneously across numerous therapeutic areas. The masterkey concept is a chemogenomics platform, since it allows to deal with a large number of potential protein targets with increased efficiency in lead generation, delivering target-specific molecules amenable to parallel optimization towards progressible pre-clinical candidates. This opportunity of systematisation of drug discovery strategies follows from the acceptance that biology as well as chemistry knowledge gained from one target can be transferred to "adjacent" targets from the same gene family. Even though systematization might require significant commitment of time and resources, it allows enormous efficiencies to be gained through economies of scale, provided that the target families are of significant size, richness, and diversity in therapeutic value, which is undoubtedly given for the protein kinase family. Most importantly, an accumulation of target class-specific know-how is created over time whereby past experiences allow rapid attack on new members of the target cluster of interest.

The core elements of the kinase-biased masterkey concept are so-called privileged structures that emerge from a sophisticated molecular design and optimization process that encodes for a target family-wide structural commonality in ligand binding. The combination of a kinase family-wide

Table 2. QSPR Models for ADME Prediction of Compounds

Summary of ADME models ADME parameter	No. of molecules	Final model type and number of descriptors	External validation Q2 (No. of molecules in the external validation set)
logP	625	PLS, 37 descriptors	0.856 (300)
logS _w	1381	ANN, 39 descriptors	0.863 (81)
logBB	99	PLS, 46 descriptors	0.712 (19)
HIA%	98	ANN, 27 descriptors	0.627 (18)
Human Oral Bioavailability	276	ANN, 39 descriptors	0.638 (76)
MDR1 (Calcein)	101	PLS, 18 descriptors	0.471 (25)

in ATP-binding, or alternatively, in allosteric inhibition modes

- generation of lead structures and pre-clinical candidates for selected kinases by specific derivatization of a once generated masterkey.
- launch into the entire kinase family by design and synthesis of kinase-biased compound libraries templated on a privileged structure by means of automated synthesis.

APPLICATION OF *KINATOR*TM FOR THE 2ND GENERATION KINASE INHIBITORS

The kinase family-biased masterkey concept is intentionally designed to rigorously exploit the kinase-intrinsic potential for lead discovery and optimization in that once established inhibitor concepts are repeatedly applied for various representatives of the family without compromising the required selectivity. In this context, the *KinaTor*TM technology emerges as a strategic tool for successful implementation of the corresponding research structure. The strategic relevance of the *KinaTor*TM technology in the area of lead finding and optimization is twofold. Firstly, it provides the ultimate selectivity panel for any ongoing optimization program on distinct kinase targets, and secondly, more important in the context of privileged structure-based inhibitor design, it identifies novel and unexpected kinase targets for an investigated compound series templated on a privileged structure.

An immediate extrapolation of once established chemistries, structure-activity, and structure-property relationships

for the primary target onto a newly identified off-target with proven validation state will give multiple opportunities for head-starts, bypassing numerous iterations in early discovery chemistry. It is the conceptual combination of the proteomics tool *KinaTor*TM with an intelligent and modern medicinal chemistry concept that will elicit a cascade of drug discovery and development projects throughout the kinase family across any therapeutic boundaries.

Summarizing, the major gain in lead discovery and optimization efficiency is achieved by repeatedly using established and steadily growing knowledge on a target family in all involved areas of biology and chemistry. Optimized technical procedures for e.g. protein production, purification, assay development, and screening can be used for numerous members from a target family of interest. A considerable percentage of a compound collection with built-in target family bias, preferentially based on tailor-made privileged structures [129], will show activity against distinct members of the target enzyme or receptor cluster, with emerging structure-activity and structure-selectivity relationships, respectively. Multiple use of the target family-directed biology and chemistry resources is definitely more efficient than starting from scratch for each new discovery project, thus accelerating lead finding and optimization campaigns considerably.

In the chemogenomics approach, increased productivity and shorter timelines are achieved by a strict re-use of well-designed chemistry concepts, based on the mutual overlap between privileged structure based pharmacophore space and the structural and physicochemical requirements of the

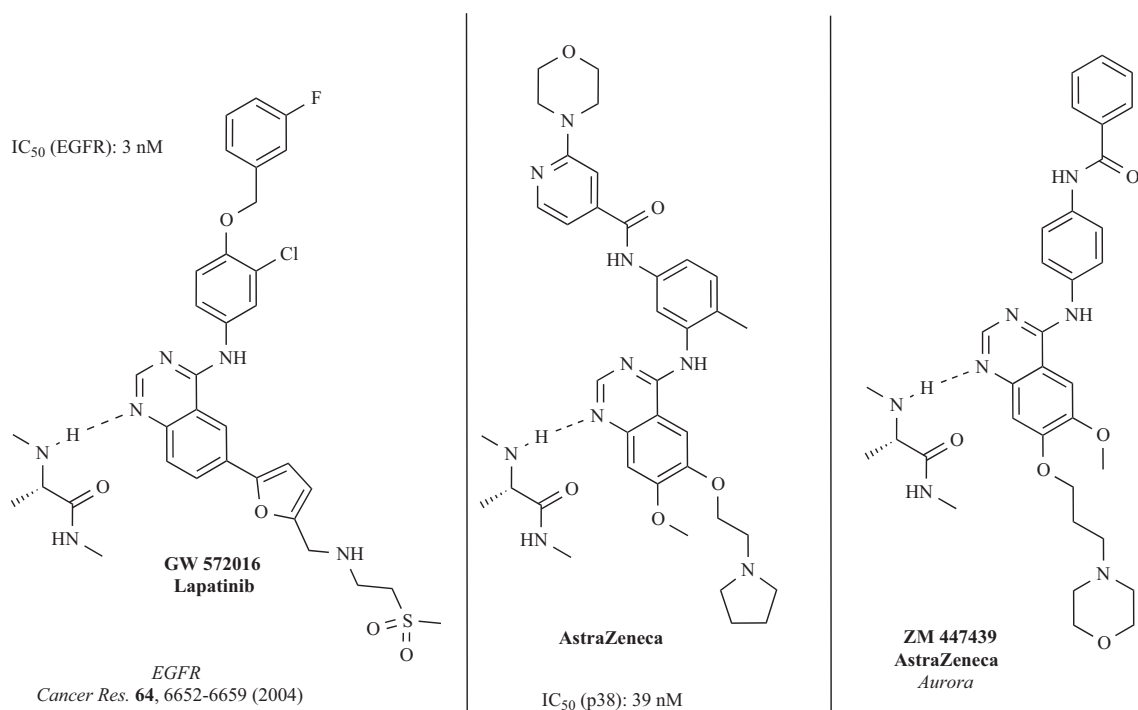


Fig. (18).

ligand binding site of target family members. This overlap is the privileged structure-encoded information content that is optimized for complementarity towards the target family-wide commonality in molecular recognition. A clear structural understanding of this relation is required to leverage the intrinsic potential of a target family approach with associated multiple therapeutic scopes.

CONCLUSION

Signal transduction therapy has become a very important area of drug research since most of our diseases are related to intra or intercellular signaling disorders, in other words to systemic regulatory disorders. Deregulated protein kinase activity can result from genetic alterations acquired early in tumorigenesis and remains an essential aspect of tumor cell physiology throughout disease progression.

Kinases are probably the most important signaling enzymes, which represent about 20% of the druggable genome. Currently, more than 70 kinases are known which are validated target molecules for signal transduction therapy. Therefore, in the drug discovery process, the selection of relevant, susceptible protein kinase targets, together with searches for lead drug candidates and optimization of these lead molecules for potency, selectivity and pharmacological properties have become a crucial approach.

However, we have to be aware of the fact that in the protein kinase area, it will be very much unlikely to find a unique compound that specifically inhibits a selected single protein kinase, leaving the other more than 500 protein kinases unaffected. On the other hand, with sufficient structural biology information about the binding mode and possible interactive sites and related pharmacophore modeling, it may be possible to develop protein kinase inhibitors which can have a "reasonable side effect profile." With the recently developed kinator technology, the selectivity profile of the kinase inhibitors can be thoroughly analyzed.

To improve kinase selectivity of certain inhibitors - for example in the area of neurodegenerative disease related kinase targets - developing compounds that bind preferentially to the inactive forms of protein kinases, or which prevent one protein kinase from activating another, can represent a promising approach. Detailed understanding of the catalytic and regulatory properties of kinases can contribute significantly to develop novel kinase inhibitors including more specific allosteric inhibitors.

However, the currently developed kinase inhibitors are mostly ATP-competitive small-molecule inhibitors that block the enzymatic activity of kinases and thereby interfere with phosphorylation of cellular substrates. The therapeutic inactivation of an essential protein kinase creates selective pressures, for tumor cells to evolve a variety of routes to resistance. These include producing a drug-resistant variant of the targeted protein, substituting its cellular function by upregulating alternate pathways, and increasing the expression and function of transporters involved in drug efflux.

A general drawback of target-specific monotherapy therefore derives from the fact that a single genetic alteration conferring target resistance to an individual tumor cell can eventually lead to relapse. The ability of kinases to mutate in response to the selective pressure created by drug treatment provides a strong rationale for hitting more than one essential target at the same time in the tumor cells [27]. Multi-targeted therapy can be achieved with either a combination of medicines or single 'promiscuous' drugs that act on a set of disease-relevant proteins. Protein kinases, which share a relatively conserved ATP-binding site, are amenable to the latter concept of targeted poly-pharmacology.

The multiple targeted compounds can be very unselective; this might interfere with normal cellular function and result in dose-limiting toxicity. To keep potential adverse side effects to a minimum, compounds must ideally possess a multi-target selectivity that is restricted to cancer-relevant protein kinases and must be ineffective at least against the so called untouchable kinases. This challenge might be addressed using proteomic techniques, which make use of immobilized kinase inhibitors for the affinity purification of cellular drug targets followed by sensitive mass spectrometry for subsequent protein identification.

Therefore, mechanistic and structural insights into the molecular aspects of drug-target resistance provide a rationale for the selection and design of back-up compounds for drug development that show potent activity against mutant kinase alleles and might also be generally less susceptible to resistance formation.

It is essential to expand the definition of a disease-relevant target to include the whole range of functional mutant phenotypes. In this context, it might be conceivable that a set of distinct small-molecule inhibitors with complementary activities towards desensitized mutant alleles ensures effective inhibition of any resistant kinase variants that possibly emerge during targeted therapy. In this scenario, resistance formation against one targeted drug could always be therapeutically addressed with an alternative drug for the same cellular target. Moreover, the simultaneous inhibition of several cellular targets by poly-pharmacological intervention might have even greater potential in preventing the emergence of drug resistance in human malignancies.

Potent multi-target inhibitors with a reasonable selectivity profile can be developed by rational drug design where even possible gate-keeper or other resistance producing mutations can be taken into account.

The challenge for rational drug design is to develop such multi-target inhibitors by recognizing differences and similarities in the topology, chemical and electrostatic environment and specific binding modes of different kinases. Kinases that are in the same family have high amino acid homologies and hence similar active site topologies. Therefore, structure-based drug design (SBDD) utilizing X-ray crystallography and molecular modeling subsequent to focused library screening, provides the best approach to obtain selective kinase inhibitors. This allows rational design of small molecules that have a high degree of complementarities to the target active site.

In the ligand-based design approach, the Nested Chemical Library TM (NCL) technology can be used to generate extended pharmacophore models.

Nevertheless, 3D structure-based drug design will continue to provide a key strategy to generate and/or optimize promising lead compounds, and it is expected that future protein kinase drug discovery will exploit a variety of high-powered in silico tools to computationally visualize, analyze, and probe the molecular properties and interactions of novel small-molecule inhibitors with their desired therapeutic target(s). Integration of such in silico tools (e.g., molecular modeling and docking approaches and virtual screening) with structural biology (e.g., X-ray and/or NMR studies), with sophisticated medicinal chemistry, chemoinformatics, and bioinformatics, provides great possibility for developing novel potent kinase inhibitory drugs.

It is believed that the above described rational drug design strategies against validated kinase targets represent a very efficacious approach for further very important results in signal transduction therapy of various diseases.

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