Bioorganic & Medicinal Chemistry Letters 28 (2018) 2585–2592

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Digest

Inducing protein-protein interactions with molecular glues

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ARTICLE INFO

Article history:
Received 8 February 2018
Revised 16 April 2018
Accepted 18 April 2018
Available online 19 April 2018

Keywords:
Induced protein interactions
Molecular glues
Targeted protein degradation

ABSTRACT

The drugable proteome is limited by the number of functional binding sites that can bind small molecules and respond with a therapeutic effect. Orthosteric and allosteric modulators of enzyme function or receptor signaling are well-established mechanisms of drug action. Drugs that perturb protein-protein interactions have only recently been launched. This approach is more difficult due to the extensive contact surfaces that must be perturbed antagonistically. Compounds that promote novel protein-protein interactions promise to dramatically expand opportunities for therapeutic intervention. This approach is precedented with natural products (rapamycin, FK506, sanglifehrin A), synthetic small molecules (thalidomide and IMiD derivatives) and indisulam analogues.

Most of the medicines used to treat human disease exert their therapeutic effect by directly modulating the function of one or a small number of protein targets. Typically, these proteins are enzymes, transporters or receptors which are functionally activated or inhibited by the drug. Small molecule therapies typically engage well defined, hydrophobic and enclosed binding sites in direct competition with substrates, ligands or cofactors, or alternatively as allosteric modulators of protein function. Monoclonal antibody therapies interact with cell-surface or secreted proteins, most often as antagonists of protein function. Recent estimates of protein target space covered by current therapeutic agents suggest that only 667 human protein targets exist for the 1194 FDA approved drugs.[1,2] That target space constitutes a small fraction of the estimated 3000 disease-associated genes, the ca. 3000 members of the drugable genome or the ca. 600–1500 drugable, disease-associated targets that are proposed to exist in the human proteome.[3] By contrast, the human genome is estimated to contain ca. 25,000 protein-encoding genes.

Protein-protein interactions govern many fundamental processes in cells through diverse functions that include chaperoning, regulating enzyme activity, scaffolding and transmitting cellular signals. As such, dysfunctional protein interactions are implicated in a number of disease states such as neurodegeneration, cancer, autoimmune diseases and rare genetic diseases. It has been estimated that ca. 130,000 protein-protein interactions exist within the human cell, representing vast opportunity for therapeutic intervention if effective strategies could be devised for modulating this interactome.[4] Significant attention has focused on inhibiting protein-protein interactions, with recent success being demonstrated with marketed agents, such as navitoclax and lifitegrast, and several investigational drugs in clinical trials.[5] Approaches to stabilize protein-protein interactions or promote the formation of novel protein complexes have been less well studied. The topic of protein interaction networks with an emphasis on methods for finding modulators of protein-protein interactions was recently reviewed.[6] An additional paper covering small molecule inducers of protein interactions appeared during editorial review of this manuscript.[7] This review will focus on natural products and synthetic small molecules that promote new protein-protein interactions through the "molecular glue" effect, which can occur through direct binding interactions between both protein targets with the small molecule at the protein-protein interface, or through allosteric modification of protein structure that promotes formation of the new multiprotein complex.

Natural products: Stuart Schreiber remarked that macrocyclic natural products have a unique ability to function as "molecular glues" by bringing together two proteins that on their own, have very little or no affinity for one another.[8] Cyclosporine, a 33 atom macrocyclic natural product, possesses immunosuppressant activity by simultaneously binding to cyclophilin and calcineurin. Ternary complex formation effectively inhibits the phosphatase activity of calcineurin, which leads to IL-2 activation. Proof of ternary complex formation was shown by X-ray crystallography.[9] Rapamycin (**1**) (Fig. 1), another immunosuppressive macrocycle, also inhibits IL-2 signaling by making a ternary complex with two proteins, the FK506-binding protein (FKBP12) and the FKBP-rapamycin-associated protein (FRAP) also known as the Mechanistic Target of Rapamycin (mTOR).[10] In both cases, X-ray structures show that the macrocycles make significant interactions with both proteins

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https://doi.org/10.1016/j.bmcl.2018.04.046
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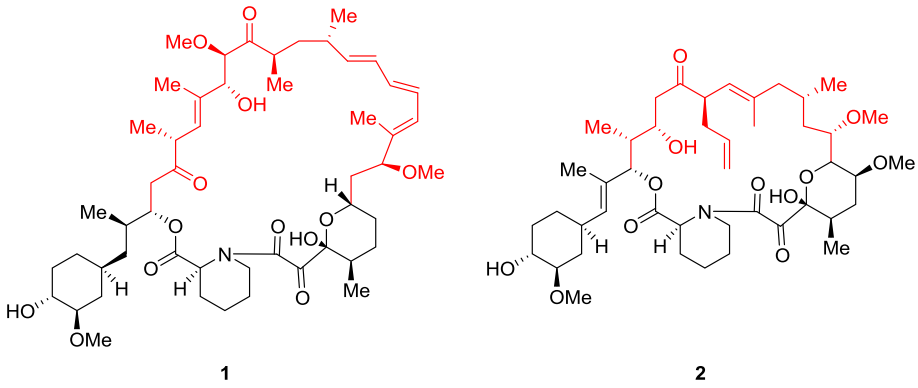

Fig. 1. Rapamycin (**1**) and FK506 (**2**). The black portion of the structure denotes the constant region that is important for binding to the presenter protein, FKBP12; and the red portion of the structure denotes the variable region that influences target specificity, FRAP and calcineurin, respectively.

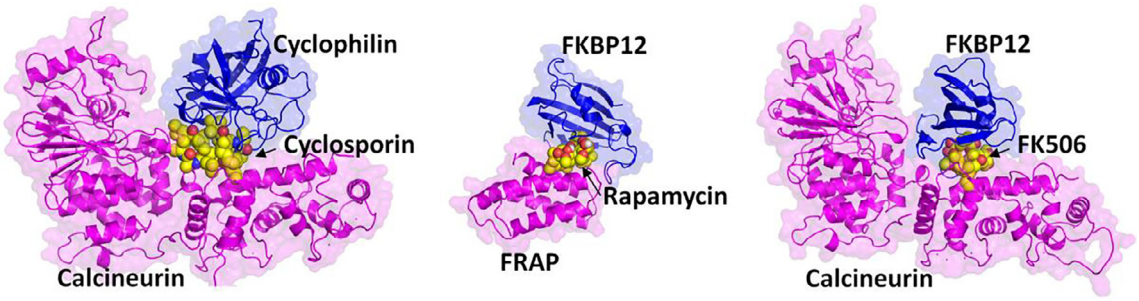

Fig. 2. Crystal structures of natural product induced protein complex formation: calcineurin-cyclosporin-cyclophilin[9] (left), FRAP-rapamycin-FKBP12[10] (middle) and calcineurin-FK506-FKBP12[11] (right).

and also help facilitate interactions between them. FK506 (**2**) also forms a ternary complex with FKBP12 and calcineurin which has the effect of inhibiting the remote phosphatase catalytic site on calcineurin.[11] Typically the contact surfaces involved in natural protein–protein interactions are large (~1500–3000 Å^2) compared to those involved in protein–small-molecule interactions (~300–1000 Å^2).[12] However, as an example the Rapamycin, FKBP12, mTOR ternary complex has a total buried surface area of 1550 Å^2, of which ~780 Å^2 represents the protein-protein contact surface between FKBP12 and mTOR, while the rapamycin-mTOR ligand-protein contact surface amounts to the remaining ~790 Å^2 (Fig. 2). In general, natural products contribute to about 25–50% of the total buried surface area in ternary complexes that have been crystallized.

A synthetic dimer of FK506 called FK1012 has also been showed to bring receptors together to initiate signaling events in cells. FK1012 was shown to dimerize the T-cell receptor MZF3E, a chimeric receptor comprising the intracellular domain of the ζ chain and three copies of FK506-binding protein (FKBP), to initiate T-cell receptor signaling that is dependent on calcineurin and NF-AT.[13] This result demonstrated that molecular glues can induce protein-protein interactions to recreate the natural TCR signaling within a cell. A second demonstration of protein-protein dimerization using FK1012 was shown using Src kinases. Inactivated constructs of Fyn, Lyn and Lck were prepared by replacing their myristoylation-targeting peptides with FKBP. The addition of FK1012 induces dimerization and activation of these Src kinases.[14] While Src kinase signaling was not observed, dimerization led to activation of transcription factors similar to those regulated by antigen receptor dimerization.

Considering that different ligands for FKBP exert their biological effects by divergent mechanisms, studies were performed to identify ligands of cyclophilin (other than cyclosporin) that might also exhibit different modes of action.[15] Screening microbial broth extracts identified a new class of compounds called sanglifehrins. Sanglifehrin A (SFA, **3** in Fig. 3), a mixed polyketide and non-ribosomal peptide synthase natural product, has picomolar affinity for its receptor cyclophilin A. Studies showed that IMPDH2 (Inosine monophosphate dehydrogenase2) is the specific target of the cyclophilin A-SFA binary complex in vitro. Forming this ternary complex does not inhibit the catalytic activity of IMPDH2 but modulates cell growth through interaction with the CBS (cystathionine-β-synthase) domain of IMPDH2.[16]

Small molecule-assisted receptor targeting (SMARTs): The science behind these ternary binding complexes was the catalyst behind the formation of Warp Drive Biosciences,[17] a company that seeks to take advantage of macrocycles which bind to accessory proteins such as cyclophilin and FKPB12 to target proteins previously considered to be intractable due to a lack of small molecule binding sites. Their technology called SMART™ (Small Molecule-Assisted Receptor Targeting) takes advantage of the excellent properties

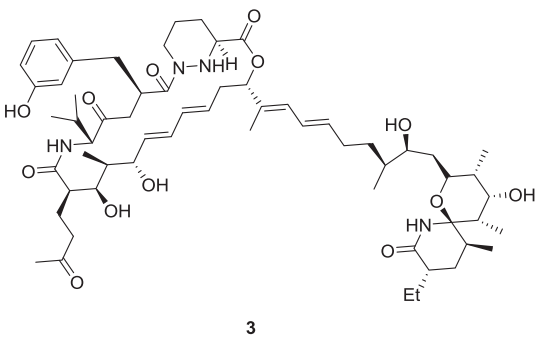

Fig. 3. Sanglifehrin A (**3**).