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Chemical modulators working at pharmacological interface of target proteins

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ABSTRACT

For last few decades, the active site cleft and substrate-binding site of enzymes as well as ligand-binding site of the receptors have served as the main pharmacological space for drug discovery. However, rapid accumulation of proteome and protein network analysis data has opened a new therapeutic space that is the interface between the interacting proteins. Due to the complexity of the interaction modes and the numbers of the participating components, it is still challenging to identify the chemicals that can accurately control the protein–protein interactions at desire. Nonetheless, the number of chemical drugs and candidates working at the interface of the interacting proteins are rapidly increasing. This review addresses the current case studies and state-of-the-arts in the development of small chemical modulators controlling the interactions of the proteins that have pathological implications in various human diseases such as cancer, immune disorders, neurodegenerative and infectious diseases.

1. Introduction

For many years, researchers have been exploiting the enzymes or receptors for the discovery of small molecule drugs as therapeutic agents. They usually utilize the holes or pockets at the active site of enzymes or the ligand binding site of receptors to regulate the activity of target molecules. However, together with recent progress in genomics and proteomics, protein–protein interactions provide new way of finding novel bioactive molecules acting on their interfaces.

One of the obstacles to discover the enzyme inhibitors is the lack of structural diversity among the similar proteins in the same folding families. If a bioactive molecule can bind both to a target and similar protein which has a different function, it will generate a side effect. On the other hand, protein–protein interactions provide a variety of target points to regulate their function as well as the structural diversity at the interaction surfaces. Nevertheless, it has been considered as difficult task for researchers to discover inhibitors acting on protein–protein interactions, because of the several reasons such as lack of small-molecule starting points for drug design, large surface areas through which proteins are thought to interact with one another, and typical flatness of the binding interfaces. However, the analyses of the protein–protein interfaces by X-ray crystallography and site-directed mutagenesis has suggested that many binding interfaces consist of compact, centralized region of residues—called as hot-spots—which is crucial for the affinity of the interaction.[1] Moreover, NMR-based fragment discovery and computer-aided molecular design enabled researchers to use the neighboring pocket effectively to improve the potential drug molecules.[2] Now we have increasing number of successful drug candidates inhibiting protein–protein interaction as discussed in this review. Here we would like to review these approaches in a view of therapeutic area with some representative case studies.

2. Implications of protein–protein interactions in the regulation of human diseases

Protein is one of the most essential biomolecules which mediate a variety of cellular processes through complex interaction networks. Protein–protein interactions are the functional units to regulate these biological processes and therefore being recognized as important classes of therapeutic targets. Recent studies on human proteome have suggested that there are 650,000 interactions within human interactome,[3] although the predictions vary from a lower number ca. 40,000.[4] This complexity of interaction network provides a variety of binding interfaces which can be exploited as novel targets for the selection of a functional regulator based on their interface structures. Moreover, the structural diversity of the interfaces may need more unique inhibitors rather than focused chemical library for the enzyme inhibitors. Even though there are many technical hurdles and difficulties to obtain inhibitors of protein–protein interactions, successful applications of new drug discovery targeting the interfaces between proteins are rapidly growing.[5,6] Many protein–protein interactions involved in cell signaling are related to specific diseases such as cancer, immune disorder, neurodegenerative disease, and infectious disease. Thus, researches to

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identify critical interactions of specific proteins implicated in pathological processes have great impact not only to understand etiology but also to drug discovery.

For the treatment of cancer, interactions of several crucial molecules involved in apoptosis such as p53 binding N-terminal domain of HDM2, BAK binding Bcl-2, and the polo-box binding C-terminal domain of PLK1, are actively being studied to prevent abnormal cell growth. For the immunological disorders such as autoimmune disease, cytokines and their receptors are being studied for the discovery of the modulators of T cell proliferation and immune responses. In neurodegenerative diseases, protein–protein interactions in the apoptosis are being targeted to prevent neuronal cell death. In another approach, attempts to inhibit protein–protein interactions to prevent neurotoxic aggregation of tau or amyloid β are being carried out. For infectious diseases, interactions between viral or bacterial and host proteins can be targeted. Some viral proteins which should assemble during their life cycle were exploited to discover antiviral drugs.

3. Investigation of protein–protein interaction modulators

Here we have reviewed the current state of arts in the development of small molecule modulators of protein–protein interaction that show therapeutic potential (Table 1).

3.1. Target disease: Cancer

3.1.1. p53–HDM2 inhibitor

p53 is a tumor suppressor protein which has a crucial role in DNA repair mechanism.[7] It has a variety of anti-cancer function and plays a role in apoptosis of damaged cells. p53 can induce activation of DNA repair proteins and cell cycle arrest in response to the DNA damage. If the p53 gene is damaged, tumor suppression is severely reduced. It is reported that around 50% of human tumors have p53 mutation.[8]

Functionally, p53 is a transcription factor that activates many pro-apoptotic genes.[9] p53 promotes apoptosis and eliminate the damaged cells upon exposure of genomic stress, and protects malignant transformation of biological tissues.[10] Thus, numerous biological studies on p53 have been carried out to find out an effective way of cancer treatment. In normal condition, p53 level is kept low, and is continuously produced and degraded. The degradation of p53 is conducted through the ubiquitination mediated by E3 ubiquitin ligase HDM2 (human protein double minute 2). HDM2 binds to the tumor suppressor p53 and mediates its degradation by the proteasome that results in a loss of p53 activity. p53 is activated by various types of stresses such as DNA damage by UV or chemical agents, oxidative stress, and osmotic shock. The phosphorylation of N-terminal domain of p53 by the protein kinases including the MAPK family is known to mediate this p53 activation through inhibiting HDM2-binding. A 15 residue α-helical structure of p53 is bound to the hydrophobic surface of HDM2, and their complex structure has been solved by X-ray crystallography.[11,12] Inhibition of HDM2 binding to p53 increases the stability and biological activity of p53.

High throughput screening and medicinal chemistry researches to find out small molecule inhibitors of HDM2–p53 interaction were performed by pharmaceutical industry, such as Hoffmann-La Roche and Johnson & Johnson. Potent inhibitors of HDM2–p53 interaction were discovered as Nutlin-1, 2 and 3 (Fig. 1a). Among them, Nutlin-3 inhibits the HDM2–p53 interaction with an IC_{50} of 90 nM, reactivating p53-dependent cell cycle arrest and apoptosis. The other class of inhibitor, benzodiazepinedione (Fig. 1a and b), was discovered by Johnson & Johnson, which inhibited cancer cell proliferation. These compounds also showed a synergistic effect with doxorubicin against tumors. Isseava et al. of National Cancer Institute (NCI) has reported another molecule named RITA (2,5-bis(5-hydroxymethyl-2-thienyl) furan; Reactivation of p53 and Induction of Tumor cell Apoptosis), which binds to p53 rather than HDM2. RITA successfully disrupts the HDM2–p53 interaction in vitro and in vivo, suppressing tumor growth in mouse model.[13] These small molecule inhibitors of HDM2–p53 interaction are recognized as a promising example for anti-cancer drug discovery based on protein–protein interaction.[10,13,14]

3.1.2. Compound affecting tubulin polymerization

Microtubules (MT) are large cylindrical protein polymer composed of alternating heterodimer of α- and β-tubulin. MT plays an important role in maintaining cell shape and cellular movement. As MT is a critical regulator in cell division, there are several natural and synthetic compounds affecting protein–protein interactions of tubulin which have significant clinical activity in cancer treatment.[15,16] A natural product taxol stabilizes microtubules by strengthen protein–protein interactions, leading to the inhibition of cell division. Several compounds acting on taxol binding site of MT were discovered and are under clinical investigation.[17–19] In contrast, colchicine and its analogues destabilize microtubules. Colchicine interacts with β-tubulin at the interface between two tubulin monomers. However, these compounds showed multi-drug resistance problem, which prompted researchers to discover new class of compounds. Vincristine, vinblastine, and BPR 0L075 (Fig. 1c) are new generation tubulin modulators which destabilize microtubules by binding to colchicine-binding site.[15,16,20] These compounds show anti-tumoral and anti-angiogenic activities and have effects on multi-drug resistant cell line that opens a new opportunities for anti-cancer drug discovery.

3.1.3. BCL–BAK inhibitor

B-cell lymphoma-2 (BCL2) and BCL-XL are anti-apoptotic proteins which regulate cell death. BCL2 and BCL-XL inhibit apoptosis by binding to the 16-residue BH3 domain from pro-apoptotic protein BAK.[21] Thus, small molecule inhibitors of protein–protein interaction between these BCL2 family proteins and BAK are predicted to induce apoptosis in cancer cells. The NMR structure of BCL-XL and BAK complex showed that the BAK-derived peptide forms an α-helix at the hydrophobic surface of BCL-XL.[21] Based on the structural information and so-called 'SAR by NMR' method, which exploits two dimensional ^{1}H–15N heteronuclear single quantum coherence (HSQC) spectroscopy and fragment based compound discovery, several inhibitors acting on hydrophobic helical domain of BCL-XL were identified.[2] Optimization of the selected compounds was performed by NMR-structure-guided medicinal chemistry.[22] Among them, ABT-737 (Abbott Laboratories) binds to BCL-XL with a high affinity (K_i = 0.6 nM) (Fig. 1d and e), and its anti-tumor activity was confirmed by cell-based assays and tumor xenograft animal models. These compounds showed synergistic effect on cancer treatment with several other chemotherapeutic agents and radiotherapy. Another small molecule inhibitor of BCL2, GX015-070 (Gemin X Biotechnologies Inc.) (Fig. 1d), has been introduced and is being tested in Phase I and Phase II clinical trials against solid tumors and haematological malignancies.[23] These examples provided successful proof-of-concept for the inhibitors of protein–protein interaction as an effective therapeutic strategy in cancer.

3.1.4. PLK1–PBIP1 inhibitor

Serine/threonine-protein kinase PLK1 (polo-like kinase 1) is a 66 kDa enzyme that plays a pivotal role in cell proliferation and is considered a proto-oncogene, whose overexpression is often observed in tumor cells.[24] During interphase, PLK1 localizes to centrosomes, and in early mitosis, it associates with mitotic spindle

Table 1
PPI modulators in various target diseases

PPI modulator	Target	Mechanism/function	Reference
Cancer			
• Nutlin-1,2,3 • Benzodiazepindione	p53/HDM2	- Active antagonists of the p53–HDM2 interaction - Bind to HDM2 in the p53-binding pocket - Activate the p53 pathway (cell cycle arrest, apoptosis)	12,8,37,9,58
• RITA	p53/HDM2	- Bind to N-terminal region of p53 - Activate p53 function in tumors	11
• ABT-737 • Obatoclax • (GX015-070) • HA14-1 • BH31-1, 2 • Antimycin A3	Bcl-XL, Bcl-2/pro-apoptotic BCL2 proteins	- Bind to Bcl-2/ Bcl-X_L - Blocking the inhibition of BID-mediated cytochrome c release through binding to the BH3-binding groove of BCL2 - Induce apoptosis selectively in cancer cells	35,12,8,19,59
• XIAP inhibitor • XIAP-DIABLO inhibitor	XIAP/caspases XIAP/DIABLO	- Bind to the BIR3 domain of the XIAP - Inhibit the interaction between XIAP and caspases/DIABLO - Induce apoptosis	12,8,60,61
• IIA4B20 • IIA6B17	Myc/Max	- Interfere with Myc/Max dimerization - Interfere with Myc-induced oncogenic transformation	62,63
• PKF115-584 • CGP049090	Tcf4/β-catenin	- Inhibition of the Tcf4/β-catenin association - Reduce cell growth and survival	37,64
• FJ9	FRZ/DVL (Frizzled/Dishevelled)	- Inhibition of the FRZ-7 and the PDZ domain of DVL interaction - Suppress β-catenin-dependent tumor growth	8,65
• ICG-001	β-Catenin/CBP	- Bind to CBP - Growth reduction and induction of apoptosis in transformed colon cells	8,37,66
• Inhibitors of the CDK2/Cyclin A recruitment site	CDK2/Cyclin A	- Result in cell death - In combination with - CDK2/Cyclin A inactivation	67
• RAS–RAF inhibitor (sulindac-derived inhibitor)	RAS/RAF	- Inhibition of the RAS/RAF interaction - Block cell division (anti-proliferation)	8
• Chetomin	P300/HIF-1α	- Disrupt the formation of the p300/HIF1α complex - Inhibition of tumor growth	37,68
• NCGC00046775	HSP90/HOP	- Binding at a key position on the TPR2A interaction interface - Inhibit the interaction of the C-terminal peptide of Hsp90 and the TPR2A domain of HOP - Active in vivo, in reducing the levels of the cancer-promoting, Hsp90-dependent client protein HER2 and in cell killing	69,70
• Poloxin • PLHSpT	PLK1 PBD	- Inhibition of the protein–protein interactions mediated by the PLK1 PBD - Interfere with correct localization of PLK1 - Lead to mitotic arrest and apoptosis of cancer cells	27,69,71
• Compound **1a**	UBC13/Uev1	- Inhibit the formation of UBC13/Uev1 heterodimers - Blocking lysine 63- - Dependent polyubiquitylation of PCNA - Inhibition of NFκ-B activity and compromise of DNA repair - Inhibit invasiveness, clonogenicity and tumor growth	72,73
• 4EGI-1	eIF4E/eIF4G	- Bind to eIF4E - Disrupt eIF4E/eIF4G association - Inhibit cellular expression of - oncogenic proteins encoded by weak mRNAs - Exhibit activity against - multiple cancer cell lines	2,74
• Pateamine A	eIF4A/eIF4G	- Bind to and enhance the intrinsic enzymatic activities of eIF4A - Inhibit eIF4A–eIF4G association - Promote the formation of a stable ternary complex between eIF4A and eIF4B - Induce stress granule formation and inhibit translation initiation	2,75
• BPR0L075 • Vincristine • Vinblastine	Tubulin	- Bind to the colchicine-binding site of tubulin - Inhibit tubulin polymerization - Arrest the growth of cancer cells at the G_2–M phase - Induce apoptotic cell death	13,14,18,76,77
• NSC23766	Rac1/GEF (TrioN, Tiam1)	- Inhibition of the Rac1/TrioN, Tiam1 interaction - Inhibit Rac1 activation - Reduce formation of lamellipodia - Reduction in tumor invasiveness	76,78
• Adamanolol • Wrenchnolol	Sur-2/ESX	- Bind to Sur-2 - Inhibit the interaction between the two cancer-linked nuclear proteins (Sur-2/ESX) - Inhibit ESX-dependent transcription, cell growth in HER2 expressing cells, Her2 expression	76,79,80
Immune diseases			
• GPM1	Gp96/AIMP1	- Bind to Gp96 - Suppression of cell surface gp96 through increased ER retention - Reduce the incidence and severity - of SLE-associated phenotypes	31
• Ro26-4550 • SP-4206	IL2/IL2Rα	- Bind to IL2 - Inhibit interaction between IL2 and IL2 receptor	35,12,37

(*continued on next page*)

Table 1 (*continued*)

PPI modulator	Target	Mechanism/function	Reference
		- Control T-cell proliferation - Suppression of immune responses - (e.g.: Organ transplant rejection)	
• B7/CD28 competitive antagonists (ligand for B7-1)	B7-1/CD28	- Disrupt the interaction of co-stimulatory molecules on T cells - (CD28) with B7 molecules on antigen presenting cells - Reduce transplant rejection as well as autoimmune diseases	35
• Lovastatin • BIRT 377 • *p*-Arylthio-cinnamide • 1,4-Diazepane-2,5-dione	LFA1/ICAM	- Bind to LFA1 - Inhibit the interaction between LFA1 and ICAM - Reduce inflammation and autoimmune disease	35,12
• BBS-2 • PPA250	iNOS	- Bind to iNOS - Inhibit dimerization of iNOS - Reduce immune response	35,12
• SP304	TNFα	- Bind to the intact biologically active TNFα trimer - Promote subunit dissociation to rapidly inactivate the cytokine - Inhibit TNF receptor 1 (TNFR1) - binding to TNF-α	12,4,81
• TJU103	CD4/MHC class II	- Inhibit autoreactive $CD4^+$ T cells by disrupting the function of the CD4 molecule during activation - Reduce the pathological symptoms of acute experimental allergic encephalomyelitis	82,83
• Suramin	CD40/CD154	- Inhibition of the CD40/CD154 interaction - Show immune-suppressive effects	84,85
Infectious diseases			
• GS 4104 (oseltamivir, TAMIFLU™)	Neuraminidase	- Bind to neuraminidase - Serving as a competitive inhibitor towards sialic acid - Inhibit virion release	86
• Hexapeptide inhibitor	HIV-1 protease	- Disrupt the dimer assembly of HIV-1 protease - Inhibit the HIV-1 protease activity	38
• Pyridyl-pyrimidine • Indoloquinolizinone	ZipA/FtsZ	- Bind to ZipA - Inhibition of ZipA and FstZ interaction - Inhibit the bacterial cell division (antibiotics)	12,37,44
• SDZ NIM 811	HIV-1Gag/cyclophilin A	- Disruption of HIV-1 Gag/cyclophilin A interaction - Inhibit the HIV-1 replication	87
• Maraviroc (CCR5 antagonist)	HIV-1/CCR5	- Inhibit HIV-1 infection mediated by CCR5 - Reduction in viral load (antiretroviral agent)	88
• Glycoprotein 120-mimic peptide (CT319)	HIV-1 glycoprotein 120 (gp120)/Tat	- Interfere with Tat/gp120 interaction - Inhibiting HIV-1 entry and propagation of the infection	89
• BP5	UL42/Pol-derived peptide (Herpes simplex virus DNA polymerase)	- Inhibition of the interaction between UL42 (processivity subunit) with a Pol (UL30,catalytic subunit)-derived peptide - Suppression of HSV replication	37,90
• AL18, AL21	UL54/UL44 (Human cytomegalovirus DNA polymerase)	- Inhibition of the interaction between the catalytic subunit UL54 and accessory subunit UL44 of HCMV DNA polymerase - Inhibition of HCMV replication	37,91
• SB2	RNAP/σ^{70}	- Inhibition of the interaction between $\alpha_2\beta\beta'$ complex (the core RNAP enzyme) and σ subunit - Exhibit antibacterial activity	37,92
• Inandione inhibitor	HPV11 E1/E2	- Bind to E2 transactivation domain - Inhibition of the HPV11 E1/E2 interaction - Suppression of the HPV DNA - Replication	4,93,94
• PB1–PB2 inhibitor	PB1–PB2	- Inhibition of the PB1–PB2 subunit interaction of the influenza virus RNA polymerase - Suppression of the viral transcription activity	49,50
Neurodegenerative diseases			
• Ro 028-2750	NGF (Nerve growth factor)/ $p75^{NTR}$	- Bind to NGF - Inhibit binding to $p75^{NTR}$ (pro-apoptotic receptor) - Control neuronal differentiation, apoptosis and neurite outgrowth	35
• Tat-NR2B9c	NDMAR/PSD-95	- Interfering with the interaction between NDMARs and PSD-95 - Interrupt signaling downstream from NMDARs that leads to neuronal death - Reduce focal ischemic brain damage and improve neurological function	95,96
• PGL-135	Huntingtin	- Suppress the self-assembly of huntingtin	97,98
• PGL-137 (benzothiazole derivatives)	Huntington's disease (HD)	- Inhibit the accumulation of polyQ-containing huntingtin aggregates	
• Alzhemed	Amyloid-β	- Bind to soluble amyloid-beta peptide - Inhibit the formation of neurotoxic aggregates that lead to amyloid plaque deposition in the brain	97,99
• Rifampicin	α-Synuclein	- Inhibit α-synuclein fibrillation and disaggregate existing fibrils - Therapeutic potential for - Parkinson's disease	97,100
• M119	Gβγ	- Bind to Gβγ - Selectively modulate functional Gβγ-effector interactions in vitro	97,101
• Phenothiazines	Tau	- Blocking the tau–tau binding interaction	102,103

Table 1 (*continued*)

PPI modulator	Target	Mechanism/function	Reference
		- Facilitate the proteolytic degradation of tau aggregates and prevent the further propagation of tau capture in AD (Alzheimer's disease)	
Others			
• Inhibitor of the TR-CoR interaction	TR/CoR (Thyroid hormone receptor/Coregulator)	- Irreversibly inhibiting the coactivator binding of a nuclear receptor and suppressing its transcriptional activity - Potential to treat hyperthyroidism without affecting thyroid hormone levels	37,104
• KG-501 (allosteric inhibitor of CBP)	CREB/CBP (cAMP response element binding protein/CBP)	- Disrupt the CREB/CBP complex and attenuated target gene induction in response to cAMP agonist - Potential therapeutic benefit to type II diabetes	37,105
• BI-78D3	JNK-1/JIP-1	- Binding to the JIP-1 docking site on JNK-1 - Restore insulin sensitivity in insulin-insensitive mice	69,106

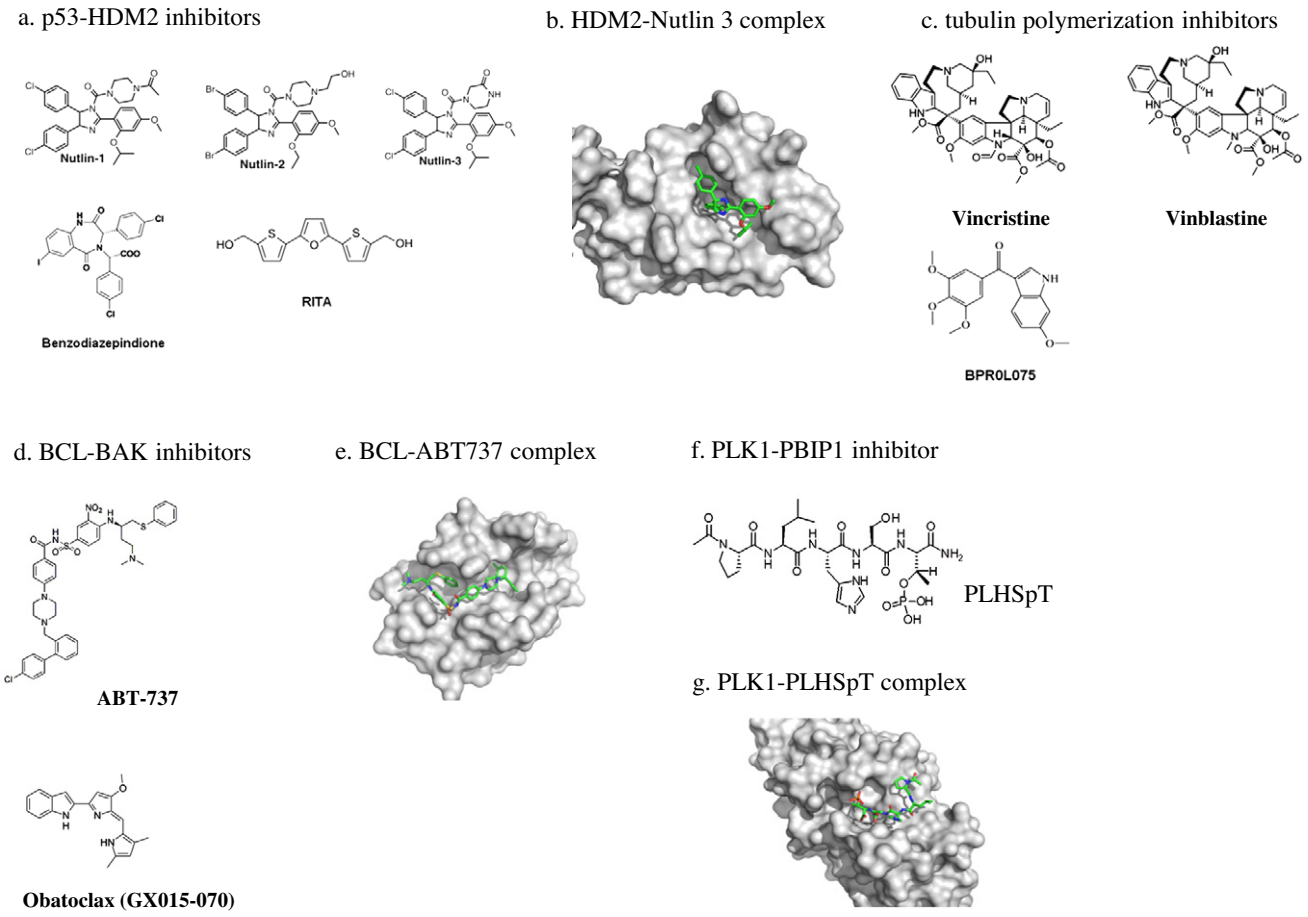

Figure 1. PPI modulators for cancer. (a) Structures of chemical inhibitors working at the interface between HDM2 and p53. (b) Docking structure of HDM2 and Nutlin 3. (c) Structures of chemicals that inhibit tubulin polymerization. (d) Structures of chemicals blocking the interaction between BCL and BAK. (e) Complex structure of BCL and the inhibitor, ABT737. (f) Structure of PLHSpT blocking the interaction between PLK1 and PBIP. (g) Complex structure of PLK1 and PLHSpT.

poles. Plk1 triggers G2/M transition, and supports the functional maturation of the centrosome in late G2/early prophase and establishment of the bipolar spindle.[25] PLK1 consists of an N-terminal kinase domain and two C-terminal polo-box binding domains (PBDs) (residues 411–489 and 511–592 in Plk1).[26] The noncatalytic polo-box domain (PBD) of Plk1 forms a phosphoepitope-binding module for protein–protein interaction, which is essential in targeting its catalytic activity to specific subcellular structures critical for mitosis. Interaction between PLK1 and PBIP1 is essential for the recruitment of PLK1 to the interphase and mitotic kinetochores.[27] The polo-boxes have functions for both auto-inhibition and subcellular localization.[25]

Several attempts to discover specific small molecule inhibitor of PLK1–PBIP1 interaction are being actively carried out with the promising specificity profiles. Recently, Bang et al. reported the diverse phosphopeptides, including PLHSpT from the T78 motif of a centromere protein PBIP1 binds specifically to PBD in a cleft formed between the PB1 and the PB2 motifs by forming direct and bridged hydrogen bonds (Fig. 1f and g). The His 538 and Lys 540 residues from PB2 are essential for electrostatic interactions with the negatively charged phosphate group of the phosphorylated Thr (pThr) residue, whereas the Trp 414 residue from PB1 is central for the selection of the Ser residue at the pThr-1 position (−1 indicates the relative position of the Ser residue from the pThr

residue) by engaging in hydrogen bonding and van der Waals interactions.[28]

Many kinase inhibitors have failed in preclinical or clinical development due to the lack of selectivity that induces intolerable side effects. Until now, several reviews discussed about the recent development of small-molecule Plk1 inhibitors. It has been well documented that peptide-derived inhibitors, such as peptide mimetics or peptoids, are extremely potent and highly specific, thus avoiding many of their disadvantages that small molecule inhibitors often have. Also, they do not accumulate in organs or suffer from drug–drug interactions as many small molecules do. In this regard, targeting the polo-box domain of Plk1 with the peptides such as PLHSpT (Fig. 1f and g) may serve as an attractive approach to overcome many of the problems associated with inhibitors targeting the kinase domain of Plk1. More recently, the molecular and structural basis of various small-molecule and peptide-derived inhibitors that target either the kinase domain or the PBD was reviewed.[29]

3.2. Target disease: Immune diseases

3.2.1. Gp96–AIMP1 inhibitor

Gp96 is a HSP90 family heat shock protein which presents in endoplasmic reticulum. While gp96 works as an intracellular chaperon in endoplasmic reticulum, it is also involved both in innate and adaptive immune responses. Chronic surface exposure of gp96 is associated with dendritic cell activation and SLE (systemic lupus erythematosus)-like phenotype in mice. SLE is a systemic autoimmune disease in which body's immune system attacks cells and tissues resulting in inflammation and tissue damage.[30] Although corticosteroids drugs are usually treated to SLE, they cause many side effects. Thus, the therapeutic targets that can specifically control SLE are on high demand and gp96 has been suggested as one of the potential SLE targets.

Recently, AIMP1/p43 (ARS-interacting multi-functional protein 1, also known as p43) was found to bind gp96 in endoplasmic reticulum and inhibit the extracellular translocation of gp96.[31] From the chemical library screening for the chemicals that can interfere with the interaction between AIMP1 and gp96, a chemical designated, GPM1 (Fig. 2a), was identified to suppress surface translocation of gp96.[32] GPM1 binds to the C-terminal dimerization domain of gp96 and enhances oligomerization by conformational change, which functionally mimics AIMP1 (Fig. 2b). The oligomerization of gp96 leads to the KDEL receptor mediated retrograde transport to ER. GPM1-induced suppression of cell surface gp96 reduces immune cells including dendritic cells, B cells, and memory T cells. Treatment of GPM1 relieved SLE-associated symptoms such as glomerulonephritis, proteinuria, and accumulation of anti-nuclear and -DNA antibodies in SLE model mice. This is a successful demonstration that a small molecule compound can regulate the localization of gp96, and can be exploited as a therapeutic target to treat autoimmune disease like SLE.[32]

3.2.2. IL2–IL2Rα inhibitor

The interleukin-2 (IL2) is a cytokine which mediates T-helper immune response. It facilitates the proliferation and differentiation of T cells, and many clinical data showed that IL2 and its receptor mediate immune disorders including autoimmune disease. IL2 itself can be used in immunotherapy to treat cancer, and an inhibitor of IL2 showed an effect as immune suppressor for transplant patients.[33] Binding of IL2 with trimeric IL2 receptors (α, β, and γ) forms a quaternary complex. X-ray and NMR structures of IL2 were reported with several inhibitors.[34,35]

A series of small molecule inhibitors of IL2 were discovered by Hoffmann La Roche and Sunesis Pharmaceuticals.[1] A fragment based approach was used to identify IL2 antagonist using an array of biophysical techniques such as NMR, AUC, and SPR.[36] In this approach, chemistry, biophysics and structural biology were used cooperatively for the optimization of the compounds. Interestingly, most small molecule inhibitors of IL2–IL2 receptor interaction bind to the hot spot on the surface of IL2, rather than IL2 receptor. Ro26-4550 was the first biophysically characterized inhibitor of a cytokine–receptor interaction with moderate affinity (IC_{50} = 3–6 μM) (Fig. 2c). SP4206 is a more potent IL2 inhibitor (K_i = 60 nM) and

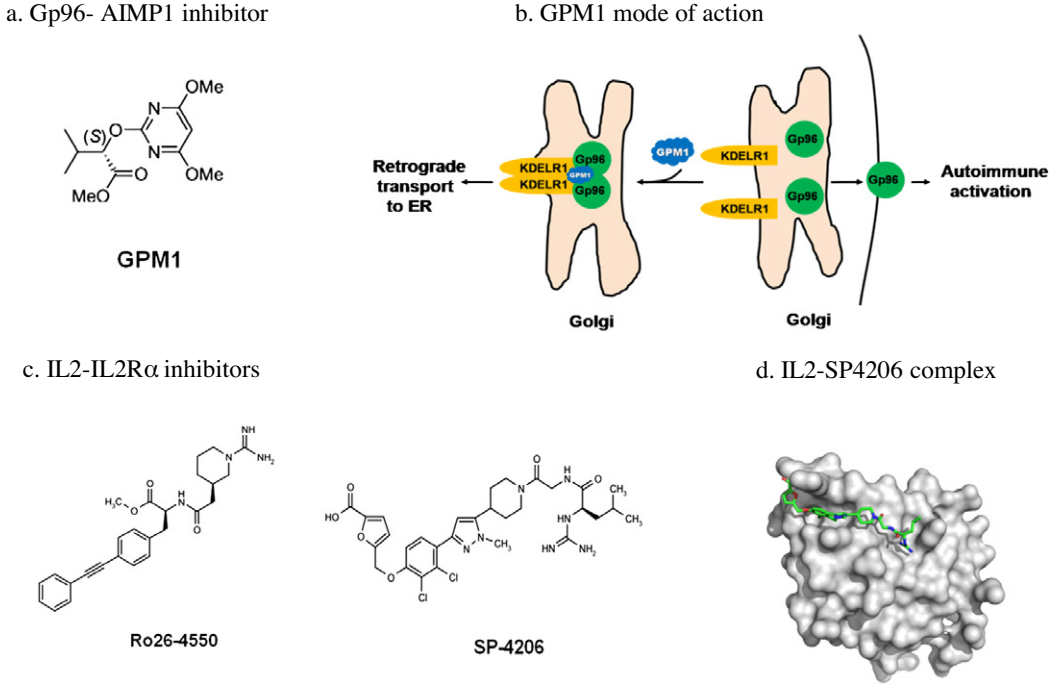

Figure 2. PPI modulators for immune diseases. (a) Chemical structure of GPM1 that inhibits the interaction between AIMP1 and gp96. (b) GPM1 functionally mimics AIMP1 and enhances the retrograde transport of gp96 to endoplasmic reticulum. (c) Structures of chemical inhibitors against IL2 and IL2Rα interaction. (d) Complex structure of IL2 and SP4206.

showed strong inhibition of the interaction between IL2 and α chain of IL2 receptor (Fig. 2c and d), and effects on organ transplant rejection.[37]

3.3. Target disease: Infectious diseases

3.3.1. Inhibitors of viral enzyme assembly

Viral enzymes often form oligomeric assembly, which can be a target for antiviral agents. For example, HIV protease is a dimeric enzyme which has an active site composed of residues from each monomer. Monomers of HIV protease are not active. Thus several small molecule inhibitors were designed to disrupt the dimer assembly. These molecules were derived from a peptide sequence existing at the dimer interface. A hexapeptide inhibitor of HIV protease has IC_{50} of 12 μM, and modification of this peptide provided more potent inhibitors.[38] For example, introduction of hydrophobic group enhanced the inhibition by 50 folds,[38] and cross-linking of the interfacial peptides provided a series of inhibitors with IC_{50} ranging from 2 μM to 380 nM.[39] Besides of HIV protease, the other viral enzymes were also targeted including reverse transcriptase, integrase, and DNA polymerase.[40–42] These data demonstrated a promising method for drug discovery targeting the subunit assembly of viral enzymes.

3.3.2. ZipA–FtsZ inhibitor

Z-interacting protein A (ZipA) is a membrane-anchored protein in gram negative bacteria forming septal ring of cell walls in cell division process. FtsZ is a tubulin-like GTPase which binds to ZipA for the formation of cell wall. The interaction between ZipA and FtsZ is essential for the cell division of gram negative bacteria such as *Escherichia coli*.[37,43] Inhibition of the association of FtsZ with ZipA is recognized as new target for antimicrobial development. A high throughput screening of chemical library yielded a series of compounds including pyridylpyrimidine (K_i = 12 μM) and indoloquinolizinone[14,44] (Fig. 3a). This compound binds to the hydrophobic interface on ZipA (Fig. 3b). These attempts have led to the discovery of a series of novel antibiotics.

3.3.3. Inhibitors of subunits interaction in influenza virus RNA polymerase

The RNA polymerase of Influenza A virus carries out a number of essential processes in the viral life cycle. The RNA polymerase consists of three distinct subunits, PA, PB1 and PB2. All of the three subunits are essential for both viral transcription and replication.[45,46] PB1 contains the polymerase active site, PB2 carries the capped-RNA recognition domain, and PA is involved in assembly of the functional complex.[47,48] The N-terminal tip of PB1 binds to the C-terminus of PA, and loss of this interaction abolishes RNA polymerase activity and viral replication. On the other hand, the C-terminal helices of PB1 bind to the N-terminal helices of PB2, and this subunit interface is essential for transcription initiation.

Most current influenza drugs such as GS4104 (trade name TAMIFLU™) (Fig. 3c) [86] target either neuraminidase (NA) or haemagglutinin (HA), but resistant variants to these drugs are already emerging. Due to continuous mutations of the surface proteins, appearance of new strains can cause new pandemics. On the other hand, the binding interfaces of RNA polymerase subunits are completely conserved among avian and human influenza viruses. Based on the 3D structures of PB1–PA and PB1–PB2 complexes,[49,50] Park et al. extensively screened small molecule inhibitors against these interactions (personal communication). These compounds showed inhibition of viral transcription activity. Although the effects of these compounds were not tested in clinical study yet, these approaches suggested a promising target for novel anti-influenza drugs against all strains of influenza A virus.

3.4. Target disease: Neurodegenerative diseases

3.4.1. NGF–$p75^{NTR}$ inhibitor

Nerve growth factor (NGF) is a small secreted protein, regulating growth, maintenance and survival of neurons.[1] Depending on the interaction of NGF with two different neurotropin receptors, TrkA (pro-growth) and $p75^{NTR}$ (pro-apoptotic), neuronal cells undergo neurite growth or programmed cell death.[51] While immature NGF preferentially binds to $p75^{NTR}$, protease-cleaved NGF shows higher affinity to TrkA receptor. Small molecule inhibitors of NGF–$p75^{NTR}$ interaction have been shown to prevent neuronal degeneration[52] and promote survival signaling through $p75^{NTR}$-dependent manner.[53] These anti-apoptosis drug can be applied for the treatment of neurodegenerative disease such as Alzheimer's disease. A small molecule inhibitor of NGF–$p75^{NTR}$, Ro028-2750, which binds to NGF (IC_{50} = ~1 μM), was discovered[51] (Fig. 4a). It showed selective dissociation of NGF–$p75^{NTR}$ over NGF-TrkA. Ro028-2750 binds to NGF dimer and induces a conformational change, resulting in a defect of binding to $p75^{NTR}$. This compound provides a good tool for studying the selective regulation of

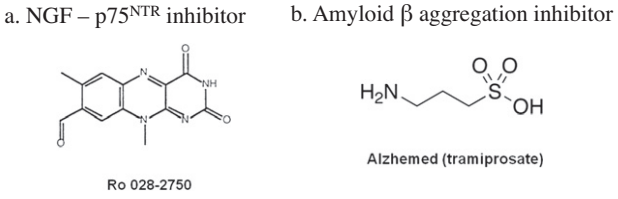

Figure 4. PPI modulators for neurodegenerative diseases. (a) Structure of $p75^{NTR}$ inhibitor binding to NGF. (b) Structure of Alzhemed blocking aggregation of amyloid.

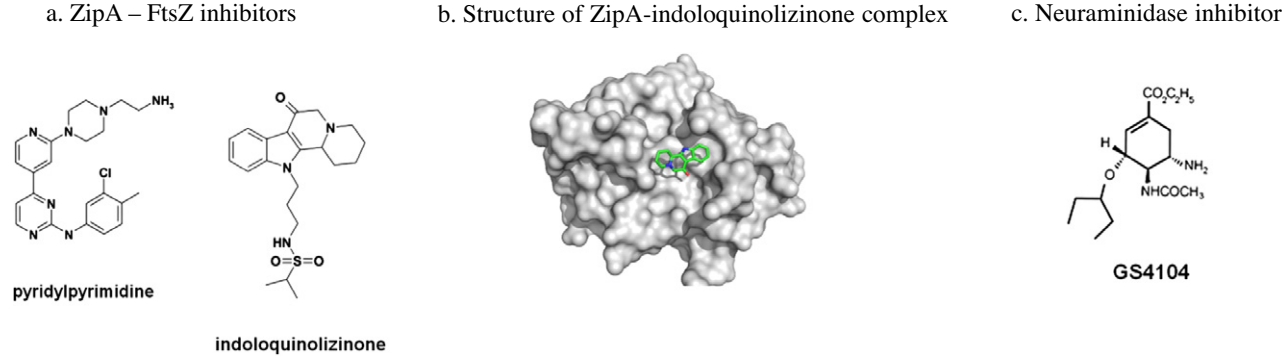

Figure 3. PPI modulators for infectious diseases. (a) Chemical inhibitors against the interaction between a membrane-bound protein, ZipA, and tubulin-like GTPase, FtsZ in gram negative bacteria. (b) Complex structure of ZipA and indoloquinolizinone. (c) Structure of chemical inhibitor, GS4104 binding to viral neuramidase.

$p75^{NTR}$ over TrkA to investigate the functions of $p75^{NTR}$ in neuronal differentiation, apoptosis and neurite growth.

3.4.2. Amyloid β aggregation inhibitor

Amyloid β (Aβ) is a ~40 amino acids peptide, which is a main constituent of amyloid plagues of Alzheimer's disease patients. Aβ is formed by cleavage of amyloid precursor protein (APP) by α-, β-, and γ-secretases. The most common isoforms are Aβ40 and Aβ42. While the shorter form is more common of the two, Aβ42 is more closely related to disease progress. Recent studies have uncovered a mechanism of neuronal cell damage due to the soluble oligomer of amyloid β, rather than the plaques itself.[54] The soluble oligomer of Aβ causes a formation of neuronal tangle, oxidative stress and finally neuronal cell death to facilitate the pathogenesis of Alzheimer's disease.[55] Thus, inhibition of the Aβ aggregation is recognized as an important target for the Alzheimer's drug discovery. Identification of Aβ aggregation blockers has been extensively carried out.[56,57] Anti-aggregation agents such as apomorphine or homotaurine (Alzhemed; tramiprosate) (Fig. 4b) can bind to soluble amyloid β peptide and inhibit the aggregation of neurotoxic oligomers. Alzhemed binds to the soluble amyloid β peptide and isolate the monomeric Aβ from aggregation. One of the interesting indications is that a hormone melatonin can interact with amyloid β and inhibit its aggregation. Melatonin binds to the dimeric state of soluble Aβ, and inhibits the fibril formation. The effect of melatonin on the prevention of amyloid deposition was supported by the experiments in transgenic mice when melatonin was administrated in early life. These approaches provided valuable examples for the treatment of Alzheimer's disease by the small molecule inhibitors of Aβ interaction.

4. Conclusion and perspectives

Recent accumulation of the information on protein–protein interaction enabled us to understand detailed mechanism of pathophysiology with cellular signal transduction networks, and find a new way of therapeutic drug discovery. Together with structural information of proteins derived by X-ray crystallography and NMR spectroscopy, development of high throughput technologies such as HTS, fragment-based approach, computer-aided molecular simulation has made the finding of small molecule modulators of protein–protein interaction more feasible and applicable. The structural diversity of protein–protein interfaces may provide more specific regulation of the target proteins, which suggests lower side effects and higher therapeutic efficacy. In addition to the interfaces of binding, there is another possibility targeting allosteric sites. Moreover, not only the interaction between the two proteins, but also a more diverse interactions such as protein–nucleic acids and protein–lipid interactions can be investigated. These approaches may provide new opportunities of disease treatment and drug discovery in future.

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