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European Journal of Cancer Vol. 38 Suppl. 5 (2002) S19–S27

European Journal of Cancer

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Imatinib: a selective tyrosine kinase inhibitor

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Abstract

The understanding of the pathophysiology of a large number of cancer types provides a strategy to target cancer cells with minimal effect on normal cells. Protein phosphorylation and dephosphorylation play a pivotal role in intracellular signaling; to regulate signal transduction pathways, there are approximately 700 protein kinases and 100 protein phosphatases encoded within the human genome. In cancer, as well as in other proliferative diseases, unregulated cell proliferation, differentiation and survival frequently results from abnormal protein phosphorylation. Although it is often possible to identify a single kinase that plays a pivotal role in a given disease, the development of drugs based upon protein kinase inhibition has been hampered by unacceptable side effects resulting from a lack of target selectivity. With the growing understanding of the molecular biology of protein tyrosine kinases and the use of structural information, the design of potential drugs directed towards the bind adenosine triphosphate (ATP)-binding site of a single target has become possible. These advances have transferred emphasis away from the identification of potent kinase inhibitors and more towards issues of target selectivity, cellular efficacy, therapeutic effectiveness and tolerability. In this paper, the relationship between molecular biology and drug discovery methods, as utilized for the identification of anticancer drugs, will be illustrated.

Keywords: Imatinib; Protein kinase inhibition; Targeted therapy; Intracellular signaling

1. Introduction

The long-sought goal of treating cancer by eliminating only neoplastic cells, with little or no effect on normal cells, has until recently been elusive. Cancer is not one disease but more than 200 and, although many of these share mechanisms of carcinogenesis across tumor types, different neoplasms have widely varying pathophysiologies and underlying biochemistries. To exploit these differences, molecular biology and genetics have been used to identify and characterize components of the mitogenic signaling pathway of normal and neoplastic cells, thereby providing much insight into the biochemistry of many cancers. From this understanding, together with epidemiological evidence, protein kinases have been implicated as being crucial to the generation and maintenance of the transformed phenotype in many types of tumors [1]. Therefore, a key to increasing the responsiveness of a particular tumor type is to phenotype the neoplasm according to the expression of a particular target and then to direct therapy against that target. With such an approach, coupled with the ability of researchers to design tyrosine kinase inhibitors that are both effective and suitable for chronic therapy, we are becoming increasingly successful in specifically targeting cancer cells.

2. Target profile

Protein tyrosine kinases are part of a family of enzymes that bind adenosine triphosphate (ATP) and catalyse the transfer of the γ-phosphate to the hydroxy group of a tyrosine residue on a protein. These cytosolic phosphorylated

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sites can then serve as binding sites for other substrates, which in turn may be phosphorylated as part of a signal transduction cascade. The kinases themselves are usually activated either by phosphorylation or, in the case of receptor protein tyrosine kinases, by the interaction of a protein with a receptor located within the extracellular domain of the kinase. The drug discovery process to find a tyrosine kinase inhibitor that specifically blocks a deregulated signal transduction pathway is not trivial. Because of the difficulties associated with the competitive inhibition of protein–protein interactions by low-molecular-weight compounds [2], inhibition of either ligand or substrate binding is unattractive, and modulating the activity of kinases by targeting their catalytic site with ATP-competitive inhibitors is the most promising approach for drug intervention. However, the human genome encodes up to 800 serine/threonine and tyrosine kinases [3], all of which bind ATP in a highly conserved manner within their catalytic domains. Therefore, finding a drug that specifically targets the ATP-binding site of a single kinase is a challenging task. Consequently, we are currently satisfied with molecules that possess an inhibitory profile whereby they inhibit the target kinase with sufficient selectivity towards other often closely related kinases, to give an acceptable side-effect profile. (Although this discussion is limited to low-molecular-weight compounds, inhibiting the activity of receptor tyrosine kinases with antibodies, such as the anti-vascular endothelial growth factor (VEGF) neutralizing monoclonal antibody bevacizumab, is also a viable approach [4]).

3. Drug discovery strategies for tyrosine kinase inhibitors

The drug discovery strategy for tyrosine kinase inhibitors, like that for many drug targets, often commences with the high-throughput screening of compound archives, followed by lead optimization for potency, selectivity and biopharmaceutical properties. Unfortunately, in the absence of structural biology input to help drive this process, it is often a hit-and-miss, time-consuming affair, and tyrosine kinases are no exception.

ATP binds to protein kinases within a deep cleft formed between two lobes of the folded protein and the interconnecting hinge region (Fig. 1). The triphosphate group is constrained by a glycine-rich loop and bound by a conserved array of basic amino-acid residues, which, to-

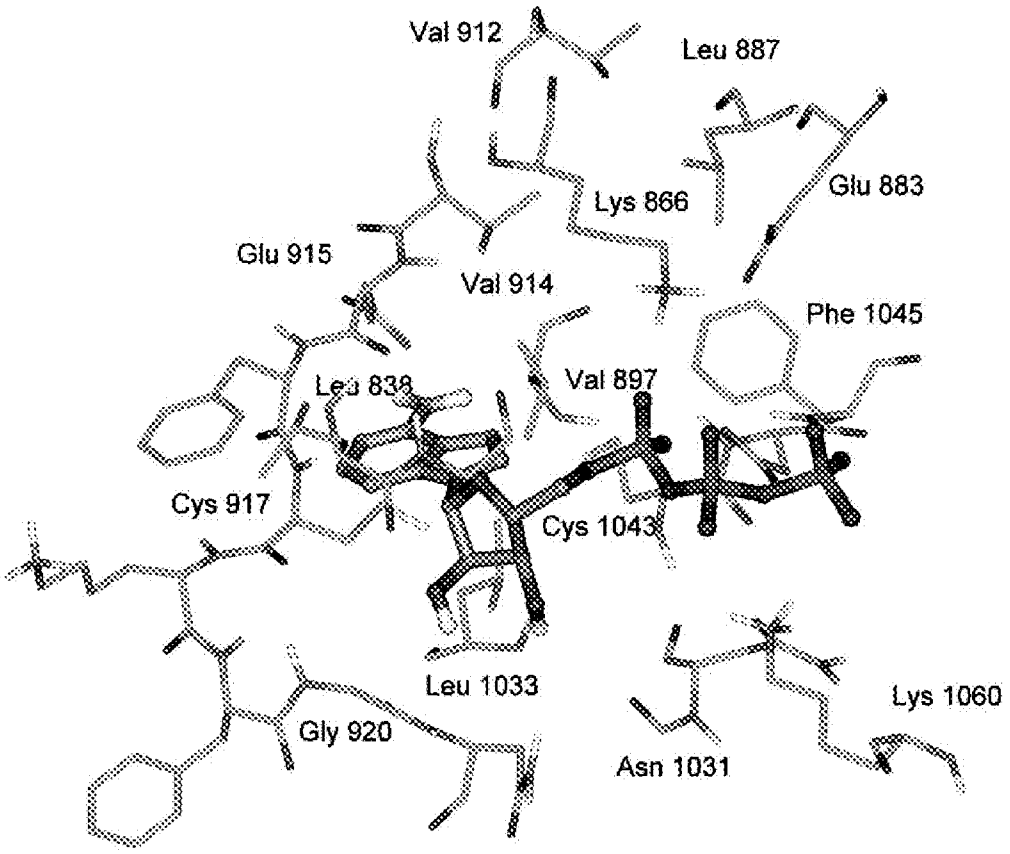

Fig. 1. Representation of a homology model of ATP liganded to VEGFR-2, based upon the ATP-FGFR-1 crystal structure [5]. The adenine ring is sandwiched between Ala864 (not shown) and Leu1033 of the N- and C-terminal lobes of the protein, while N-1 and N-7 interact with the hinge region via bidentate H-bond interactions with the backbone NH of Cys917 and carbonyl of Glu915, respectively.

gether with an invariant aspartic acid residue (part of the DFG triad) that deprotonates the phosphoacceptor hydroxy group, are involved in the catalytic process. The adenosine binding interactions are less conserved, and apart from a bidentate donor-acceptor H-bonding motif involving the N-1 of the adenine ring and its N-7 amino group with the backbone NH and carbonyl groups of residues in the hinge region, the interactions with the nucleoside are of a lipophilic/van der Waals nature. Because highly polar molecules would be required to target the triphosphate/catalytic site, which would have poor oral absorption, cellular permeability and pharmacokinetic properties, almost all ATP-competitive kinase inhibitors target the adenosine recognition motif of the ATP-binding site. However, while the ATP-binding site is highly conserved throughout kinases, the architecture of these proteins in the regions proximal to the ATP-binding pocket does afford some key diversity. The case of the tyrosine kinase inhibitor imatinib (Glivec®, formerly STI571, Novartis Pharma AG, Basel, Switzerland) provides a good example to illustrate these points.

3.1. Discovery of imatinib as an inhibitor of Bcr-Abl

In chronic myelogenous leukemia (CML), a reciprocal balanced chromosomal translocation in hematopoietic stem cells (HSCs) produces the BCR-ABL hybrid gene, which encodes the oncogenic Bcr-Abl fusion protein. The ABL gene encodes the tightly regulated non-receptor protein tyrosine kinase cAbl, which plays a fundamental role in regulating cell proliferation, adherence and apoptosis. In contrast, the BCR-ABL fusion gene encodes a constitutively activated kinase. This transforms HSCs to produce a phenotype exhibiting deregulated clonal proliferation, reduced capacity to adhere to the bone marrow stroma and a reduced apoptotic response to mutagenic stimuli, which enable them to accumulate progressively more malignant transformations. The resulting granulocytes fail to develop into mature lymphocytes and are released into the circulation, leading to a deficiency in the mature cells and an increased susceptibility to infection. This etiology renders Bcr-Abl as a highly attractive target for drug intervention in CML. Unlike the membrane-bound receptor tyrosine kinases, such as those of the platelet-derived growth factor receptor (PDGFR) family, Bcr-Abl is a cytosolic kinase that is constitutively active and does not depend upon ligand binding and subsequent receptor dimerization for activation. Consequently, Bcr-Abl activity cannot be modulated by means of receptor-based inhibitors. Furthermore, since Bcr-Abl phosphorylates a large number of substrates, thereby upregulating several intracellular signaling pathways (Fig. 2), the most obvious approach to blocking the activation of this enzyme cascade is by means of an ATP-competitive inhibitor of the Bcr-Abl kinase activity.

The value of large, diverse compound archives was illustrated when screening at Novartis resulted in the identification of the pyrimidine *A* as a structurally attractive lead molecule for optimization against cAbl (Fig. 3, Table 1) [6]. Structure-activity studies led to *N*-acyl and *N*-aroyl amide derivatives, such as *B* in which replacement of the imidazole with a benzamido group maintained the activity of the compound as a kinase inhibitor. Subsequently, introducing a methyl substituent *ortho* to the pyrimidinyl-amino group abrogated PKC-α and CDK1 serine/threonine

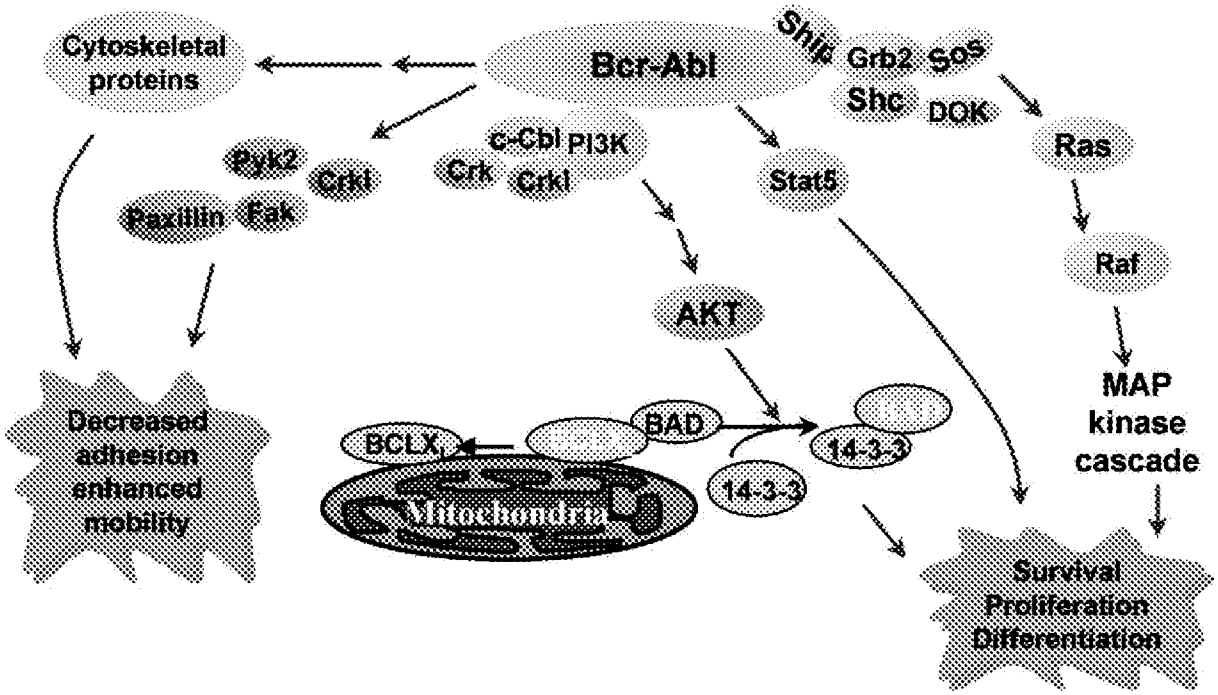

Fig. 2. Diagrammatic representation of some of the key pathways in the Bcr-Abl signaling cascade.

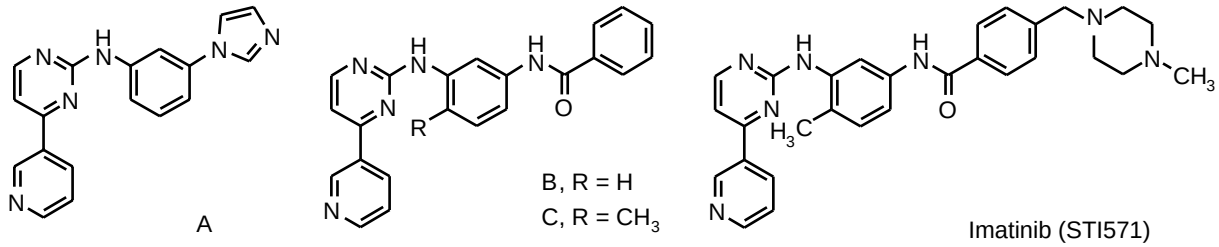

Fig. 3. The structure of imatinib and of the lead compounds from which it was derived.

Table 1
Inhibitory profile against a panel of protein kinases

Kinase	Inhibitory concentration (IC_{50} nM)			
	A	B	C	Glivec
cAbl	3300 ± 1100	2800 ± 850	361 ± 48	188 ± 18
Kit	1100 ± 200	1100 ± 180	785 ± 140	413 ± 23
PDGFR-ß	390 ± 58	870 ± 110	400 ± 72	386 ± 111
VEGFR-2 (Human KDR)	1400 ± 210	1300 ± 310	10,000	10,000
EGFR (HER-1; Erb B)	>10,000	>10,000	>10,000	>10,000
FGFR-1	2500	>10,000	>10,000	>10,000
CMet	n.d.	>10,000	>10,000	>10,000
IGF-R	>10,000	>10,000	>10,000	>10,000
CDK1/cyclinB	92 ± 4	200 ± 37	>10,000	>10,000
CSrc	1700 ± 100	>10,000	>10,000	>10,000
PKC-α	1000	1200	72,000	>10,000

Data represent the mean ±SEM ($n \geq 3$) drug concentrations required to inhibit enzyme activity by 50% (IC_{50} value; nM) at ATP concentrations optimized for each kinase. Alternative nomenclature for the kinases is given in parenthesis. n.d. = not determined.

kinase activity and enhanced potency against cAbl (Fig. 4) [7,8]. This increased potency is probably a result of the preferred orthogonal orientation of the pyrimidine and phenyl rings on compound *C*, whereas the loss of activity against PKC-α and CDK1 might be the result of a steric clash with Met470 and Leu134, respectively [9]. Whereas these amides are highly insoluble and lack oral bioavailability, introducing suitably placed hydrophilic moieties such as the *N*-methylpiperazine group, which had been successfully utilized for such purposes in the area of quinolinone antibiotics [10], resulted in the discovery of imatinib.

Imatinib is a potent, competitive inhibitor of cAbl, having a K_i value of 85 ± 19 nM [11], with an attractive profile in which it also inhibits cKit and PDGFR-β, but is devoid of significant activity against most other kinases. In contrast to compounds such as *C*, imatinib, formulated as the methane sulphonate salt, possesses good aqueous solubility (approximately 50 mg/l at pH 7.4) and inhibits Bcr-Abl autophosphorylation in cellular assays, as well as the proliferation of Bcr-Abl-dependent cell lines [12,13]. Imatinib is well absorbed following oral administration and displays an excellent pharmacokinetic profile, such that in humans single daily doses of ≥400 mg maintain mean plasma concentrations above 1.46 μM [14].

Imatinib is well tolerated and, in a Phase I study in CML patients, 98% of subjects in chronic phase showed a complete hematological response with 54% showing a cytogenic response [14]. The success of this drug is a result of precise targeting of the underlying cause of CML, with a molecule tuned to have an excellent inhibitory profile against the target kinase and with biopharmaceutical properties optimized to achieve drug levels at the site of action sufficient to inhibit Bcr-Abl with good efficacy.

Despite the efficacy of imatinib, some advanced-stage patients in blast crisis develop drug resistance and relapse. Recent studies show that many relapsed patients carry point mutations in the kinase domain of Bcr-Abl, such as the Thr315Ile mutation first observed by Sawyers and co-workers [15]. To understand the molecular mechanism by which such mutations might cause resistance to imatinib, we are studying the three-dimensional structure of a complex between the drug and the cAbl kinase domain of Bcr-Abl by X-ray crystallography and have related this to the mutant proteins. Recombinant human cAbl kinase (amino acids 218–500) was produced using the baculovirus/insect cell expression system, with a proteolytically cleavable His-tag attached at the *N*-terminus to aid in purification. Expression in Sf9 insect cells in the presence of imatinib led to the isolation of an exclusively unphosphorylated cAbl-imatinib complex. Crystallization, followed by data collection using synchrotron radiation provided a high-resolution (2.4 Å) molecular structure of imatinib bound within the ATP-binding site of the kinase

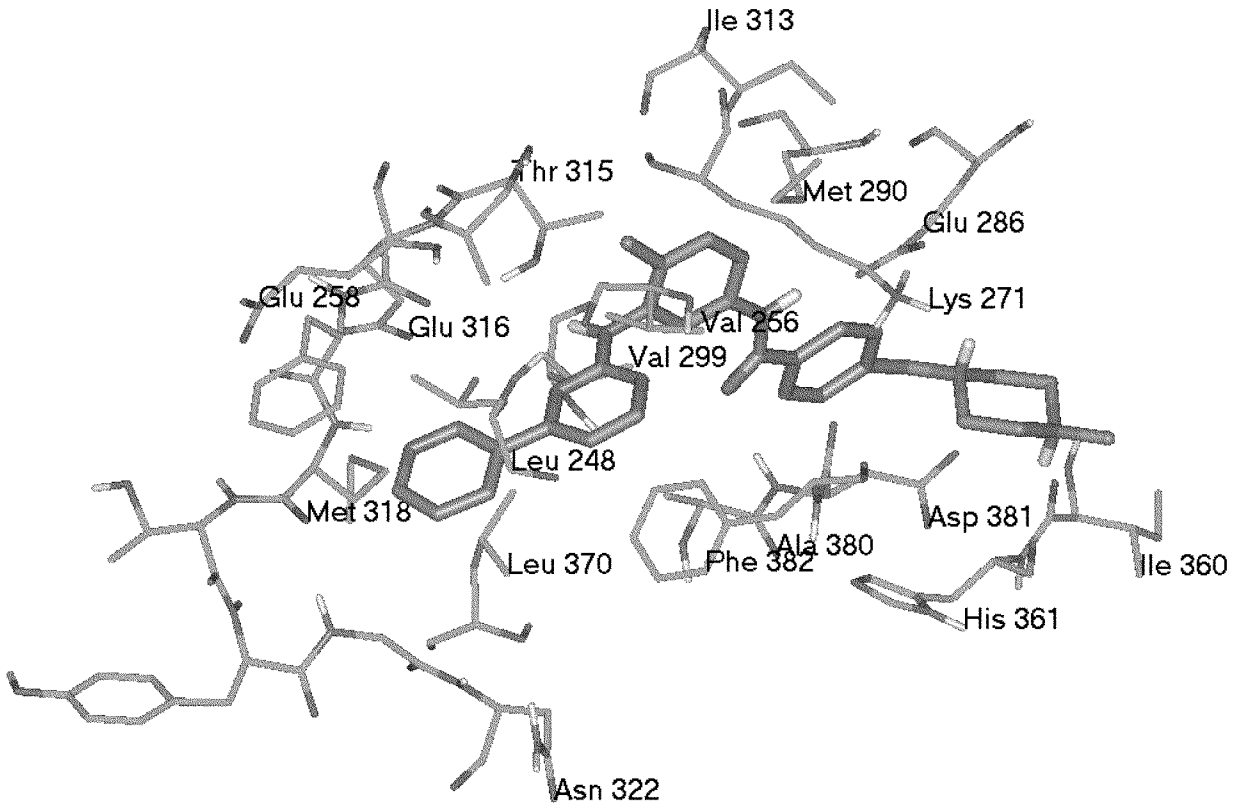

Fig. 4. Cartoon showing details of the interactions between imatinib and the kinase domain of human cAbl, based upon the a 2.4 Å resolution crystal structure.

domain of Abl. A key finding from this structure is that imatinib binds to an inactive conformation of the kinase, in which the N-terminal part of the activation loop containing the highly conserved DFG motif is folded into the ATP-binding site. This inactive conformation, which is unable to bind ATP, has previously been observed by Kuriyan and co-workers [16] in the complex between murine cAbl and a variant of imatinib. The unusual conformation of the activation loop may be illustrated by comparing the position of Phe1045 of the VEGFR-kinase DFG motif shown in in Fig. 1 with that of the corresponding residue Phe382 in the imatinib structure shown in Fig. 4. In this conformation, the size and shape of the binding site differs from that normally observed for tyrosine kinases liganded to either ATP or the majority of co-crystallized inhibitors. In particular, the hydrophobic pocket observed in most kinases, often referred to as the selectivity pocket (or hydrophobic region I) due to its variation in shape and volume amongst different kinases [17,18], is much larger than usual and extends right across the enzyme. Imatinib utilizes this enlarged selectivity pocket, and it seems likely that this unusual mode of binding is the key for the tyrosine kinase selectivity profile of the compound, with only those kinases for which such a conformation is available being susceptible to inhibition. In the crystal structure, imatinib binds to cAbl via a series of H-bond interactions and van der Waals contacts: the pyridine-N accepts an H-bond from the backbone-NH of hinge residue Met318; the anilino-NH donates an H-bond to the side-chain of Thr315; the amide-NH donates an H-bond to the side-chain of Glu286; the amide-carbonyl accepts an H-bond from the backbone-NH of Ala380; and the protonated *N*-methylpiperazine forms H-bonds to the backbone-carbonyl groups of Ile360 and His361 (Fig. 4). Van der Waals contacts/lipophilic interactions also play a major part in binding, such as in the case of the pyrimidine ring, which is sandwiched between the side-chains of Val299, Ala269, Val256 and Phe382; as well as the pyridine, which is surrounded by the side-chains of Leu248, Leu370, Met318 and Phe317.

In addition to providing a rationale for the selectivity of the compound, the molecular structure of the complex between imatinib and cAbl can also be used to explain why patients possessing mutant Bcr-Abl kinases are less sensitive to imatinib therapy. Gorre *et al.* [15] attributed this loss of potency to the loss of the H-bond interaction between the anilino-NH of imatinib and the side-chain of Thr315, compounded by a steric clash between the Ile315 side-chain and the NH-phenyl ring. Our structural studies have added further support to this, confirming the importance of the Thr315 binding interaction and confirming that imatinib does indeed bind to human cAbl in a mode similar to that observed by Schindler *et al.* for the variant [16]. Additional confirmation comes from the sensitivity of the recombinant kinases toward imatinib, which

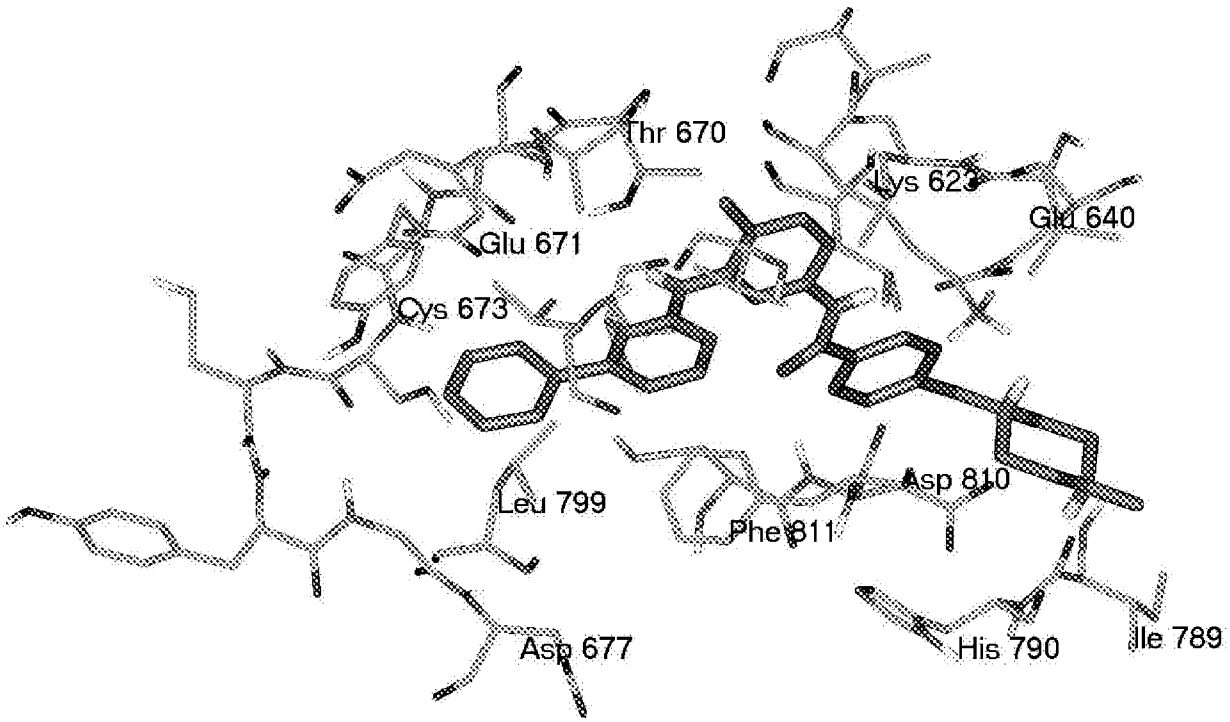

Fig. 5. Representation of a model of imatinib liganded to the kinase domain of c-Kit, based upon the imatinib-cAbl crystal structure.

inhibits the transphosphorylation (poly-AlaGluLysTyr as substrate) catalysed by wild-type cAbl with an IC_{50} value of 237 ± 93 nM, whereas under the same conditions the Thr315Ile cAbl mutant is not affected at concentrations below 10,000 nM. Some measure of the role of steric hindrance in this desensitization can be assessed by the observation that imatinib inhibits the Thr315Val mutant cAbl with an IC_{50} value of 3100 ± 1100 nM.

For the treatment of CML blast crisis, mutants such as Thr315Ile present new targets for drug discovery; from these studies, it is clear that structural biology can be a powerful tool for use in the design of new inhibitors of Bcr-Abl that maintain inhibitory activity of mutant kinases.

3.2. Imatinib as an inhibitor of Kit and PDGFR-β

In addition to inhibiting cAbl, imatinib inhibits the unrelated Kit and PDGFR-β kinases. These enzymes are members of the receptor tyrosine kinase family, possessing five immunoglobulin-like motifs in the extracellular

Table 2
Protein kinase inhibitors in drug development, grouped according to structural class

Identity [a]	Target kinase [b]	Company/institution	Indication	Status [c]
Herceptin™ (Trastuzumab)	HER2 ab	Genentech	Cancer	Launched
Imatinib	Bcr-Abl	Novartis	Cancer	Launched
LY333531	PKC	Eli Lilly	Ocular	CP III
PKC412	Flt-3; PKC	Novartis	Cancer/Ocular	CP II
IMC-C225 (Cetuximab)	EGF ab	Imclone	Cancer	CP III
Iressa™ (ZD1839)	EGFR	AstraZeneca	Cancer	CP III
Tarceva™ (OSI-774)	EGFR	OSI/Genentech	Cancer	CP III
PKI166	EGFR, HER2	Novartis	Cancer	CP II
CI 1033	EGFR, HER2/3/4	Pfizer/Warner Lambert	Cancer	CP I
Semaxanib™ SU5416	VEGFR	Pharmacia/SUGEN	Cancer	CP III
Bevacizumab	VEGF ab	Genentech	Cancer	CP III
SU6668	PDGFR/VEGFR	Pharmacia/SUGEN	Cancer	CP I
PTK787 (ZK222584)	VEGFR-2	Novartis (Schering AG)	Cancer	CP II
ZD6474	VEGFR/EGF	AstraZeneca	Cancer	CP I
IMC-IC11	VEGFR ab	Imclone	Cancer	CP I
VX-745	P38	Vertex	Arthritis	Discontinued
CEP-701	Trk	Cephalon	cancer	CP II

[a] See Fig. 6 for structures; [b] ab = antibody; [c] CP = clinical phase.

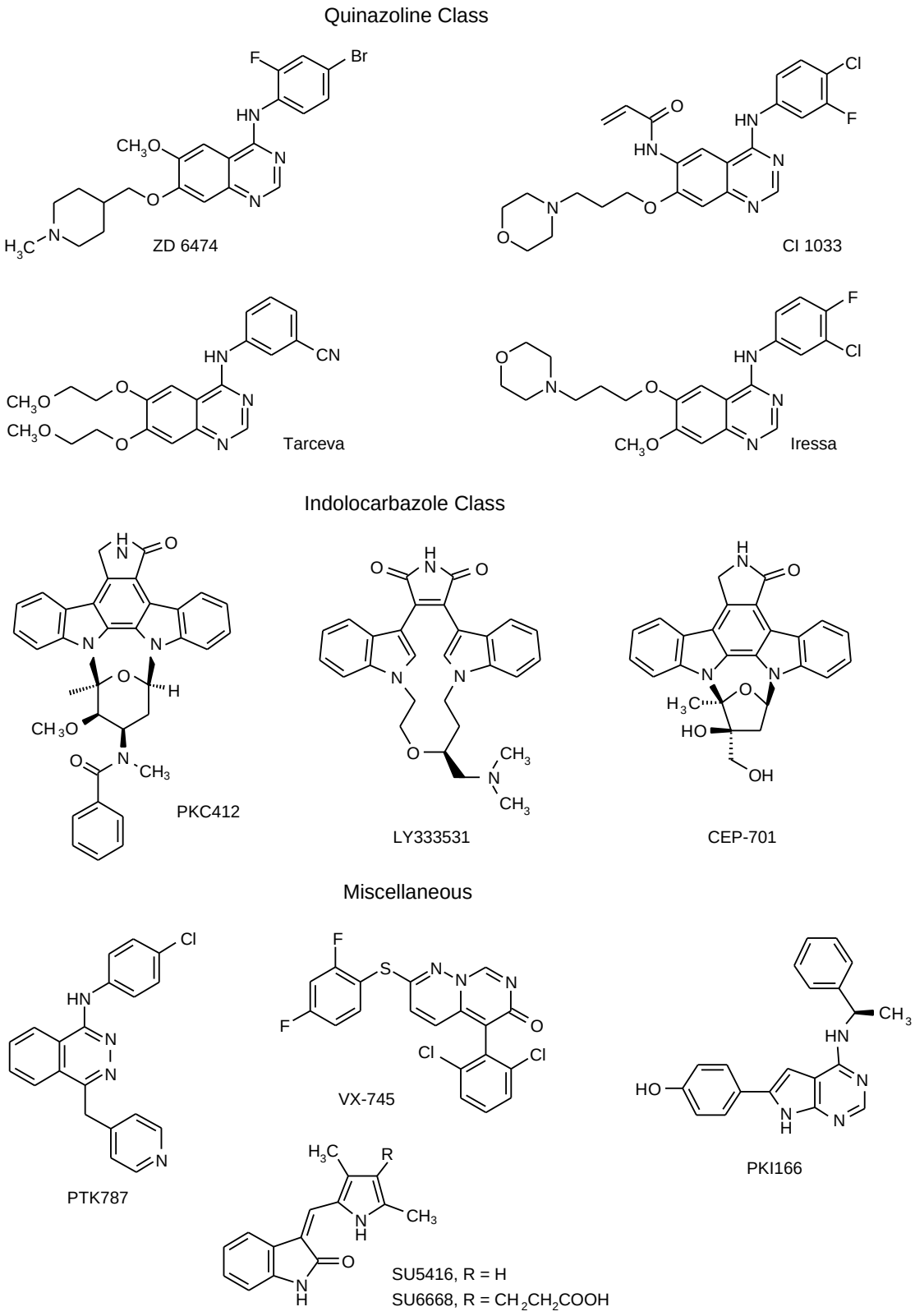

Fig. 6. Structures of protein kinase inhibitors in clinical development (excluding imatinib, which is shown in Fig. 3).

domain and a cytoplasmic, split-kinase domain. Upon binding ligands to receptors located within their extracellular domains, the intracellular kinase domains of these transmembrane protein kinases become activated, binding ATP and catalysing the phosphotransfer reaction to initiate an intracellular signaling cascade. Under normal circum-

stances, Kit activity is modulated by stem cell factor (SCF) protein which, upon binding to the extracellular domain of two proximal Kit receptors, leads to their dimerization and concomitant activation of their kinase domains. Dysregulation of Kit has been implicated in the etiology of a number of cancers, including acute myelogenous leukemia (AML), some lung cancers, gliomas, testicular cancer and gastrointestinal stromal tumor (GIST). The latter tumors are believed to be predominantly the result of Kit mutation, leading to the constitutive activation of this kinase [19]. Imatinib shows remarkable efficacy in the treatment of GIST [20]. This indicates that the utility of imatinib and/or related compounds might extend well beyond CML, so that once again structural biology studies of cAbl, Kit and PDGFR-β promise to be powerful tools to help design potential new therapies.

Extrapolating from our understanding of the molecular interactions between imatinib and cAbl, an analogous model has been developed for the interaction between imatinib and Kit (Fig. 5). This model is consistent with the structure-activity relationships observed for analogs of imatinib. A key binding interaction between imatinib and Kit is believed to include an H-bond between the anilino-NH and Thr670, directly parallel to that with cAbl, with additional H-bonds between the amide-NH, the amide-carbonyl and the pyridine with backbone elements of Glu640, Asp810 and Cys673, respectively. Further studies in this direction are ongoing.

4. Current status of protein kinase drug discovery

A key to successful drug discovery in cancer is the selection of a highly relevant, drugable target, such as Bcr-Abl in the case of CML and Kit in the case of GIST. However, this needs to be coupled with efficient lead finding followed by careful optimization of the lead molecule for potency, selectivity, efficacy and biopharmaceutical properties. Thus, in the case of imatinib, the excellent pharmacokinetic properties of the molecule are crucial to its efficacy in patients.

It is also important to recognize that in the protein kinase area, researchers are unlikely to find truly specific kinase inhibitors. However, with sufficient structural biology information, we are able to fine-tune the kinase inhibitory profile of given compounds. In this respect, imatinib has an excellent profile, leading to exceptional efficacy against several targets at very well-tolerated doses. However, in achieving such a selectivity profile, we are left open to tumors developing resistance through kinase mutation. A potential approach to combating such mutants is to treat patients with a combination of agents that interact differently with the target at the molecular level. Eventually, however, since many tumors accumulate a number of genetic alterations and mutations can arise in response to drug treatment even in the case of clonal diseases, effective long-term cancer therapy is still likely to require cocktails of drugs, each aimed at a specific target.

Imatinib is a forerunner among a number of kinase inhibitors currently under development within the pharmaceutical industry (Table 2). However, whereas almost all of the low-molecular-weight compounds have originated from leads obtained from high-throughput screening of compound archives (Fig. 6), structural biology studies of complexes between kinases and ATP-competitive inhibitors are providing crucial insight for the rational design of new compounds having a particular selectivity profile. Furthermore, the emerging technology of in silico high-throughput docking of database libraries to protein structures (derived from either homology models of binding sites or, more reliably, from X-ray crystal structures) promises to greatly increase the availability of attractive lead molecules suitable for optimization for kinase inhibition.

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