

Design and Development of Signal Transduction Inhibitors for Cancer Treatment: Experience and Challenges with Kinase Targets

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Abstract: The last several years have seen major progress towards the goal of translating our growing understanding of the molecular basis of cancer into drugs with improved therapeutic activity and selectivity. Tremendous advances have been made but significant obstacles remain. In this review we assess our experience in the design and development of signal transduction drugs for cancer treatment, with a specific focus on small molecule kinase inhibitors. The druggability of cancer kinome targets is exemplified by imatinib, gefitinib, erlotinib and many other emerging agents. We assess the current status of the design of potent and selective kinase inhibitors, which has benefited greatly from high throughput screening and structure-based approaches. A diverse range of kinase inhibitory scaffolds is now available based on these methods. Multi-parameter optimisation now focuses as much on pharmacokinetic and metabolic properties as it does on target potency and selectivity. Development of a 'molecular audit trail' requiring assays to demonstrate mechanism of action *in vitro* and *in vivo* is essential. Current issues include our relatively poor ability to predict the level of kinase selectivity in the intact cell, uncertainties around the most desirable selectivity profile, and the emergence of drug resistance.

Key Words: Cancer, kinase, inhibitor, structure-based design, selectivity, pharmacodynamics.

INTRODUCTION

The progress that has been made in understanding the molecular causation of cancer has been remarkable over the last few years. Although our molecular comprehension remains incomplete, we now have sufficient knowledge to develop drugs that should be more effective and less toxic than those that are commonly used to treat malignancy [1,2]. The development of new cancer drugs is now based very solidly on the identification of genes that are responsible for driving malignancy and the elucidation of the signal transduction pathways that they hijack. There is now extraordinary potential to develop signal transduction inhibitors (STIs) that are tailored to the precise molecular abnormalities that can be identified in individual patients. Prospects for personalised medicine for cancer patients are now very real. In this sense, oncology is leading the way in the exploitation of the fruits of the human genome sequence for patient benefit.

Tremendous advances have been made but significant obstacles remain. In this review we assess the experience that has been gained in the design and development of signal transduction drugs for cancer treatment. The focus is on small molecule kinase inhibitors since this is the area in which the greatest progress has been made and in which we have personal experience. In the first part of the article we review the progress and challenges with respect to the technical objective of designing kinase inhibitors. We show how this has benefited greatly from high throughput screening and structure-based approaches. The druggability of cancer

kinome targets is exemplified by imatinib, gefitinib, erlotinib and many other emerging agents under preclinical and clinical development. We describe the various kinase inhibiting scaffolds that are available. We emphasise the importance of modern multi-parameter optimisation which focuses as much on pharmacokinetic and metabolic properties as it does on target potency and molecular selectivity. We advocate the development of assays that provide a 'molecular audit trail' that can be used *in vitro*, in animal models and in patients to demonstrate inhibition of the molecular target and the downstream consequences of target modulation. We highlight current issues, including our rather poor ability to predict the extent of kinase inhibition and selectivity in the intact cell. In the final part of the review we address issues in the clinical development of kinase inhibitors, including the need for robust and informative biomarkers, the importance of developing assays for predicting molecular dependence and hence for identifying patients who will benefit from a particular agent, and the perennial problem of drug resistance. We also highlight the challenges in the development of rational combination therapies that are likely to be essential in most cancers, both to overcome the problem of drug resistance and also to obtain optimal effects against malignancies that are driven by multiple abnormalities.

SIGNAL TRANSDUCTION KINASE INHIBITOR DISCOVERY

As for any drug discovery effort, the first step in developing a small molecule targeted to a particular signal transduction event is the discovery of a hit ligand as a starting point for medicinal chemistry. Many formats are suitable for high-throughput screening (HTS) of large compound libraries for inhibition of isolated kinases, with non-radioactive assays using fluorescence or luminescence read-outs emerging as preferred technologies [3,4]. Assays configured to find inhibitors of the catalytic transfer of the γ -

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phosphate of ATP to substrate by an activated enzyme, where the small molecule displaces ATP from its binding site, are most common. Screens of this type have led to the discovery of useful chemotypes for kinase inhibition, but this is not the only mechanism by which kinase activity may be blocked. Several successful STIs act by stabilisation of the inactive form of the kinase e.g. imatinib [5], sorafenib [6]. With the burgeoning of structural studies on the enzymes, it has been noted that there may be more distinction between the inactive conformations of the members of the class than is seen for the relatively conserved ATP binding sites of the activated enzymes, suggesting that inhibitors targeting the inactive forms might offer advantages in selectivity [5]. Small molecules may also, less commonly, act competitively with respect to ATP through allosteric binding sites, or interfere with the interaction of the kinase with an upstream regulator. One way to search for multiple modes of kinase inhibition within an assay is to recapitulate part of a signal transduction pathway *in vitro*, as was demonstrated for the RAF/MEK/ ERK cascade [7]. Such approaches require post-screening deconvolution of the mechanism of action of the hits. At a further remove, cell-based screens that measure phenotypic changes as the ultimate output of a complete signal transduction cascade, e.g. reporter gene transcription, may similarly encompass a range of inhibitory mechanisms, but may also be limited by issues of throughput and hit deconvolution [8]. One proposed strategy to minimise extensive post-screening deconvolution is to select compound libraries based on 'privileged structures' [9], drug-like molecules where existing knowledge suggests potential biochemical targets for the structural types, as was demonstrated for a phenotypic screen of the RAS-RAF pathway [10,11].

The generation of hits from a screening campaign is necessarily followed by careful evaluation of their potential as medicinal chemistry start points. In particular, confirmation of activity, biochemical characterisation of the inhibitors and appropriate counterscreens are needed to rule out false positives that may have been generated through interference of the small molecules with the assay readout [12], or through non-specific inhibition, especially by aggregation driven by poor solubility [13,14]. Hits may also need to be disregarded for gross toxicological liabilities, such as chemically reactive functional groups or membership of known pharmacological classes [15]. Assessment of 'drug-likeness' and 'lead-likeness' in terms of the physicochemical properties of the compounds [16,17,18,19] can be enhanced by consideration of ligand efficiency, where comparison is made of the strength of the observed biological activity relative to that anticipated for the hit chemical structure [20,21].

A substantial number of diverse chemotypes have provided kinase inhibitors relevant to oncology that have reached, or are close to, clinical investigation (Figs. 1 and 2), and many of these were first identified by screening approaches, including 4-anilinoquinazolines [22], oxindoles [23], ureas [24,25,26] and 2-phenylamino-pyrimidines [27,28]. Other molecular starting points have arisen from natural product kinase inhibitors such as flavonoids [29] and staurosporine [30], or from studying heterocycles with close

structural analogy to the purine of ATP, like olomoucine [31] and seliciclib [32]. Rational design has been productive in extending diversity within these established inhibitor classes. Following the success of the first signal transduction kinase inhibitors in oncology and other disease areas, protein kinases are well established as attractive, 'druggable' molecular targets [33,34,35,36,37].

PRECLINICAL DEVELOPMENT OF SIGNAL TRANSDUCTION KINASE INHIBITORS AND THE CONTRIBUTION OF STRUCTURE-BASED DESIGN

Necessarily, the development of some of the molecules now most advanced along the drug discovery pathway was set in train before the first publication of the X-ray crystal structure of a kinase catalytic domain (PKA) in 1991 [38]. The subsequent rapid growth in publicly available structural information has profoundly changed the way kinase inhibitor medicinal chemistry is approached, such that the majority of drug discovery programs now reported use some element of structure-based design to inform the ongoing medicinal chemistry.

The ATP-binding site and catalytic machinery is highly conserved across the kinase super-family, facilitating the development of common medicinal chemistry strategies [39,40]. For active kinases, ATP binds in a fold between the N- and C-terminal domains, with the heteroaromatic adenine ring forming characteristic bidentate hydrogen bonding to the peptide backbone of the hinge-region linking the two domains (Fig. 3). The ability to mimic these interactions is at the core of inhibitor binding for the majority of kinase inhibitor structural classes (Fig. 1). As well as conserved binding pockets for the ribose and triphosphate groups of ATP, a number of non-conserved features are also discernable: in particular, the presence of a hydrophobic pocket buried further into the binding cleft and a solvent exposed surface, neither accessed by ATP, which vary in size and composition between different kinases, and offer opportunities for selective inhibitor binding [39,40]. Another component determining the binding site structure is the 'gate-keeper' residue, which delineates the entrance to the hydrophobic pocket. The glycine-rich loop, which forms part of the phosphate binding groove, is a relatively flexible region of the protein, but nonetheless forms productive binding interactions with several inhibitor classes [40].

The switch between active and inactive forms of a kinase is controlled by the movement of an activation loop [39,40]. In the inactive conformation, this sits across the ATP binding site and blocks the access of ATP. Activation, often by phosphorylation of residues on the activation loop, causes it to move to an open, extended conformation exposing the ATP binding site. In the inactive form, additional hydrophobic binding sites are exposed by the positioning of the loop that are not present in the active form. Although the inactive kinase structures are more diverse than the active forms, common patterns of inhibitor binding can be identified, in which hydrogen bonding to the hinge-region remains an important component [5,6,39,40,41].

Importantly, kinase activity is regulated by other domains within the protein, and these may also represent valid targets

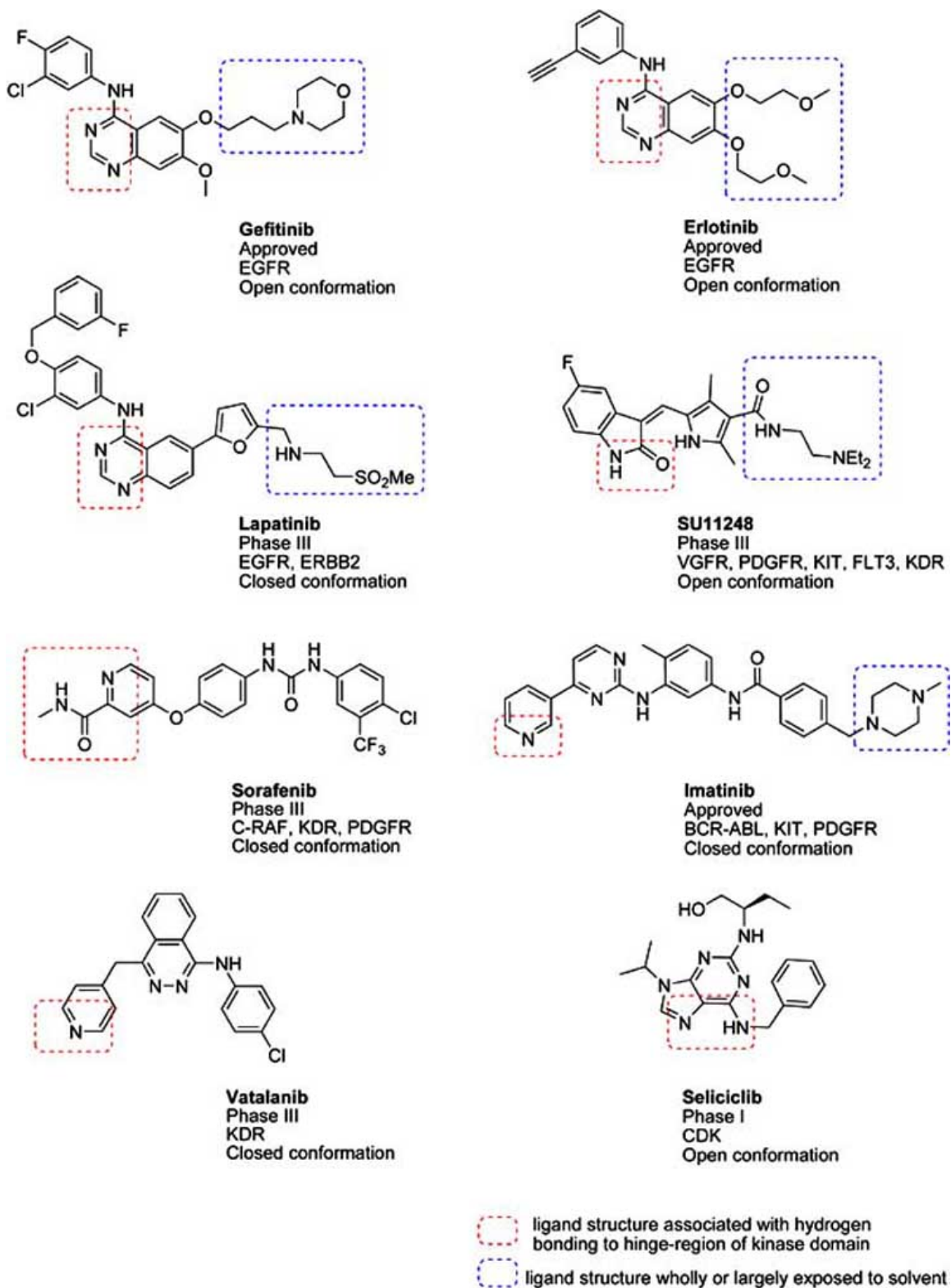


Fig. (1). Selected clinically investigated signal transduction kinase inhibitors that bind to the kinase domain.

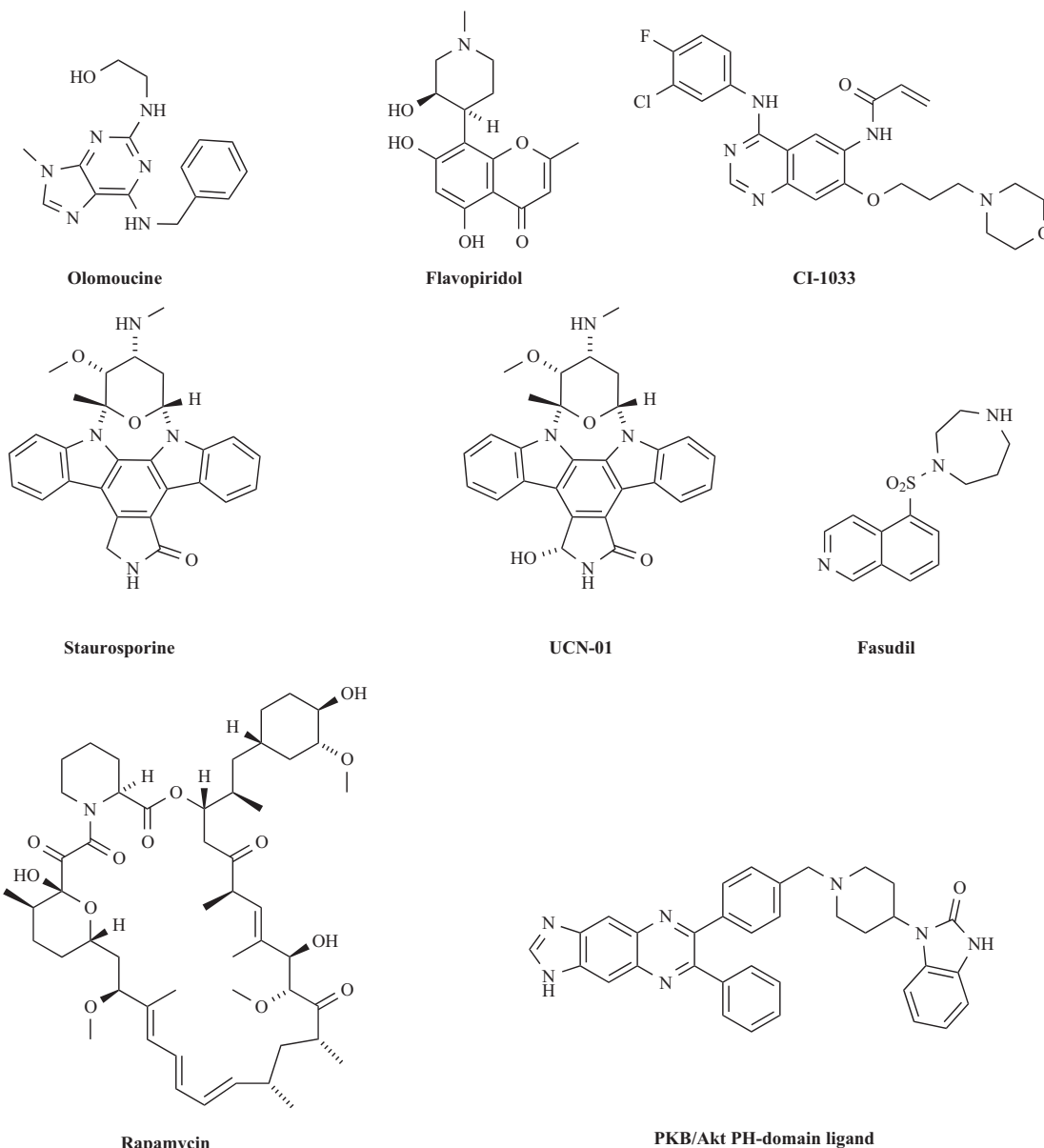


Fig. (2). Additional compounds referred to in text.

for small molecule intervention. For example, a series of small molecule inhibitors that bind to the pleckstrin homology (PH) domain of Akt/PKB prevent activation of the kinase by disrupting the interaction with phosphatidylinositols [42,43]. The natural product rapamycin is an effective inhibitor of signal transduction through the PKB-mTOR-p70S6K-S6 pathway through a more complicated mechanism in which a binary complex of the inhibitor and a regulatory cyclophilin binds to and inhibits mTOR kinase activity [44,45]. Such alternatives to direct competition with ATP offer other avenues for seeking highly selective inhibition of signal transduction kinases.

The BCR-ABL tyrosine kinase inhibitor imatinib was developed before X-ray crystallography showed the high selectivity of the inhibitor to arise from binding to, and stabilising of, an inactive conformation of the enzyme [5,46]. The development of imatinib shows how empirical medicinal chemistry can transform the specificity of a lead series from inhibition of one protein target to another [47]. The 2-phenylamino-pyrimidine chemotype was originally identified in a screen for PKC inhibition, and subsequent counter-screening of compounds proceeding from this hit identified a subset of amide-substituted structures with activity against the chimeric BCR-ABL kinase. Abolition of the residual

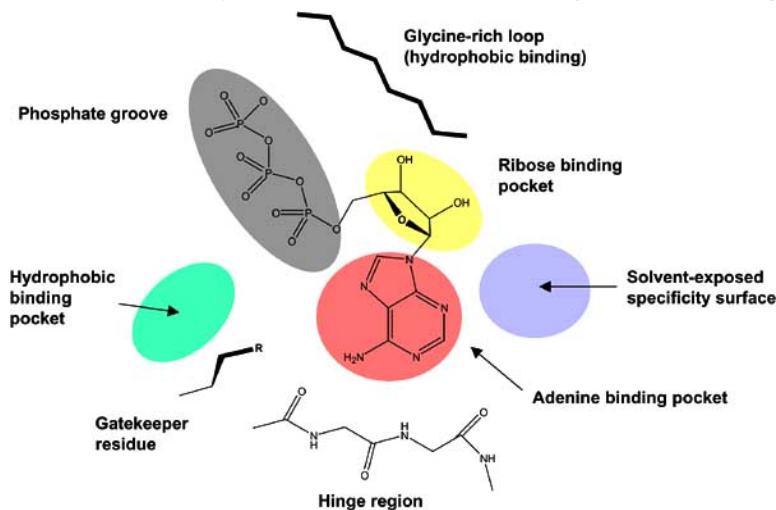


Fig. (3). Schematic of typical ATP-binding site showing ATP binding and additional non-conserved regions.

PKC inhibition was achieved by introduction of a conformationally restricting methyl substituent to provide tyrosine kinase selective ligands. Further optimisation was required to increase the water solubility and hence the oral bioavailability of the compounds, in this case by addition of a solubilising side-chain, leading ultimately to imatinib. Interestingly, subsequent structural studies have shown that the putative piperazine solubilising group in fact interacts strongly with the protein through specific hydrogen bonds [5,41,46].

The presence of substantial chemical functionality specifically to aid solubility of the inhibitory compounds, usually through salt formation at a basic site, is a common feature of approved and late-stage kinase inhibitors (see Fig. 1). For example, crystallographic studies quite early in the development of the oxindole class of ATP-competitive inhibitors identified suitable positions for substitution on the prototype SU6668 [48]. Appending of appropriate amines not only improved solubility and cellular activity, but also altered the selectivity profile of the inhibitors, leading to SU11248 [49]. Simplistically, this dual structure-activity can be seen to arise from the nature of the non-conserved specificity surface in the active form of kinase domains which is also partially exposed to solvent (Fig. 3). Importantly, the nature of the solubilising groups, in particular the acidity or basicity, can also control the degree of plasma protein binding (PPB), and hence the distribution and metabolism of the compounds *in vivo* [49]. For example, very high binding in serum to α -acid glycoprotein has proved limiting for clinical efficacy of the flavonoid kinase inhibitor UCN-01 [50].

The C-RAF inhibitor was also developed [51] before structural studies with the related kinase B-RAF revealed the molecule to bind to an inactive conformation of the enzyme [6]. A urea hit from HTS of 200,000 compounds was explored by traditional medicinal chemistry strategies involving single-point changes to the chemical structure, which established the class as a genuine medicinal chemistry series but led to only modest improvements in potency. A

breakthrough was achieved by applying a combinatorial approach to prepare a 1000-member library of ureas with multiple-point variations in structure, enabling synergistic structural variations to be detected. In the light of subsequent structural studies with sorafenib, one of the key substitutions of a 4-pyridoxy-group can be seen to extend into and bind to the conserved hinge-region of the kinase domain. However, in the absence of structural studies on the early compounds in this series, variations in the binding mode between weak and potent compounds cannot be ruled out. With a more potent lead established, the conservative single-point variation of structure was successful in further improving the inhibitor properties to give sorafenib [52]. Subsequent work has identified suitable positions for the addition of solubilising groups to the structure of sorafenib and analogues [53].

In the case of the 4-anilinoquinazoline inhibitor chemotype that forms the basis of the inhibitors gefitinib, erlotinib and lapatinib, the initial discovery of the core chemical template was informed to some degree by structural considerations [22]. Using knowledge of the catalytic mechanism of the enzyme, screening for ATP-competitive activity against EGFR tyrosine kinase was conducted on a library enriched in compounds containing functionality capable of mimicking the γ -phosphate of ATP and/or the tyrosine residue of the peptide substrate. The hits from this approach defined a 2-D pharmacophore that was used to select compounds for assay from a larger library of more diverse small molecules, leading to the prototype 4-anilinoquinazoline CAQ [22]. Early in the evolution of the 4-anilinoquinazolines, the 4-fluoro-3-chlorophenyl motif present in gefitinib emerged as a preferred group to confer metabolic stability and thus enhanced *in vivo* activity. In common with imatinib, and SU11248, the addition of a basic solubilising group was necessary to optimise the physicochemical and pharmacokinetic properties of the series, leading ultimately to gefitinib [54]. The pharmacophoric features of gefitinib are conserved in the structurally related inhibitor erlotinib [55], and in other investigational inhibitors from this chemical class [37,56]. This is reflected

in the similarity of kinase binding modes observed for 4-anilinoquinazolines, such as erlotinib bound to EGFR kinase [57]. The understanding of the binding mode of this chemotype has been used to develop irreversible inhibitors of the ERBB2 tyrosine kinase, such as CI-1033, by placing chemically reactive Michael acceptor groups at positions adjacent to a unique cysteine residue in the ribose binding pocket [58,59]. The inhibitor lapatinib deviates significantly from the simple pharmacophore, and has been found to bind to a closed conformation of the kinase, with a substantially different binding mode [60]. The additional bulky substitution of the aniline appears to drive the switch in binding modes, which is also associated with a slower inhibitor off-rate that may be an important component of the *in vivo* activity of the compound.

Increasingly, structure-based approaches are becoming integral to the discovery of new inhibitor chemotypes, and the conserved nature of the active conformation of protein kinases presents both opportunities and challenges. The adaptation of existing lead series to new targets is both feasible and attractive, and the rational design of focussed chemical libraries based on the binding modes observed in co-crystallography studies has received considerable attention, with much success [61,62]. One potential challenge for commercial drug development is the rapidly growing intellectual property coverage of known inhibitor chemotypes that this engenders, and the consequent narrowing of these chemical areas for commercial exploitation. For example, the emergence of sorafenib as one of the first urea-based inhibitors has been followed by a large number of other urea-containing ligands, some of which have been progressed to the clinic [63,64]. Fragment-based screening is one methodology that has proved successful in discovering new kinase inhibitor structural types [65,66]. In one manifestation, this uses physical chemistry techniques such as protein-ligand co-crystallography or NMR to screen for weakly binding, very low molecular weight fragments that fulfil the conserved interactions with the protein kinase deduced by structural biology [66]. Such methods offer the opportunity for *de novo* construction of new inhibitor scaffolds.

FEATURES OF THE MEDICINAL CHEMISTRY OPTIMISATION OF SIGNAL TRANSDUCTION KINASE INHIBITORS

In common with all drug discovery, medicinal chemistry optimisation of STIs is a complex task of simultaneous multi-parameter optimisation [67]. The candidate drug will likely occupy chemical space at the intersection of many one-dimensional structure-activity relationships (SAR), for potency, selectivity, ADME properties, toxicity etc, some of which are mutually reinforcing whilst others are antagonistic. There is often a need for rational compromise in the fulfilment of these individual criteria to achieve the overall goal of an efficacious, well-tolerated drug [68]. As yet, there are no convincing means of using 'design rules' to completely circumvent empirical optimisation of therapeutic ratio. However, the use of design criteria early in the discovery process to establish starting points with a higher probability of success is now well established, based on assessments of 'drug-like' physicochemical and biological

properties and the scope for optimisation within these properties that a certain hit structure presents [16,17,18,19]. Special attention has been paid to elucidating the physicochemical properties and structural features of orally bioavailable drugs [69,70,71]. This is likely to be of direct relevance to STIs where the possibility of a cytostatic effect necessitating chronic administration of the drug exists. Interestingly, it has been observed that the relatively few current clinically investigated, orally bioavailable kinase inhibitors exhibit on average higher molecular weight (MW) and lipophilicity than the consensus for oral drugs across the complete spectrum of diseases [72]. Thus the usual attrition rates during development associated with larger, lipophilic molecules do not appear to operate so strongly for this inhibitor class. The signal transduction kinase inhibitors now in the most advanced stages of clinical examination may have been developed from starting points identified before design criteria such as Lipinski's 'Rule-of 5' [16] were firmly established, and several required addition of functionality (and thus MW) late in development specifically to improve solubility and ADME properties, as detailed above. There are examples of lower MW kinase inhibitors, such as the RHO kinase inhibitor fasudil (MW=291), approved for i.v. administration for the treatment of brain haemorrhage [73], which possesses a basic amine that is required for binding interactions to the kinase, but which also governs the physicochemical behaviour of the ligand. It might be anticipated that structure-based design and fragment-based screening approaches could be used to identify more such dual-purpose, lower MW scaffolds. Recent adaptation of the isoquinoline sulfonamide chemotype to provide ATP-competitive inhibitors of Akt/PKB, where a basic nitrogen is critical for kinase binding, exemplifies this further [74,75]. It is also noteworthy, however, that structural biology studies show inactive kinase conformations exposing a larger potential contact area between ligand and protein. Thus inhibitors that bind effectively and stabilise these conformations, like sorafenib and imatinib, may have an inherent need for larger molecular size [39,41].

Optimisation of the ADME properties of STIs for cancer treatment includes achieving control of tissue distribution and adequate tumour levels, together with an understanding of the formation and pharmacology of metabolites. The requirement to minimise potential drug-drug interactions through avoidance of cytochrome P450 inhibition or induction is general for all medicines [76,77], but is particularly relevant to STIs in oncology where co-administration of the drug in combination with other STIs or cytotoxic agents, or indeed with other non-oncology therapies, is highly likely [78]. It is also desirable to undertake early screening of lead structures for known toxicological liabilities likely to prevent development, such as the blockade of hERG ion channels leading to QT prolongation [79].

MOLECULAR AUDIT TRAIL

A major issue that presents in the preclinical development of STIs is the problem of correlating phenotypic endpoints in cellular and whole animal assays, i.e. linking measures of efficacy with the potency of the inhibitor at its molecular targets. Since signal transduction networks are

highly interconnected and may show compensatory behaviour following antagonistic challenge, it is desirable early in inhibitor development to establish an 'audit trail' of molecular pharmacology to demonstrate that a single-point STI intervention causes downstream effects that persist and are closely coupled to phenotypic responses [80,81]. Such considerations do not only apply to preclinical assays, but raise important issues concerning the selection of patient tumour types for relevant clinical study of selective STIs, and the definition of suitable end-points for such clinical trials [35,36,37].

To effectively address these issues, a preclinical assay cascade for selective STIs might take the following form: Determination of biochemical activity and selectivity, followed by measurement of specific effects of target inhibition in cellular assays at multiple points downstream of the molecular target [82]. Evidence of similar, multiple pharmacodynamic effects in animal models (typically human tumour xenografts) should be sought and correlated to the observed therapeutic response [83]. Ideally, these pharmacodynamic readouts should be transferable to human clinical samples [84]. However, it is also necessary to maintain a pragmatic approach to the development of new STIs, to account for the inevitable gaps in the understanding of the cellular signalling networks that can only be fully addressed for new therapeutic targets when novel selective pharmacological agents become available. The drug discovery effort to optimise therapeutic ratio and provide effective therapies at tolerated doses, and the molecular pharmacology effort to characterise the mechanism of action of the agent necessarily progress in parallel [80].

SIGNAL TRANSDUCTION KINASE SENSITIVITY AND INHIBITOR SELECTIVITY

The therapeutic selectivity of a signal transduction kinase inhibitor exhibited *in vivo* can be seen as a convolution of parameters, some of which are properties of the drug molecule while others are features of the biochemistry of the tumour. Following the pioneering work by Cohen and colleagues, the measurement of the biochemical selectivity of inhibitors against large panels of kinases has become well established and provides a coherent approach for categorising ligand properties [85]. However, the question of which are the most relevant kinases to screen against in any therapeutic context is not always easily answered. Structure-based approaches have informed the understanding of inhibitor selectivity. For example it has been observed that differences in ATP-binding site structure and the SAR of inhibitors only become significant when the sequence identity of the kinase catalytic domains falls below approximately 60% [72]. Such considerations should be useful in selecting a truly representative panel of dissimilar and/or similar kinases for biochemical selectivity screening.

The biochemical selectivity, while important, is not necessarily the dominant factor in determining the selectivity of action of inhibitors in the cellular context. A detailed framework for interpreting kinase inhibitor selectivity has been put forward by Shokat [86]. The inherent differences in enzyme kinetic performance, e.g. $K_{m, ATP}$, between even closely related kinases can lead to situations where

apparently unselective inhibitors (as measured by IC_{50} determinations in biochemical assays) will have selective effects in the cell. Moreover, the phenotypic outcome of signal transduction inhibition depends on the relative responsiveness of the downstream pathways to changes in a particular kinase activity. This 'gearing' may vary not only between kinases in a given cell, but also between the same kinase in tumours of different genotype. What level of biochemical selectivity for inhibition is required of an inhibitor to generate a controlled, selective response in tumour tissue depends on the combination of these factors. There is therefore a need for more cellular readouts of inhibitor selectivity, that is, evidence of the consequences of specific kinase inhibition in the cell. Proteomic techniques have been successful in rapidly building wide profiles of inhibitor activities. For example, the immobilisation of a gefitinib analogue on a sepharose matrix led to the identification of more than 20 previously unknown kinase targets of the drug from affinity chromatography of HeLa cell lysates [87]. A recent alternative approach to pan-kinase profiling measured the competitive binding of clinical kinase inhibitors and immobilised non-specific ligands to phage-tagged kinase fusion proteins, constructing an interaction map for each inhibitor [88]. The extension of this methodology to include inhibitor-resistant mutant kinases provides a useful means of screening for inhibitor analogues capable of overcoming resistance arising from kinase mutation.

CLINICAL DEVELOPMENT ISSUES

There are a number of challenges facing the clinical development of STIs for cancer treatment. The successes with the imatinib, gefitinib and erlotinib have shown us that the cancer kinome is druggable and that valuable clinical benefit can be gained. At the same time, experiments with these and other emerging agents has taught us that we still have a long way to go before we are able to develop and utilise the new molecular therapeutic agents in the optimal fashion.

One major lesson is the importance of selecting patients based on evidence of likely molecular dependence and oncogene addiction [36]. In addition to the well known preferential activity of trastuzumab in ERBB2-positive breast cancer, the association between somatic mutations in EGFR and the dramatic clinical responses to gefitinib and erlotinib in non-small cell lung cancer has had a major impact on the field [89]. Furthermore, secondary mutations in the catalytic domain have been shown to lead to resistance. Similar mutations in the BCR-ABL and KIT kinases lead to resistance to imatinib in chronic myeloid leukaemia and gastrointestinal stromal tumours respectively [90]. The experience summarised above has taught us two things. Firstly that greater activity is seen for a signal transduction inhibitor where there is evidence of molecular dependence on the target, as shown by mutation or translocation; and secondly that resistance can develop as a result of mutation in the catalytic domain. Alternative inhibitors can be identified that are active against the mutant kinases [90].

A prospective example of the concept of selecting patients based on molecular dependence is the use of

inhibitors of the PI3 kinase pathway in the treatment of tumours with activation of the pathway [2]. Patients that might be especially sensitive would include those with cancers with activating mutations in the p110 α catalytic domain of PI3 kinase, those with loss of the PTEN tumour suppressor gene, and those with increased phosphorylation of phospho-PKB by these or other means.

Another recent example is the demonstration that cancer cell lines with oncogenic B-RAF are very much more sensitive to MEK inhibitors than those with RAS mutations or those that are wild-type for both genes [91]. In addition to selecting patients for molecular dependence and recognising the key role of mutations in predicting for both sensitivity and resistance, it is essential to emphasise the critical importance of biomarkers that can be used to show proof of principle for target inhibition and to select the optimal dose and administration schedule [80,81].

Probably the greatest challenge facing the development of molecular therapeutics is the likely need to inhibit several oncogenic targets in order to overcome cancers that are driven by several abnormalities, as well as to prevent or neutralize the development of drug resistance [92,93]. There are various ways to do this. One is cocktails of highly targeted agents that are constructed according to the molecular make-up of the individual cancer. Another approach is to use so-called multi-targeted kinase inhibitors, such as sorafenib. This 'poly-pharmacology' approach offers promise, although very considerable challenges remain to achieve full control over the inhibitory profile in the design of such multi-targeted compounds [94]. However, the wealth of structural biology information available for kinases and the inherent similarity of the ATP-binding site between enzymes, as discussed above, suggests that this is an area where progress is likely [93]. A further strategy is to use inhibitors of cancer support systems that control many oncogenic players and pathways in malignancy. Current examples of this approach that are in the clinic include inhibitors of histone deacetylases and the Hsp90 molecular chaperone [92,95].

The rational selection and development of combination treatments is extremely challenging [96]. Better model systems and approaches are required for this. It seems likely that success will be achieved by building combinations in a rational way based on knowledge of the molecular abnormalities in particular cancers, together with an understanding of the feedback loops that apply upon blockade of a given pathway. However, high throughput random combinational approaches may also be successful.

CONCLUDING REMARKS

Proof of concept has now been established for the development of STIs for cancer treatment. In addition to the successes already referred to here, a large number of exciting new agents acting on novel oncogenic targets are now in preclinical development. New targets continue to be identified, for example by high throughput genome resequencing of cancer cells and gene expression microarray profiling [97,98]. The greatest success to date has been obtained with kinase targets. The application of a powerful set of techno-

logies, including both high throughput screening and structure-based design, allows us to develop potent and selective drugs acting on new targets in a rapid and efficient manner. At the same time, modern multi-parameter optimisation is incorporated into the inhibitor development process to ensure that pharmacokinetic and metabolic properties are optimised at the same time as biochemical and cellular activities.

Turning to clinical development, the selection of patients with molecular dependence on the target or pathway is critical, as is the need for biomarkers of drug action. Particular challenges are associated with the development of resistance and the design of rational combinations, both to overcome drug resistance and also to optimally treat cancers driven by multiple oncogenic abnormalities.

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