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The Gatekeeper: Friend or Foe in Identifying the Next Generation of Kinase Inhibitors

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Dedicated to Prof. Dr. D. Hoppe on the occasion of his 65th birthday

The epidermal growth factor receptor (HER-1, ErbB-1, EGFR) belongs to the family of receptor tyrosine kinases that regulate cell growth, survival, differentiation, and motility via several signal transduction pathways. Malfunction or overexpression of EGFR could lead to cancer development and progression.

Currently, gefitinib and erlotinib represent promising first-generation small-molecule kinase inhibitors with a proven efficacy against EGFR-dependant tumors.[1] In addition, the monoclonal antibodies cetuximab (Erbitux, Merck KGaA, Darmstadt, Germany) and trastuzumab (Herceptin, Hoffmann-La Roche Ltd., Basel, Switzerland), which target the HER-1 and HER-2 subtypes, respectively, are in clinical use.

Recently, scientists from Novartis and the Scripps Research Institute in San Diego published data on selective small-molecule kinase inhibitors derived from the 4,6-diaminopyrimidine scaffold (Figure 1).[2] Although pyrimidines are known to behave as promiscuous binders, the authors claim compound **1** inhibits EGFR with an IC_{50} value of 21 nM and is selective, at a concentration of 10 μM, against a set of 55 additional kinases tested in a panel. Surprisingly, none of the other synthesized isomeric 2,4-pyrimidine derivatives bearing the same side chains displayed any activity below 10 μM against EGFR. These entities were considerably less selective when tested against the same set of 55 kinases.

At first sight this could just be another brief article that describes the identification of small-molecule kinase inhibitors.

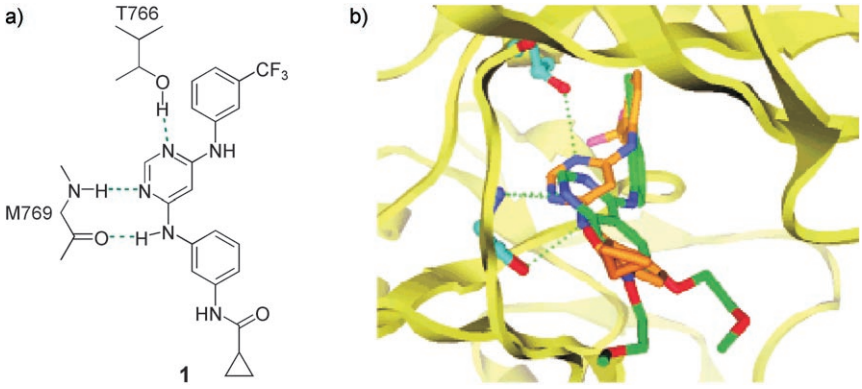

Figure 1. a) Docking model of compound **1** bound to the hinge region of the EGFR kinase domain. b) Superimposition of model of **1** (orange) with erlotinib (green) in the ATP site of the EGFR kinase domain. Reprinted with permission from reference [2]. Copyright© 2006 American Chemical Society.

However, besides their rounded approach covering medicinal chemistry and enzyme engineering, the article highlights at least three very important future challenges: the identification of novel scaffolds, improving the selectivity of inhibitors, and targeting kinase mutation. All of these hinge on the importance of the gatekeeper residue in kinases.

The authors note a surprising dearth in structurally distinct and selective inhibitors, despite significant research activity. This high degree of structural similarity among tentative small-molecule ATP-competitive kinase inhibitors is due to the fact that all kinases rely on the common ligand ATP. Binding to the highly conserved hinge region is essential, but selectivity will mostly be achieved by exploiting additional pockets, as exemplified by gleevec, BIRB-796, and many other compounds. Nevertheless, the scaffold is generally responsible for significant binding to the hinge region through the formation of multiple hydrogen bonds, whereas thoughtful variation of the decorating building blocks is necessary to achieve selectivity and to allow rapid tuning of sensitive pharmacokinetic properties, such as solubility, metabolic stability, and CYP inhibition. In most cases the development of novel scaffolds might be driven rather by prior art and the urgent need to gain patent protection.

Docking studies of the synthesized molecule **1** were performed in the EGFR binding niche derived from the EGFR–erlotinib co-crystal structure (PDB code 1M17). Molecular modeling suggests binding to the hinge region through the interaction of N1 and 6-NH with M769. Moreover, the authors rationalize the high selectivity and affinity through the formation of an additional hydrogen bond between N3 and the hydroxy group of the T766 gatekeeper residue. This is in accordance with other SAR data and represents a valuable alternative to the water-mediated hydrogen bond between N3 and the side chain of the T830 residue, as shown for other derivatives.[3] Recently, researchers from Roche also revealed a strong interaction between a bound inhibitor and the T106 gatekeeper residue in p38 MAP kinase by X-ray crystallographic analysis,[4] explaining its high selectivity. Prior to these

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findings, variation of the gatekeeper has been examined by genetic engineering to render certain kinases more susceptible to specific inhibitors, thus allowing detailed pathway studies.[5,6]

Compound **1** was potent against clinically observed EGFR mutants L858R (IC_{50} = 63 nM) and L861Q (IC_{50} = 4 nM); gefitinib itself is also active against cell lines expressing the L858R mutation, such as H1975.[7] Indeed, this specific mutation might account for a high prevalence of EGFR-related tumors. As compound **1** is directed toward the gatekeeper, it is not surprising that this compound does not inhibit the EGFR T790M[1] mutant due to steric congestion.

Generally, tumors bearing the T790M and L858R dual mutation retain catalytic kinase activity, but are resistant against gefitinib treatment as observed in patients with a relapse in the course of the treatment.[8] Besides T790M for EGFR, T351I (bcr-abl), T674I (PGDF), and T670I (c-kit) are the most frequent gatekeeper mutations behind the resistance observed in the course of treatment,[7,9] which indicates that future drug candidates should be tailored to the acquired mutation to provide second-line therapy for patients with a relapse. Recent findings from the clinic describe EKB-569 from Wyeth as an irreversible EGFR inhibitor with some clinical activity in patients who were initially responsive against gefitinib,[10] but who relapsed after a certain period of time. The observed activity of EKB-569 might be rationalized by irreversible Michael-type addition of the mutated gatekeeper cysteine residue to the side chain of the inhibitor, possibly followed by altered EGFR trafficking.[11]

The "one-size-fits-all" approach in clinical oncology is an outdated treatment paradigm. Today it is already clear that two subjects diagnosed with lung cancer might require completely different therapy in the clinic. Clearly, future preclinical research in oncology will have to follow up on this and might result in therapies being conducted in a completely new manner.

In the future, health authorities will continue to be obliged to provide the best therapy for each individual patient, and the patient, in turn, will have to deal with the facts of knowing which therapy might work and which might not work as a result of the tumor's genetic origin and current characteristics. However, the accompanying ethical complications that arise from the denial of specific therapies to certain patient populations owing to alleged ineffectiveness of treatment must be considered thoughtfully and cannot be underestimated.

The targeted use and refinement of current treatment would be a fundamental step toward a customized therapy. In the long term, this kind of personalized medicine might even result in lower overall health costs relative to the current regimen, despite enormous initial investments.

In addition, stratifying clinical studies by the mutational status of patients' material prior to clinical trials could enable the identification of responders in the patient population, and thus lower development costs and decrease time to market significantly. Under such circumstances, even small-market niches with limited patient populations might appear lucrative.

In summary, medicinal chemistry has already demonstrated its efficiency in designing selective kinase inhibitors, although the necessary degree of specificity to gain clinical efficacy remains uncertain. Clearly, optimizing drug candidates preferably against acquired kinase mutations has emerged as a challenging task which must be addressed to allow future next-line therapy. The biological origin of gatekeeper mutations, whether already present in the primary tumor or acquired in the course of the therapy, still remains unknown. Other facets such as oncogene addiction of certain tumor types are also discussed;[12] even the preferred downstream pathways in active EGFR mutations are not fully known yet. Efforts in elucidating such highly regulated biochemical processes and the development of molecular probes have to be intensified to gain a fundamental understanding of the underlying mode of action to deliver meaningful answers and to develop treatment regimens accordingly.

Curing cancer will persist as the overall goal. However, taming the disease by therapeutic manoeuvres and trying to understand the molecular genetics of cancer by closer interaction of preclinical research with clinical oncology is already a major step in this direction. In the anti-infective business, fighting bacteria also remains a moving target, but drug therapy can be escalated depending on the mutational status of the strain. This might be another comfortable scenario for future oncology therapies. In the end, smart research will always have to compete with nature's creativity.

Keywords: antitumor agents · drug design · gatekeeper · inhibitors · mutation

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[1] *Residue numbering is somewhat inconsistent across the literature, but EGFR mutants T766M and T790M are considered identical.*

