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The road less traveled: modulating signal transduction enzymes by inhibiting their protein–protein interactions

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The biological functions of intracellular signaling enzymes typically depend on multiple protein–protein interactions (PPI) with substrates, scaffolding proteins, and other cytoplasmic molecules. Blocking these interactions provides an alternative means to modulate signaling activity without fully ablating the catalytic activity of the target. Several recent reports describe small-molecule antagonists that target PPI sites on signaling enzymes. These findings suggest that such sites may often be druggable. However, the hypothesis that targeting such sites might confer on the resulting inhibitors improved properties of efficacy and/or tolerability, while appealing, remains largely untested.

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Introduction

Drug-like small-molecule inhibitors of PPI targets have generally been difficult to achieve [1–3,4•,5]. The past several years, however, have seen a steady increase in the number of direct small-molecule PPI inhibitors appearing in the literature [6–8] including two that have progressed to clinical testing in humans [9,10]. In particular, there has been considerable success developing inhibitors against PPI that involve either a contiguous peptide strand or an α-helix on one protein binding to a shallow groove on the other [1,9–17,18]. A hallmark of such interactions is that they can be inhibited by a fairly short peptide that recapitulates the sequence of the key strand or helix that mediates binding.

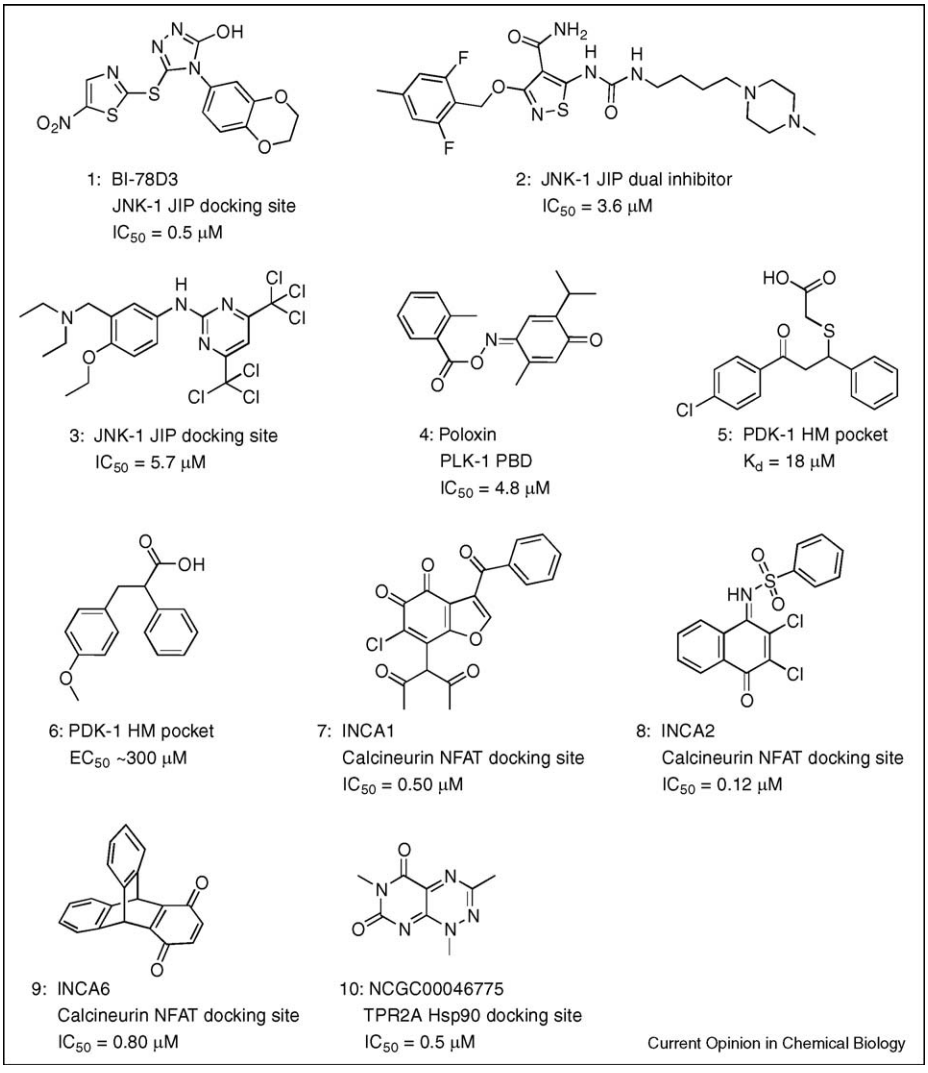
In this review, we focus on a potentially important application for small-molecule inhibitors of PPI that has received attention only recently. That is, modulators of cell signaling that act not by blocking the active site of signaling enzymes, but by inhibiting their interactions with substrates and scaffolding proteins. The activity of signaling enzymes such as kinases and phosphatases is regulated through their expression levels, subcellular localization, participation in protein–protein complexes, and/or by the phosphorylation state of the enzymes themselves [19••]. There could be several advantages to targeting these regulatory mechanisms rather than the enzyme's catalytic activity. For example, PPI inhibitors could be more selective than ATP-mimetic kinase inhibitors, since ATP-binding sites tend to be well conserved. Targeting a regulatory site might also bring improved biological selectivity, if the enzyme's activity is pathological with regard to some substrates but not others. Finally, there could be synergy between an active site and a PPI inhibitor of the same enzyme, such that combination therapy might achieve therapeutic inhibition at dose levels that are better tolerated by the patient. Thus we see the usual druggability issue turned on its head: are there situations in which targeting the more difficult PPI site is advantageous compared to the typically more tractable enzyme active site?

Several examples of small molecules that block PPI sites on signaling enzymes have been reported recently (Figure 1). The cases described here represent early lead-discovery efforts, but provide important proof-of-concept that such sites can be effectively targeted. The resulting inhibitors present an opportunity to determine whether inhibiting a PPI interface on a given signaling enzyme results in a biological outcome that is distinct from inhibiting the active site, and whether this distinction promises an improved balance between activity and toxicity.

Inhibition of JNK/JIP binding

The c-Jun N-terminal kinases JNK-1, JNK-2, and JNK-3 have been the focus of intensive drug-discovery research for multiple indications [20]. In particular, JNK-1 has been implicated in insulin sensitivity, and is a promising target for type II diabetes [21,22]. JNK-1 is activated by upstream kinases through binding to a scaffold protein known as JNK-interacting protein (JIP)-1. The same JIP-1 docking site on JNK-1 also recruits downstream targets of JNK, including c-Jun and activating transcription factor (ATF)-2. Other mitogen activated protein kinases, such as extracellular signal-regulated kinase (ERK)-2, also contain an analogous substrate-docking site [23•]. Figure 2A shows the cocrystal structure of JNK-1 with an 11-mer peptide

Figure 1



Chemical structures of compounds that inhibit the protein–protein interactions of signal transduction enzymes. IC_{50} values for compounds 1–4, and 7–10 were determined by inhibition of the protein/peptide interaction. The K_D value for compound 5 was determined by isothermal calorimetry. The EC_{50} value for compound 6 was estimated from assays for enzymatic activity. References are provided in the text.

derived from JIP-1 that binds with a dissociation constant (K_D) of 0.4 μM [24]. A cell-permeable version of the peptide inhibits JNK-1 activity in cells and in an animal model of diabetes, providing proof-of-concept for inhibiting JNK-1 activity by targeting this protein–protein interaction [22]. Two recent publications describe small-molecule inhibitors that target the JNK-1/JIP-1 interaction [25••,26••].

Stebbins *et al.* screened a 30 000-compound library for inhibitors of the binding a JIP-derived peptide to JNK-1 [25••]. A compound designated BI-78D3 (1; Figure 1) was shown to inhibit peptide/JNK-1 binding selectively with an inhibition constant (IC_{50}) of 0.5 μM. The authors demonstrated that BI-78D3 bound to the JIP-1-docking site on JNK-1 using enzyme kinetics, site-directed mutagenesis, paramagnetic relaxation NMR, and computational modeling. As expected for this mechanism of action, BI-78D3 did not inhibit phosphorylation of a short peptide substrate that does not contact the docking site, but did inhibit phosphorylation of the docking-dependent protein substrate ATF2. In cells, BI-78D3 inhibited phosphorylation of the JNK-1 substrate c-Jun. BI-78D3 was active in a preliminary animal model, reducing ConA-induced release of the liver enzyme ALT that is driven in part by JNK signaling [27]. In a second model with insulin-insensitive mice, BI-78D3 treatment led to a reduction in blood glucose two to three hours after insulin treatment. This experiment suggested that inhibiting JNK-1/JIP-1 could restore insulin sensitivity, as has been shown separately for the isolated JIP domain [22]. How