Protein–protein interactions as targets for small-molecule therapeutics in cancer

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Small-molecule inhibition of the direct protein–protein interactions that mediate many important biological processes is an emerging and challenging area in drug design. Conventional drug design has mainly focused on the inhibition of a single protein, usually an enzyme or receptor, since these proteins often contain a clearly defined ligand-binding site with which a small-molecule drug can be designed to interact. Designing a small molecule to bind to a protein–protein interface and subsequently inhibit the interaction poses several challenges, including the initial identification of suitable protein–protein interactions, the surface area of the interface (it is often large), and the location of ‘hot spots’ (small regions suitable for drug binding). This article reviews the general approach to designing inhibitors of protein–protein interactions, and then focuses on recent advances in the use of small molecules targeted against a variety of protein–protein interactions that have therapeutic potential for cancer.

Fundamental processes in living cells, such as intracellular signal transduction and maintenance of cytoskeletal architecture, are largely controlled by proteins, often acting in concert with other protein partners through protein–protein interactions (PPIs). Inappropriate protein–protein recognition can contribute fundamentally to many diseases, including cancer. Selective, small-molecule modulation of PPIs is therefore an area of growing interest to pharmaceutical science (Refs 1, 2, 3, 4, 5, 6, 7, 8, 9).

Despite the pivotal role of PPIs in many processes relevant to cancer development, targeting PPIs as a therapeutic strategy in cancer is still in its infancy, and examples of modulators of protein complexes as potential therapeutic agents are few relative to the more widely studied area of small-molecule enzyme (e.g. kinase) inhibitors (Ref. 10). The reasons for the dominance of kinase (and other enzyme) inhibitors as targets for modern cancer therapeutics are centred on the enzyme substrate-binding site. In the case of enzyme inhibition, the design methodology for these compounds is well established, and active sites are usually contained within deep clefts that clearly define the ligand-binding site and contain the critical amino acid residues required for ligand interaction. Furthermore, all
molecules in a biological system are bathed in an aqueous environment, and enzyme active-site clefts tend to shield binding sites from water molecules that can otherwise hinder ligand interactions.

Small-molecule drug discovery against protein–protein targets is more challenging than for conventional small-molecule protein targets but represents an innovative direction for 21st century medicine. Although the field has already been the subject of several reviews (for a selection see Refs 1, 2, 3, 4, 5, 6, 7, 8, 9), in this article we aim to give a broad overview of emerging small-molecule PPI inhibitors in the anticancer field.

The concept of protein–protein interaction and ‘hot spots’
Among the reasons for the lack of modulators of protein complexes as therapeutic agents has been the notion that protein interfaces represent difficult or intractable targets because of the generally large and noncontiguous nature of PPI surfaces. This perceived problem is compounded by the lack of high-throughput screening technologies and paucity of small molecules in compound libraries with the size and functionality to modulate protein-complex targets selectively. In recent years, however, several reports have appeared that challenge the dogma that PPIs represent difficult targets to modulate using small molecules. In particular, the concept of PPI ‘hot spots’ has aided the development of small molecules that interact with a small area of an entire protein–protein interface to exert high-affinity and selective binding (Ref. 11).

The initial challenge in developing PPI inhibitors is the discovery of specific PPIs and in turn identification of those that are ‘druggable’. Two types of PPI can be defined: transient and tight. Tight interactions can be identified more easily, through biochemical co-purification; in contrast, transient interactions have been characterised by site-directed mutagenesis and molecular modelling as it is often difficult to co-crystallise transient partners (Ref. 12). Computational techniques have also been used to identify PPIs and their inhibitors, and have been recently reviewed (Ref. 13). Following the identification of a suitable PPI, the next step involves study of the binding interface and ligand design. This is also challenging compared with designing a ligand to interact with an active-site cleft. Interaction sites are typically relatively flat with a large surface area, although shallow grooves are sometimes seen. Ligand-binding sites may be exposed only after conformational changes concomitant with binding of protein partners (Ref. 14).

To understand better the nature of protein–protein interfaces, a study of 75 PPIs established a ‘standard size’ of 1600 ± 400 Å² for an interaction surface. The smallest interfaces were approximately 1150 Å² and the largest interfaces identified were up to 4660 Å² (Ref. 15). Given the large sizes involved, it would be difficult to target a small molecule to the whole interface. Similarly, large peptide-based inhibitors can be less effective since a single continuous peptide sequence may poorly match the binding surface (Ref. 12). Fortuitously, within a binding interface only a small number of highly conserved amino acid residues – ‘hot spots’ – are crucial for the interaction, and thus these are often the target for drug design. Hot spot residues within a protein–protein interface are less obvious to identify than, for example, important residues within an enzyme active site. Although a laborious process, site-directed mutagenesis has been commonly employed to locate hot spot residues, leading to a definition of a hot spot as ‘a residue that, when mutated to Alanine, gives a distinct drop in the binding constant, typically ten-fold or higher’ (Ref. 16). Phage display has also been used to detect hot spots (Ref. 17).

Based on the analysis of currently identified hot spots, the amino acids tryptophan (Trp), tyrosine (Tyr) and arginine (Arg) occur frequently, often surrounded by hydrophobic regions. These amino acids are thought to exclude solvent from the important interacting residues and are favoured as they can form multiple interactions. For example, despite structural similarity, phenylalanine is three times less likely to occur in a hot spot than Tyr, presumably due to the ability of Tyr to form an additional hydrogen bond. Hot spots contain leucine, methionine, serine, threonine and valine residues less frequently (Ref. 11). However, a review of the thermodynamic aspects of PPIs concludes that hydrophobic interactions are also a key driving force for PPIs, and that the complex thermodynamics of PPIs are ultimately a function
of how protein–protein binding changes the internal structure of the molecule(s) (Ref. 18).

Scope of the review
Over the past decade, the principal exploitation of PPIs has been through therapeutic antibodies, such as Herceptin, which are showing great promise as a new class of drugs in cancer treatment (Ref. 19). Therapeutic antibodies are large proteins that interact directly, for example, with membrane-bound receptor proteins, such as growth factor receptors, to elicit a biological response. Although an important class of cancer therapeutic agents exploiting protein–protein recognition, therapeutic antibodies (or other ‘biologics’) are not included in this review; instead, we discuss the emerging class of small-molecule inhibitors of PPIs, focusing on their application to anticancer therapy. We consider established clinical agents that fall into the PPI modulator class (such as tubulin inhibitors), but concentrate on agents in preclinical development (such as the p53–MDM2 inhibitors). The mechanism of action of small-molecule PPI inhibitors differs from that of the antibodies in that a small, nonphysiological compound designed to bind preferentially to a PPI site blocks the binding of a further protein, preventing the formation of a protein dimer/multimer that would otherwise be essential for regulating a biological process.

Protein–protein interactions as therapeutic targets in cancer

Microtubules
One of the most successful therapeutic targets in cancer treatment is tubulin. There are several natural, semisynthetic and synthetic compounds that have significant clinical activity by affecting tubulin PPIs. They have been extensively reviewed elsewhere (Refs 20, 21, 22, 23), but we summarise the topic here for completeness.

Microtubules are large cylindrical proteins – 25 nm in diameter and up to several μm in length (Ref. 21). A microtubule is composed of copolymers of two monomers – α- and β-tubulin – binding in an alternating manner. Microtubules undergo constant dynamic polymerisation and depolymerisation at both ends of the filament (referred to as plus and minus ends), although the process is faster at the plus end (Refs 20, 21, 24). Microtubule dynamicity is required for several biological processes including maintenance of cell shape, cellular signalling and movement; however, their best-studied role is in cell division (Ref. 21). Several drugs exert their antitumour effect by interfering with tubulin PPIs and are classified according to the site to which they bind on microtubules.

A crystal structure of the taxane analogue taxol (a natural product from the Pacific yew tree Taxus brevifolia) bound to tubulin indicates that it binds within a hydrophobic pocket. It is suggested that taxol stabilises microtubules by strengthening PPIs, thus inhibiting cell division (Ref. 20). Several synthetic taxane derivatives are under clinical investigation. In addition, nontaxane compounds are known to bind at the taxane site and several are under preclinical investigation, including the epothilones (Ref. 25), eleutherobins (Ref. 26), discodermolides (Ref. 27), sarcodicyins and laulimalides (Ref. 21).

By contrast to taxol, colchicine (a natural product from the plant genus Colchicum) and its analogues destabilise microtubules. Although the crystal structure of the tubulin–colchicine complex is not available, studies suggest that colchicine interacts with β-tubulin at the interface between two tubulin monomers (Refs 28, 29, 30).

Vincristine and vinblastine (natural products from the periwinkle plant Catharanthus roseus), and new-generation analogues vinflunine and vinorelbine, also destabilise microtubules (Ref. 31). Vinblastine has been shown to interact with β-tubulin (Refs 20, 23, 32). Other natural products, including rhyzoxin, cryptophycins and dolastatin 10, have similar effects to the vincas (Ref. 33).

p53
The tumour suppressor protein p53, known informally as ‘the guardian of the genome’, has been extensively studied in the field of cancer. It protects biological tissues from malignant transformation and forms a central part of the DNA-damage response. It is estimated that around 50% of all human tumours have mutations in the p53 gene (TP53), implying that cancer cells sustain viability by reducing the biological activity of p53 (Refs 34, 35, 36).

Human p53 contains 393 amino acids arranged in four functional domains. The N-terminal
interacts positively with transcription machinery and with MDM2, an E3 ubiquitin ligase. The second domain constitutes the DNA-binding region, and harbours 90% of the reported p53 mutations. The third domain is responsible for the formation of the p53 tetramer. The current model of p53 function suggests that DNA interactions occur with a tetramer form of p53 (actually a dimer of a dimer) (Refs 34, 36, 37).

Several events are known to induce the activity of p53, including DNA damage, hypoxia, oncogene overexpression and viral infections (Refs 36, 38, 39). Numerous biological functions have been attributed to p53 such as apoptosis (Refs 40, 41) and senescence (Ref. 42), G1 arrest, cell cycle control, DNA repair (Ref. 43) and angiogenesis inhibition. Among the most important of these functions is apoptosis (programmed cell death), a conserved process that mediates the elimination of a cell upon exposure to genomic stress. The complete mechanism through which p53 mediates apoptosis is still under study; it is mediated primarily but not exclusively by p53. Following a stressful event, p53 activates through its N-terminus many pro-apoptotic genes such as POXA and PUMA. Another suggested mechanism is linked to the mitochondria, whereby p53 binds to the mitochondrial membrane resulting in the formation of pores and the release of cytochrome c; this in turn activates caspases, which degrade the nucleus resulting in cell death (Refs 44, 45).

Under normal conditions, cellular levels of p53 are barely detectable: it has a short half-life of 10–20 min as a result of a PPI with MDM2 that results in the negative regulation of p53 (Fig. 1) (Refs 34, 35). MDM2 catalyses the binding of ubiquitin to p53, thereby directing it to degradation by the proteasome (Ref. 46). In a typical feedback loop, p53 increases the transcription of MDM2 (Ref. 47). Under stressful conditions, p53 concentrations are increased due to a reduction in its MDM2-mediated degradation and a concurrent increase in p53 transcription.

As increased p53 activity has an antitumour effect, there have been many attempts to elevate the biological activity of p53 by increasing its accumulation in the cell. The main strategies employed have been stabilisation of p53 or protection from ubiquitination and proteasomal degradation, primarily by blocking the p53–MDM2 interaction (Refs 48, 49, 50). Close examination of the p53–MDM2 interface reveals that it is the side chains of only three key p53 amino acids that make most of the interactions within a hydrophobic groove in the surface of MDM2 (Figs 1 and 2). In an X-ray-crystallography-derived model of the p53–MDM2 interaction shown in Figure 2a, a p53-derived peptide (green) is shown complexed with an MDM2 fragment (surface render) containing the hydrophobic binding cleft. The p53 fragment forms an α-helix from which the side chains of the three amino acids Leu26, Trp23 and Phe19 (shown left to right) project into the binding cleft, promoting the hydrophobic interaction. The hot spot is a good target for drug design – the strategy being to design a drug that mimics the conformation and interaction of the three p53 residues.

**Small-molecule inhibitors of p53 PPIs**

Although a p53–MDM2 PPI inhibitor has yet to reach clinical trials, several small-molecule inhibitors with significant potency have been reported in the literature. Most of these molecules are useful experimental tools for research. Chalcones (compounds derived from 1,3-diphenylprop-2-en-1-one) were early-reported molecules with such activity. They interfere with p53–MDM2 interactions by binding to the p53 transactivation domain with relatively low potency: IC_{50} were in the range 50–250 μM (IC_{50} is the concentration of inhibitor required to achieve 50% inhibition) (Ref. 53). The natural product chlorofusin is a fungal metabolite containing a peptide segment that was discovered by screening over 53,000 microbial extracts. It inhibits the p53–MDM2 interaction with an IC_{50} of 4.6 μM; however, it is a large (molecular weight of 1363.7), structurally complex molecule that is less practical for drug development or clinical use than a smaller molecule (Ref. 54). A more drug-like molecule called RITA (‘reactivation of p53 and induction of tumour cell apoptosis’) (Fig. 3a) was similarly discovered by screening a molecular library against cancer cell lines. It too has micromolar potency and was reported to interfere with p53–MDM2 interactions by binding to p53 (Ref. 55), although more-recent nuclear magnetic resonance (NMR) studies

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argue that RITA does not in fact block p53–MDM2 binding in vitro (Ref. 56).

The nutlins are a series of highly promising molecules for p53–MDM2 inhibition and were discovered by high-throughput screening followed by structure-based optimisation (for an example structure, see Fig. 3b). The nutlin inhibitors are similarly thought to increase p53 levels, by blocking MDM2-mediated degradation. The PPI of p53 and MDM2 is mediated by the side chains of three p53 amino acids (Leu, Trp and Phe) that are recognised by MDM2. The nutlin inhibitors mimic the binding of these amino acid side chains and preferentially bind to MDM2. This physically blocks the interaction of the two proteins, thus releasing p53 that is free to exert a beneficial antitumour effect. This schematic illustration is derived from the X-ray data presented in Fig. 2a, and comparison of Figs 1 and 2a should be instructive. Abbreviation: MDM2, ‘mouse double minute 2’ homologue.

The BCL2 family

The B-cell lymphoma 2 (BCL2) family of proteins is composed of more than 20 members (Refs 58, 59, 60). Some members are anti-apoptotic [e.g. BCL2, BCL2L1 (Bcl-xL), BCL2L2 and MCL1], whereas others are pro-apoptotic [e.g. BAX, BAK1, BID and BCL2L11 (BIM)]. The family members cooperate through PPIs to mediate the intrinsic apoptotic pathway (Refs 61, 62). Anti-apoptotic BCL2 family members protect cells from apoptosis by inhibiting the action of pro-apoptotic members (Ref. 63). Three-dimensional NMR studies of anti-apoptotic BCL2 proteins revealed the presence of a hydrophobic groove that acts as a binding site for the BH3 peptide domain of pro-apoptotic BCL2 members (Ref. 64). Agents designed to target the binding grooves of anti-apoptotic BCL2 proteins are predicted to induce apoptosis in cancer cells (which overexpress these anti-apoptotic proteins) by antagonising their protective effect.

Over the past ten years, several nonpeptidic small-molecule inhibitors to anti-apoptotic BCL2 proteins have been introduced and some of them are already in clinical trials as...
single-agent chemotherapy. For example, GX015-070 (Obatoclax) is a small-molecule BCL2 inhibitor developed by Gemin X Biotechnologies Inc. currently being tested in multiple Phase I and Phase II clinical trials against both solid tumours and haematological malignancies (Ref. 66). The development of BCL2-modulatory small molecules as potential anticancer therapeutics has been reviewed in recent years (for examples, see Refs 65, 66), and discussion here is limited to two examples that have pioneered interest in this field and provided a proof-of-principle for inhibition of BCL2 PPIs as a viable therapeutic strategy in cancer.

### Small-molecule inhibitors of BCL2 PPIs

ABT-737 (Fig. 3c) was discovered by high-throughput NMR-based screening of a chemical library to identify small molecules that bind to the BH3-binding groove of BCL2L1 (Ref. 66). ABT-737 was found to bind to BCL2, BCL2L1 and BCL2L2 with high affinity ($K_i \leq 1$ nM). ABT-737 did not itself induce release of cytochrome $c$ from mitochondria, but inhibited BCL2 protection at concentrations $\leq 10$ nM by blocking the inhibition of BID-mediated cytochrome $c$ release through binding to the BH3-binding groove of BCL2. This binding disrupted the PPI between BCL2L1 and pro-apoptotic BCL2 proteins.

ABT-737 was found to be active both as a single agent or when used in combination with other chemotherapies or radiation, where it reduced the EC$_{50}$ (the half maximal effective concentration – the concentration that produces a 50% effect compared with the maximum) of several antitumour agents by 50% or more. As a single agent, it displayed potent cytotoxicity in several lymphomas and small-cell lung cancer (SCLC), where it had an EC$_{50}$ of 0.13–0.85 $\mu$m.

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**Figure 2. Structural analysis of the interactions of MDM2 with p53 and with a nutlin inhibitor.**

(a) X-ray crystallographic models of the p53–MDM2 interaction (Ref. 51). A fragment of MDM2 is shown rendered as a solvent-accessible solid surface and coloured by atom type (carbon, grey; oxygen, red; nitrogen, blue; sulphur, yellow). The p53 fragment is rendered as a green ribbon showing only the side-chains of Leu26, Trp23 and Phe19 rendered as sticks and coloured by atom type (carbon, green; nitrogen, blue). These three nonpolar side-chains project into a hydrophobic (i.e. predominantly carbon-containing) pocket in the surface, thus stabilising the interaction between p53 and MDM2. (b) X-ray crystal structure of the MDM2–nutlin-inhibitor complex (Ref. 57). The MDM2 fragment is again shown as a solvent-accessible solid surface, from a similar perspective. Binding to the hydrophobic pocket is a nutlin inhibitor, rendered as sticks and coloured by atom type (carbon, green; nitrogen, blue; oxygen, red; bromine, purple). Note that the hydrophobic (i.e. mostly carbon-containing) groups project into the binding pocket, mimicking the p53 side-chains and that additional groups in the nutlin inhibitor form further interactions. Abbreviation: MDM2, ‘mouse double minute 2’ homologue.
Chemical structures of protein–protein interaction (PPI) inhibitors

Figure 3. Chemical structures of protein–protein interaction (PPI) inhibitors. (See next page for legend.)
when used in lymphoma cell lines carrying the t(14:18) chromosomal translocation. As an inhibitor of anti-apoptotic BCL2, ABT-737 exhibited pro-apoptotic activity and induced apoptosis in a concentration-dependent manner in patient-derived chronic lymphocytic B-cell leukaemia cells. In vivo, ABT-737 caused complete regression of established SCLC tumour xenografts as a result of apoptotic cell death. ABT-737 induced apoptosis selectively in cancer cells without affecting normal cells, where a significant increase in caspase-3 was noticed in tumour cells but not in liver, heart or intestinal normal cell lines (Ref. 67).

Peptide inhibitors of BCL2 PPIs
The concept of hydrocarbon-stapled helical peptides that bind with increased affinity to protein targets such as BCL2 has recently been pioneered; these ‘stapled’ peptides provide enhanced protease-resistance and cell permeability compared with their nonstapled counterparts. The chemical ‘stapling’ strategy involves the insertion of αααα-disubstituted non-natural amino acids containing side-chains with an olefinic terminus at two carefully chosen peptidic positions to flank either three or six amino acids. The chemoselective ruthenium-catalysed olefin metathesis reaction was used to ‘staple’ together the two alkene tethers to provide peptides that maintain helical structure, but with enhanced stability. A stapled peptide of the BH3 domain from the pro-apoptotic BID protein was found to specifically activate the apoptotic pathway to kill leukaemia cells, and inhibit the growth of human leukaemia xenografts in vivo (Ref. 68).

XIAP–DIABLO
‘Inhibitors of apoptosis proteins’ (IAPs) are endogenous caspase inhibitors that bind and inhibit caspases-3, -7 and -9. Eight members of the IAP family are known so far (Ref. 69), of which X-linked inhibitor of apoptosis protein (XIAP) is the best characterised in terms of its inhibitory mechanism. XIAP has three BIR domains (BIR-1–3) and a RING finger that binds and inhibits caspases through specific PPIs (Ref. 70). The BIR-3 domain of XIAP inhibits caspase-9, while BIR-1 and -2 are more specific for caspases-3 and -7 (Refs 71, 72, 73).

Endogenous inhibition of the IAP family can occur via DIABLO (also known as Smac), a protein released from the mitochondria in response to apoptotic stimuli, which directly interacts with XIAP (Refs 72, 73). This, together with the finding that the levels of different IAP family members were elevated in several tumours, indicated the possibility of targeting the IAP family to induce apoptosis and treat cancer (Refs 74, 75, 76). More-recent studies in this area have focused on the role of TNF-α signalling in mediating apoptotic cell death, since IAP antagonists were found to induce TNF-α-dependent apoptosis (Refs 77, 78, 79).

Since small-molecule inhibitors of XIAP have been recently reviewed (Refs 80, 81), we will focus here on one relevant example from the group of compounds derived from DIABLO.

### Figure 3. Chemical structures of protein–protein interaction (PPI) inhibitors.

(Legend; see previous page for figure.)

(a) RITA is a simple and potentially drug-like thiophene-furan-thiophene derivative. (b) The nutlin series of MDM2–p53 PPI inhibitors is exemplified here by nutlin 3; the dihydroimidazole core (i.e. the central five-membered nitrogen-containing ring) is fixed and variation is introduced within the substituents – for example, nutlin 3 is a chlorine analogue. (c) ABT-737 is a prototypical compound of the PPI inhibitors that target anti-apoptotic BCL2 family members. (d) This inhibitor of the anti-apoptotic XIAP is a tripeptide composed of alanine, an unnatural cyclohexyl amino acid and proline; the N-terminus is modified with a methyl group and the C-terminus is modified with a bulky tetrahydronaphthalene derivative. (e–g) The structures of these GRB2 SH2 inhibitors clearly show the β-turn-like nature of these compounds. Note the addition of a restraining olefin (double bond) bridge in (g). (h) This Raf–Raf inhibitor is derived from the nonsteroidal anti-inflammatory drug sulindac (i); both structures are provided to show the comparison. (j) FJ9 is a small-molecule indole-based inhibitor of the interaction between the receptor FRZ-7 and the PDZ domain of DVL, thereby inhibiting oncogenic WNT signalling. (k) ICG-001 is a small molecule that downregulates β-catenin–TCF signalling by specifically binding to the coactivator CBP. Abbreviations: BCL2, B-cell lymphoma 2; CBP, cyclic AMP response element-binding protein; DVL, dishevelled; FRZ, frizzled; GRB2, growth factor receptor bound protein 2; MDM2, ‘mouse double minute 2’ homologue; SH2, Src-homology 2; TCF, T-cell factor; WNT, ‘wingless-type MMTV integration site family’; XIAP, X-linked inhibitor of apoptosis protein.

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peptides (DIABLO ‘mimetics’) that inhibit the interaction between the BIR-3 domain of XIAP and DIABLO. DIABLO-mimetic XIAP inhibitors act by binding more strongly to the BIR-3 domain of XIAP, inhibiting the caspase binding to XIAP at this site; they thus antagonise the inhibitory effect of XIAP and induce apoptosis. For example, a series of tripeptides that bind to the XIAP BIR-3 domain with nanomolar affinities have been reported (Ref. 82). The most active compound of the series (Fig. 3d) showed a wide range of potencies against a diverse panel of human cancer cell lines, with the highest activity (nanomolar EC₅₀) against the human breast cancer cell lines BT-549 and MDA-MB-231, the melanoma cell line SK-MEL-5, and the acute promyelocytic leukaemia cell line HL-60. Interestingly, there was no correlation between the potency of this compound and the expression of XIAP. Measuring caspase-3 activation in MDA-MB-231 cells treated with the tripeptide showed an up to eightfold increase in caspase-3 activity in a dose-dependent manner. Apoptosis was induced by the tripeptide in the absence of an identified apoptotic stimulus. In vivo testing (MDA-MB-231 flank breast cancer xenograft model) showed that a 20 mg/kg/day dose of the compound inhibited tumour growth rate by 50–60%.

**SH2 domains**

Many membrane-associated proteins are coupled to kinases, and their activation is essential for cellular signalling. Src-homology 2 (SH2) domains are noncatalytic, highly conserved domains of approximately 100 amino acids that are an essential feature in many cellular signalling proteins: they recognise hot spots that contain a phosphorylated tyrosine residue and adjacent residues, facilitating the assembly of transient protein pairs that subsequently participate in signal transduction. One of the best-studied SH2 domains is that of growth factor receptor bound protein 2 (GRB2). GRB2 links signalling from receptor proteins to the RAS signal transduction pathway, ultimately controlling the mitogen-activated protein (MAP) kinase cascade. This important regulatory pathway is vital for cell division and an excellent target for novel cancer therapies (Refs 83, 84). Increased GRB2 SH2 signalling is seen in cancer, and so inhibiting the PPI between GRB2 and tyrosine-phosphorylated signalling proteins will have antiproliferative effects. Several tyrosine-containing, peptide-based inhibitors have been reported but have stability and delivery problems. However, compounds containing a phosphorylated tyrosine residue were shown to be optimal for potent PPI inhibition. The development of nonpeptide small-molecule inhibitors has been reported; the phosphorylated compound in Figure 3e represents a typical member of a series of compounds designed to inhibit Grb2 SH2 PPIs (Ref. 85).

However, the phosphotyrosine group of this compound led to practical difficulties, and a bioisosteric replacement of the phosphate group was investigated to improve the stability/delivery profile of this class of compounds. When the phosphate group was replaced with a dicarboxylic acid (Fig. 3f), reasonable activity was retained, suggesting that a similar number of hydrogen bonds with the target protein were formed. Related compounds within this series were shown to penetrate cells and inhibit downstream MAP kinase activation, establishing good rationale for these compounds as potential cancer therapeutics (Ref. 86). Their potency is believed to be related to the ability to mimic secondary structure elements in the PPI. Several PPI hot spots have been found in β-turns (Ref. 87), and the Grb2 SH2 domain recognises a phosphorylated tyrosine hot spot that adopts the conformation of a β-turn. The cyclohexyl amino acid residue in this inhibitor series (Fig. 3e–g) helps to restrain the molecule in a similar, β-turn-like conformation (Ref. 88). The concept has been extended with the addition of an olefin (i.e. carbon–carbon double bond) link (Fig. 3g) to further stabilise the β-turn conformation by incorporating it into a macrocyclic ring. This compound produced a 100-fold enhancement in terms of binding to the protein target, but no cellular activity was observed (Refs 89, 90).

**The RAS–RAF interaction**

RAS is a GTPase important to several signal transduction cascades. Normally, RAS binds to RAF, a kinase that subsequently activates the MAP kinase signalling pathway that is an important proliferative pathway in the cell. RAS is mutated and hence deregulated in approximately 30% of human cancers (Ref. 91).
Inhibitors of the RAS–RAF interaction are thus likely to block cell division and be of interest in anticancer therapeutics. Small-molecule inhibitors have been identified – notably a furano-indene derivative (Fig. 3 h). This compound was derived by rational medicinal chemistry from a metabolite of the nonsteroidal anti-inflammatory drug (NSAID) sulindac (Fig. 3i), following the observation that it had anticancer properties associated with the RAS pathway. Although inhibition of the RAS–RAF interaction by this compound was modest ($IC_{50} = 30 \mu M$), this simple small molecule provides a significant advance over previous attempts to disrupt the interaction (Refs 6, 92). Related investigations reported that a 28 amino acid peptide comprising the hot spot region of RAS inhibited the RAS–RAF interaction with an improved $IC_{50}$ of $3 \mu M$. However, although an order of magnitude less active, the nonpeptide, sulindac-derived inhibitor (Fig. 3 h) remains a more drug-like molecule better suited for further development (Ref. 93).

**WNT pathway PPI targets**

Multiple mechanisms activate the WNT signalling pathway in cancer. For example, in response to WNT ligands, inactivation of GSK-3β leads to inhibition of degradation of the β-catenin-turnover complex, leading to inappropriate transcriptional activation. Inappropriate activation of the WNT pathway is associated with stimulation of proliferation and prevention of apoptosis in a number of human cancers (Ref. 94), and the discovery of inhibitors of WNT pathway PPIs is a growing area of interest. Two PPI targets within the WNT signalling pathway are considered here, where small-molecule inhibitors with antitumour properties have been described.

**Frizzled–Dishevelled**

The Frizzled (FRZ)–Dishevelled (DVL) interaction is of crucial importance in relaying WNT signalling to the β-catenin-turnover complex. Different WNT signalling molecules use different subtypes of FRZ receptor. Particularly promising with respect to cancer drug design is the observation that WNT signalling via FRZ-7 is associated with oncogenesis, and FRZ-7 is reported to be overexpressed in both tumour cell lines and tumour tissues. FRZ-7 interacts directly with the PDZ domain of the DVL family of proteins (overexpressed in a variety of cancer cells); hence specific interference of the FRZ-7–DVL PPI could represent a promising target for cancer therapy. A small-molecule inhibitor (FJ9; Fig. 3 j) of the interaction between FRZ-7 and the PDZ domain of DVL has been identified, which was able to suppress β-catenin-dependent tumour cell growth (Ref. 95). Significant antitumour effects of FJ9 were observed, including induction of apoptosis in human cancer cell lines and in vivo inhibition of tumour growth of mouse xenograft (H460) models. FJ9 is one of the first small-molecule inhibitors to show antitumour activity through selective inhibition of PPIs mediated by PDZ domains.

**β-Catenin–CBP**

Mutations in one of the components of the β-catenin complex regulated by WNT signalling, the adenomatous polyposis coli (APC) gene product, occur in most colon cancers and lead to inappropriate activation of β-catenin-responsive genes in colon cancer. β-catenin regulates transcription in a complex with T-cell factor (TCF), whose formation involves the coactivator cyclic AMP response element-binding protein (CBP). ICG-001 (Fig. 3 k) is a small molecule that downregulates β-catenin–TCF signalling by specifically binding to CBP. ICG-001 was also found to selectively induce apoptosis in transformed (but not normal) colon cells, and have in vitro activity in colon carcinoma cells (growth reduction and induction of apoptosis). Notably, ICG-001 was also found to have efficacy in the Min mouse and nude mouse xenograft models of colon cancer (Ref. 96).

**Summary and conclusions**

The study of PPIs has gathered considerable pace in recent years. Initial experimental difficulties have been overcome and practical applications, for example drug design, can now be considered. Drug design has traditionally been directed towards well-characterised targets, such as enzyme active sites; however, a greater understanding of PPIs and how to target them is opening up new horizons in the future of drug development. A large number of diseases could potentially be treated by PPI inhibition due to the myriad PPIs that occur within the
cell. Progress in the cancer arena has been particularly strong. In most cases PPI inhibition has provided an alternative approach to exploit an existing, known target. However, it seems highly likely that entirely novel PPI-unique therapeutic targets may soon emerge. Many of the early successes targeting PPIs involved the use of peptide inhibitors, but peptides (and large molecules, such as chlorofusin, with molecular weights >500) have poor bioavailability and are generally unsuitable as drug molecules. Although it initially appeared unlikely that small molecules could successfully inhibit PPIs the findings reviewed in this article clearly demonstrate that low molecular weight compounds can successfully block PPIs and thus represent a new emerging class of therapeutic agents.

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For general information on all aspects of cancer (science, treatment and research) visit:
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http://www.cancerhelp.org.uk/

All published protein structures, including several discussed in this article, are available for viewing, download and information at the protein databank:
http://www.rcsb.org/pdb

A protein–protein interaction Wikibook (a free, open content textbook that anyone can edit) can be viewed at:
http://en.wikibooks.org/wiki/Proteomics/Protein_-_Protein_Interactions

Commercial websites containing information relating to compounds reported in this article:
http://www.geminx.com (Phase 2 clinical trial of GX015-070 small-molecule BCL2 inhibitor)

Features associated with this article

Figures
Figure 1. A conceptual diagram of a protein–protein interaction (PPI) inhibitor targeting a PPI (p53–MDM2) hot spot.
Figure 2. Structural analysis of the interactions of MDM2 with p53 and with a nutlin inhibitor.
Figure 3. Chemical structures of protein–protein interaction (PPI) inhibitors.

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