

JB Commentary

Encountering unpredicted off-target effects of pharmacological inhibitors

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With the emergence of chemical biology, the use of pharmacological inhibitors in biological research has been expanding. SP600125 is a low-molecular weight compound that has been widely used to inhibit c-Jun-N-terminal kinase (JNK). A recent publication by Tanemura *et al.* (*J. Biochem.* 145:345–354, 2009) indicated that SP600125 also inhibits phosphatidylinositol 3-kinase (PI3K) in an isoform-selective fashion: it efficiently inhibited the delta isoform of p110 catalytic subunit (p110 δ), which is primarily expressed in leucocytes, but neither of the ubiquitously expressed isoforms, p110 α and p110 γ . Here, I discuss what we learn from such unpredicted off-target effects of pharmacological inhibitors.

Keywords: Inhibitor/Kinase/JNK/off-target/PI3K.

Abbreviations: GIST, gastrointestinal stromal tumour; JNK, c-Jun N-terminal kinase; PI, phosphatidylinositol; PI3K, phosphatidylinositol 3 kinase.

Recently, cell-permeable low-molecular weight inhibitors have been used extensively as probes in order to study intracellular events. These compounds are convenient tools for preliminary screening of signalling pathways involved in various cellular responses. In addition, their effects are usually rapid onset (arising immediately after addition) and rapid offset (disappearing quickly after removal). Thus, they can be used for transient inhibition of target molecules, which is not possible when using a gene knockdown approach (1). Unfortunately, most of these compounds lack strict selectivity. If the target proteins had unique catalytic functions and active site structures, achieving specificity would not be a great challenge. In the case of kinases, however, there are several hundred members encoded within the human genome,

and they all share similar structural frameworks in their active sites. Most kinase inhibitors target the ATP-binding sites in order to abrogate enzymatic activity. Therefore, it is easy to see why kinase inhibitors are not very specific, and why off-target effects are inevitable.

In clinical drugs, off-target effects are sometimes advantageous (2). Gleevec (Imatinib) was first developed for treatment of chronic myelogenous leukaemia as an oral inhibitor of BCR-Abl, a fusion kinase generated by chromosomal translocation (3). Currently, it has also been approved for treatment of gastrointestinal stromal tumour (GIST), because it inhibits the receptor tyrosine kinases c-kit and platelet-derived growth factor receptor α , mutations of which are implicated in the pathogenesis of GIST (4). The off-target effects of Gleevec have thus extended its range of clinical applications. Nexavar (Sorafenib) was originally developed as a Raf-kinase inhibitor, but subsequently shown to be a multi-kinase inhibitor that suppresses tumour angiogenesis through inhibition of receptor kinases for proangiogenic growth factors (5). Nexavar is now used for treatment of patients with advanced renal carcinoma and unresectable hepatocellular carcinoma; its therapeutic efficacy is conferred through multiple mechanisms (6).

In research use, however, off-target effects sometimes lead to misinterpretation of data, especially if researchers are not aware of them. Accordingly, the selectivity of kinase inhibitors has been examined using recombinant kinases (7–10), giving us valuable information on widely used kinase inhibitors. At the National Centre for Protein Kinase Profiling (<http://www.kinase-screen.mrc.ac.uk/>), the specificities of kinase inhibitors are assessed using the Protein Kinase Panel, which is composed of ~120 kinases. This number is not small, but nearly 80% of protein kinases are still excluded from the panel. In addition, *in vitro* specificities may be somewhat different from *in vivo* specificities. The *in vitro* system employs standardized conditions for enzyme, substrate and ATP concentrations, analogous to ‘standard temperature and pressure’ in physical chemistry; these conditions might not reflect the situation in live cells. Some target kinases may not be expressed in some cellular contexts, and even if expressed, a given kinase may not be activated.

SP600125 was originally developed as anti-cancer agent (11), and was subsequently shown to inhibit c-Jun-N-terminal kinases (JNKs) (12). This compound is widely used as a selective inhibitor of JNKs in order to examine involvement of the JNK pathway in cell-signalling processes. However, it is also known to inhibit other kinases. Bain *et al.* (8, 9) examined the effects of SP006125 on protein kinases other than JNKs, and found that 20 out of 66 protein kinases examined were inhibited to a similar or greater extent than JNKs. These include p70 ribosomal protein S6 kinase (S6K1), a kinase involved in protein synthesis.

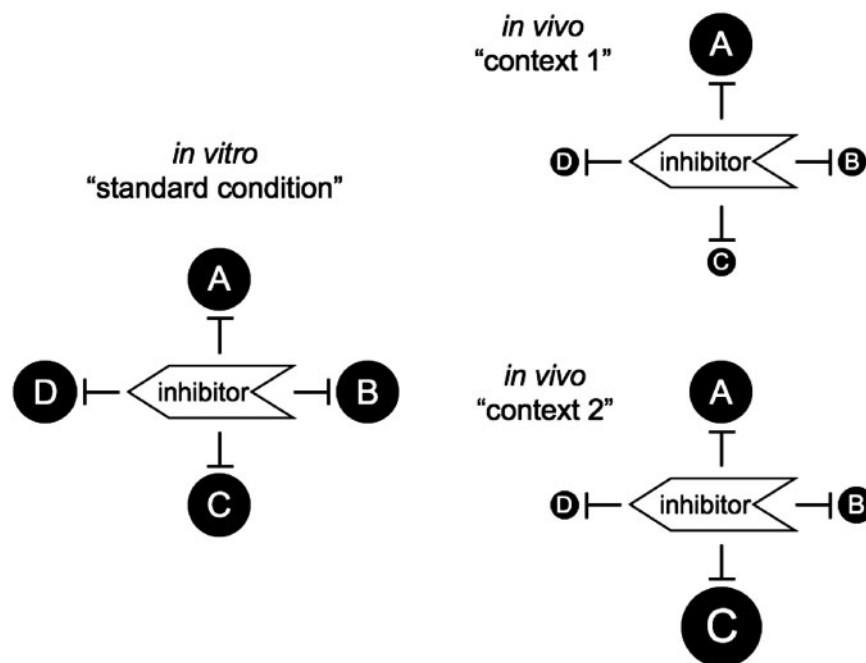


Fig. 1 Apparent target specificities of chemical inhibitors can be dependent on the context within target cells. Even if a chemical compound inhibits several targets (A–D) under the *in vitro* ‘standard condition’, it may appear to be specific to target A (context 1) or dually specific to targets A and C (context 2) when other targets are expressed or activated at very low levels *in vivo*.

Consistently, a high concentration of SP600125 (50 μ M) inhibited cap-dependent translation, while shRNA for JNKs did not (13). In spite of such information, researchers continue to use SP600125, probably because it is selective for practical purposes. In addition, the compound can be reliably used to exclude possible involvement of JNKs when it fails to affect cellular events of interest (1).

A recent paper by Tanemura *et al.* (14) provides us with a good example of the way in which careful experiments can unveil unpredicted targets of chemical inhibitors. The authors first found that a standard concentration of SP600125 (10 μ M) efficiently inhibited mast cell responses after stimulation of Fc receptor for immunoglobulin E (Fc ϵ RI). However, they also noticed that serotonin release preceded JNK activation following Fc ϵ RI stimulation. The finding raised the possibility that SP600125 inhibits mast cell responses through pathway(s) other than the JNK pathway. Therefore, they studied mast cells prepared from mice lacking MKK7, the kinase that directly activates JNKs (15). In mast cells derived from MKK7 knockout mice, JNKs were not activated after stimulation of Fc ϵ RI; however, cellular responses, including degranulation as well as induction of cytokines, were not abrogated. The authors next searched for the crucial target(s) of SP600125 involved in transduction of Fc ϵ RI signalling, and found that SP600125 inhibits phosphatidylinositol 3-kinase (PI3K) activity activated by Fc ϵ RI stimulation.

PI3K catalyses phosphorylation of the three positions of phosphatidylinositol (PI) or its phosphorylated derivatives, PI(4)P and PI(4,5)P₂. The enzyme is involved in signal transduction pathways downstream of various transmembrane receptors. How is it that the

effect of SP600125 had not yet been discovered, even though the compound has been widely used? Part of the explanation is that SP600125 inhibits only one specific isoform of PI3K, the δ -isoform that is exclusively expressed in leucocytes, but not the α - or γ -isoforms that are ubiquitously expressed. SP600125 inhibits the δ -isoform of PI3K to roughly the same extent as wortmannin, although a higher concentration is required.

This work tells us that the apparent specificities of chemical inhibitors can depend on cellular contexts (Fig. 1). Our knowledge of the behaviour of chemical inhibitors *in vivo* is incomplete. Therefore, we must avoid misinterpretation of our experimental data by examination that is careful as well as fundamental. Guidelines for the use of kinase inhibitors have recently been proposed (1).

Conflict of interest

None declared.

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