

# Protein Kinase Inhibitors for the Treatment of Inflammation – An Overview

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**Abstract:** Protein kinases are key factors in signal transduction, playing a pivotal role in the initiation, propagation and regulation of immunologic responses. In contrast to protein-protein interaction they are considered to be “drugable” by small molecular weight inhibitors. Thus kinases moved into the focus as promising drug targets for the therapy of inflammatory and autoimmune diseases. Whereas some kinase inhibitors are in clinical development already, most others are in early stages of research or still require validation. Recently, major progress has been made in elucidating the complete human kinome, in understanding molecular mechanisms of protein kinase action in inflammation as well as in regard to technologies suitable for *in vitro* and *in vivo* target validation, inhibitor screening and its structural refinement. Starting with some general points, this review summarises some recent findings and developments and prepares the stage for the subsequent review articles published in this hot topic issue. It is the purpose to highlight both the opportunities and the issues associated with kinases as drug targets and to enable the reader to keep up with this fast developing field, that is of substantial interest for basic and applied scientists.

**Key words:** Drug targets, Inflammation, Immune regulation, Inhibitors, Kinases.

## INTRODUCTION

There is still a high medical need for better therapy for many inflammatory diseases, such as eczema and psoriasis, rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis and others. Many well established drugs on the market fail in patients with severe disease or have major side effects [1]. Recently, significant progress has been made with the launch of some biologics such as cytokine neutralising antibodies (e.g. Anti-TNF alpha, [2]). These approaches are limited by application via injection, whereas most patients prefer oral drugs. Consequently, there is a continuous search for promising new drug targets.

Protein kinases are key factors in signal transduction, playing a pivotal role in the initiation, propagation and regulation of immunologic responses. In contrast to protein-protein interaction they are considered to be “drugable” by small molecular weight inhibitors. Thus kinases moved into the focus as promising drug targets for the therapy of inflammatory and autoimmune diseases which is reflected by several excellent reviews [1-6]. Whereas some kinase inhibitors are in clinical use and development already, most others are in early stages of research or still require validation. Hence, the question whether protein kinases are the major drug targets of the twenty-first century [7] is still awaiting an answer.

Recently, major progress has been made in elucidating the complete human kinome, in understanding molecular mechanisms of protein kinase action in inflammation as well

as in regard to technologies suitable for *in vitro* and *in vivo* target validation, inhibitor screening and its structural refinement. The human genome revealed the existence of 518 expressed kinase genes [8], designated as kinome, and defines the combinatory biochemical space in which protein kinase inhibitors could act, although it can not be excluded *per se* that these small molecule inhibitors target other proteins as well. In the model organism mouse, where constitutive and conditional gene deletion by homologous recombination in embryonic stem cells is established, 510 orthologs of the human kinases were found [9]. The mouse knockouts of orthologs to human protein kinases are a valuable tool for validation of a specific human protein kinase as pharmacological target. However, the absence of a protein has often effects different from that due to the presence of an inhibited molecule. Knock-in of kinase-dead or hypomorphic kinase mutations may solve this problem. Furthermore, in inflammatory mechanisms and cytokine spectra there are some differences between mouse and men which limit the mouse model and require further technologies (see articles of Gust and Bonin and of Gausterer *et al.*, this issue).

Target validation seems one of the most critical steps for the successful exploration of protein kinase inhibitors. Taking into account that more than ten thousand human proteins can exist in phosphorylated forms in living cells - in many cases even with multiple phosphorylations - , it becomes clear that each of the 518 expressed human protein kinases has in average more than 20 different physiologically relevant substrates. In addition, some substrate proteins can show pleiotropic biological action interfering with different physiological functions in parallel. Hence, even inhibition of a single protein kinase by an absolutely specific inhibitor could lead to many different physiological effects. On the

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other hand structurally and functionally related kinases can show different susceptibility to specific kinase inhibitors and the effect of inhibition of the one kinase may be compensated by the non-targeted related kinase. Probably, only a small subset of the human kinome is suited as target for small molecule inhibitors and only a few kinases will be useful targets in inflammation.

There is a wide variety of different small molecule protein kinase inhibitors which have been in use in biochemical research labs for many years [3]. Most of these inhibitors act in an ATP-competitive manner and their potency depends on the intrinsic affinity of the inhibitor for the ATP-binding pocket and residues in its vicinity and on the specific affinity of the kinase for ATP [10]. Since the ATP-concentration in living cells ranges between one to five micromolar, the *in vivo* potency of an inhibitor can only be estimated from *in vitro* experiments using comparable ATP-concentrations. Many kinases are regulated by ligand-binding or phosphorylation and are thought to exist in two conformations, an inactive and an active with different affinities for ATP making it also important to which conformation the inhibitor is binding. Interestingly, a few kinase inhibitors, such as the c-abl tyrosine kinase inhibitor Gleevec or the p38 MAPK inhibitor BIRB-796, extend their interaction with the kinase to further regions of the catalytic core including the activation loop and stabilise the inactive conformation of the enzyme leading to higher potency [5, 6]. There are also kinase inhibitors which do not competitively interfere with ATP-binding. The best example is the group of PD98059-related compounds which binds to the inactive, dephosphorylated conformation of MAPK-kinase 1 preventing its activation by phosphorylation [7, 8].

Beside potency, the specificity of an inhibitor is of equal importance (see article of Schäfer, this issue). All compounds available so far inhibit more than one kinase of the kinome subset tested [9, 10]. Some of these inhibitors, such as staurosporine, show a broad spectrum inhibiting more 100 protein kinases. Recently, 16 clinically relevant (approved or in development) kinase inhibitors were tested for its specificity against 119 protein kinases spread over the entire kinome [17]. It was found that even the "specific" inhibitors Gleevec and BIRB-796 cross-inhibit phylogenetically unrelated kinases with significant potency indicating that inhibitor specificity is still the most important issue. This notion is also supported by proteomic approaches using immobilised small molecule inhibitors for affinity purification of target kinases [18]. In consequence the limited selectivity of most kinase inhibitors available so far can lead to very heteroge-

neous clinical profiles with regard to effects and side effects in patients.

It is the aim of the following chapters of this issue to summarize the current knowledge on protein kinases as targets in inflammation by giving state of the art overviews on particular fields and particular signalling pathways. Both receptor tyrosine kinases (see Koch and Tamura, in this issue) and soluble tyrosine kinases (see Melcher *et al.*, in this issue) contribute and mitogen- and stress-activated kinases cascades (see Hitti and Kotlyarov, in this issue) as well as transcriptional activation by NFkB (see Kracht, in this issue) are involved.

## REFERENCES

- [1] Asadullah, K.; Volk, H.D.; Sterry, W. *Trends Immunol.*, **2002**, *23*, 47-53
- [2] Schottelius, A.; Asadullah, K.; Sterry, W. In *Cytokine and anti-cytokine therapy in Dermatology*; Asadullah, Volk, Sterry, Eds.; in press
- [3] Cohen, P. *Curr. Opin. Chem. Biol.*, **1999**, *3*, 459-465.
- [4] Kumar, S.; Boehm, J.; Lee, J.C. *Nat. Rev. Drug Discov.*, **2003**, *2*, 717-726.
- [5] Orchard, S. *Curr. Opin. Drug Discov. Devel.*, **2002**, *5*, 713-717.
- [6] Saklatvala, J. *Curr. Opin. Pharmacol.*, **2004**, *4*, 372-377.
- [7] Cohen, P. *Nat. Rev. Drug Discov.*, **2002**, *1*, 309-315.
- [8] Manning, G.; Whyte, D.B.; Martinez, R.; Hunter, T.; Sudarsanam, S. *Science*, **2002**, *298*, 1912-1934.
- [9] Caenepeel, S.; Charydzak, G.; Sudarsanam, S.; Hunter, T.; Manning, G. *Proc. Natl. Acad. Sci USA*, **2004**, *101*, 11707-11712.
- [10] Knight, Z.A.; Shokat, K.M. *Chem. Biol.*, **2005**, *12*, 621-637.
- [11] Pargellis, C.; Tong, L.; Churchill, L.; Cirillo, P.F.; Gilmore, T.; Graham, A.G.; Grob, P.M.; Hickey, E.R.; Moss, N.; Pav, S.; Regan, J. *Nat. Struct. Biol.*, **2002**, *9*, 268-272.
- [12] Schindler, T.; Bornmann, W.; Pellicena, P.; Miller, W.T.; Clarkson, B.; Kuriyan, J. *Science*, **2000**, *289*, 1938-1942.
- [13] Alessi, D.R.; Cuenda, A.; Cohen, P.; Dudley, D.T.; Saltiel, A.R. *J. Biol. Chem.*, **1995**, *270*, 27489-27494.
- [14] Sebolt-Leopold, J.S.; English, J.M. *Nature*, **2006**, *441*, 457-462.
- [15] Bain, J.; McLauchlan, H.; Elliott, M.; Cohen, P. *Biochem. J.*, **2003**, *371*, 199-204.
- [16] Davies, S.P.; Reddy, H.; Caivano, M.; Cohen, P. *Biochem. J.*, **2000**, *351*, 95-105.
- [17] Fabian, M.A.; Biggs, W.H. 3<sup>rd</sup>; Treiber, D.K.; Atteridge, C.E.; Azimioara, M.D.; Benedetti, M.G.; Carter, T.A.; Ciceri, P.; Edeen, P.T.; Floyd, M.; Ford, J.M.; Galvin, M.; Gerlach, J.L.; Grotzfeld, R.M.; Herrgard, S.; Insko, D.E.; Insko, M.A.; Lai, A.G.; Lelias, J.M.; Mehta, S.A.; Milanov, Z.V.; Velasco, A.M.; Wodicka, L.M.; Patel, H.K.; Zarrinkar, P.P.; Lockhart, D.J. *Nat. Biotechnol.*, **2005**, *23*, 329-336.
- [18] Godl, K.; Wissing, J.; Kurtenbach, A.; Habenberger, P.; Blencke, S.; Gutbrod, H.; Salassidis, K.; Stein-Gerlach, M.; Missio, A.; Cotten, M.; Daub, H. *Proc. Natl. Acad. Sci. USA*, **2003**, *100*, 15434-15439.