Title

Peptidyl-Proline Isomerases (PPIases): Targets for Natural Products and Natural Product-Inspired Compounds

Journal

Journal of Medicinal Chemistry, 59(21)

ISSN

0022-2623

Authors

Dunyak, Bryan M
Gestwicki, Jason E

Publication Date

2016-11-10

DOI

10.1021/acs.jmedchem.6b00411

Peer reviewed

HHS Public Access

Author manuscript

J Med Chem. Author manuscript; available in PMC 2017 November 10.

Published in final edited form as:

J Med Chem. 2016 November 10; 59(21): 9622–9644. doi:10.1021/acs.jmedchem.6b00411.

Peptidyl-Proline Isomerases (PPIases): Targets for Natural Products and Natural Product-Inspired Compounds:

Miniperspective

Bryan M. Dunyak[†,‡] and **Jason E. Gestwicki**[*,‡]

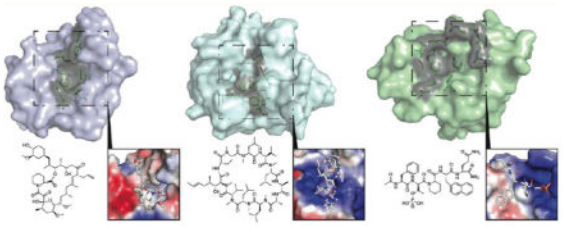
[†]Department of Biological Chemistry, University of Michigan Medical School, 1150 W. Medical Center Drive, Ann Arbor, Michigan 48109, United States

[‡]Department of Pharmaceutical Chemistry, University of California at San Francisco, 675 Nelson Rising Lane, San Francisco, California 94158, United States

Abstract

Peptidyl-proline isomerases (PPIases) are a chaperone superfamily comprising the FK506-binding proteins (FKBPs), cyclophilins, and parvulins. PPIases catalyze the cis/trans isomerization of proline, acting as a regulatory switch during folding, activation, and/or degradation of many proteins. These “clients” include proteins with key roles in cancer, neurodegeneration, and psychiatric disorders, suggesting that PPIase inhibitors could be important therapeutics. However, the active site of PPIases is shallow, solvent-exposed, and well conserved between family members, making selective inhibitor design challenging. Despite these hurdles, macrocyclic natural products, including FK506, rapamycin, and cyclosporin, bind PPIases with nanomolar or better affinity. De novo attempts to derive new classes of inhibitors have been somewhat less successful, often showcasing the “undruggable” features of PPIases. Interestingly, the most potent of these next-generation molecules tend to integrate features of the natural products, including macrocyclization or proline mimicry strategies. Here, we review recent developments and ongoing challenges in the inhibition of PPIases, with a focus on how natural products might inform the creation of potent and selective inhibitors.

Graphical Abstract



[*]Corresponding Author: Phone: 1-415-502-7121. jason.gestwicki@ucsf.edu.

Notes

The authors declare no competing financial interest.

INTRODUCTION

Peptidyl-prolyl isomerases (PPIases) are a superfamily of molecular chaperones that play widespread roles in protein folding and regulation through isomerization of proline residues.[1,2] Unlike other chaperones,[3] PPIases do not utilize cofactors, such as ATP, to drive their activity; rather, they bind their "clients" using a shallow and promiscuous interface that is thought to favor proline isomerization through conformational selection. As discussed below, this deceptively simple mechanism is critical to the folding and function of numerous "clients". Indeed, genetic studies have shown that PPIases are essential to the function/folding of proteins important in cancer, neurodegenerative disorders, viral infection, and psychiatric disorders.[4–7]

Although the active site of PPIases is shallow and "undruggable", nature has repeatedly found ways of creating potent PPIase inhibitors, as exemplified by the macrocycles FK506, rapamycin, and cyclosporin. These natural products have been key probes for understanding PPIase function and were even used to identify members of the PPIase family.[8] Previous reviews have summarized the structure and function of PPIases,[9,10] their roles in disease,[11–14] and the history of natural products as inhibitors.[15–19] Here, we only briefly discuss these topics before focusing on understanding how natural products have informed recent developments in the search for selective, potent PPIase inhibitors.

PROLINE ISOMERIZATION IN PROTEIN FOLDING AND FUNCTION

Peptide bonds in proteins are dominated by the trans conformation due to the steric clashes that occur at the α carbon in the cis orientation. However, proline is different (Figure 1A). The cyclized side chain of proline samples both the cis and trans conformations, typically in a ratio of ~20% cis to ~80% trans. Spontaneous isomerization of the Xaa-Pro bond is slow (on the time scale of milliseconds to seconds), creating a particular challenge to protein folding because the majority of folding events occur on the microsecond-to-millisecond time scale.[20] Thus, proline isomerization can be rate limiting, requiring PPIases to alleviate the bottleneck (Figure 1B).[21,22] Beyond folding, this special feature of proline has been exploited as a regulatory switch in signal transduction. For example, oncogenic p53 is activated after binding of the PPIase Pin1, enhancing malignancy in transformed cells.[23,24]

SUPERFAMILY OF PPIases

PPIases are a superfamily consisting of the immunophilins and parvulins. In turn, the immunophilin family is further subdivided into the FK506-binding proteins (FKBPs) and cyclophilins. In humans, there are 18 FKBPs, 24 cyclophilins, and 3 parvulins.[25,26]

Each of the PPIases contains at least one PPIase domain. This domain is composed of antiparallel β-sheets that position a short α-helix. A shallow groove between the α-helix and β-sheets forms a solvent-exposed active site that binds to the target proline (Figure 2). Generally, the proline makes contact with an aromatic residue on the floor of the pocket.[27] In the prototypical PPIases FKBP12, cyclophilin A (CypA), and Pin1, this interaction is facilitated by Trp-59, Phe-113, and Phe-134, respectively. It is not entirely clear how this contact contributes to the catalytic mechanism, but some studies suggest that the PPIases

may stabilize a twisted-amide transition state.[28,29] In this model, stabilization is conferred by hydrophobic residues within the pocket alongside crucial hydrogen bonds formed with the proline backbone and adjacent residues (Figure 3A). In CypA, this interaction is carried out by Arg-55, which forms hydrogen bonds with the proline carbonyl oxygen in the trans state and proline amide nitrogen in the cis state.[30] These interactions have been studied by multitemperature crystallography, [31] revealing the dynamical nature of the active site and supporting the idea that these contacts might favor isomerization. In contrast, less is known about how members of the FKBP class perform their function. Mutagenesis experiments have implicated Asp-37 and His-87 in forming the hydrogen bond network between the pocket and bound client.[32] In Pin1, there is an active site Cys-113 that was initially thought to operate via covalent nucleophilic attack on the client. However, follow-up studies demonstrated that the cysteine could be mutated to serine (C113S) or aspartate (C113D) without loss of function, suggesting that hydrogen bonding is more important.[33] This model is further supported by the observation that Cys-113 is replaced by Asp-74 in the related family member, parvulin-14.[34] Beyond this residue, a substantial hydrogen-bonding network involving His-59 and His-157 helps stabilize interactions with clients in Pin1.[34] Thus, all of the PPIases feature a key, floor residue that is supported by hydrophobic interactions and a hydrogen-bonding network, although the exact identity of the residues often varies substantially. The best inhibitors of the PPIase access all of these regions (Figure 3B), which is a challenge given the extended dimensions of the pocket.

FK506-Binding Proteins

The FKBPs are a subfamily of 18 proteins and they are named for their apparent molecular mass. The 12 kDa family member, FKBP12 (gene *FKBP1A*), is composed of just a PPIase domain. It is widely expressed but especially abundant in skeletal and cardiac muscle, the central nervous system, peripheral nerves, and blood cells.[35,36] The cellular roles of FKBP12 are not entirely clear. Despite its abundance, FKBP12$^{-/-}$ knockout mice exhibit a relatively mild phenotype, including cardiac muscle defects and some behavioral changes.[37,38] These observations suggest that FKBP12 activity may potentially be partly redundant with other PPIases.

Other members of the FKBP family have, in addition to at least one PPIase domain, other modules. These other domains provide additional functions and often serve as scaffolds between the PPIase clients and other cellular pathways. For example, FKBP51 (gene *FKBP5*) and FKBP52 (gene *FKBP4*) share 70% sequence identify and possess three domains, two PPIase domains (FK1 and FK2) and a C-terminal tetratricopeptide repeat (TPR) domain.[6] The FK1 domain has PPIase activity, and it binds both rapamycin and FK506 (FKBP51: $K_D^{Rapa} \approx 3.7$ nM, $K_D^{FK506} \approx 104$ nM. FKBP52: $K_D^{Rapa} \approx 4.2$ nM, $K_D^{FK506} \approx 23$ nM)[39] with similar affinity as FKBP12. The function of the FK2 domain is still largely unknown. FK2 does not bind FK506 or rapamycin, and it does not have measurable isomerase activity in vitro. Through their TPR domains, FKBP51 and FKBP52 interact with the unstructured C-terminal tail of heat shock protein 90 (Hsp90), which links them to steroid hormone receptor maturation.[40–42] Interestingly, despite their high homology to each other, FKBP52 positively regulates the stability of androgen, glucocorticoid, and progesterone receptors while FKBP51 acts as a negative regulator. Through this activity,

FKBP52 has been implicated as a target in hormone-driven tumors, such as prostate cancer, colorectal adenocarcinomas, breast cancer, and myelomas.[6] The functional differences between FKBP51 and FKBP52 have been isolated to differences in the dynamics of FK1.[43] In addition to steroid hormone signaling, FKBP51 and FKBP52 have been shown to interact with intrinsically disordered proteins, such as tau.[44] In Alzheimer's disease models, FKBP51-Hsp90 is associated with tau retention, while FKBP52-Hsp90 is linked to tau degradation through the proteasome.[45,46] However,

FKBP52 is also associated with promoting tau aggregation[47,48] and inhibitors have shown promise in tauopathy models.[49] FKBP38 (gene *FKBP8*) has a single PPIase domain, which is normally inactive. However, this family member also has a TPR domain and a putative calmodulin-binding motif. Increases in Ca^{2+} trigger a conformational change that recruits calmodulin and activates the PPIase. In response to intracellular Ca^{2+}, activated FKBP38 then becomes competent for binding to the antiapoptotic protein, Bcl-2. The exact role of FKBP38 in the regulation of Bcl-2 remains enigmatic, with conflicting results suggesting that FKBP38 may either promote or inhibit apoptosis.[50]

Cyclophilins

Similar to the FKBPs, cyclophilins contain at least one PPIase domain and are widely distributed across tissue types.[51] The most abundant cyclophilin, cyclophilin A (gene *PPIA*), was named after the discovery of its ability to bind cyclosporin A ($K_D \approx 6$ nM).[52,53] CypD, a mitochondrial cyclophilin, has been implicated in mitochondrial permeability transition (MPT) pore opening, allowing for the release of proapoptotic factors into the cytosol.[54] It is thought that CypD binds the adenine nucleotide translocase (ANT) machinery directly. Under stress conditions, this facilitates a Ca^{2+}-mediated conformational change in ANT, altering the role of ANT from a nucleotide transporter to an open pore.[55] Analogous to FKBP51/52, Cyp40 contains a TPR domain and interfaces with Hsp90, facilitating complex formation with the estrogen receptor to modulate steroid hormone receptor maturation and trafficking.[56]

Parvulins

The parvulins make up the smallest family of PPIases, consisting of: peptidyl-prolyl cis/trans isomerase NIMA interacting protein 1 (Pin1), parvulin-14 (Par14), and parvulin-17 (Par17). The parvulins were originally discovered as a novel PPIase family in *Escherichia coli*.[57] Human parvulins can be subdivided into two groups by substrate specificity. Par14 and Par17, products of alternative transcription initiation of the parvulin gene,[58] are similar to the prokaryotic parvulins and exhibit the conserved isomerase and chaperoning activity characteristic to most PPIases. The exact cellular functions of Par14 and Par17 are still cryptic. Par14 has been found in both the cytosol and nucleus, reportedly binding DNA[59] and assisting in rRNA processing for ribosome biogenesis.[60] Less is known about Par17, although it has been shown to promote microtubule assembly[61] and it contains a mitochondrial targeting sequence, where it is implicated in DNA binding.[62] Pin1, the third member of the parvulin family, is unique among all PPIases because it only recognizes prolines that are adjacent to phosphorylated Ser/Thr residues (i.e., the pS/T-P motif). Pin1 also has an N-terminal WW domain, which shares a similar client profile to the PPIase

domain. The WW domain does not possess intrinsic isomerase activity, and it is not clear why it has an overlapping substrate preference. It is thought that the WW domain may help recruit Pin1 to relevant clients, but the hand-off mechanism is unknown. What is clear is that Pin1 plays essential roles in cell division. For example, Pin1 regulates the activity of the phosphatase Cdc25, coordinating it with the cell cycle.[63] Pin1 also stabilizes the regulatory protein, cyclin D1, preventing its degradation and mediating its nuclear localization through the conformational switch of pThr286-Pro287.[64]

MECHANISMS OF BIFUNCTIONAL PPIase LIGANDS

Two of the PPIases, FKBP12 and CypA, were discovered as the molecular targets of the natural products FK506, cyclosporin, and rapamycin, in a pregenomic example of pharmacology leading to the discovery of a protein function.[52,65–67] Subsequent work showed that FK506 forms a ternary complex between FKBP12 (and other FKBPs[68]) and the phosphatase, calcineurin. Interestingly, cyclosporin and rapamycin have a conceptually similar way of binding their targets. Cyclosporin brings together CypA and calcineurin, while rapamycin facilitates the ternary complex between FKBPs and mTOR.[69] These molecules are able to bind both targets and are thus naturally “bifunctional”; rapamycin binds FKBPs with one chemical face and mTOR with another, nonoverlapping motif (Figure 4) to facilitate the formation of a stable ternary complex (Figure 4C). In each case, binding two proteins at the same time is critical to the natural product’s immunosuppressive activity. For example, FK506 blocks the dephosphorylation and activation of the transcription factor NF-AT by using the steric bulk of FKBP12 to limit accessibility to the calcineurin active site.[70] It would be difficult for a small molecule to do this effectively on its own because of the shallow, open nature of the phosphatase pocket. Similarly, rapamycin blocks the G1 to S phase transition and prevents proliferation of activated T-cells by using FKBP12 to limit accessibility of substrates to mTOR.[71–73] Thus, the PPIase (i.e., FKBP12) is not necessarily the target that drives the biological affect; it is simply “along for the ride”. Over the past 2 decades, this strategy has been exploited in dozens of chemical biology strategies, in which synthetic, bifunctional molecules, which are based on the natural products, are used to control protein dimerization, stability, localization, and function.[74–79]

ROLES OF PPIases IN DISEASE

More recently, the PPIases themselves have emerged as targets in their own right and for a wide range of diseases.[80] The breadth of diseases linked to PPIases is staggering, likely because they have so many potential clients. For example, FKBP12 has been shown to modulate the activity of ryanodine receptor, inositol 1,4,5-triphosphate receptor, TGF-β receptor 1, and activin type 1 receptor.[81,82] Similarly, as mentioned above, FKBP51 and FKBP52 are critical for maturation and activity of the steroid hormone receptors, such as androgen receptor (AR) and glucocorticoid receptor (GR),[83] FKBP38 is linked to the antiapoptotic proteins Bcl-2/Bcl-xL matrix metalloproteinase 9 (MMP9),[50] FKBP25 regulates casein kinase II and nucleolin,[84] and FKBP13 and FKBP65 control elements of the secretory pathway for protein trafficking and collagen biosynthesis.[85,86] Similarly, the cyclophilins are involved in collagen remodeling,[87] function of the steroid hormone receptors,[56,88] and activation of numerous transcription factors.[51] The cyclophilins also play

key roles in calcium signaling and homeostasis, through activities on multiple clients.[55,89] CypA binds the SH2 domain of Itk and regulates production of $CD4^+$ T_h2 cytokines.[90] Highly expressed in vascular smooth muscle cells, CypA may be excreted as an extracellular growth factor as a response to oxidative stress to mediate ERK1/2[91] and CD147[92] activation. Other cyclophilins, such as CypD, have been implicated in Alzheimer's disease through a direct interaction with Aβ.[93] In addition to these roles, cyclophilins have been shown to be critical host-factors that are co-opted by viruses.[13] For example, CypA regulates multiple steps in the human immunodeficiency virus type 1 (HIV-1) life cycle and is repackaged into new virion particles during HIV replication and release. During hepatitis C virus (HCV) infection, CypA and CypB bind the regulator protein NS5A and RNA polymerase NS5B, which are critical for HCV replication. The breadth of clients showcases how the PPIase active site must be shallow and malleable to accommodate a range of sequences and topologies around the central proline. In addition to the challenges this feature creates in target engagement, it also makes it harder to envision the development of selective, clinical biomarkers for each PPIase.

After its discovery as a crucial regulator of cell cycle progression, Pin1 has since been shown to be involved in numerous phosphorylation-dependent regulatory mechanisms. Pin1 works in tandem with "proline-directed" kinases and phosphatases, binding and isomerizing the peptide backbone of the pS/T-P motifs. This activity is especially important in the context of pS/T-P motifs, as they have been shown to isomerize 8-fold slower than their nonphosphorylated counterparts (Figure 5).[94] For example, Pin1-mediated conformational changes act as a switch that is required for specific cellular functions, such as activating some transcription factors, facilitating dephosphorylation or degradation of some clients, and targeting specific proteins to their subcellular localization.[95] Accordingly, Pin1 has been linked to a particularly widespread set of clients, including transcription factors, oncogenes, and proteins that form amyloids.[96] However, what determines a Pin1 "client" has been called into question.[97] Indeed, putative Pin1 binding sites have been challenging to substantiate, such as the unconventional bivalent interaction as seen with protein kinase C[98] or the role in controlling phosphorylated tau function for microtubule assembly and stabilization.[99]

Through the ability to control the folding or function of so many clients, PPIases have been suggested as potential drug targets for cancer, viral infection, neurodegeneration, and other diseases. Indeed, Pin1 is one of the most widely overexpressed proteins in all cancers,[100] including prostate, brain, breast, ovary, cervical, and melanomas.[101] So what are the hurdles to creating potent, selective, and clinically useful PPIase inhibitors? More importantly, what are the solutions?

CHALLENGES TO THE DEVELOPMENT OF PPIase INHIBITORS

Shallow Binding Site

The PPIase binding site is shallow, creating problems with generating low molecular mass inhibitors with sufficient potency. As discussed below, the key may be to incorporate features of macrocyclic natural products. Indeed, nature has repeatedly used this strategy to overcome the problem.

Similarity between Paralogs

There is structural and sequence similarity between the active sites of the PPIases (see Figure 3), making it more difficult to generate paralog-selective inhibitors. This conservation is likely why known inhibitors often bind to multiple members of the PPIase families;[102] for instance, FK506 binds with tight affinity to FKBP12, FKBP38, and FKBP51 (see below). As we will discuss, recent work has suggested that subtle structural differences between paralogs might be sufficient to garner selectivity in some cases. However, we suggest that more structures, likely combined with molecular modeling, are needed to better understand (and exploit) the differences between members of the PPIase families.

Lack of Clinical Benchmarks

There is little clinical data available on PPIase inhibitors. The widely used drugs, FK506, cyclosporin, and rapamycin, certainly bind to members of the PPIase family, yet they manifest their immunosuppressive activity through other pathways. Other “pure” PPIase inhibitors have been explored in peripheral neuropathy trials, but there is uncertainty as to which PPIase (if any) might have been involved (see below). In some ways, this is a “catch-22” problem that will only be resolved by selective, potent inhibitors. However, additional clinical studies that measure the levels or contribution of specific PPIases in disease, such as has been done most extensively with Pin1 in cancer, would certainly inspire and inform continued preclinical development of inhibitors.

Lack of Target Validation in Disease

In the previous sections, the observations that link PPIases to cancer, neurodegenerative disease, peripheral neuropathy, and viral infection were briefly mentioned. However, we feel that more could be done to validate these proteins as promising drug targets. With modern CRISPR methods, it seems feasible and important to replace PPIases with hyperactive or activity-dead mutants to specifically ask whether isomerase activity is required. PPIase proteins often have scaffolding function, owing to their multidomain nature. In some cases, the isomerase activity per se may not be relevant to the biological activity. For example, treatment with FK506 does not block the function of FKBP51 in GR maturation because FKBP51’s PPIase domain appears to play an ancillary or complex role in that process.[103,104] In neurodegenerative disease, low Pin1 expression has been correlated to increased neuronal vulnerability and enhanced degeneration of neurons and neurofibrillary tangle formation during AD-like pathologies.[105] Pin1 is often sequestered into paired helical filaments (PHF) of hyperphosphorylated tau,[106] while soluble Pin1 is deactivated by oxidative stress at its catalytic cysteine.[107] Thus, activating, not inhibiting, Pin1 might be the best approach for neurodegenerative diseases.[100] More genetic studies may clarify how much activity, either above or below normal, might be needed to achieve a therapeutic effect. Another benefit of additional genetic studies on PPIases will likely be that we better understand the overlap and redundancies between paralogs. Like in the kinase field, it seems possible that the best clinical approach might include some aspect of “polypharmacology”; in other words, simultaneous inhibition of multiple PPIases that share disease-relevant clients.

In the next sections, we discuss recent successes in the synthesis of PPIase inhibitors. We also go into additional detail on the remaining challenges for each of the major classes of PPIases. We then conclude with some projections for where the field might go next.

INHIBITORS OF FKBPs

Much of what we know about the active site and inhibition of FKBPs comes from pioneering studies using FK506. FK506 **(1)** binds FKBP12 with an affinity of ~0.4 nM, with the key contacts made in a wide region of the molecule that includes the cyclohexylethenyl group through the pipecolate α-ketoamide and into the pyranose ring (Figure 6). This region makes contact with FKBP12 in a broad pocket that includes Trp-59, Phe-113, and Phe-134, where the pipecolate appears to mimic the proline that is present in most native clients.[108,109]

Using this information, Armistead et al. and Holt et al. developed the early synthetic (e.g., non-natural product) FKBP12 inhibitors, in which the pipecolate core of FK506 is retained but the pyranose ring is replaced by various cyclic alkanes and substituted alkyl substituents to improve rigidity (Figure 6).[110,111] The best of these molecules contained a *tert*-pentyl substitution off the α-ketoamide, resulting in **2**, which retained a significant portion of the affinity for FKBP12 ($K_d \approx 660$ nM). Subsequent derivatization of the pipecolic ester to represent the cyclohexylethenyl group afforded the phenylpropyl ester **3** ($K_i \approx 110$ nM) and trimethoxyphenylpropyl ester **4** ($K_i \approx 12$ nM). Finally, stereospecific substitution at the carbinol center produced **5** and **6** ($K_i \approx 7$ nM and 300 nM, respectively). Crystal structures confirmed that the compounds adopt a binding pose similar to FK506, largely driven by the pipecolyl ring interaction with Trp-59. The cyclohexylethenyl chain, replaced by the phenethyl fragments in the synthetic molecules, sits in the same hydrophobic groove, engaging in hydrophobic interactions with Ile-56 and Tyr-82. The most significant deviation from the FK506 pose occurs in the pyranose replacement, where a hydrogen bond with Asp-37 is lost. Nonetheless, native salt bridges are maintained and the *tert*-pentyl group makes productive van der Waals contacts. Consistent with their design, these compounds lack immunosuppressant activity because they can no longer bind calcineurin.

Additional optimization of this core scaffold by ARIAD Gene Therapeutics ultimately resulted in **7**[112] (Figure 7A), also known as SLF, or synthetic ligand for FKBP. SLF was designed to retain most of the affinity of **5** ($K_i \approx 20$ nM) while also including a pendent carboxylate that could be used to add additional functionalities that point away from the FKBP surface (Figure 7B,C). In this way, SLF became a “go-to” molecule for creating chemical inducers of dimerization (CIDs).[74,113] Typical CIDs were assembled by coupling SLF either to itself (**8**)[112] or to molecules with high affinity for promising drug targets, such as amyloids (**9**)[114] or HIV protease (**10**; Figure 8).[115] Thus, the CIDs would act as artificial versions of rapamycin or FK506, bringing the bulk of FKBPs to targets. CIDs also retained some of the interesting pharmacology of the natural products. For example, they accumulated in tissues with high FKBP12 expression, especially cells that express both protein partners.[116] CIDs have also been developed to alter protein stability, as has been done by linking thalidomide (**11**) to induce FKBP12 degradation.[117]

While the CIDs are a powerful platform for chemical biology, they largely use PPIases as steric bulk, much like the natural products. To revisit the creation of dedicated PPIase inhibitors, a group at Guilford Pharmaceuticals developed improved ligands, such as **12** (GPI-1046), and assayed them for neurotrophic activity (Figure 9).[118] One goal of that work was to reduce molecular mass and improve ligand efficiency and cellular potency. While **12** only had an apparent $K_i \approx 7.5$ nM, neurite outgrowth was promoted down to 1 pM, with half-maximal stimulation at 58 pM. The less stable ester was replaced with a thioester or a ketone to produce **13** ($K_i \approx 11.0$ nM, neurite outgrowth $EC_{50} < 0.01$ nM, $EC_{min} \leq 0.03$ pM)[119] and **14** (equipotent to GPI-1046).[120] Intrigued by the reason for the large disconnect between binding affinity and cellular potency, Fischer and colleagues found that only modest isomerase inhibition was required to achieve potency in peripheral nerve injury models such that potency can be 100- to 1000-fold greater than apparent affinity.[121] This relationship between isomerase activity and neuronal function is promising, as it suggests that even modest disruption of PPIase activity might produce robust outcomes.

It is not yet clear which FKBP family member, if any, is the relevant target for neurotrophic activity, and there remains significant controversy. Hippocampal cultures from FKBP12$^{-/-}$ mice treated with FK506 displayed enhanced neurite outgrowth, suggesting other FKBP family members, such as FKBP52, are involved.[122] More recently, selective inhibitors of FKBP38 (see below) have also demonstrated efficacy in neurotrophic models so that paralog might also be a candidate. However, it remains unclear which PPIase is the best target. As mentioned above, it seems that additional target verification studies in vivo are needed.

To this point in the review, we have discussed inhibitors of FKBPs that were derived from FK506 as the starting point. Attempts at finding new scaffolds have been less successful. For example, a screen of secondary metabolites yielded cycloheximide as a modest inhibitor of FKBP12 activity ($K_i \approx 3.4$ μM; Figure 10). Modification of the core scaffold produced the equipotent *N*-ethylethanoate derivative **15** (ME-CHX). Critically, the *N*-ethylethanoate substitution decreased cytotoxicity by 150-fold, a known issue with cycloheximide-mediated inhibition of ribosomal translation elongation.[123] Compounds **12**, **15** and an analog with improved metabolic stability, **16**, were screened against a panel of purified FKBPs. Surprisingly, they demonstrated at least 6-fold selectivity for FKBP38.[121] Inhibition of FKBP38 is known to suppress its proapoptotic functions in neuroblastoma cells, so the neurotrophic activity of **12**, **15**, and **16** might originate, in part, from FKBP38 inhibition.[124] Recently, this work has been expanded to test the cycloheximide scaffold and its derivatives as novel antibacterial agents.[125]

This relative selectivity for FKBP38, while still somewhat modest, is surprising because selectivity for individual members of the FKBP family has been considered a major challenge. Hausch and colleagues designed a series of compounds based on **7**, which is approximately 100- to 500-fold more potent for FKBP12 than FKBP51 or FKBP52, providing a promising avenue toward this goal.[39,126] Initially, this group generated a small series of compounds in which the pipecolate core, cyclohexylethenyl motif, and pyranose ring region were systematically varied. No significant potency was gained, supporting the idea that the binding pocket is highly conserved. An alignment of FKBP12 with the structures of FKBP51 (Figure 11) and FKBP52 showed that the 40s and 80s loops were the

only regions with significant deviation, likely giving rise to the selectivity for FKBP12. In addition, the authors noted that FKBP51 had a Leu at position 119 in the 80s loop, while FKBP52 had a Pro. The crystal structure of **7** in complex with FKBP51 was analogous to that of similar ligands bound to FKBP12, with the exception of the *tert*-pentyl group in the pyranose region, which was rotated 180° to engage the 80s loop. With the observation that this region of the binding pocket is expanded in FKBP51, the authors focused on the *tert*-pentyl substituent. Replacement of the *tert*-butyl with a cyclohexyl moiety mimicking FK506 (**17***) increased potency 2-fold versus **7**. Synthesis of α-keto acids for coupling to the pipecolate core provided final compounds as a mixture of diastereomers (denoted *). Replacement of the phenoxyacetic acid with phenoxyethylmorpholine further improved potency by 2-fold and afforded a submicromolar inhibitor of FKBP52 (**18***). To determine the effect of stereochemistry, **19** and **20** were purified and found to be equipotent (Figure 11). Thus, stereochemistry does not seem to alter the binding preference of the pyranose group, possibly due to flexibility of the 80s loop. Despite the relatively weak affinities, this study was one of the first reported SAR analyses to explore paralog selectivity.

Expanding on this work, synthetic derivation was abandoned in favor of a "bump-hole"[127] approach previously employed to engineer highly specific ligands for mutant FKBP12^{F36V} variants (**21**, Shld1).[75,128] Ethyl substitution in the α carbon position of the α-keto amide results in over 1000-fold selectivity versus FKBP12^{WT} and subnanomolar affinity for the mutant, FKBP12^{F36V} (Figure 12). Adapting **21** to FKBP51^{F67V} and FKBP52^{F67V}, the pendent ethyl was substituted in favor of a slightly bulkier vinyl group and the pipecolate core was replaced by 4,5-dehydropipecolate (**22**).[129,130] While testing derivatives of **22** against mutant FKBP51 and FKBP52, it was discovered that some complementary ligands (**23**, **24**) still had modest affinity for FKBP51^{WT}. From analysis of the crystal structure, the α carbon allyl substitution induces a conformational rearrangement of the active site, with Phe-67 rotating down to contact Lys-58 and Lys-60. This induced fit conformation occurs to a lesser extent in FKBP52, as the bulky Thr-58, Trp-60, and Val-129 are relatively occluded from full rotation. A small series of compounds was designed with increased bulk at the α carbon position. Cyclohexenyl derivatives **25** and **26** bound FKBP51 in the low nanomolar range in the same binding mode as the weaker derivatives. Reduction to the cyclohexyl resulted in a further increase in potency to single digit nanomolar with over 10 000-fold selectivity for FKBP51 over FKBP52. Significantly, these are the first ligands to be highly selective for FKBP51 without appreciable FKBP12 binding. Additionally, induced fit ligands are approximately 20-fold higher affinity than the natural ligand FK506 ($K_i \approx 93$ nM). Consistent with the known mechanism of FKBP51, inhibition by **28** blocked FKBP51-mediated attenuation of the GR negative feedback loop, resulting in a decrease of secreted corticosterone in an animal model. This work presents a promising therapeutic strategy for new antidepressants in patients with hyperactive stress hormone secretion networks or hyperinducing FKBP51 gene variants.[131] Follow-up studies with these compounds further validated the FKBP51 dependence in various pathologies. For example, in a melanoma model, abnormal activation of the transcription factor NF-κB is modulated by FKBP51 activation of IKK. Inhibition by **27** and **28** prevented IKK degradation and blocked NF-κB nuclear translocation.[132] By use of inflammation and neuropathic pain models, **28** was shown to be effective at reducing hypersensitivity resulting from glucocorticoid receptor

signaling/activation and subsequent FKBP51 up-regulation. Significantly, FKBP51 blockade did not prevent acute pain responses and may be an effective strategy for novel analgesics to treat longlasting pain.[133]

Optimization of this scaffold was subsequently performed to address the low ligand-efficiency and poor physicochemical properties that limit CNS penetration of the previous series (Figure 13).[134] Because the core pipecolate possessing the cyclohexyl ring had good activity, a series of aminoamides were appended off of the pipecolic acid. Replacement of the ester moiety resulted in a large reduction of molecular weight while retaining modest potency and selectivity for FKBP51 (**29**). Derivation in this position highlighted a geminal dimethylamide (**30**) with a substantial increase in potency, and this chemical functionality was further explored. A series of carbocycles was also prepared, affording the high affinity analog **31**. This compound was only slightly less potent than **28**, with 20-fold selectivity against FKBP12 and no appreciable binding to FKBP52. Notably, the molecular weight of **31** was dramatically reduced, with improvements in ligand efficiency and CNS-related physicochemical properties.

INHIBITORS OF CYCLOPHILINS

To date, most of the inhibitors of cyclophilins are based on the natural product cyclosporin A (**32**, CsA, Figure 14) using semisynthetic approaches.[135] NIM811 (**33**), originally developed by Sandoz, was discovered as a biosynthetic derivative of **32** containing an isoleucine substitution at position 4.[136] Addition of the isoleucine group in this position is sufficient to block binding to calcineurin and prevent immunosuppression. In a model of HIV infectivity, **33** was found to be ~5-fold more effective than **33**, with favorable pharmacokinetics in animals. Similarly, **33** was found to be effective in models of HCV infection, demonstrating 5-fold increased activity over **32** and equipotent across genotypes. Compound **33** was recently found effective in a phase 2 clinical trial in patients given interferon combination therapy but ultimately discontinued due to weaker antiviral activity when compared to alisporivir.[137] Alisporivir (**34**) is a nonimmunosuppressive **32** derivative that is semisynthetically modified with an *N*-methylalanine substitution at position 3 and *N*-ethylvaline replacement at position 4.[138] Alisporivir potently inhibits HCV replication and was shown to be effective in patients co-infected by HIV and HCV.[139] Like **33**, coadministration of alisporivir with interferon and/or ribavirin is often required for maximum effectiveness. At the time of this writing, multiple clinical trials of alisporivir are ongoing. In addition, SCY-635 (**35**) has been developed as a novel cyclosporin thioether with a favorable pharmacodynamics profile. Preclinical testing showed **35** to potently inhibit HCV replication in vitro. It is also orally bioavailable and partitions into hepatocytes upon administration. Like other cyclosporins, **35** is synergistic with other antivirals, including ribavirin and interferon.[140] In an effort to better understand the role of extracellular cyclophilins, derivatives of CsA have been developed to be cell-membrane-impermeable.[141] One example, MM284 (**36**), was derivatized by modification of the MeBmt residue with a carboxy benzimidazole.[142] This compound retained high Cyp inhibition, yet lacked immunosuppressive activity. In models of inflammation, extracellular **36** was effective at blocking Cyp-mediated leukocyte chemotaxis. Further, this action was shown to be selective

for CD147 and treatment of **36** had no effect on $CD147^{-/-}$ mice, highlighting a role for the Cyp–CD147 interaction in inflammation.

More recently, small molecule inhibitors of the cyclophilins have been developed as an alternative to the synthetic challenges of **32**. Another goal of those studies is to create paralog-selective inhibitors because cyclosporin itself has a K_D between 2 and 10 nM for most members of the family. As mentioned above, CypD is an especially promising target, as this PPIase plays roles in calcium signaling. To find specific CypD inhibitors, a small library was synthesized based on a quinoxaline scaffold (Figure 15).[143] Three compounds from this series had moderate activity in biochemical assays. Compounds **37** and **38** were equipotent for CypA and CypD with K_i values of ~3 μM, while **39** preferentially bound CypD over 10-fold tighter than CypA (2 μM vs 21 μM). In a mitochondrial swelling model, **39** was the most potent and it was able to block pore opening at 100 μM. While preliminary, this work suggests that the quinaxaline scaffold might be a reasonable starting point for more potent and selective inhibitors. Toward that goal, Valasani et al. used the available structure–activity relationships to build a three-dimensional pharmacophore model of the CypD active site. Then, a test set of thiazolo[3,2-*a*]pyrmidine and sulfonamide derivatives was docked into the refined CypD structure.[144] The highest scoring ligand (**40**) was synthesized and found to bind to CypD with K_D = 149 nM.[145] Similar to the quinoxaline-based inhibitors, **40** was able to prevent Ca^{2+}-dependent mitochondrial swelling in a dose-dependent fashion.

An alternative scaffold based on aryl 1-indanyl ketones,[146] also reported to target Pin1 (Figure 16),[147] has been shown to differentiate between CypA and CypB. Compounds **41** and **42** primarily inhibit CypA, with 5- to 10-fold less activity for CypD. However, these compounds have similar affinity for CypA and Pin1. Other analogs, such as **43** and **44**, did not enhance selectivity. For example, **43** had CypA $K_i \approx 1.2$ μM and CypB $K_i = 2.1$ μM. Although it had weak potency, compound **45** was the only compound to show a preference for CypB (CypB $K_i \approx 63$ μM and CypA K_i = no inhibition). Taken together, this study highlights the challenges in developing selective inhibitors of the cyclophilins.

Computational modeling has proven to be particularly effective in the discovery of high affinity cyclophilin ligands, likely due to the availability of numerous, high-resolution crystal structures. In one approach, virtual screening was performed using a crystal structure of CypA in complex with **32** (PDB code 1CWA).[148] On the basis of the binding interactions of **32**, a pharmacophore model was designed and screened for in the ACD-3D database. A subset of compounds was selected and docked against CypA, identifying a common diarylurea scaffold amenable to synthetic optimization (representative hit **46**; Figure 17). IC_{50} values of hit compounds were experimentally determined, with **46** inhibiting CypA isomerase activity at ~300 nM. After a brief SAR analysis, bulky electronegative groups in the phenylurea para-position were found to be well-tolerated (e.g., **47**), improving the IC_{50} up to 20-fold (14 nM). A similar derivative based on a benzylurea scaffold with the benzyl phenyl ether moiety replaced by an ethyl acetate group was crystallized with CypD, confirming the computational model (Figure 17).[149] The urea core forms productive hydrogen bonds with the side chains of Gln-105 and Asn-144, critical interactions observed in the virtual screen. Optimization of this scaffold to improve solubility further validated this

binding orientation and demonstrated a high affinity 2-arylpyrrolidine substitution with high affinity (**48**).[150] In an alternative approach, a purely de novo drug design strategy was attempted using an iterative ligand building method (LigBuilder 2.0).[151] The central urea linker from **46** was chosen as a seed and oriented into the CypA binding pocket. Procedural generation and optimization were then performed to build the ligand on either side of the linker. Of the top scoring molecules generated, 38% shared a common scaffold, which was then selected for follow-up (**49**; Figure 18). Resynthesis of **49** and evaluation in a CypA isomerase assay in vitro demonstrated inhibition with an IC_{50} of 32 nM, which was slightly more potent than **32** ($IC_{50} \approx 41$ nM). Surprisingly, almost any replacement of the 9*H*-fluorene ring resulted in a dramatic loss of potency. For example, a naphthyl substitution decreased activity in the isomerase assay to over 10 μM. In contrast, the 2,6-dihydroxyphenyl ring was amenable to substitution with the 2,6-dichloro (**50**) and 2-chloro-6-fluoro (**51**) substitutions substantially increasing potency to 2.6 nM and 1.5 nM, respectively. In general, halogens and electronwithdrawing groups were necessary for binding, while electron-donating substituents resulted in a loss of activity. One interesting observation was that large asymmetric substitutions on the phenyl ring were well tolerated, such as the benzyl ether (**52**, $IC_{50} \approx 12$ nM). Docking studies suggest that this series of compounds likely reach across the substrate recognition site in CypA (see Figure 3). The 9*H*-fluorene interacts with a pocket neighboring the proline-binding site, with the acylurea linker acting as a bridge and making backbone hydrogen bonds to Ala-103. In the proline pocket, the phenyl ring favorably stacks above Phe-113, positioning ring substitutions to engage with a hydrophobic cleft above His-126. Similar to **46** and **47**, solubility is a likely concern with this scaffold. Additional SAR improvements can likely be made in the 9*H*-fluorene pocket and through investigation into the favorable binding of the benzyl ether substitution, hopefully improving the physicochemical properties of this series. Continued structural and SAR studies of these scaffolds are necessary, with these compounds representing a promising lead for future small molecule cyclophilin therapeutics.

INHIBITORS OF PARVULINS

Due to the role of Pin1 in numerous human diseases including cancer and neurodegeneration, finding high affinity, cell permeable inhibitors has been an ongoing pursuit.[152] Discovered as the first Pin1 inhibitor, juglone (**53**, Figure 19) functions by covalent modification of Cys-113 in the active site.[153] Juglone is produced by the black walnut tree, and its inhibition of Pin1 activity has been suggested to contribute to the toxicity of black walnuts. However, the exact binding interaction of **53** is unclear and this compound has proven to have other cellular targets, likely acting nonspecifically.[154] To date, no inhibitors are known to mimic this mode of inhibition. Interestingly, **53** does not inhibit the FKBPs or cyclophilins, whereas Pin1 has not been shown to have any appreciable affinity for the natural products, rapamycin, FK506, or cyclosporin (**1**, **32**). This observation is perhaps surprising, due to the similarity between active sites across the PPIases. However, Pin1 is unique among the PPIases because it binds to phosphopeptides through a positively charged surface. Thus, Pin1 inhibitors must navigate this feature of the pocket by including highly electronegative substituents. The challenge is that these same features often reduce membrane permeability. Other natural product derivatives (Figure 19) have been shown to

inhibit Pin1 while also engaging the phosphate-binding region, providing an alternative approach toward novel scaffolds. Screening a panel of phosphate-binding proteins, **54** demonstrated selectivity for Pin1 and was inactive against the other proteins. Compound **54** is the synthetic corticosteroid prodrug dexamethasone-21-phosphate and modestly inhibits Pin1 activity (K_D = 2.2 μM).[155] By NMR and X-ray crystallography, **54** was shown to bind across the proline pocket to the cationic groove formed by Lys-63, Arg-68, and Arg-69. Using a similar screening strategy, the vitamin A derivative all-trans-retinoic acid (**55**) was found to be a submicromolar inhibitor of Pin1 (K_D = 0.8uM) and bound similarly to **54**.[156] The cyclohexene ring is anchored into the proline binding pocket with the alkene chain extending the carboxylic acid into the cationic phosphate-binding region. Treatment with **55** suppresses proliferation of mouse embryonic fribroblasts and leads to Pin1 degradation but has no effect in Pin1 knockout cells or those harboring inactivating mutations in the binding site. In a model of acute promyelocytic leukemia (APL), **55** facilitates degradation of the fusion oncoprotein promyelocytic leukemia-retinoic acid receptor α (PML-RAR-α) and was shown to be due to the inhibition of the stabilizing effect of Pin1. This was further demonstrated in a breast cancer model where Pin1 is highly expressed and possesses pro-oncogenic activity. Inhibition of Pin1 by **55** limited tumorigenicity of MDA-MBA-231 cells injected into mice in a dose-dependent manner. Inhibitors based on these natural products may be a promising starting point for future therapeutics similar to the evolution of FKBP12 ligands.

Because of the challenges with the Pin1 site, early inhibitors were primarily based on peptides. Although these molecules have not been useful as probes in cells or animals, they have illustrated the features of the pocket. In one approach, a cellulose membrane-bound combinatorial library of 5-mer N-acetylated peptides incorporating unnatural amino acids surrounding a phosphothreonine residue was screened against the PPIase domain of Pin1 and bound hits were detected by Western blot. The strongest hit contained a pipecolate core not unlike those found to strongly inhibit FKBPs. Optimization of the peptide resulted in **56** (Ac-Phe-D-Thr(PO_3H_2)-Pip-Nal-Gln-NH_2; Figure 20), which had a $K_i \approx 18$ nM. Switching the phosphothreonine residue to the L-isomer (*) resulted in a 30-fold loss in activity ($K_i \approx$ 550 nM), and removing the phosphate group ablated the interaction. Addition of the WW domain in the full-length protein did not alter binding preference.[157] This result is important because it suggests that the WW domain does not act as a sink for the compounds. Crystallization of the L- and D-peptide versions of **56** yielded surprising results, with nearly identical conformations of the stereoisomers. In both structures, the electronegative phosphate group was anchored into the phosphate recognition pocket, contacting Lys-63 and Arg-69. The pipecolic core rested in the proline-binding pocket, making hydrophobic contacts with Leu-122, Met-130, and Phe-134. The naphthylalanine side chain extends up and stacks on a hydrophobic shelf formed by Leu-122 and the top of the Met-130 side chain.[158] On the basis of these structures, the reasons for the large difference in binding affinity are not obvious. Strikingly, the Pin1 active site bound to these peptides adopted a conformation that is reminiscent of FKBP when bound to rapamycin, with both pipecolic rings in nearly identical orientations. However, rapamycin has no measurable affinity for Pin1, so the affinity of the peptides for Pin1 must originate through the phosphate-binding surface.

The Pei group employed an alternative strategy based on cyclic peptides.[159] Cyclic peptides have the advantage of being relatively resistant to proteolytic degradation. Also, they are conformationally rigid, which can increase affinity for the target and enhance membrane permeability,[160,161] which is likely how cyclosporin retains good pharmacokinetic properties despite its high molecular mass. The Pei group's library consisted of the tripeptide core containing an electronegative amino acid (DpThr, D-pSer, Glu, D-Asp) and an isomerizable hydrophobic (Pro, D-Pro, L-Pip, L-*N*-methyl-Ala, L-*N*-methyl-Leu, L-*N*-Methyl-Phe) and a general hydrophobic, aromatic, or positively charged residue. Flanking residues of the tripeptide consisted of amino acids resistant to proteolysis, and then the ring was cyclized with anywhere between zero to three D-Ala residues (Figure 21). These compounds were screened on-resin for binding to Pin1, and from this screen, 51 peptides were isolated. Hit compounds overwhelmingly comprised D-pThr-Pip-Xaa in the tripeptide core, with a hydrophobic residue in the third position. There was no preference for cyclic ring size or flanking residues to the core tripeptide, suggesting that the binding site is fully occupied by the core. Four peptides with the D-pThr-Pip motif were chosen for resynthesis. Importantly, these peptides were cleaved from resin and purified to measure affinity in solution. As expected, the D-pThr-Pip-Nal tripeptide was again the most potent, with flanking residues having minimal effect. The analogous D-pSer-Pro-Nal tripeptide core was significantly less potent by nearly 20-fold, and the glutamate-L-*N*-methyl-Phe bioisostere did not bind. To increase the likelihood of membrane permeability, an octaarginine sequence was then appended to the cyclic peptides via a disulfide bond that is reduced in the cytosol (Figure 21). Consistent with Pin1 inhibition, cells treated with **57** (1 μ*M*) underwent cell cycle arrest and the stability of negatively regulated Pin1 clients was increased. More recently, the cyclo(FΦRRRRQ (with Φ = L-napthylalanine) motif and its bicyclic versions have been developed as cell-penetrating peptides that efficiently escape the endosome and deliver small peptides to the cytosol.[162] This bicyclic peptide approach was applied to the previously described cyclic peptides targeting Pin1, resulting in the development of **58**, which bound Pin1 with $K_D \approx 72$ nM (Figure 22). Treatment of HeLa cells with this peptide resulted in inhibited cell growth and increased stability of Pin1 clients. Subsequent library generation and medicinal chemistry optimization resulted in a nonphosphorylated derivative, replacing residues from the N-terminal with D-Fpa-Fpa and Phe, respectively (Figure 22, green residues, $K_D = 120$ nM).[163] This compound was active in cell-based assays, and future structural studies will hopefully elucidate the important binding interactions independent of the phosphate-binding site.

Motivated by the binding interaction between CypA and the nonribosomal peptide **32**, Duncan et al. used a phage panning technique to develop cyclic peptides that bind Pin1 without the requirement of the charged phosphate group.[164] The phage library contained 1.2×10^9 heptamers flanked by terminal cysteine residues, which should form disulfide bonds and cyclize on the oxidizing surface of the phage. After multiple rounds of selection, a common core of Tyr-Pro-Glu-Val was identified. By use of this sequence, a representative peptide was chosen (**59**, CRYPEVEIC) due to its good solubility and rapid cyclization (Figure 23). In an assay for Pin1 inhibition, **59** was able to inhibit isomerase activity at 0.52 μ*M*, whereas the linear peptide **60** inhibited at nearly 100-fold higher concentration (44 μ*M*). NMR titration experiments with **59** revealed chemical shift perturbations throughout the

active site, with significant peak broadening of residues Cys-113 and Ser-154. In contrast, residues in the basic pocket, such as Lys-63 and Arg-68, were not affected, suggesting that they do not interact with the glutamic acids. NMR was also used to explore the effects of cyclization on the conformation of the peptide. The low RMSD that was obtained in the total structural ensemble from the $^{1}H–^{1}H$ NOESY NMR experiment suggested a constrained ring system that is tightly folded, likely providing the high binding affinity of even the nonphosphorylated peptide. Future studies will be required to further understand the interaction of Pin1 with **59** and potential ways that this starting point may be leveraged in drug discovery efforts. This scaffold is particularly interesting because it does not seem to rely on interactions with the phosphate-binding cleft for tight affinity.

Pfizer initiated a program to discover small molecule Pin1 inhibitors. Guided by the Pin1 crystal structure, FKBP ligands were modeled in the active site and inhibitors designed (Figure 24). The first compound, **61**, was found to be a low micromolar inhibitor ($K_i \approx 1.7$ μM), which was promising for a de novo lead.[165] Attempts to optimize the charge by replacing the phosphate with a sulfate (**62**) resulted in a significant drop in potency (9.5 μM). Reinstalling the phosphate and appending a propylbenzene to the carbinol center improved potency to 0.8 μM and inhibited cell cycle progression of CA46 cells at 15 μM (**63**). However, these compounds were not ligand efficient, so the scaffold was abandoned. Instead, a combinatorial library of 200 molecules was screened for examples that would interact in place of the pipecolyl ester using the phenylalaninol phosphate as the minimum binding epitope. This screen identified two actives, the biarylamides **64a** and **65a**, which inhibited Pin1 at 100 nM and 179 nM, respectively. Cocrystallization of **65c** (Figure 25) revealed a new binding pose, with the compound rotated to allow the phenyl group to dock into the hydrophobic proline pocket and placing the benzothiophene on the hydrophobic shelf formed by Leu-122 and Met-130. Analysis of this new interaction in the binding pocket revealed a small cavity at the meta-phenyl position and a set of analogs were designed to place a small hydrophobic group in this position. Any small substitution at this position resulted in a significant boost in binding affinity, with the 3-fluoro substitution improving potency by 10- to 30-fold. However, these compounds were inactive in whole cell assays, likely due to the poor permeability of the phosphate group. Subsequent derivatization of the benzothiophene **65a** and **65b** to replace the phosphate group with either a primary alcohol or carboxylic acid inactivated the compound. However, the carboxylic acid replacement in **64a** did retain some activity ($K_i \approx 28$ μM), and this compound was the subject of a limited SAR series.[166]

Fluorine substitution at the 3-phenyl position improved potency 2-fold, and a modest extension of the linker to the carboxylic acid added another 2-fold improvement (Figure 26). However, this increase was limited to the smaller hydrophobic substitutions on the phenyl ring, whereas the affinity of larger 3-trifluoromethyl and 3-chloro groups was not improved. The larger groups likely push the phenyl ring slightly out of the pocket; thus linker extension of the carboxylic acid moves this group far into the basic groove of the phosphate recognition pocket. In addition to modifications of the phenyl ring system, phosphate bioisosteres, such as tetrazoles, acylaminothiazoles, and acylsulfonamides, were tried in replacement of the carboxylic acid. Unfortunately, only the tetrazole retained the same

activity as the carboxylate. To further examine the effect of linker lengths, a series of compounds with extensions between the amido carbon and phenyl ring were synthesized (**66**–**68**, Figure 27). Extending the phenyl ring deeper into the prolyl pocket resulted in a marginal increase of potency (18 μM, 0.89 μM, and 1.87 μM, respectively), likely due to rigidifying the linker. Replacing the saturated alkane to the more rigid alkene afforded the largest gain in binding affinity (~20-fold), but adding an unsaturation was detrimental (2-fold reduction compared to the single alkene).

While many of the derivatives of **64a** afforded enhanced binding affinity and Pin1 inhibition even down to the nanomolar range (**67**), these compounds were still inactive in cell-based assays. An analysis of the pharmacokinetic properties of **67** and its analogs demonstrated acceptable cell permeability and a favorable ADME profile, suggesting that the lack of cellular activity is likely due to other factors, such as limited potency (binding to Pin1 without interruption of isomerase activity), failure to compete with native clients, or off-target binding. Although disappointing, this work provided some of the first membrane permeable, low molecular mass inhibitors of Pin1. Working off this scaffold, it was hypothesized that a larger aromatic ring system in place of the phenyl group could be used to retain binding in the proline pocket while also making additional hydrogen bonds with Cys-113 and Ser-154.[167] These residues are important for the catalytic isomerase activity of Pin1 but might only be reached by a larger ring system extending out of the proline pocket.

Consistent with this idea, a series of benzimidazole derivatives was found to potently inhibit Pin1, with the fluoro derivatives **69c** and **70c** binding at 80 nM and 76 nM, respectively (Figure 28). Similar to other structure–activity relationships, hydrophobic halogen substitutions (**69a**,**c** and **70a**,**c**) at the 3-position were significantly more potent than methyls (**69b** and **70b**). The crystal structure of **69c** in complex with Pin1 resolved a hydrogen bond network spanning the entire Pin1 active site (Figure 28). In this structure, the benzimidazole bridges both Cys-113 and Ser-154, donating a hydrogen bond to Ser-154 and receiving a hydrogen bond from Cys-113, which likely facilitates the increased potency of **69c** versus the 3-fluorophenyl derivatives (Figure 27). Unfortunately, none of these compounds had cellular activity, despite the improved binding affinity for Pin1. It was hypothesized that export by P-glycoprotein efflux pumps may be responsible. Further, the amidyl carboxylate moiety was implicated in recognition by P-glycoprotein, so a series was synthesized in which the amides were replaced by dihydrothiazoles (Figure 29). These molecules retained potency for Pin1 and demonstrated surprising good antiproliferative activity in cancer cells. The naphthyl dihydrothiazole **71**, which mimics **69c**, lost potency for Pin1 by over 10-fold but still had antiproliferative activity in HT29 colorectal cells. Optimization of the naphthyl substituent afforded the 9-methylfluorene (**72**), which improved in vitro potency to 320 nM and was able to inhibit cell cycle progression at 4.2 μM. An additional scaffold was prepared containing a 2,6-dichlorophenethyl substitution on the dihydrothiazole ring (**73**). This compound further enhanced potency against Pin1 isomerase activity, but it was approximately equipotent in the viability assays. Increasing the rigidity of the alkane chain between the 2,6-dichlorophenyl and dihydrothiazole core (**74**) resulted in a 3-fold loss in Pin1 inhibition but improved antiproliferative activity to 1.9 μM (~2-fold). The dihydrothiazoles represent a novel class of cell permeable PPIase inhibitors. Further

optimization of the interactions between **72**–**74** and peripheral residues of the Pin1 binding site may be a possible strategy.

Using a different, NMR-based screening approach, a group at Vernalis discovered small fragments as ligands for Pin1 (Figure 30).[168] They used inhibitors **56** and **65a** to saturate the binding site, followed by screening of approximately 1200 fragments in displacement assays. From the screen, the most potent active was an indole-2-carboxylic acid (**75**) with an $IC_{50} \approx 16\ \mu M$. The crystal structure of **75** bound to Pin1 showed that the indole contacts the bottom of the proline-binding site. In addition, a key hydrogen bond was made between the indole nitrogen and Cys-113, positioning the carboxylic acid into the phosphate recognition pocket. Starting from **75**, an SAR campaign tested substitutions around the phenyl ring and the effects of heteroatom replacements. The nitrile benzimidazole **76** was found to be the most potent, with $IC_{50} \approx 10\ \mu M$. This compound was similar to those found by the Pfizer group (**69a**–**c**, **70a**–**c**) and bound in a similar orientation. Because no significant gain in potency was afforded, the original hit was expanded to **77**, which allowed for further modification of the scaffold. Specifically, the intent was to engage with the Pin1 hydrophobic shelf formed by Leu-122 and Met-130. Although **77** was less potent than **75**, its superior ligand efficiency suggested that this approach may be fruitful. Focusing on substitutions of the α carbon in the carboxylic acid, an ethyl substitution was made (**78**) to test whether binding was preserved (Figure 31). Indeed, **78** bound with an $IC_{50} \approx 78\ \mu M$, a 10-fold improvement over the parent compound **77**. Interestingly, replacement of the ethyl group with a benzothiophene derivative (**79**) was almost identical to that of the Pfizer group's **70a–c**, although arrived at by a distinct approach. Compound **79** had an IC_{50} of 0.83 μM in the PPIase assay (but bound to Pin1 with a $K_D \approx 10\ \mu M$ by SPR), so it was not as potent as **70a–c.** This observation suggests that the lack of substitution on the benzimidazole ring may be important. Lastly, the most potent compound in the series was **80** with $IC_{50} \approx 0.025\ \mu M$, which was designed to interact with the hydrophobic shelf adjacent to the active site. Unfortunately, none of the compounds in this series were able to inhibit cell cycle progression in PC3 prostate cancer cells. It was thought that the benzimidazole may be too polar and not sufficiently membrane permeable. To reduce the polar surface area, analog **81** was synthesized with the goal of increasing membrane permeability. This compound was approximately 100-fold weaker with $IC_{50} \approx 2.6\ \mu M$, but it inhibited cell cycle progression in the PC3 model at 12.5 μM. Importantly, it also prevented cyclin D1 expression, which would be expected for Pin1 inhibition. To further modify this scaffold, Nakagawa et al. focused on the region of the molecule that resides in the proline-binding pocket.[169] After a limited SAR series, **82** was found to be the most potent inhibitor ($IC_{50} \approx 3.0\ \mu M$) and contains an aliphatic azocane substituent. This group imparts some conformational flexibility and reduces aromatic planarity common to most other Pin1 scaffolds. Critically, the ring size was shown to be crucial for orientating the carboxylic acid into the phosphate-binding site.

Due to the relative lack of cellular activity from their first screen, the Vernalis group performed a second fragment screen of 900 compounds (Figure 32). These fragments were tested for the ability to inhibit Pin1 isomerase activity on a model substrate.[170] Compounds were screened at 40 mM, leading to 40 initial actives. In a follow-up NMR screen, only 2 of the 37 novel hits were genuine binders (**83** and **84**), and the nearest neighbor **85** had $IC_{50} \approx$

180 μM. Optimization of this imidazole fragment led to a series of compounds that were substituted at the 3-position (**86**; Figure 33), a site shown previously to be effective in engaging the proline pocket. This fragment had $IC_{50} \approx 20$ μM, while replacement of the 3-chloro for a 3-fluoro as well as conversion of the methylimidazole to a carboxylic acid (**87**) further enhanced potency (0.32 μM). Unfortunately, these compounds also lacked cellular activity. Subsequent modification at the 3 position resulted in a series of amide substituted 3-chlorophenylimidazole inhibitors with modest affinity for Pin1 (**88**, $IC_{50} \approx 2.0$ μM) that were cell penetrant and able to inhibit cell division and Pin1-mediated cyclin D1 expression at low micromolar levels. Furthermore, these compounds had a unique binding mode in Pin1, flanking Lys-63 and Arg-69 in the phosphate recognition pocket. It is worth noting that these fragments do not engage the hydrophobic shelf, as seen with other potent Pin1 inhibitors (**64**–**74**). Additional modifications, perhaps from the phenyl ring, may allow for dramatic improvements based on previous structural insights.

CONCLUSION

PPIases are challenging drug targets. Their active sites are shallow, hydrophobic, and often degenerate between family members. In this way, inhibition of the PPIase active site has many of the same challenges as other protein–protein interaction sites.[171–173] Despite these complications, the natural products FK506, rapamycin, and cyclosporin bind PPIases with tight affinity and, in some cases, with some paralog specificity. Indeed, analogs of the natural products, including everolimus and pimecrolimus, have been approved for immune and oncology indications and have been explored in aging models.[173] However, there have not been any approvals for molecules that exert biological activity through inhibition of a PPIase. One of the major hurdles has been pharmacokinetics and membrane permeability. The natural products use the special properties of macrocycles, such as intramolecular hydrogen bonds and cellular partitioning,[160,174] to engender “druglike” properties into a large scaffold that might normally be considered too large. The macrocycles are, therefore, able to cover more “real estate” and access the important sites on the PPIase: the proline pocket, hydrophobic shelf, and the hydrogen-bonding network. Thus, one problem with smaller molecules might be the disconnect between their low molecular mass and the far-flung features of the active site. For similar reasons, the field of protein–protein interactions has long realized that macrocycles provide a privileged scaffold for building inhibitors.[175] More specifically, it has become clear that there are multiple categories of protein–protein interactions, which are defined based on the buried surface area and the affinity of the interaction.[176–178] Those contacts that are limited to small surfaces (<200 $Å^2$) and high affinity (<200 nM) have been found to be suitable for inhibition by molecules that largely conform to Lipinski’s rules. Successful inhibitors of these interactions often bind directly to the site of the protein–protein interaction and orthosterically compete for the native partner. In contrast, protein–protein interactions that cover larger areas (>200 $Å^2$) and with weaker affinity (>200 nM) have been less amenable to inhibition by molecules less than 500 Da. Rather, these contacts are often best approached with either macrocycles or allosteric inhibitors that bind a distal site and cause conformational changes in the active site.[179] The interactions between PPIases and their clients are more similar to the broad, weak

interactions of the latter category. Therefore, macrocycles or allosteric inhibitors might provide a more viable path forward.

One of the major questions in the field of PPIase inhibitors has been whether it is possible to create paralog-selective molecules. Developments in the past 3 years have provided renewed enthusiasm, and we anticipate that continued exploration of these scaffolds will further improve paralog selectivity. However, an alternative to these molecules may be to create compounds that inhibit PPIases by targeting their other domains. It is becoming increasingly clear that other domains in PPIases contribute to their functions. Molecules that bind these regions might avoid the degenerate, shallow features of the PPIase domains to generate selectivity. Regardless of which strategy is ultimately successful, molecules that selectively inhibit individual PPIase paralogs will serve important roles as chemical probes (or tool molecules), even if they are not therapeutics. For example, selective inhibitors might isolate the intertwined biological roles of related PPIases, such as FKBP12, FKBP51, and FKBP52.[180] Such tools are important because genetic deletion often results in compensation and obscures the roles of the paralog. In contrast, chemical probes allow temporal control and selective disruption of individual functions, such as isomerase activity. Thus, chemical probes could be used to identify the clients that are most reliant on individual paralogs. Selectivity will also benefit clinical progression. For example, the roles of certain PPIases in disease (e.g., FKBP51 in neuropathic pain or depression) are not yet clear, and a combination of selective molecules and genetic methods (e.g., CRISPR) could finally validate these targets.

The unique and singular roles of Pin1 in regulating the function of phosphorylated clients have prioritized this PPIase in recent drug discovery efforts. Yet Pin1 has proven to be one of the most difficult PPIases to inhibit. The basic nature of the active site has presented distinct challenges absent in the other PPIases. Of the strategies reported to date, those small molecules evolved from carboxylic acid fragments seem to be most effective in mediating cell cycle arrest and destabilization of Pin1 clients, likely because they balance Pin1 binding affinity with the need for membrane permeability. However, this complication in inhibitor design may ultimately prove beneficial by enhancing the possibility of paralog specificity. An underexplored strategy might exploit the Cys-113 in Pin1 to create covalent, irreversible inhibitors. This residue has been shown to be reactive and is modified by the natural product juglone. Furthermore, the potential involvement of Cys-113 in the catalytic mechanism means that covalent modification would be expected to directly inactivate Pin1. In addition, interactions with the cysteine could be slowly reversible, because Pin1 inhibitors would not necessarily have to continually occupy the binding pocket. Even if such molecules were not advanced as therapeutics, they might serve as chemical probes.

What is next for PPIase inhibitors? One lesson is that chemical series, exemplified by **7** and **56**, that start from peptides or natural products lend themselves to relatively rapid evolution of affinity. In other words, scaffolds that have incorporated features of FK506, rapamycin, and cyclosporin, such as the proline isosteres in **57** and cyclization in **59**, tend to produce tight affinity, even early in the medicinal chemistry campaign. While synthetic inhibitors have shown surprising efficacy and some selectivity, an overview of the field suggests to us that the natural products can still teach us about PPIase inhibition. Moreover, recent work

has shown that the PPIases have a wide array of clients, suggesting that they evolved to have malleable, broad, and promiscuous active sites. Therefore, compounds with nontraditional mechanisms, such as covalent inhibitors and those that take advantage of induced fit, may provide a way to garner high affinity with relatively low molecular weight. Thus, we favor a mindset in which PPIases inhibitors are subject to the same development “guidelines” as inhibitors of protein–protein interactions.

Acknowledgments

The authors thank Mitchell Mutz and members of the Gestwicki group for helpful comments. Our work on PPIases has been supported by NIH (Grants AI098431 and NS059690).

ABBREVIATIONS USED

ANT	adenine nucleotide translocase
APL	acute promyelocytic leukemia
AR	androgen receptor
CypA	cyclophilin A
FKBP	FK506-binding protein
GR	glucocorticoid receptor
MMP9	matrix metallopeptidase 9
MPT	mitochondrial permeability transition
mTOR	mammalian target of rapamycin
NF-AT	nuclear factor of activated T cell
PML-RAR-α	oncoprotein promyelocytic leukemia-retinoic acid receptor α
PPIase	peptidyl-proline isomerase
rmsd	root-mean-square error
TGF-β	transforming growth factor β
TPR	tetratricopeptide repeat domain

References

1. Fischer G, Bang H, Mech C. Determination of enzymatic catalysis for the cis-trans-isomerization of peptide binding in proline-containing peptides. Biomed Biochim Acta. 1984; 43:1101–1111. [PubMed: 6395866]
2. Galat A. Peptidylprolyl cis/trans isomerases (immunophilins): Biological diversity–targets–functions. Curr Top Med Chem. 2003; 3:1315–1347. [PubMed: 12871165]
3. Saibil H. Chaperone machines for protein folding, unfolding and disaggregation. Nat Rev Mol Cell Biol. 2013; 14:630–642. [PubMed: 24026055]

4. Theuerkorn M, Fischer G, Schiene-Fischer C. Prolyl cis/trans isomerase signalling pathways in cancer. Curr Opin Pharmacol. 2011; 11:281–287. [PubMed: 21497135]
5. Frausto SD, Lee E, Tang H. Cyclophilins as modulators of viral replication. Viruses. 2013; 5:1684–1701. [PubMed: 23852270]
6. Storer CL, Dickey CA, Galigniana MD, Rein T, Cox MB. FKBP51 and FKBP52 in signaling and disease. Trends Endocrinol Metab. 2011; 22:481–490. [PubMed: 21889356]
7. Koren J 3rd, Jinwal UK, Davey Z, Kiray J, Arulselvam K, Dickey CA. Bending tau into shape: The emerging role of peptidylprolyl isomerases in tauopathies. Mol Neurobiol. 2011; 44:65–70. [PubMed: 21523562]
8. Siekierka JJ, Hung SH, Poe M, Lin CS, Sigal NH. A cytosolic binding protein for the immunosuppressant FK506 has peptidyl-prolyl isomerase activity but is distinct from cyclophilin. Nature. 1989; 341:755–757. [PubMed: 2477714]
9. Schmid FX. Prolyl isomerase: Enzymatic catalysis of slow protein-folding reactions. Annu Rev Biophys Biomol Struct. 1993; 22:123–142. [PubMed: 7688608]
10. Fischer G, Tradler T, Zarnt T. The mode of action of peptidyl prolyl cis/trans isomerases in vivo: Binding vs. catalysis. FEBS Lett. 1998; 426:17–20. [PubMed: 9598969]
11. Yeh ES, Means AR. Pin1, the cell cycle and cancer. Nat Rev Cancer. 2007; 7:381–388. [PubMed: 17410202]
12. Cao W, Konsolaki M. FKBP immunophilins and Alzheimer's disease: A chaperoned affair. J Biosci. 2011; 36:493–498. [PubMed: 21799260]
13. Nigro P, Pompilio G, Capogrossi MC. Cyclophilin A: a key player for human disease. Cell Death Dis. 2013; 4:e888. [PubMed: 24176846]
14. Butterfield DA, Abdul HM, Opii W, Newman SF, Joshi G, Ansari MA, Sultana R. Pin1 in Alzheimer's disease. J Neurochem. 2006; 98:1697–1706. [PubMed: 16945100]
15. Gaali S, Gopalakrishnan R, Wang Y, Kozany C, Hausch F. The chemical biology of immunophilin ligands. Curr Med Chem. 2011; 18:5355–5379. [PubMed: 22087830]
16. Carlson EE. Natural products as chemical probes. ACS Chem Biol. 2010; 5:639–653. [PubMed: 20509672]
17. Heitman J, Movva NR, Hall MN. Proline isomerases at the crossroads of protein folding, signal transduction, and immunosuppression. New Biol. 1992; 4:448–460. [PubMed: 1515410]
18. Koehn FE. Therapeutic potential of natural product signal transduction agents. Curr Opin Biotechnol. 2006; 17:631–637. [PubMed: 17049224]
19. Rosen MK, Schreiber SL. Natural products as probes of cellular function: Studies of immunophilins. Angew Chem, Int Ed Engl. 1992; 31:384–400.
20. Kim PS, Baldwin RL. Specific intermediates in the folding reactions of small proteins and the mechanism of protein folding. Annu Rev Biochem. 1982; 51:459–489. [PubMed: 6287919]
21. Schonbrunner ER, Mayer S, Tropschug M, Fischer G, Takahashi N, Schmid FX. Catalysis of protein folding by cyclophilins from different species. J Biol Chem. 1991; 266:3630–3635. [PubMed: 1825312]
22. Andreotti AH. Native state proline isomerization: An intrinsic molecular switch. Biochemistry. 2003; 42:9515–9524. [PubMed: 12911293]
23. Zheng H, You H, Zhou XZ, Murray SA, Uchida T, Wulf G, Gu L, Tang X, Lu KP, Xiao ZX. The prolyl isomerase Pin1 is a regulator of p53 in genotoxic response. Nature. 2002; 419:849–853. [PubMed: 12397361]
24. Girardini JE, Napoli M, Piazza S, Rustighi A, Marotta C, Radaelli E, Capaci V, Jordan L, Quinlan P, Thompson A, Mano M, Rosato A, Crook T, Scanziani E, Means AR, Lozano G, Schneider C, Del Sal G. A Pin1/mutant p53 axis promotes aggressiveness in breast cancer. Cancer Cell. 2011; 20:79–91. [PubMed: 21741598]
25. He Z, Li L, Luan S. Immunophilins and parvulins. Superfamily of peptidyl prolyl isomerases in Arabidopsis. Plant Physiol. 2004; 134:1248–1267. [PubMed: 15047905]
26. Somarelli JA, Lee SY, Skolnick J, Herrera RJ. Structurebased classification of 45 FK506-binding proteins. Proteins: Struct, Funct Genet. 2008; 72:197–208. [PubMed: 18214965]

27. Fulton KF, Jackson SE, Buckle AM. Energetic and structural analysis of the role of tryptophan 59 in FKBP12. Biochemistry. 2003; 42:2364–2372. [PubMed: 12600203]
28. Rosen MK, Standaert RF, Galat A, Nakatsuka M, Schreiber SL. Inhibition of FKBP rotamase activity by immunosuppressant FK506: Twisted amide surrogate. Science. 1990; 248:863–866. [PubMed: 1693013]
29. Xu GG, Zhang Y, Mercedes-Camacho AY, Etzkorn FA. A reduced-amide inhibitor of Pin1 binds in a conformation resembling a twisted-amide transition state. Biochemistry. 2011; 50:9545–9550. [PubMed: 21980916]
30. Howard BR, Vajdos FF, Li S, Sundquist WI, Hill CP. Structural insights into the catalytic mechanism of cyclophilin A. Nat Struct Biol. 2003; 10:475–481. [PubMed: 12730686]
31. Keedy DA, Kenner LR, Warkentin M, Woldeyes RA, Hopkins JB, Thompson MC, Brewster AS, Van Benschoten AH, Baxter EL, Uervirojnangkoorn M, McPhillips SE, Song J, Alonso-Mori R, Holton JM, Weis WI, Brunger AT, Soltis SM, Lemke H, Gonzalez A, Sauter NK, Cohen AE, van den Bedem H, Thorne RE, Fraser JS. Mapping the conformational landscape of a dynamic enzyme by multitemperature and XFEL crystallography. eLife. 2015; 4doi: 10.7554/eLife.07574
32. DeCenzo MT, Park ST, Jarrett BP, Aldape RA, Futer O, Murcko MA, Livingston DJ. FK506-binding protein mutational analysis: Defining the active-site residue contributions to catalysis and the stability of ligand complexes. Protein Eng, Des Sel. 1996; 9:173–180.
33. Behrsin CD, Bailey ML, Bateman KS, Hamilton KS, Wahl LM, Brandl CJ, Shilton BH, Litchfield DW. Functionally important residues in the peptidyl-prolyl isomerase Pin1 revealed by unigenic evolution. J Mol Biol. 2007; 365:1143–1162. [PubMed: 17113106]
34. Barman A, Hamelberg D. Cysteine-mediated dynamic hydrogen-bonding network in the active site of pin1. Biochemistry. 2014; 53:3839–3850. [PubMed: 24840168]
35. Carmody M, Mackrill JJ, Sorrentino V, O'Neill C. FKBP12 associates tightly with the skeletal muscle type 1 ryanodine receptor, but not with other intracellular calcium release channels. FEBS Lett. 2001; 505:97–102. [PubMed: 11557049]
36. Lyons WE, Steiner JP, Snyder SH, Dawson TM. Neuronal regeneration enhances the expression of the immunophilin FKBP-12. J Neurosci. 1995; 15:2985–2994. [PubMed: 7536825]
37. Hoeffer CA, Tang W, Wong H, Santillan A, Patterson RJ, Martinez LA, Tejada-Simon MV, Paylor R, Hamilton SL, Klann E. Removal of FKBP12 enhances mTor-raptor interactions, LTP, memory, and perseverative/repetitive behavior. Neuron. 2008; 60:832–845. [PubMed: 19081378]
38. Shou W, Aghdasi B, Armstrong DL, Guo Q, Bao S, Charng MJ, Mathews LM, Schneider MD, Hamilton SL, Matzuk MM. Cardiac defects and altered ryanodine receptor function in mice lacking FKBP12. Nature. 1998; 391:489–492. [PubMed: 9461216]
39. Kozany C, Marz A, Kress C, Hausch F. Fluorescent probes to characterise FK506-binding proteins. ChemBioChem. 2009; 10:1402–1410. [PubMed: 19418507]
40. Davies TH, Ning YM, Sanchez ER. A new first step in activation of steroid receptors: Hormone-induced switching of FKBP51 and FKBP52 immunophilins. J Biol Chem. 2002; 277:4597–4600. [PubMed: 11751894]
41. Assimon VA, Southworth DR, Gestwicki JE. Specific binding of tetratricopeptide repeat proteins to heat shock protein 70 (Hsp70) and heat shock protein 90 (Hsp90) is regulated by affinity and phosphorylation. Biochemistry. 2015; 54:7120–7131. [PubMed: 26565746]
42. Cheung-Flynn J, Prapapanich V, Cox MB, Riggs DL, Suarez-Quian C, Smith DF. Physiological role for the cochaperone FKBP52 in androgen receptor signaling. Mol Endocrinol. 2005; 19:1654–1666. [PubMed: 15831525]
43. LeMaster DM, Mustafi SM, Brecher M, Zhang J, Heroux A, Li H, Hernandez G. Coupling of conformational transitions in the N-terminal domain of the 51-kDa FK506-binding protein (FKBP51) near its site of interaction with the steroid receptor proteins. J Biol Chem. 2015; 290:15746–15757. [PubMed: 25953903]
44. Blair LJ, Baker JD, Sabbagh JJ, Dickey CA. The emerging role of peptidyl-prolyl isomerase chaperones in tau oligomerization, amyloid processing, and Alzheimer's disease. J Neurochem. 2015; 133:1–13. [PubMed: 25628064]
45. Jinwal UK, Koren J 3rd, Borysov SI, Schmid AB, Abisambra JF, Blair LJ, Johnson AG, Jones JR, Shults CL, O'Leary JC 3rd, Jin Y, Buchner J, Cox MB, Dickey CA. The Hsp90 cochaperone,

FKBP51, increases tau stability and polymerizes microtubules. J Neurosci. 2010; 30:591–599. [PubMed: 20071522]

46. Chambraud B, Sardin E, Giustiniani J, Dounane O, Schumacher M, Goedert M, Baulieu EE. A role for FKBP52 in tau protein function. Proc Natl Acad Sci U S A. 2010; 107:2658–2663. [PubMed: 20133804]
47. Giustiniani J, Guillemeau K, Dounane O, Sardin E, Huvent I, Schmitt A, Hamdane M, Buee L, Landrieu I, Lippens G, Baulieu EE, Chambraud B. The FK506-binding protein FKBP52 in vitro induces aggregation of truncated tau forms with prion-like behavior. FASEB J. 2015; 29:3171–3181. [PubMed: 25888602]
48. Giustiniani J, Chambraud B, Sardin E, Dounane O, Guillemeau K, Nakatani H, Paquet D, Kamah A, Landrieu I, Lippens G, Baulieu EE, Tawk M. Immunophilin FKBP52 induces tau-P301L filamentous assembly in vitro and modulates its activity in a model of tauopathy. Proc Natl Acad Sci U S A. 2014; 111:4584–4589. [PubMed: 24623856]
49. Stocki P, Sawicki M, Mays CE, Hong SJ, Chapman DC, Westaway D, Williams DB. Inhibition of the FKBP family of peptidyl prolyl isomerases induces abortive translocation and degradation of the cellular prion protein. Mol Biol Cell. 2016; 27:757–767. [PubMed: 26764098]
50. Edlich F, Lucke C. From cell death to viral replication: The diverse functions of the membrane-associated FKBP38. Curr Opin Pharmacol. 2011; 11:348–353. [PubMed: 21514222]
51. Wang P, Heitman J. The cyclophilins. Genome Biol. 2005; 6:226. [PubMed: 15998457]
52. Handschumacher RE, Harding MW, Rice J, Drugge RJ, Speicher DW. Cyclophilin: A specific cytosolic binding protein for cyclosporin A. Science. 1984; 226:544–547. [PubMed: 6238408]
53. Fruman DA, Burakoff SJ, Bierer BE. Immunophilins in protein folding and immunosuppression. FASEB J. 1994; 8:391–400. [PubMed: 7513288]
54. Crompton M. The mitochondrial permeability transition pore and its role in cell death. Biochem J. 1999; 341(2):233–249. [PubMed: 10393078]
55. Halestrap AP, Brenner C. The adenine nucleotide translocase: A central component of the mitochondrial permeability transition pore and key player in cell death. Curr Med Chem. 2003; 10:1507–1525. [PubMed: 12871123]
56. Ward BK, Mark PJ, Ingram DM, Minchin RF, Ratajczak T. Expression of the estrogen receptor-associated immunophilins, cyclophilin 40 and FKBP52, in breast cancer. Breast Cancer Res Treat. 1999; 58:265–278.
57. Rahfeld JU, Rucknagel KP, Schelbert B, Ludwig B, Hacker J, Mann K, Fischer G. Confirmation of the existence of a third family among peptidyl-prolyl cis/trans isomerases. Amino acid sequence and recombinant production of parvulin. FEBS Lett. 1994; 352:180–184. [PubMed: 7925971]
58. Mueller JW, Kessler D, Neumann D, Stratmann T, Papatheodorou P, Hartmann-Fatu C, Bayer P. Characterization of novel elongated parvulin isoforms that are ubiquitously expressed in human tissues and originate from alternative transcription initiation. BMC Mol Biol. 2006; 7:9. [PubMed: 16522211]
59. Surmacz TA, Bayer E, Rahfeld JU, Fischer G, Bayer P. The N-terminal basic domain of human parvulin hPar14 is responsible for the entry to the nucleus and high-affinity DNA-binding. J Mol Biol. 2002; 321:235–247. [PubMed: 12144781]
60. Fujiyama-Nakamura S, Yoshikawa H, Homma K, Hayano T, Tsujimura-Takahashi T, Izumikawa K, Ishikawa H, Miyazawa N, Yanagida M, Miura Y, Shinkawa T, Yamauchi Y, Isobe T, Takahashi N. Parvulin (par14), a peptidyl-prolyl cis-trans isomerase, is a novel RNA processing factor that evolved in the metazoan lineage. Mol Cell Proteomics. 2009; 8:1552–1565. [PubMed: 19369196]
61. Thiele A, Krentzlin K, Erdmann F, Rauh D, Hause G, Zerweck J, Kilka S, Posel S, Fischer G, Schutkowski M, Weiwad M. Parvulin 17 promotes microtubule assembly by its peptidyl-prolyl cis/trans isomerase activity. J Mol Biol. 2011; 411:896–909. [PubMed: 21756916]
62. Kessler D, Papatheodorou P, Stratmann T, Dian EA, Hartmann-Fatu C, Rassow J, Bayer P, Mueller JW. The DNA binding parvulin par17 is targeted to the mitochondrial matrix by a recently evolved prepeptide uniquely present in hominidae. BMC Biol. 2007; 5:37. [PubMed: 17875217]
63. Stukenberg PT, Kirschner MW. Pin1 acts catalytically to promote a conformational change in cdc25. Mol Cell. 2001; 7:1071–1083. [PubMed: 11389853]

64. Liou YC, Ryo A, Huang HK, Lu PJ, Bronson R, Fujimori F, Uchida T, Hunter T, Lu KP. Loss of Pin1 function in the mouse causes phenotypes resembling cyclin D1-null phenotypes. Proc Natl Acad Sci U S A. 2002; 99:1335–1340. [PubMed: 11805292]

65. Liu J, Farmer JD Jr, Lane WS, Friedman J, Weissman I, Schreiber SL. Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. Cell. 1991; 66:807–815. [PubMed: 1715244]

66. Brown EJ, Albers MW, Shin TB, Ichikawa K, Keith CT, Lane WS, Schreiber SL. A mammalian protein targeted by G1-arresting rapamycin-receptor complex. Nature. 1994; 369:756–758. [PubMed: 8008069]

67. Harding MW, Galat A, Uehling DE, Schreiber SL. A receptor for the immunosuppressant FK506 is a cis-trans peptidylprolyl isomerase. Nature. 1989; 341:758–760. [PubMed: 2477715]

68. Weiwad M, Edlich F, Kilka S, Erdmann F, Jarczowski F, Dorn M, Moutty MC, Fischer G. Comparative analysis of calcineurin inhibition by complexes of immunosuppressive drugs with human FK506 binding proteins. Biochemistry. 2006; 45:15776–15784. [PubMed: 17176100]

69. Marz AM, Fabian AK, Kozany C, Bracher A, Hausch F. Large FK506-binding proteins shape the pharmacology of rapamycin. Mol Cell Biol. 2013; 33:1357–1367. [PubMed: 23358420]

70. Ho S, Clipstone N, Timmermann L, Northrop J, Graef I, Fiorentino D, Nourse J, Crabtree GR. The mechanism of action of cyclosporin A and FK506. Clin Immunol Immunopathol. 1996; 80:S40–45. [PubMed: 8811062]

71. Sehgal SN. Rapamune (rapa, rapamycin, sirolimus): Mechanism of action immunosuppressive effect results from blockade of signal transduction and inhibition of cell cycle progression. Clin Biochem. 1998; 31:335–340. [PubMed: 9721431]

72. Faivre S, Kroemer G, Raymond E. Current development of mTor inhibitors as anticancer agents. Nat Rev Drug Discovery. 2006; 5:671–688. [PubMed: 16883305]

73. Yang H, Rudge DG, Koos JD, Vaidialingam B, Yang HJ, Pavletich NP. mTor kinase structure, mechanism and regulation. Nature. 2013; 497:217–223. [PubMed: 23636326]

74. Spencer DM, Wandless TJ, Schreiber SL, Crabtree GR. Controlling signal transduction with synthetic ligands. Science. 1993; 262:1019–1024. [PubMed: 7694365]

75. Clackson T, Yang W, Rozamus LW, Hatada M, Amara JF, Rollins CT, Stevenson LF, Magari SR, Wood SA, Courage NL, Lu X, Cerasoli F Jr, Gilman M, Holt DA. Redesigning an FKBP-ligand interface to generate chemical dimerizers with novel specificity. Proc Natl Acad Sci U S A. 1998; 95:10437–10442. [PubMed: 9724721]

76. Sakamoto KM, Kim KB, Kumagai A, Mercurio F, Crews CM, Deshaies RJ. Protacs: Chimeric molecules that target proteins to the Skp1-cullin-F box complex for ubiquitination and degradation. Proc Natl Acad Sci U S A. 2001; 98:8554–8559. [PubMed: 11438690]

77. Braun PD, Barglow KT, Lin YM, Akompong T, Briesewitz R, Ray GT, Haldar K, Wandless TJ. A bifunctional molecule that displays context-dependent cellular activity. J Am Chem Soc. 2003; 125:7575–7580. [PubMed: 12812497]

78. Inoue T, Heo WD, Grimley JS, Wandless TJ, Meyer T. An inducible translocation strategy to rapidly activate and inhibit small GTPase signaling pathways. Nat Methods. 2005; 2:415–418. [PubMed: 15908919]

79. Corson TW, Aberle N, Crews CM. Design and applications of bifunctional small molecules: Why two heads are better than one. ACS Chem Biol. 2008; 3:677–692. [PubMed: 19112665]

80. Romano S, Di Pace A, Sorrentino A, Bisogni R, Sivero L, Romano MF. FK506 binding proteins as targets in anticancer therapy. Anti-Cancer Agents Med Chem. 2010; 10:651–656.

81. Harikishore A, Yoon HS. Immunophilins: Structures, mechanisms and ligands. Curr Mol Pharmacol. 2015; 9:37–47. [PubMed: 25986569]

82. Yamaguchi T, Kurisaki A, Yamakawa N, Minakuchi K, Sugino H. FKBP12 functions as an adaptor of the SMAD7-Smurf1 complex on activin type I receptor. J Mol Endocrinol. 2006; 36:569–579. [PubMed: 16720724]

83. Pratt WB, Toft DO. Steroid receptor interactions with heat shock protein and immunophilin chaperones. Endocr Rev. 1997; 18:306–360. [PubMed: 9183567]

84. Jin YJ, Burakoff SJ. The 25-kDa FK506-binding protein is localized in the nucleus and associates with casein kinase II and nucleolin. Proc Natl Acad Sci U S A. 1993; 90:7769–7773. [PubMed: 7689229]
85. Davis EC, Broekelmann TJ, Ozawa Y, Mecham RP. Identification of tropoelastin as a ligand for the 65-kD FK506-binding protein, FKBP65, in the secretory pathway. J Cell Biol. 1998; 140:295–303. [PubMed: 9442105]
86. Bush KT, Hendrickson BA, Nigam SK. Induction of the FK506-binding protein, FKBP13, under conditions which misfold proteins in the endoplasmic reticulum. Biochem J. 1994; 303(3):705–708. [PubMed: 7526846]
87. Cabral WA, Perdivara I, Weis M, Terajima M, Blissett AR, Chang W, Perosky JE, Makareeva EN, Mertz EL, Leikin S, Tomer KB, Kozloff KM, Eyre DR, Yamauchi M, Marini JC. Abnormal type I collagen post-translational modification and crosslinking in a cyclophilin B KO mouse model of recessive osteogenesis imperfecta. PLoS Genet. 2014; 10:e1004465. [PubMed: 24968150]
88. Harrell JM, Kurek I, Breiman A, Radanyi C, Renoir JM, Pratt WB, Galigniana MD. All of the protein interactions that link steroid receptor.Hsp90. Immunophilin heterocomplexes to cytoplasmic dynein are common to plant and animal cells. Biochemistry. 2002; 41:5581–5587. [PubMed: 11969419]
89. Kim J, Choi TG, Ding Y, Kim Y, Ha KS, Lee KH, Kang I, Ha J, Kaufman RJ, Lee J, Choe W, Kim SS. Overexpressed cyclophilin B suppresses apoptosis associated with ROS and Ca2+ homeostasis after ER stress. J Cell Sci. 2008; 121:3636–3648. [PubMed: 18946027]
90. Colgan J, Asmal M, Neagu M, Yu B, Schneidkraut J, Lee Y, Sokolskaja E, Andreotti A, Luban J. Cyclophilin A regulates TCR signal strength in CD4+ T cells via a proline-directed conformational switch in ITK. Immunity. 2004; 21:189–201. [PubMed: 15308100]
91. Jin ZG, Melaragno MG, Liao DF, Yan C, Haendeler J, Suh YA, Lambeth JD, Berk BC. Cyclophilin a is a secreted growth factor induced by oxidative stress. Circ Res. 2000; 87:789–796. [PubMed: 11055983]
92. Yurchenko V, Constant S, Eisenmesser E, Bukrinsky M. Cyclophilin-cd147 interactions: A new target for anti-inflammatory therapeutics. Clin Exp Immunol. 2010; 160:305–317. [PubMed: 20345978]
93. Du H, Guo L, Fang F, Chen D, Sosunov AA, McKhann GM, Yan Y, Wang C, Zhang H, Molkentin JD, Gunn-Moore FJ, Vonsattel JP, Arancio O, Chen JX, Yan SD. Cyclophilin D deficiency attenuates mitochondrial and neuronal perturbation and ameliorates learning and memory in Alzheimer's disease. Nat Med. 2008; 14:1097–1105. [PubMed: 18806802]
94. Schutkowski M, Bernhardt A, Zhou XZ, Shen M, Reimer U, Rahfeld JU, Lu KP, Fischer G. Role of phosphorylation in determining the backbone dynamics of the serine/threonine-proline motif and Pin1 substrate recognition. Biochemistry. 1998; 37:5566–5575. [PubMed: 9548941]
95. Ryo A, Liou YC, Lu KP, Wulf G. Prolyl isomerase Pin1: A catalyst for oncogenesis and a potential therapeutic target in cancer. J Cell Sci. 2003; 116:773–783. [PubMed: 12571275]
96. Driver JA, Zhou XZ, Lu KP. Pin1 dysregulation helps to explain the inverse association between cancer and Alzheimer's disease. Biochim Biophys Acta, Gen Subj. 2015; 1850:2069–2076.
97. Eichner T, Kutter S, Labeikovsky W, Buosi V, Kern D. Molecular mechanism of Pin1-tau recognition and catalysis. J Mol Biol. 2016; 428:1760–1775. [PubMed: 26996941]
98. Abrahamsen H, O'Neill AK, Kannan N, Kruse N, Taylor SS, Jennings PA, Newton AC. Peptidyl-prolyl isomerase Pin1 controls down-regulation of conventional protein kinase c isozymes. J Biol Chem. 2012; 287:13262–13278. [PubMed: 22318721]
99. Kutter S, Eichner T, Deaconescu AM, Kern D. Regulation of microtubule assembly by tau and not by Pin1. J Mol Biol. 2016; 428:1742–1759. [PubMed: 26996940]
100. Ibanez K, Boullosa C, Tabares-Seisdedos R, Baudot A, Valencia A. Molecular evidence for the inverse comorbidity between central nervous system disorders and cancers detected by transcriptomic meta-analyses. PLoS Genet. 2014; 10:e1004173. [PubMed: 24586201]
101. Bao L, Kimzey A, Sauter G, Sowadski JM, Lu KP, Wang DG. Prevalent overexpression of prolyl isomerase Pin1 in human cancers. Am J Pathol. 2004; 164:1727–1737. [PubMed: 15111319]
102. Blackburn EA, Walkinshaw MD. Targeting FKBP isoforms with small-molecule ligands. Curr Opin Pharmacol. 2011; 11:365–371. [PubMed: 21803654]

103. Owens-Grillo JK, Hoffmann K, Hutchison KA, Yem AW, Deibel MR Jr, Handschumacher RE, Pratt WB. The cyclosporin A-binding immunophilin Cyp-40 and the FK506-binding immunophilin Hsp56 bind to a common site on Hsp90 and exist in independent cytosolic heterocomplexes with the untransformed glucocorticoid receptor. J Biol Chem. 1995; 270:20479–20484. [PubMed: 7657624]
104. Davies TH, Ning YM, Sanchez ER. Differential control of glucocorticoid receptor hormone-binding function by tetratricopeptide repeat (TPR) proteins and the immunosuppressive ligand FK506. Biochemistry. 2005; 44:2030–2038. [PubMed: 15697228]
105. Liou YC, Sun A, Ryo A, Zhou XZ, Yu ZX, Huang HK, Uchida T, Bronson R, Bing G, Li X, Hunter T, Lu KP. Role of the prolyl isomerase Pin1 in protecting against age-dependent neurodegeneration. Nature. 2003; 424:556–561. [PubMed: 12891359]
106. Lu PJ, Wulf G, Zhou XZ, Davies P, Lu KP. The prolyl isomerase Pin1 restores the function of alzheimer-associated phosphorylated tau protein. Nature. 1999; 399:784–788. [PubMed: 10391244]
107. Butterfield DA, Poon HF, St Clair D, Keller JN, Pierce WM, Klein JB, Markesbery WR. Redox proteomics identification of oxidatively modified hippocampal proteins in mild cognitive impairment: Insights into the development of Alzheimer's disease. Neurobiol Dis. 2006; 22:223–232. [PubMed: 16466929]
108. Van Duyne GD, Standaert RF, Karplus PA, Schreiber SL, Clardy J. Atomic structure of FKBP-FK506, an immunophilin-immunosuppressant complex. Science. 1991; 252:839–842. [PubMed: 1709302]
109. Van Duyne GD, Standaert RF, Schreiber SL, Clardy J. Atomic structure of the rapamycin human immunophilin FKBP-12 complex. J Am Chem Soc. 1991; 113:7433–7434.
110. Armistead DM, Badia MC, Deininger DD, Duffy JP, Saunders JO, Tung RD, Thomson JA, DeCenzo MT, Futer O, Livingston DJ, Murcko MA, Yamashita MM, Navia MA. Design, synthesis and structure of non-macrocyclic inhibitors of FKBP12, the major binding protein for the immunosuppressant FK506. Acta Crystallogr, Sect D: Biol Crystallogr. 1995; 51:522–528. [PubMed: 15299839]
111. Holt DA, Luengo JI, Yamashita DS, Oh HJ, Konialian AL, Yen HK, Rozamus LW, Brandt M, Bossard MJ. Design, synthesis, and kinetic evaluation of high-affinity FKBP ligands and the X-ray crystal structures of their complexes with FKBP12. J Am Chem Soc. 1993; 115:9925–9938.
112. Keenan T, Yaeger DR, Courage NL, Rollins CT, Pavone ME, Rivera VM, Yang W, Guo T, Amara JF, Clackson T, Gilman M, Holt DA. Synthesis and activity of bivalent FKBP12 ligands for the regulated dimerization of proteins. Bioorg Med Chem. 1998; 6:1309–1335. [PubMed: 9784872]
113. DeRose R, Miyamoto T, Inoue T. Manipulating signaling at will: Chemically-inducible dimerization (CID) techniques resolve problems in cell biology. Pfluegers Arch. 2013; 465:409–417. [PubMed: 23299847]
114. Gestwicki JE, Crabtree GR, Graef IA. Harnessing chaperones to generate small-molecule inhibitors of amyloid beta aggregation. Science. 2004; 306:865–869. [PubMed: 15514157]
115. Marinec PS, Chen L, Barr KJ, Mutz MW, Crabtree GR, Gestwicki JE. FK506-binding protein (FKBP) partitions a modified HIV protease inhibitor into blood cells and prolongs its lifetime in vivo. Proc Natl Acad Sci U S A. 2009; 106:1336–1341. [PubMed: 19164520]
116. Dunyak BM, Nakamura RL, Frankel AD, Gestwicki JE. Selective targeting of cells via bispecific molecules that exploit coexpression of two intracellular proteins. ACS Chem Biol. 2015; 10:2441–2447. [PubMed: 26322864]
117. Winter GE, Buckley DL, Paulk J, Roberts JM, Souza A, Dhe-Paganon S, Bradner JE. Drug development. Phthalimide conjugation as a strategy for in vivo target protein degradation. Science. 2015; 348:1376–1381. [PubMed: 25999370]
118. Steiner JP, Hamilton GS, Ross DT, Valentine HL, Guo H, Connolly MA, Liang S, Ramsey C, Li JH, Huang W, Howorth P, Soni R, Fuller M, Sauer H, Nowotnik AC, Suzdak PD. Neurotrophic immunophilin ligands stimulate structural and functional recovery in neurodegenerative animal models. Proc Natl Acad Sci U S A. 1997; 94:2019–2024. [PubMed: 9050897]
119. Hamilton GS, Wu YQ, Limburg DC, Wilkinson DE, Vaal MJ, Li JH, Thomas C, Huang W, Sauer H, Ross DT, Soni R, Chen Y, Guo H, Howorth P, Valentine H, Liang S, Spicer D, Fuller M,

Steiner JP. Synthesis of n-glyoxyl prolyl and pipecolyl amides and thioesters and evaluation of their in vitro and in vivo nerve regenerative effects. J Med Chem. 2002; 45:3549–3557. [PubMed: 12139466]

120. Wu YQ, Wilkinson DE, Limburg D, Li JH, Sauer H, Ross D, Liang S, Spicer D, Valentine H, Fuller M, Guo H, Howorth P, Soni R, Chen Y, Steiner JP, Hamilton GS. Synthesis of ketone analogues of prolyl and pipecolyl ester FKBP12 ligands. J Med Chem. 2002; 45:3558–3568. [PubMed: 12139467]
121. Edlich F, Weiwad M, Wildemann D, Jarczowski F, Kilka S, Moutty MC, Jahreis G, Lucke C, Schmidt W, Striggow F, Fischer G. The specific FKBP38 inhibitor n-(n′,n′-dimethylcarboxamidomethyl)cycloheximide has potent neuroprotective and neurotrophic properties in brain ischemia. J Biol Chem. 2006; 281:14961–14970. [PubMed: 16547004]
122. Gold BG, Densmore V, Shou W, Matzuk MM, Gordon HS. Immunophilin FK506-binding protein 52 (not FK506-binding protein 12) mediates the neurotrophic action of FK506. J Pharmacol Exp Ther. 1999; 289:1202–1210. [PubMed: 10336507]
123. Christner C, Wyrwa R, Marsch S, Kullertz G, Thiericke R, Grabley S, Schumann D, Fischer G. Synthesis and cytotoxic evaluation of cycloheximide derivatives as potential inhibitors of FKBP12 with neuroregenerative properties. J Med Chem. 1999; 42:3615–3622. [PubMed: 10479292]
124. Edlich F, Weiwad M, Erdmann F, Fanghanel J, Jarczowski F, Rahfeld JU, Fischer G. Bcl-2 regulator FKBP38 is activated by Ca2+/calmodulin. EMBO J. 2005; 24:2688–2699. [PubMed: 15990872]
125. Rasch J, Theuerkorn M, Unal C, Heinsohn N, Tran S, Fischer G, Weiwad M, Steinert M. Novel cycloheximide derivatives targeting the moonlighting protein MIP exhibit specific antimicrobial activity against Legionella pneumophila. Front Bioeng Biotechnol. 2015; 3:41. [PubMed: 25870856]
126. Gopalakrishnan R, Kozany C, Gaali S, Kress C, Hoogeland B, Bracher A, Hausch F. Evaluation of synthetic FK506 analogues as ligands for the FK506-binding proteins 51 and 52. J Med Chem. 2012; 55:4114–4122. [PubMed: 22455444]
127. Shah K, Liu Y, Deirmengian C, Shokat KM. Engineering unnatural nucleotide specificity for rous sarcoma virus tyrosine kinase to uniquely label its direct substrates. Proc Natl Acad Sci U S A. 1997; 94:3565–3570. [PubMed: 9108016]
128. Banaszynski LA, Chen LC, Maynard-Smith LA, Ooi AG, Wandless TJ. A rapid, reversible, and tunable method to regulate protein function in living cells using synthetic small molecules. Cell. 2006; 126:995–1004. [PubMed: 16959577]
129. Feng X, Sippel C, Bracher A, Hausch F. Structure-affinity relationship analysis of selective FKBP51 ligands. J Med Chem. 2015; 58:7796–7806. [PubMed: 26419422]
130. Gaali S, Kirschner A, Cuboni S, Hartmann J, Kozany C, Balsevich G, Namendorf C, Fernandez-Vizarra P, Sippel C, Zannas AS, Draenert R, Binder EB, Almeida OF, Ruhter G, Uhr M, Schmidt MV, Touma C, Bracher A, Hausch F. Selective inhibitors of the FK506-binding protein 51 by induced fit. Nat Chem Biol. 2015; 11:33–37. [PubMed: 25436518]
131. Klengel T, Mehta D, Anacker C, Rex-Haffner M, Pruessner JC, Pariante CM, Pace TW, Mercer KB, Mayberg HS, Bradley B, Nemeroff CB, Holsboer F, Heim CM, Ressler KJ, Rein T, Binder EB. Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. Nat Neurosci. 2013; 16:33–41. [PubMed: 23201972]
132. Romano S, Xiao Y, Nakaya M, D'Angelillo A, Chang M, Jin J, Hausch F, Masullo M, Feng X, Romano MF, Sun SC. FKBP51 employs both scaffold and isomerase functions to promote NF-kappab activation in melanoma. Nucleic Acids Res. 2015; 43:6983–6993. [PubMed: 26101251]
133. Maiaru M, Tochiki KK, Cox MB, Annan LV, Bell CG, Feng X, Hausch F, Geranton SM. The stress regulator FKBP51 drives chronic pain by modulating spinal glucocorticoid signaling. Sci Transl Med. 2016; 8:325ra319.
134. Gaali S, Feng X, Hahle A, Sippel C, Bracher A, Hausch F. Rapid, structure-based exploration of pipecolic acid amides as novel selective antagonists of the FK506-binding protein 51. J Med Chem. 2016; 59:2410–2422. [PubMed: 26954324]

135. Peel M, Scribner A. Semi-synthesis of cyclosporins. Biochim Biophys Acta, Gen Subj. 2015; 1850:2121–2144.
136. Rosenwirth B, Billich A, Datema R, Donatsch P, Hammerschmid F, Harrison R, Hiestand P, Jaksche H, Mayer P, Peichl P. Inhibition of human immunodeficiency virus type 1 replication by SDZ NIM811, a nonimmunosuppressive cyclosporine analog. Antimicrob Agents Chemother. 1994; 38:1763–1772. [PubMed: 7527198]
137. Hopkins S, Gallay P. Cyclophilin inhibitors: An emerging class of therapeutics for the treatment of chronic hepatitis C infection. Viruses. 2012; 4:2558–2577. [PubMed: 23202494]
138. Hubler F, Rückle T, Patiny L, Muamba T, Guichou JF, Mutter M, Wenger R. Synthetic routes to NEtXaa^4-cyclosporin A derivatives as potential anti-HIV 1 drugs. Tetrahedron Lett. 2000; 41:7193–7196.
139. Flisiak R, Horban A, Gallay P, Bobardt M, Selvarajah S, Wiercinska-Drapalo A, Siwak E, Cielniak I, Higersberger J, Kierkus J, Aeschlimann C, Grosgurin P, Nicolas-Metral V, Dumont JM, Porchet H, Crabbe R, Scalfaro P. The cyclophilin inhibitor debio-025 shows potent anti-hepatitis C effect in patients coinfected with hepatitis C and human immunodeficiency virus. Hepatology. 2008; 47:817–826. [PubMed: 18302285]
140. Hopkins S, Scorneaux B, Huang Z, Murray MG, Wring S, Smitley C, Harris R, Erdmann F, Fischer G, Ribeill Y. SCY-635, a novel nonimmunosuppressive analog of cyclosporine that exhibits potent inhibition of hepatitis C virus RNA replication in vitro. Antimicrob Agents Chemother. 2010; 54:660–672. [PubMed: 19933795]
141. Malesevic M, Kuhling J, Erdmann F, Balsley MA, Bukrinsky MI, Constant SL, Fischer G. A cyclosporin derivative discriminates between extracellular and intracellular cyclophilins. Angew Chem, Int Ed. 2010; 49:213–215.
142. Malesevic M, Gutknecht D, Prell E, Klein C, Schumann M, Nowak RA, Simon JC, Schiene-Fischer C, Saalbach A. Anti-inflammatory effects of extracellular cyclosporins are exclusively mediated by cd147. J Med Chem. 2013; 56:7302–7311. [PubMed: 23964991]
143. Guo HX, Wang F, Yu KQ, Chen J, Bai DL, Chen KX, Shen X, Jiang HL. Novel cyclophilin D inhibitors derived from quinoxaline exhibit highly inhibitory activity against rat mitochondrial swelling and Ca2+ uptake/release. Acta Pharmacol Sin. 2005; 26:1201–1211. [PubMed: 16174436]
144. Valasani KR, Vangavaragu JR, Day VW, Yan SS. Structure based design, synthesis, pharmacophore modeling, virtual screening, and molecular docking studies for identification of novel cyclophilin D inhibitors. J Chem Inf Model. 2014; 54:902–912. [PubMed: 24555519]
145. Valasani KR, Sun Q, Fang D, Zhang Z, Yu Q, Guo Y, Li J, Roy A, ShiDu Yan S. Identification of a small molecule cyclophilin D inhibitor for rescuing Aβ-mediated mitochondrial dysfunction. ACS Med Chem Lett. 2016; 7:294–299. [PubMed: 26985318]
146. Daum S, Schumann M, Mathea S, Aumuller T, Balsley MA, Constant SL, de Lacroix BF, Kruska F, Braun M, Schiene-Fischer C. Isoform-specific inhibition of cyclophilins. Biochemistry. 2009; 48:6268–6277. [PubMed: 19480458]
147. Daum S, Erdmann F, Fischer G, Feaux de Lacroix B, Hessamian-Alinejad A, Houben S, Frank W, Braun M. Aryl indanyl ketones: Efficient inhibitors of the human peptidyl prolyl cis/trans isomerase Pin1. Angew Chem, Int Ed. 2006; 45:7454–7458.
148. Guichou JF, Viaud J, Mettling C, Subra G, Lin YL, Chavanieu A. Structure-based design, synthesis, and biological evaluation of novel inhibitors of human cyclophilin A. J Med Chem. 2006; 49:900–910. [PubMed: 16451056]
149. Gelin M, Delfosse V, Allemand F, Hoh F, Sallaz-Damaz Y, Pirocchi M, Bourguet W, Ferrer JL, Labesse G, Guichou JF. Combining 'dry' co-crystallization and in situ diffraction to facilitate ligand screening by x-ray crystallography. Acta Crystallogr, Sect D: Biol Crystallogr. 2015; 71:1777–1787. [PubMed: 26249358]
150. Shore ER, Awais M, Kershaw NM, Gibson RR, Pandalaneni S, Latawiec D, Wen L, Javed MA, Criddle DN, Berry N, O'Neill PM, Lian LY, Sutton R. Small molecule inhibitors of cyclophilin D to protect mitochondrial function as a potential treatment for acute pancreatitis. J Med Chem. 2016; 59:2596–2611. [PubMed: 26950392]

151. Ni S, Yuan Y, Huang J, Mao X, Lv M, Zhu J, Shen X, Pei J, Lai L, Jiang H, Li J. Discovering potent small molecule inhibitors of cyclophilin A using de novo drug design approach. J Med Chem. 2009; 52:5295–5298. [PubMed: 19691347]

152. Moore JD, Potter A. Pin1 inhibitors: Pitfalls, progress and cellular pharmacology. Bioorg Med Chem Lett. 2013; 23:4283–4291. [PubMed: 23796453]

153. Hennig L, Christner C, Kipping M, Schelbert B, Rucknagel KP, Grabley S, Kullertz G, Fischer G. Selective inactivation of parvulin-like peptidyl-prolyl cis/trans isomerases by juglone. Biochemistry. 1998; 37:5953–5960. [PubMed: 9558330]

154. Uchida T, Takamiya M, Takahashi M, Miyashita H, Ikeda H, Terada T, Matsuo Y, Shirouzu M, Yokoyama S, Fujimori F, Hunter T. Pin1 and Par14 peptidyl prolyl isomerase inhibitors block cell proliferation. Chem Biol. 2003; 10:15–24. [PubMed: 12573694]

155. Graber M, Janczyk W, Sperl B, Elumalai N, Kozany C, Hausch F, Holak TA, Berg T. Selective targeting of disease-relevant protein binding domains by o-phosphorylated natural product derivatives. ACS Chem Biol. 2011; 6:1008–1014. [PubMed: 21797253]

156. Wei S, Kozono S, Kats L, Nechama M, Li W, Guarnerio J, Luo M, You MH, Yao Y, Kondo A, Hu H, Bozkurt G, Moerke NJ, Cao S, Reschke M, Chen CH, Rego EM, Lo-Coco F, Cantley LC, Lee TH, Wu H, Zhang Y, Pandolfi PP, Zhou XZ, Lu KP. Active Pin1 is a key target of all-trans retinoic acid in acute promyelocytic leukemia and breast cancer. Nat Med. 2015; 21:457–466. [PubMed: 25849135]

157. Wildemann D, Erdmann F, Alvarez BH, Stoller G, Zhou XZ, Fanghanel J, Schutkowski M, Lu KP, Fischer G. Nanomolar inhibitors of the peptidyl prolyl cis/trans isomerase Pin1 from combinatorial peptide libraries. J Med Chem. 2006; 49:2147–2150. [PubMed: 16570909]

158. Zhang Y, Daum S, Wildemann D, Zhou XZ, Verdecia MA, Bowman ME, Lucke C, Hunter T, Lu KP, Fischer G, Noel JP. Structural basis for high-affinity peptide inhibition of human Pin1. ACS Chem Biol. 2007; 2:320–328. [PubMed: 17518432]

159. Liu T, Liu Y, Kao HY, Pei D. Membrane permeable cyclic peptidyl inhibitors against human peptidylprolyl isomerase Pin1. J Med Chem. 2010; 53:2494–2501. [PubMed: 20180533]

160. Rezai T, Yu B, Millhauser GL, Jacobson MP, Lokey RS. Testing the conformational hypothesis of passive membrane permeability using synthetic cyclic peptide diastereomers. J Am Chem Soc. 2006; 128:2510–2511. [PubMed: 16492015]

161. Hruby VJ, al-Obeidi F, Kazmierski W. Emerging approaches in the molecular design of receptor-selective peptide ligands: Conformational, topographical and dynamic considerations. Biochem J. 1990; 268:249–262. [PubMed: 2163604]

162. Lian W, Jiang B, Qian Z, Pei D. Cell-permeable bicyclic peptide inhibitors against intracellular proteins. J Am Chem Soc. 2014; 136:9830–9833. [PubMed: 24972263]

163. Jiang B, Pei D. A selective, cell-permeable nonphosphorylated bicyclic peptidyl inhibitor against peptidyl-prolyl isomerase Pin1. J Med Chem. 2015; 58:6306–6312. [PubMed: 26196061]

164. Duncan KE, Dempsey BR, Killip LE, Adams J, Bailey ML, Lajoie GA, Litchfield DW, Brandl CJ, Shaw GS, Shilton BH. Discovery and characterization of a nonphosphorylated cyclic peptide inhibitor of the peptidylprolyl isomerase, Pin1. J Med Chem. 2011; 54:3854–3865. [PubMed: 21545152]

165. Guo C, Hou X, Dong L, Dagostino E, Greasley S, Ferre R, Marakovits J, Johnson MC, Matthews D, Mroczkowski B, Parge H, Vanarsdale T, Popoff I, Piraino J, Margosiak S, Thomson J, Los G, Murray BW. Structure-based design of novel human Pin1 inhibitors (i). Bioorg Med Chem Lett. 2009; 19:5613–5616. [PubMed: 19729306]

166. Dong L, Marakovits J, Hou X, Guo C, Greasley S, Dagostino E, Ferre R, Johnson MC, Kraynov E, Thomson J, Pathak V, Murray BW. Structure-based design of novel human Pin1 inhibitors (ii). Bioorg Med Chem Lett. 2010; 20:2210–2214. [PubMed: 20207139]

167. Guo C, Hou X, Dong L, Marakovits J, Greasley S, Dagostino E, Ferre R, Johnson MC, Humphries PS, Li H, Paderes GD, Piraino J, Kraynov E, Murray BW. Structure-based design of novel human Pin1 inhibitors (iii): Optimizing affinity beyond the phosphate recognition pocket. Bioorg Med Chem Lett. 2014; 24:4187–4191. [PubMed: 25091930]

168. Potter AJ, Ray S, Gueritz L, Nunns CL, Bryant CJ, Scrace SF, Matassova N, Baker L, Dokurno P, Robinson DA, Surgenor AE, Davis B, Murray JB, Richardson CM, Moore JD. Structure-guided

design of alpha-amino acid-derived Pin1 inhibitors. Bioorg Med Chem Lett. 2010; 20:586–590. [PubMed: 19969456]

169. Nakagawa H, Seike S, Sugimoto M, Ieda N, Kawaguchi M, Suzuki T, Miyata N. Peptidyl prolyl isomerase Pin1-inhibitory activity of D-glutamic and D-aspartic acid derivatives bearing a cyclic aliphatic amine moiety. Bioorg Med Chem Lett. 2015; 25:5619–5624. [PubMed: 26508545]
170. Potter A, Oldfield V, Nunns C, Fromont C, Ray S, Northfield CJ, Bryant CJ, Scrace SF, Robinson D, Matossova N, Baker L, Dokurno P, Surgenor AE, Davis B, Richardson CM, Murray JB, Moore JD. Discovery of cell-active phenylimidazole Pin1 inhibitors by structure-guided fragment evolution. Bioorg Med Chem Lett. 2010; 20:6483–6488. [PubMed: 20932746]
171. Makley LN, Gestwicki JE. Expanding the number of 'druggable' targets: Non-enzymes and protein-protein interactions. Chem Biol Drug Des. 2013; 81:22–32. [PubMed: 23253128]
172. Arkin MR, Tang Y, Wells JA. Small-molecule inhibitors of protein-protein interactions: Progressing toward the reality. Chem Biol. 2014; 21:1102–1114. [PubMed: 25237857]
173. Laraia L, McKenzie G, Spring DR, Venkitaraman AR, Huggins DJ. Overcoming chemical, biological, and computational challenges in the development of inhibitors targeting protein-protein interactions. Chem Biol. 2015; 22:689–703. [PubMed: 26091166]
174. Villar EA, Beglov D, Chennamadhavuni S, Porco JA Jr, Kozakov D, Vajda S, Whitty A. How proteins bind macrocycles. Nat Chem Biol. 2014; 10:723–731. [PubMed: 25038790]
175. Driggers EM, Hale SP, Lee J, Terrett NK. The exploration of macrocycles for drug discovery–an underexploited structural class. Nat Rev Drug Discovery. 2008; 7:608–624. [PubMed: 18591981]
176. Smith MC, Gestwicki JE. Features of protein-protein interactions that translate into potent inhibitors: Topology, surface area and affinity. Expert Rev Mol Med. 2012; 14:e16. [PubMed: 22831787]
177. Thompson AD, Dugan A, Gestwicki JE, Mapp AK. Fine-tuning multiprotein complexes using small molecules. ACS Chem Biol. 2012; 7:1311–1320. [PubMed: 22725693]
178. Cesa LC, Mapp AK, Gestwicki JE. Direct and propagated effects of small molecules on protein-protein interaction networks. Front Bioeng Biotechnol. 2015; 3:119. [PubMed: 26380257]
179. Gabizon R, Friedler A. Allosteric modulation of protein oligomerization: An emerging approach to drug design. Front Chem. 2014; 2:9. [PubMed: 24790978]
180. Feng X, Pomplun S, Hausch F. Recent progress in FKBP ligand development. Curr Mol Pharmacol. 2015; 9:27–36. [PubMed: 25986570]

Biographies

Bryan M. Dunyak obtained his B.Sc. degree in Chemistry from Michigan State University in 2011, working with Dr. James Dye on the interactions and passivation of alkali metals absorbed in silica gels. He is currently a Ph.D. student in the Department of Biological Chemistry at the University of Michigan, in the laboratory of Dr. Jason Gestwicki. His thesis work is focused on peptidyl-prolyl isomerases, drug discovery, and computational modeling.

Jason E. Gestwicki earned a Ph.D at the University of Wisconsin—Madison and performed postdoctoral work at Stanford University. He started his independent career at the University of Michigan and is currently an Associate Professor in the Department of Pharmaceutical Chemistry at University of California, San Francisco. His group works on developing agonists and antagonists of molecular chaperones for use in the treatment and characterization of protein misfolding diseases.

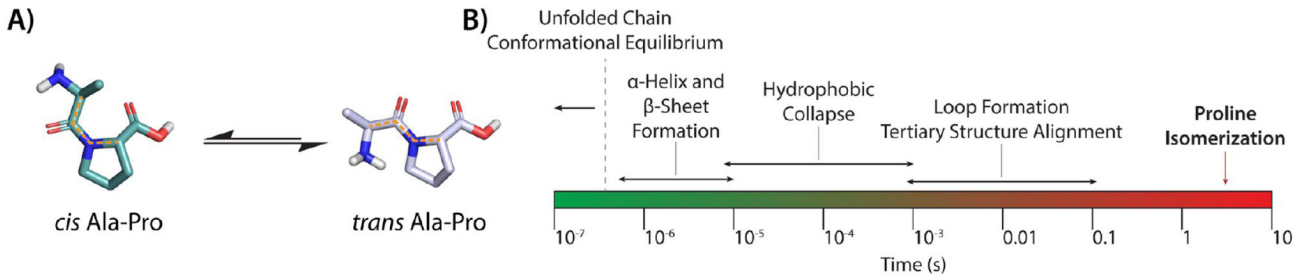


Figure 1.

Proline samples discrete cis and trans conformations, which isomerize on the time scale of milliseconds to seconds: (A) depiction of the proline conformations, with the backbone cis and trans orientations highlighted as an orange dotted line; (B) average time scales of processes important in protein folding, illustrating that uncatalyzed proline isomerization can often be a rate-limiting step.

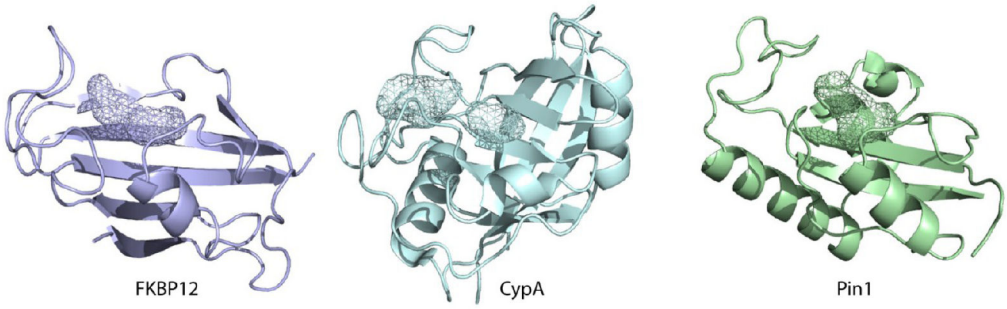


Figure 2.

Ribbon diagrams of the PPIase domains of FKBP12, CypA, and Pin1. The client binding pockets are depicted as a mesh cavity (produced in PyMol). PDB ascension codes are 1FKB, 1BCK, 1PIN, respectively. Note that the overall folds are similar, featuring a surface created by β sheets and a short helix.

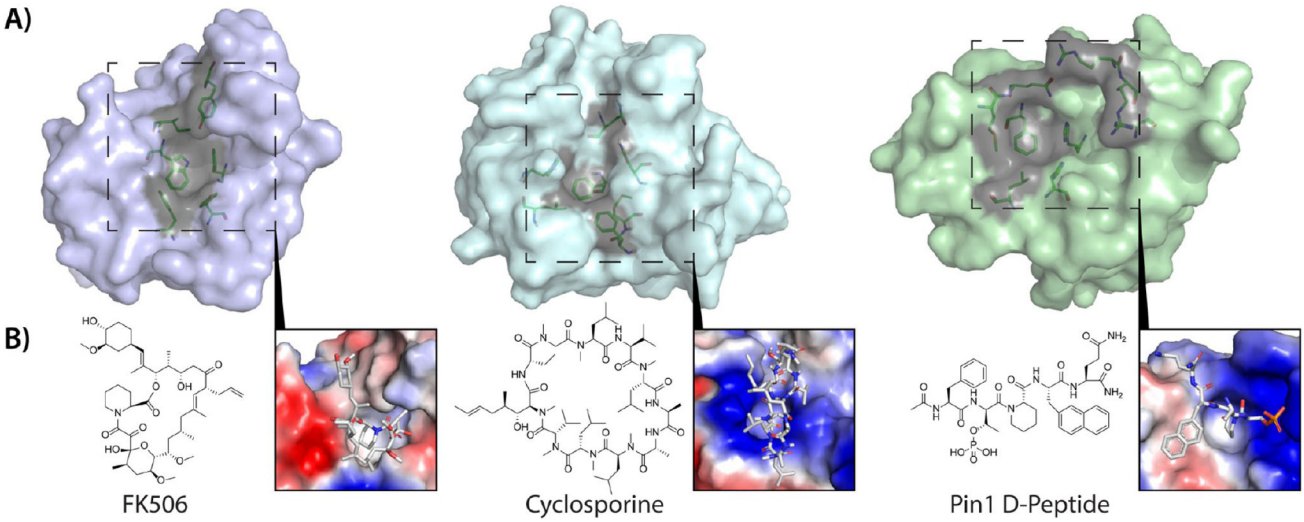


Figure 3.

PPIases have a shallow, broad active site. (A) Surface representation of the PPIase domains from FKBP12, CypA, and Pin1. The active site is shaded in gray, with critical residues shown. (B) High-affinity ligands for each PPIase are shown bound. Surface charges are as follows: blue is positive; red is negative.

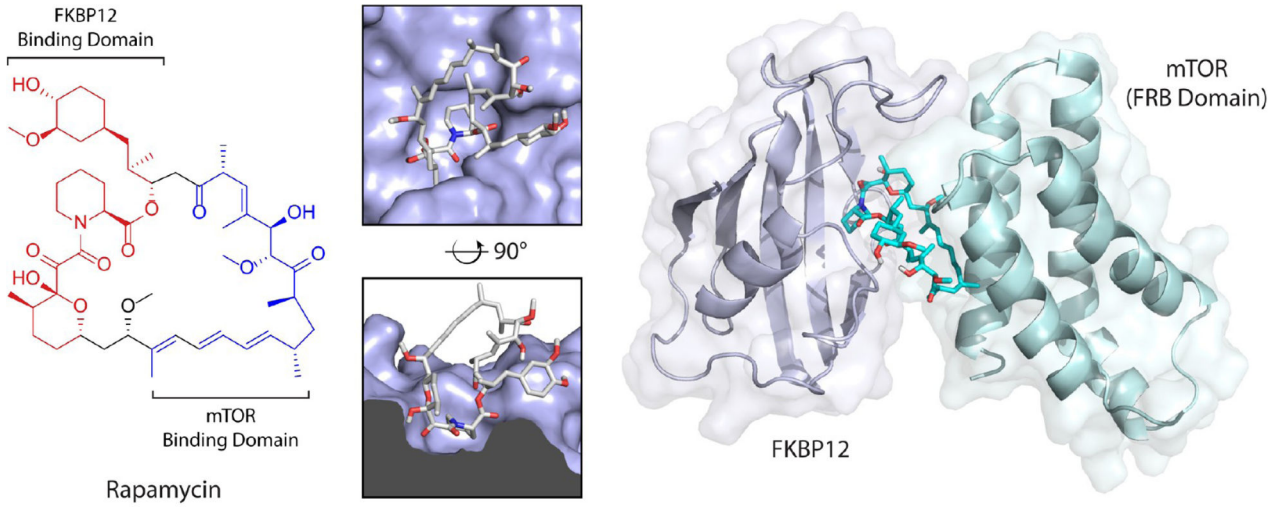


Figure 4.

Bifunctional binding mode of the natural product rapamycin. (A) Rapamycin forms a ternary complex with FKBP12 and the FRB domain of mTOR. The regions for binding FKBP (red) and mTOR (blue) are shown. (B) The pipecolyl α-ketoamide of rapamycin anchors it into the proline-binding pocket while leaving the triene exposed for interactions with mTOR. (C) Crystal structure of the FKBP12–rapamycin–mTOR ternary complex, showing the rapamycin-mediated organization of the proteins (PDB code 1FAP).

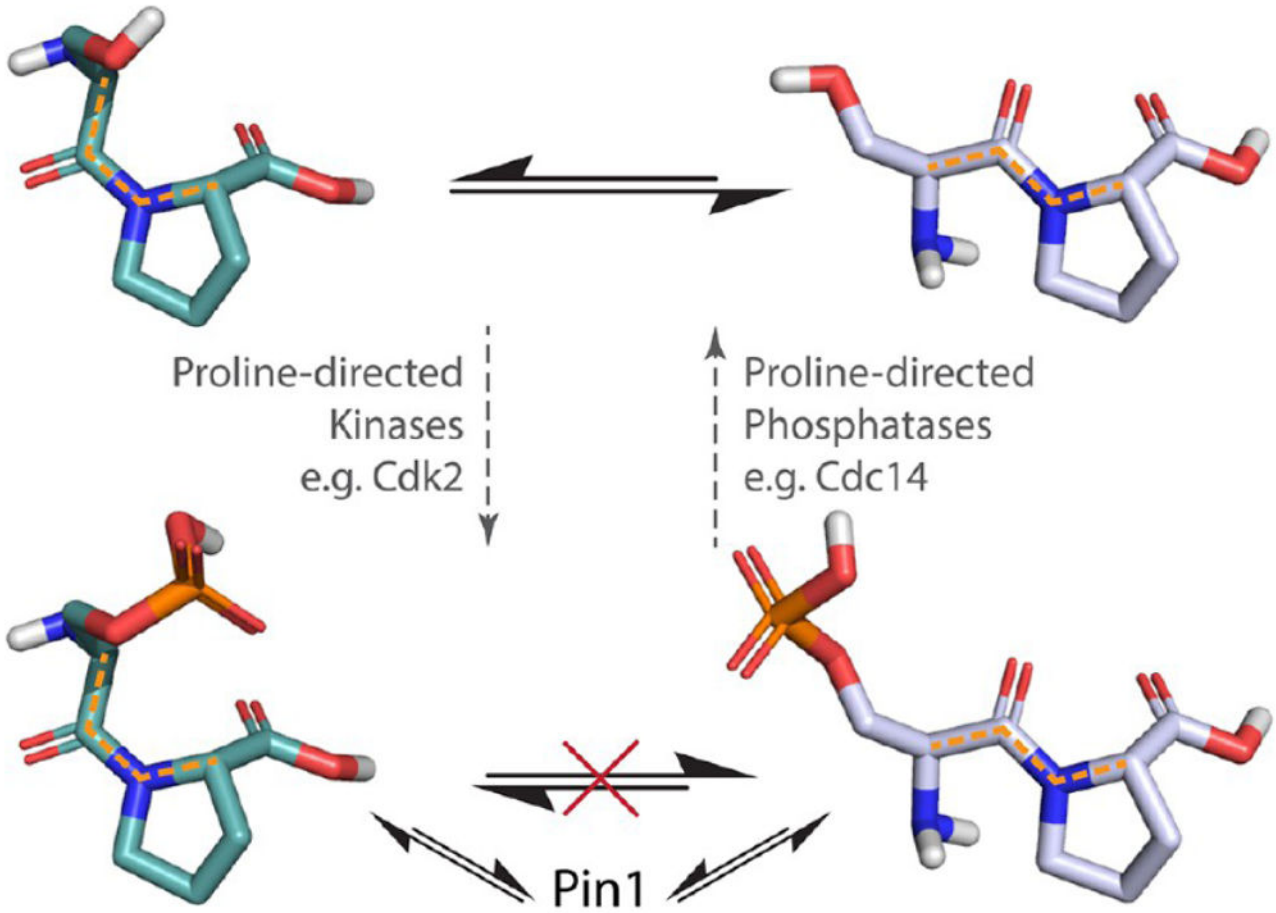


Figure 5.

Pin1 is required for conformational switching of phosphoserine-proline. Increased steric bulk and transient backbone interactions of the phosphate group dramatically slow the rate of intrinsic isomerization when compared to the unphosphorylated dipeptide. Pin1 selectively recognizes the negative charge adjacent to the proline and removes the isomerization bottleneck. The cis and trans conformations are illustrated as an orange dotted line.

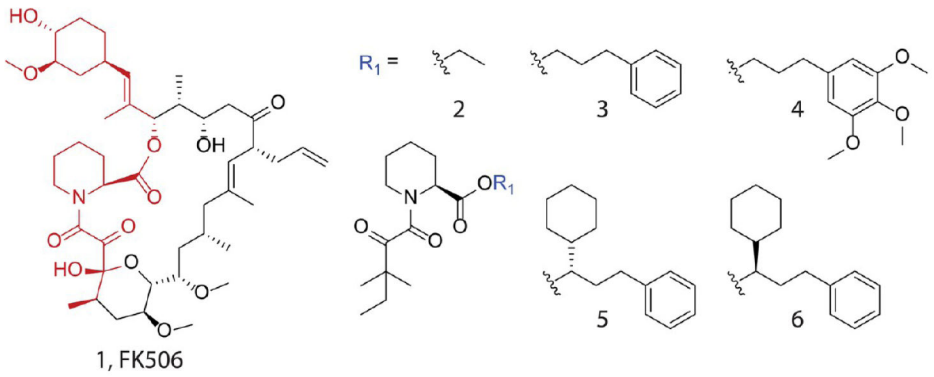


Figure 6.

Design of first high affinity, synthetic ligands for FKBP12. The FKBP12 binding motif of the natural product FK506, **1**, is highlighted in red.

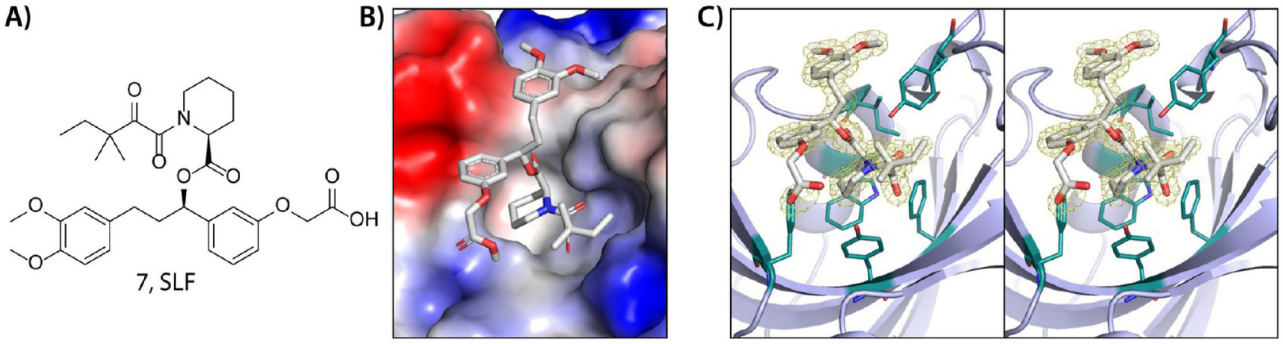


Figure 7.

Synthetic ligand for FKBP (**7**, SLF) binds with high affinity. (A) Chemical structure of SLF. (B) Binding of SLF to FKBP52 represented as an electrostatic surface (PDB code 4LAY). (C) Alternative stereoscopic representation of SLF binding to FKBP52 with the X-ray diffraction electron density map shown in yellow. Critical residues surrounding the proline-binding pocket are highlighted in green.

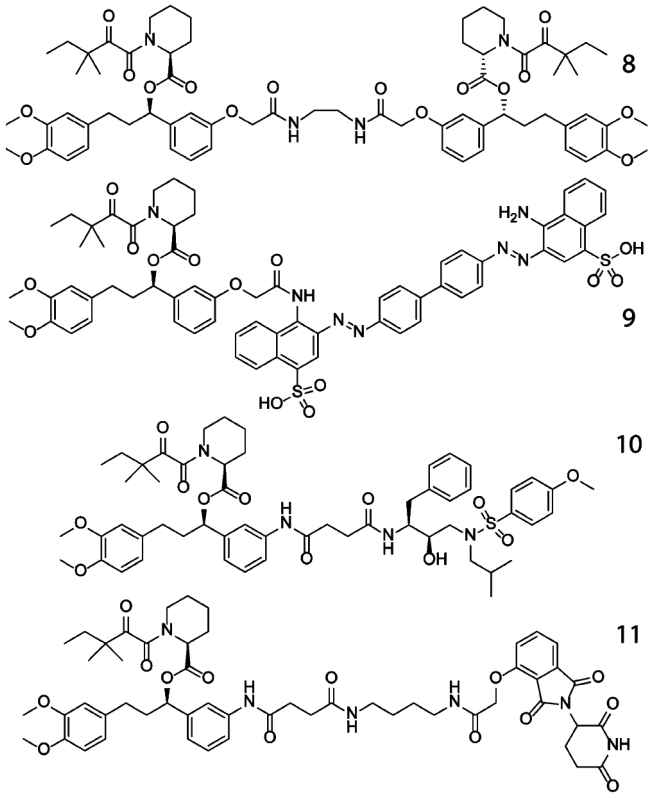


Figure 8.
Representative SLF-based chemical inducers of dimerization (CIDs).

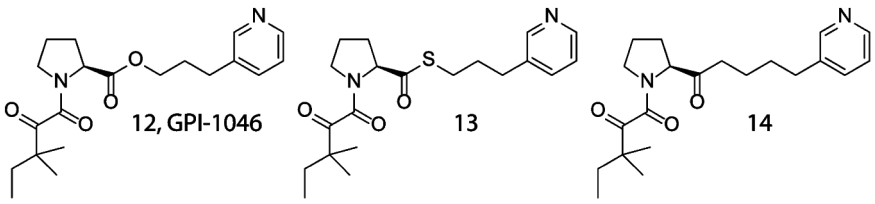
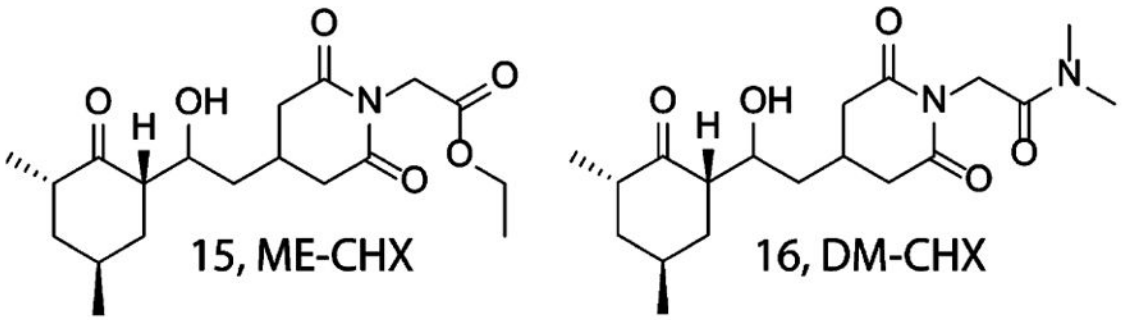


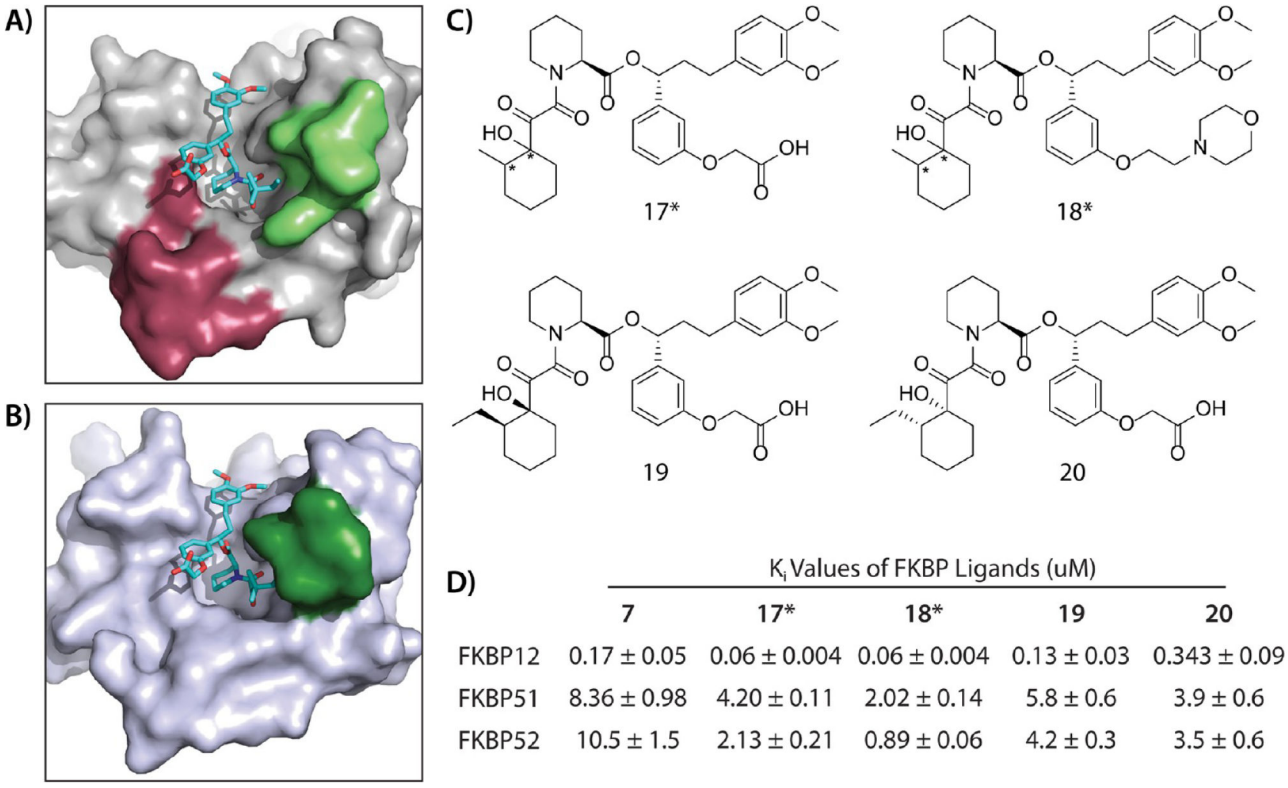
Figure 9.
Nonimmunosuppressive FKBP12 ligands.



K_i Values of FKBP Ligands (nM)

	12	15	16
FKBP12	306 ± 26	1180 ± 49	>10000
FKBP38	**48.0 ± 3.5**	**14.1 ± 0.8**	**85 ± 24**
FKBP51	447 ± 32	958 ± 70	>10000
FBKP52	936 ± 108	1690 ± 95.5	>10000

Figure 10.
Cyclohexamide derivatives potently inhibit FKBP38.



	7	17*	18*	19	20
FKBP12	0.17 ± 0.05	0.06 ± 0.004	0.06 ± 0.004	0.13 ± 0.03	0.343 ± 0.09
FKBP51	8.36 ± 0.98	4.20 ± 0.11	2.02 ± 0.14	5.8 ± 0.6	3.9 ± 0.6
FKBP52	10.5 ± 1.5	2.13 ± 0.21	0.89 ± 0.06	4.2 ± 0.3	3.5 ± 0.6

Figure 11.

Structure–activity relationship of FKBP ligands modified in the pyranose region. (A) Crystal structure of FKBP51 bound to SLF. The 40s loop (maroon) and 80s loop (pale green) are highlighted as regions that deviate from FKBP12. (B) Crystal structure of FKBP12 after alignment, with an overlay of SLF from (A). The 80s loop (dark green) is in closer proximity to the active site than in FKBP51/52. (C) Derivatives of the pyranose region to alter binding to the 80s loop and their (D) affinity to FKBPs.

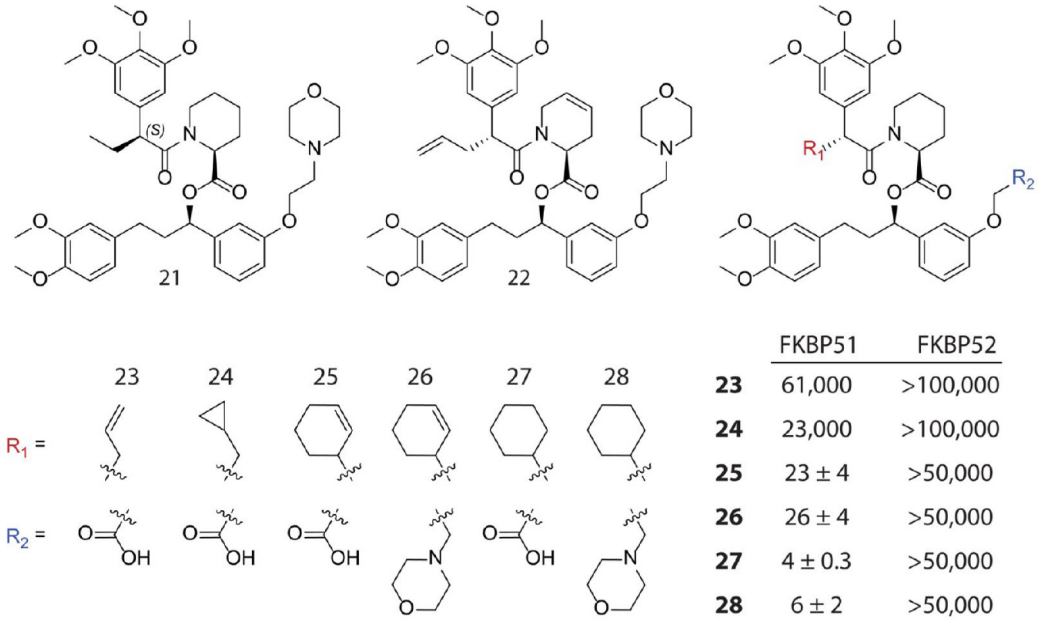
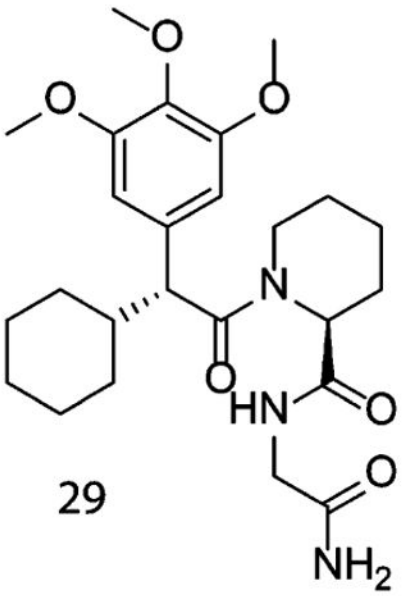
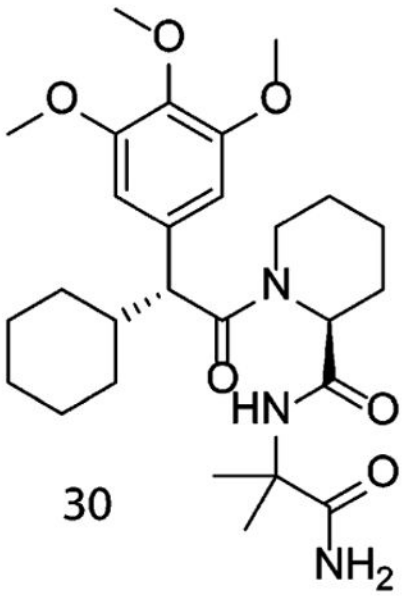
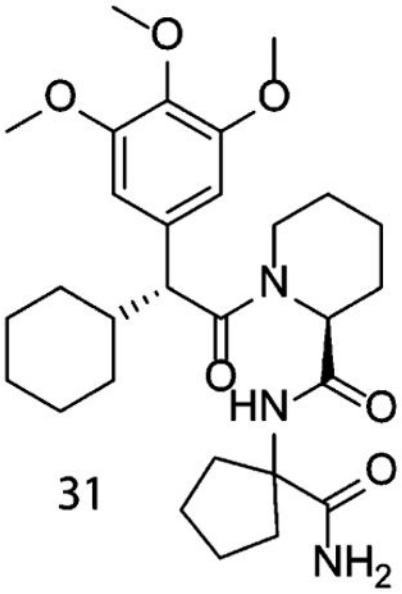


Figure 12.

Ligands designed to bind to the mutant FKBP51^{F67V} had reduced affinity for FKBP51^{WT}. It was found that FKBP51 could undergo induced fit upon binding, resulting in the discovery of high-affinity, selective inhibitors.







	FKBP12	FKBP51
29	>300	36.7 ± 3.4
30	25.1 ± 0.2	2.2 ± 0.2
31	3.8 ± 0.006	0.14 ± 0.02

Figure 13.

Optimization of specific FKBP51 inhibitors. Replacement of the ester moiety with geminally substituted amides retained affinity and selectivity for FKBP51.

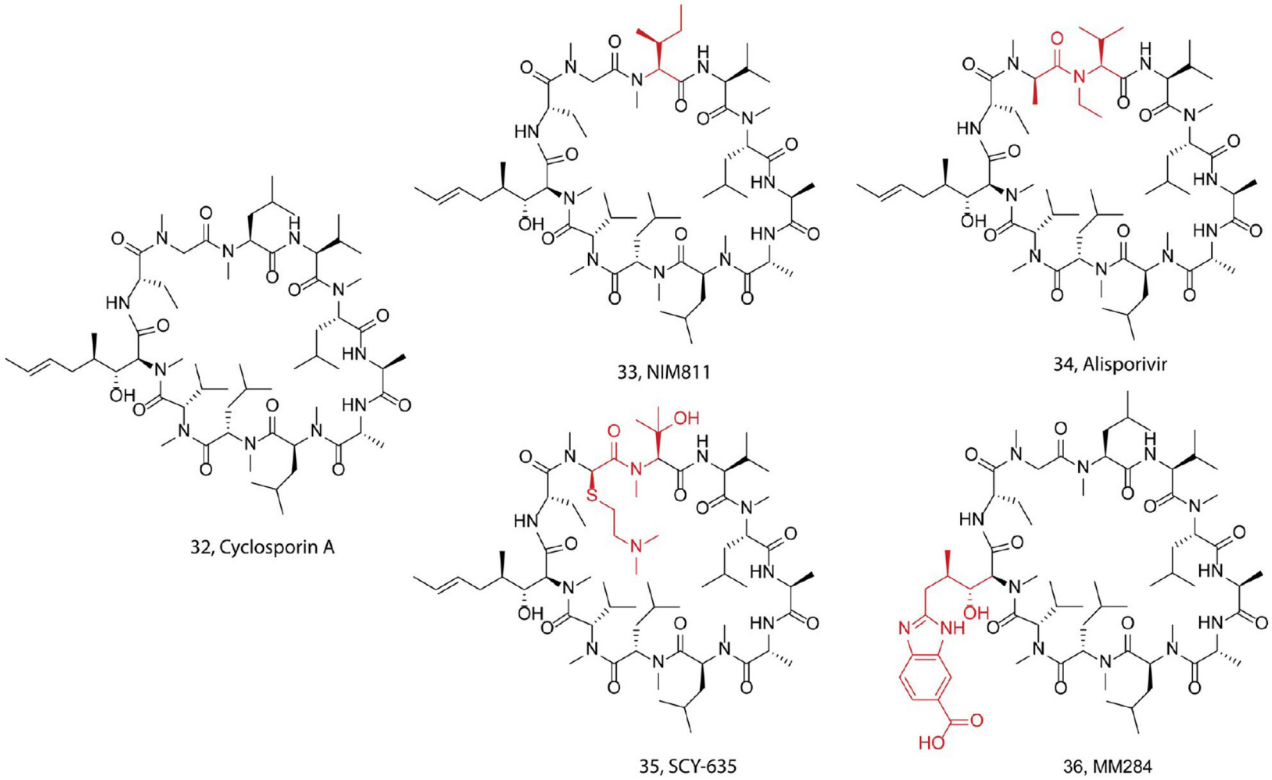


Figure 14.

Cyclosporin A (**32**, CsA) and representative derivatives. Modifications to the CsA scaffold were designed to prevent calcineurin binding by disrupting residues important for that interaction or block membrane permeability as in **36** (red).

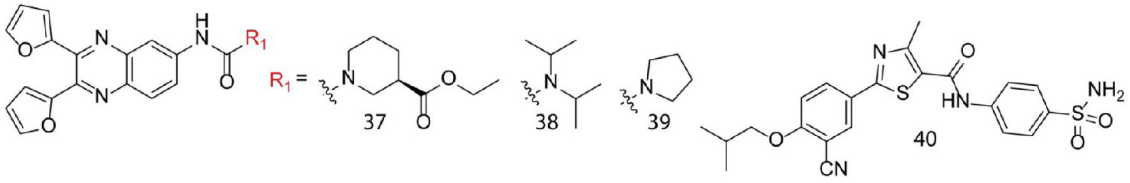
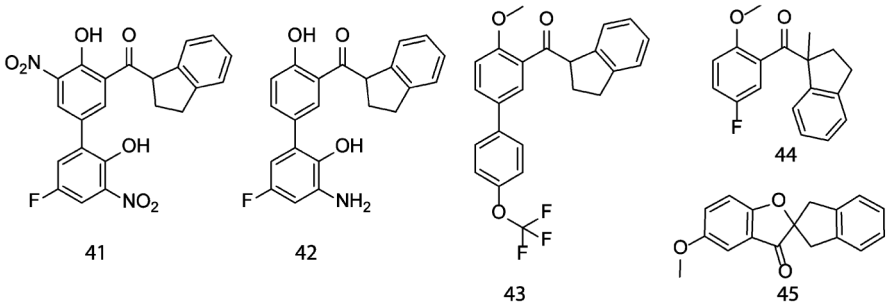


Figure 15.
Synthetic ligands designed to be selective for CypD.



	41	42
CypA	0.52 ± 0.15	0.3 ± 0.1
CypB	>100	12 ± 5
CypC	>100	>100
CypD	2.42 ± 0.76	6.28 ± 0.73
PPIL1	29 ± 7	> 100
Pin1	0.2 ± 0.1	0.4 ± 0.1

Figure 16.

Indanyl ketones designed for selective binding to cyclophilin paralogs. IC_{50} values in micromolar.

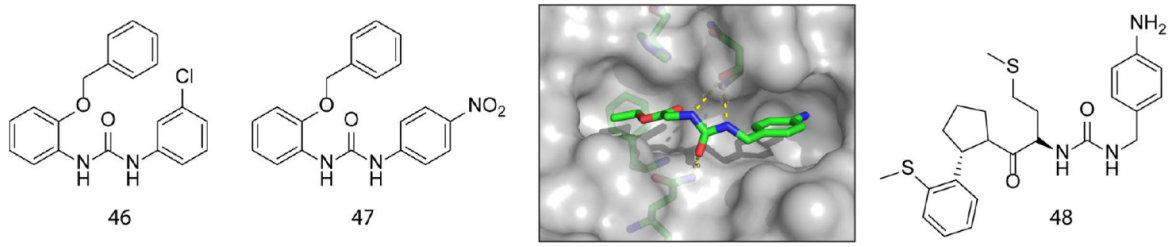


Figure 17.

Results of the computational design of CypA ligands based on a diarylurea pharmacophore. A crystal structure of this scaffold forms critical hydrogen bonds with Gln-105 and Asn-144 (dotted yellow line, PDB code 4XNC).

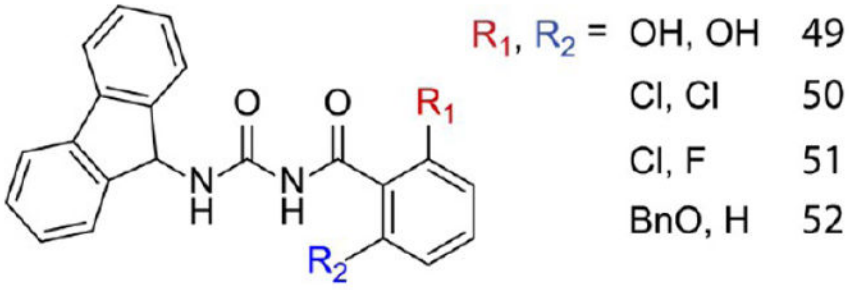


Figure 18.
Expanding the urea pharmacophore produced low nanomolar CypA inhibitors.

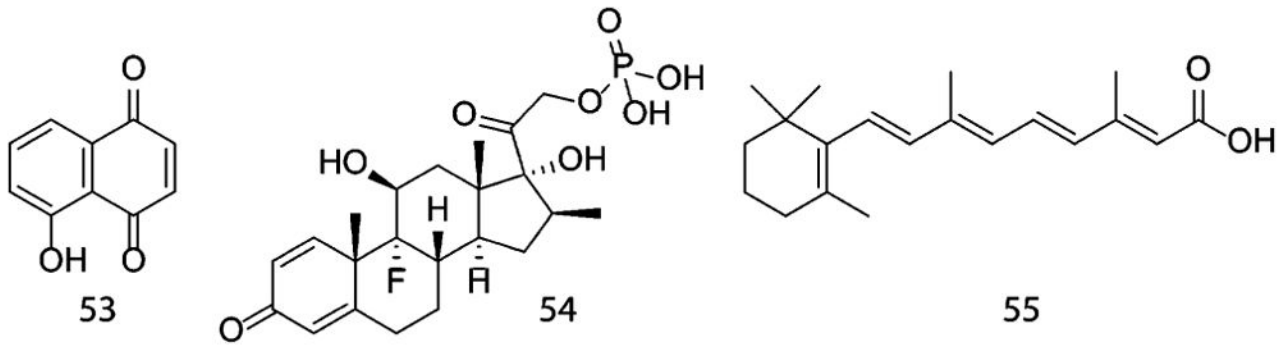


Figure 19.
Juglone is a natural product that covalently modifies Pin1.

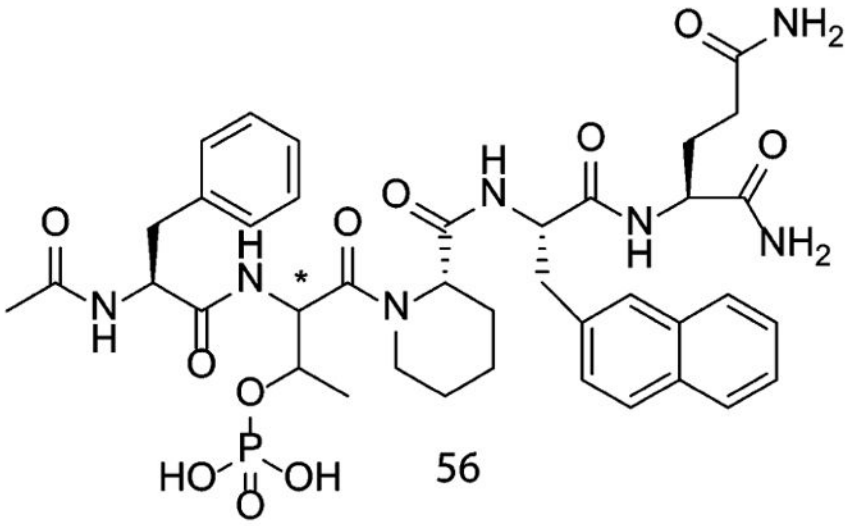


Figure 20.
Representative pipecolic acid-based phosphopeptide inhibitor of Pin1.

Peptide	K_D (uM)
cyclo(D-Ala-Sar-D-pThr-Pip-Nal-Tyr-Gln)	0.047 ± 0.0022
cyclo(D-Ala-Gly-D-pThr-Pip-Nal-Orn-Gln)	0.048 ± 0.004
cyclo(D-Ala-Ile-D-pSer-Pro-Nal-Orn-Gln)	0.92 ± 0.06
cyclo(D-Ala-Gln-Glu-Mpa-Nal-Tyr-Gln)	No Binding

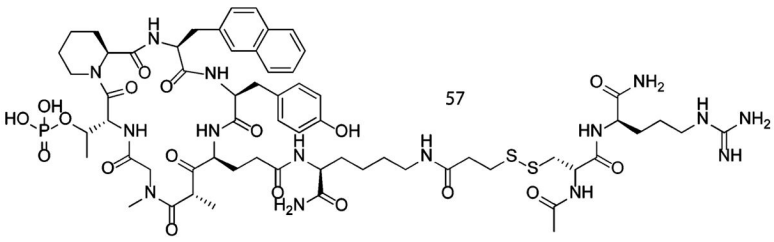


Figure 21.

Cyclic peptide Pin1 inhibitors were modified by the appendage of a membrane permeable octaarginine sequence using a disulfide linker. Reduction in the cytosol releases the free inhibitor.

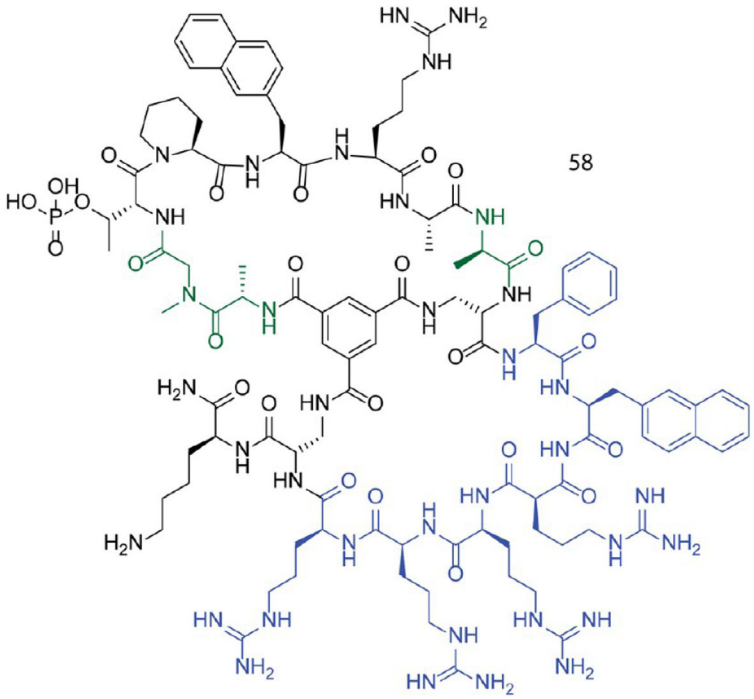


Figure 22.
Pin1 cyclic peptides were modified to yield cell-active bicyclic derivatives.

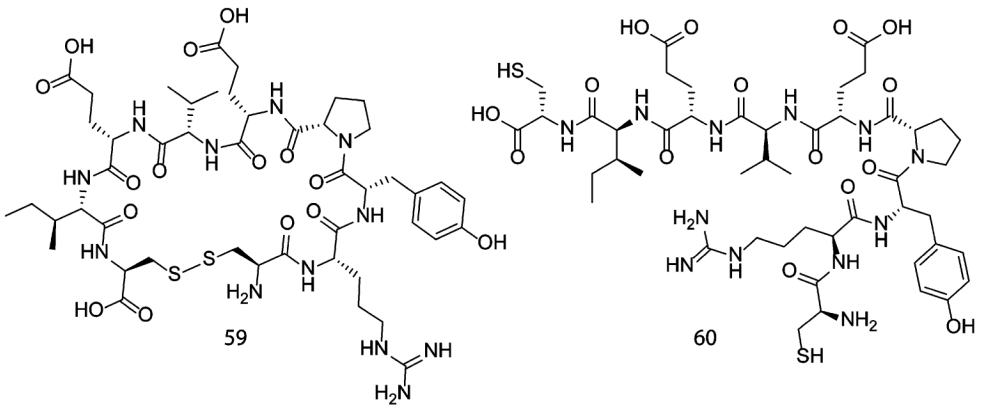
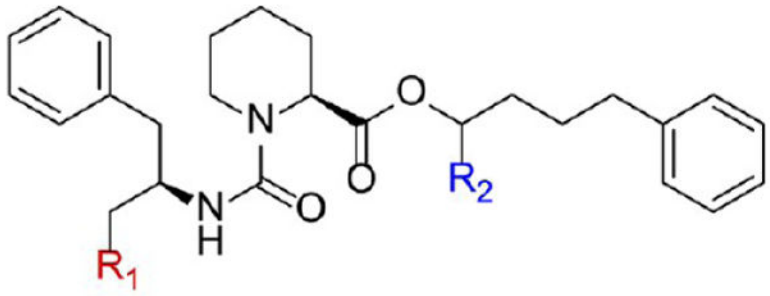


Figure 23.

High-affinity Pin1 inhibitors discovered using phage panning. Importantly, these molecules lack the electronegative groups that have hindered membrane permeability and cellular activity of other scaffolds.



R_1, R_2 =		
	$-PO_3H_2$, H	61
	$-SO_3H$, $-CH_2CH_2CH_2Ph$	62
	$-PO_3H_2$, $-CH_2CH_2CH_2Ph$	63

Figure 24.
Small molecule Pin1 inhibitors based on pipecolic acid. Note the resemblance to FKBP inhibitors, such as SLF.

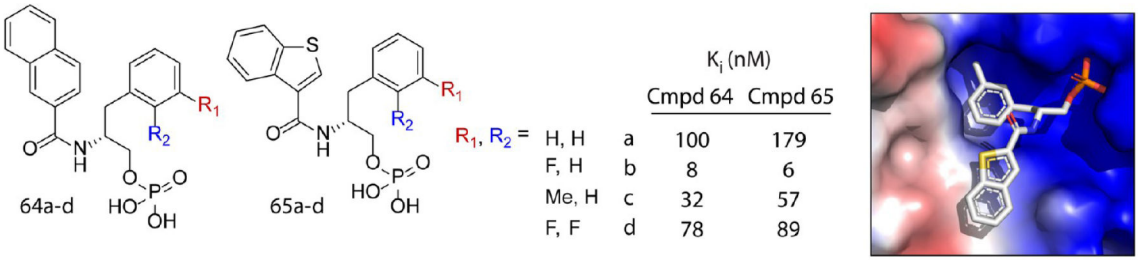
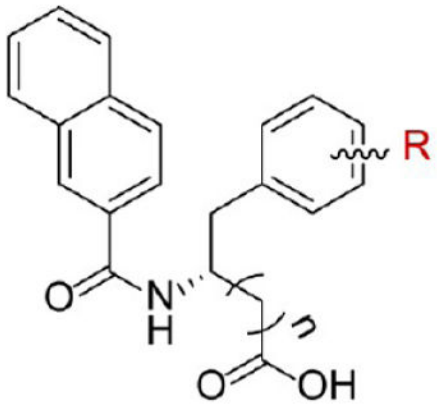


Figure 25.

Biarylamides potently bind Pin1. Cocrystallization with **65c** (PDB code 3IKG) revealed a new binding orientation with the phenyl ring oriented in the proline-binding pocket.



R	n	K_i (uM)
3-F	0	12
3-F	1	5.7
3-CF_3	0	1.8
3-CF_3	1	13
3-Cl	0	3.4
3-Cl	1	2

Figure 26.
Derivatives of **64a** were explored to investigate binding in the proline pocket.

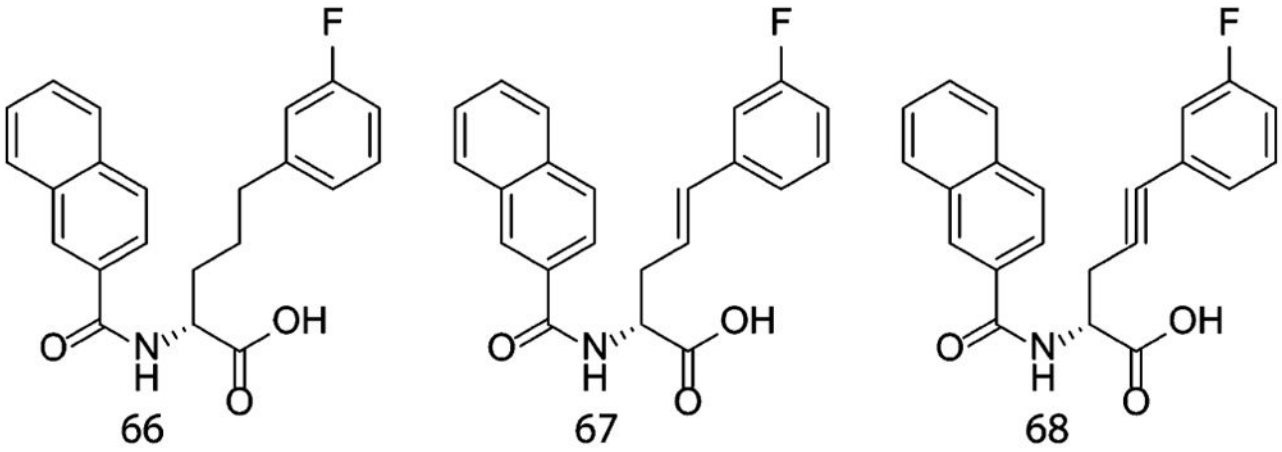


Figure 27.

Extensions of the phenyl ring were designed to increase rigidity and stabilize binding in the proline pocket.

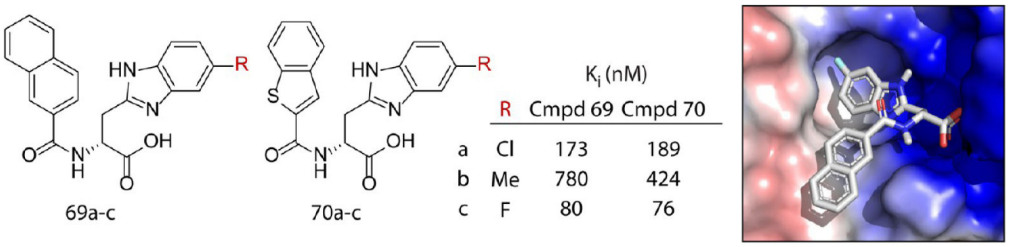
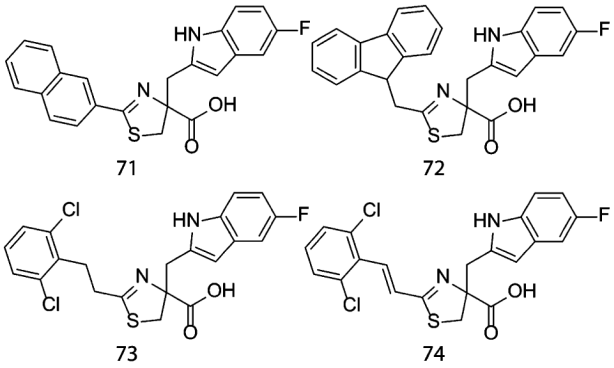


Figure 28.

A series of benzimidazoles mimic the linker length extension and potently inhibit Pin1. The most potent derivative, **63c**, binds to Pin1 in a similar orientation as **59c**. (PDB code 4TYO).



	K_i	IC_{50}
71	1.36	27
72	0.32	4.2
73	0.196	4.6
74	0.58	1.9

Figure 29.

Dihydrothiazole derivatives of the benzimidazole inhibitors were synthesized to avoid P-glycoprotein efflux. Compounds had high-affinity for Pin1 and prevented cell cycle progression in cells. All IC_{50} values are in micromolar.

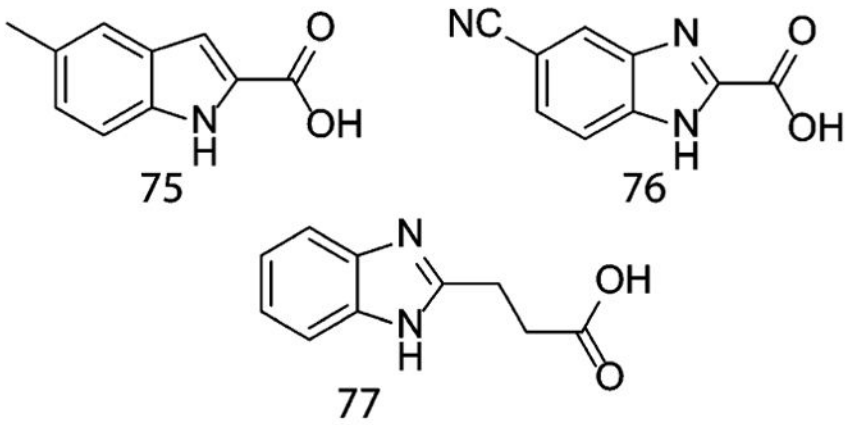


Figure 30.
Early fragment evolution of Pin1 inhibitors.

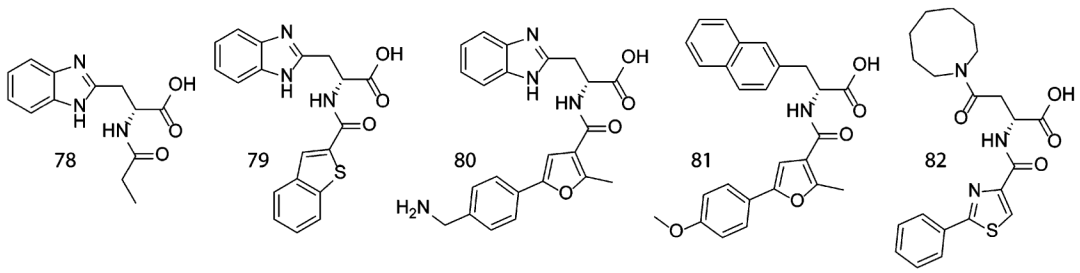


Figure 31.
Pin1 fragments designed to engage the hydrophobic shelf adjacent to the proline pocket.

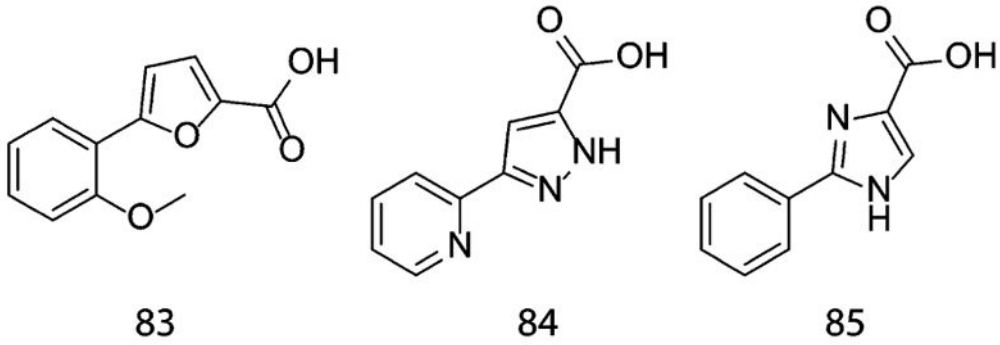


Figure 32.
Second-generation fragments for Pin1.

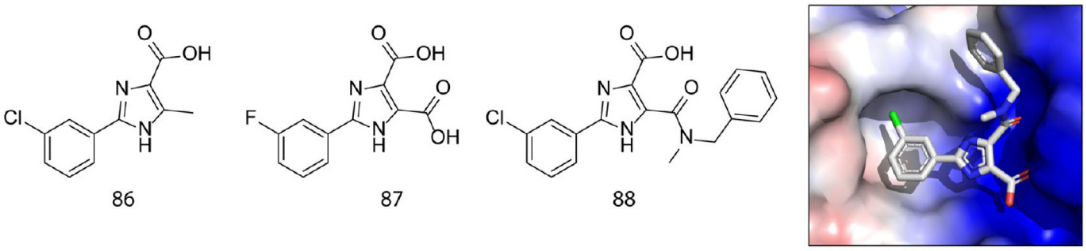


Figure 33.

Pin1 fragments expanded into the phosphate-binding pocket exhibit a new mode of binding that flanks Lys-63 and Arg-69 (PDB code 2XPB).