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Cyclophilin inhibitors as antiviral agents

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A B S T R A C T

Cyclophilins (Cyps) are ubiquitous proteins that effect the cis–trans isomerization of Pro amide bonds, and are thus crucial to protein folding. CypA is the most prevalent of the ~19 human Cyps, and plays a crucial role in viral infectivity, most notably for HIV-1 and HCV. Cyclophilins have been shown to play key roles in effective replication of a number of viruses from different families. A drug template for CypA inhibition is cyclosporine A (CsA), a cyclic undecapeptide that simultaneously binds to both CypA and the Ca2+-dependent phosphatase calcineurin (CN), and can attenuate immune responses. Synthetic modifications of the CsA scaffold allows for selective binding to CypA and CN separately, thus providing access to novel, non-immunosuppressive antiviral agents.

Keywords:
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Since the initial development of cyclosporin A (CsA) as an immunosuppressing agent for preventing allograft rejection,1 cyclophilin (Cyp) inhibitors have been explored extensively over the last four decades for treating a wide variety of medical conditions, including autoimmune diseases,² myocardial infarction ischemia reperfusion,³ and viral diseases,⁴ the latter of which will be the focus of this review. An overview of Cyp structure and function will first be presented, followed by evidence implicating the role of Cyps in viral diseases, notably HIV-1 and HCV. This will be followed by a discussion of CsA-based Cyp inhibitors, including in vivo and in vitro structure–activity relationships, leading up to a discussion of Cyp inhibitors currently in development for the treatment of hepatitis C virus.

Cyclophilins: The cyclophilin (Cyp) class of proteins shares a common enzymatic activity, that of a peptidyl–prolyl isomerase (PPIase), which effects a cis–trans isomerization of amide bonds N-terminal to proline (Pro) residues, and thus play a regulatory role in protein folding.⁵ At least 20 members of the Cyp class have been identified in humans,⁶ with sizes ranging from small, single domain Cyps (CypA, CypB, CypC, CypD, CypJ, PPII1, PPIA4L, and USA-Cyp) to large multi-domain proteins in which a catalytic PPIase activity is linked to various additional activities (Fig. 1).⁷ CypA is the most abundantly expressed member of the class, representing the majority of the PPIase activity in a cell, with CypB being the only other Cyp found above trace level. In addition to the Cyps, two other families of proteins, FKBPks and parvulins (Fig. 1), have been described with similar PPIase activity.⁸ Inhibition of the PPIase activity of FKBPks by either FK506 or rapamycin was shown to be required for the immunosuppressive activity of these compounds.⁹ The parvulins are responsible for the cis–trans isomerization of PO3H2Ser (PO3H2Thr)-Pro bonds; however, they are much less well studied.¹⁰ One key member of the parvulin class, Pin1, interacts with phosphoproteins involved in the cell cycle of some cancer cells;¹² however, the lack of potent and selective inhibitors for parvulins has limited research in this area.¹³,¹⁴,¹⁵

Cyps show widely differing expression profiles, with CypA being found predominantly in the cytoplasm, while more restricted localization is observed for CypB (endoplasmic reticulum), CypD (mitochondrial inner membrane), CypE (nucleus), and RanBP2 (nuclear pore).⁶ Studies directed at the functional characterization of CypA, CypB, CypD and Cyp40 have been reported over the last 20 years,¹⁰ however, native roles for the majority of the class, and potential roles in disease states, have not yet been elucidated.

Several mechanisms by which Cyps catalyze the cis–trans isomerization of an Xaa-Pro bond have been proposed; however, the currently accepted mode of catalysis is based on distortion of the planar amide bond (Fig. 2).¹² Binding of a Pro-containing substrate in a manner that twists the amide bond leads to a lower barrier to rotation due to loss of amide resonance.¹³ An important hydrogen bond between an active site residue (side chain of Arg55 in CypA) and the prolyl amide nitrogen facilitates the formation of a pyramidal nitrogen, which is a key feature of the isomerization.¹⁴ Notably, mutation of Arg55 results in a ‘catalytically inactive’ form of CypA, which has been used extensively as a mechanistic probe.¹⁵ Crystal structure analysis of several Pro-containing substrate peptides bound to human CypA revealed that all ligands evaluated bound with the Xaa-Pro bond in the cis conformation and with no
distortion from planarity. Since most Xaa-Pro amide bonds reside preferably (5- to 6-fold preference) in the trans conformation, Ke proposed that the principle role of Cyps is to facilitate a trans to cis isomerization. While the PPIase activity of Cyps has been well studied, several additional roles for Cyps have also been described. Binding of either CypA or CypB to CD-147/EMMPRIN, an extracellular receptor for CypA, leads to activation of MAP kinase pathways and induction of matrix metalloproteinase (MMP) production. A role for CypA as an inflammatory chemotactic cytokine, acting through the CD-147/EMMPRIN receptor, was revealed using a murine model of asthma in which blocking the CypA-CD-147 interaction with anti-CD-147, or CsA, resulted in a significant decrease in neutrophil and eosinophil migration, and improved lung pathology. Further roles for extracellular Cyps in renal fibrosis and ischemia-reperfusion injury have been described with CD-147/EMMPRIN being a key activating receptor.

The discovery that CypA is a requisite binding protein for the immunosuppressive activity of CsA served to place CypA firmly within the sphere of immunology research. The demonstration that this binary CypA:CsA complex inhibits the calcium-dependent phosphatase activity of calcineurin (CN), leading to inhibition of key activators of T-cells, provides a compelling explanation of the immunosuppressive activity of CsA. The discovery of new CsA analogs that retained potent binding to CypA but prevented ternary complex formation with CN allowed the biology of Cyps to be probed independent of immunosuppressive activities. Furthermore, the use of knockdown and gene-silencing techniques have revealed a rich and expanding biology associated with Cyps, with virology emerging as an important focus of research.

Human immunodeficiency virus-1 (HIV-1), the parasitic virus causing AIDS, depends heavily on host cellular machinery for its survival, replication, and infectivity. CypA is the first cellular protein ever found to be incorporated into HIV-1 virions. Subsequently, a significant number of publications have suggested several roles of CypA in the HIV life cycle, all of which are independent of CN inhibition. Initial reports of CsA inhibiting HIV-1 surfaced in 1988, although the first report specifically implicating CypA involvement in the HIV-1 life cycle was published five years later when it was reported that endogenous CypA colocalizes with the HIV-1 Gag protein in the cytoplasm, binding specifically to a Pro-rich sequence in the HIV-1 capsid domain of Gag. X-ray crystallography later revealed that residues 85–93 of HIV-1 capsid bind to the active site of CypA, and subsequent studies have shown that virions produced in the presence of Gag mutated in the CypA binding region, or under pressure from competitive CypA inhibitors, were less infectious than wild type virions.

CypA is then incorporated into nascent HIV-1 virions during the assembly and budding process. As these HIV-1 virions now infect new T cells, CypA from the virion delivered to the new cell...
facilitates uncoating of the capsid and supports sufficient reverse transcription of the viral genome. More recent studies, however, have suggested that CypA in the infected target cell, and not CypA from HIV-1 virions, is more important to HIV-1 infectivity. It has been further shown that CypA in the target cell increases HIV-1 infectivity by inhibiting host cell restriction factors mounted as part of an innate immune response that ordinarily combats invading retroviruses. Reducing the CypA-capsid interaction either by mutations altering binding of CypA to capsid, or by the introduction of a CypA inhibitor such as CsA, has been shown to decrease HIV-1 susceptibility to TRIM5α restriction. Other recent studies have suggested that CypA binds to HIV-1 viral protein R (Vpr), which undergoes cis–trans isomerization of Pro residues in its N-terminal region. This activation by CypA ultimately governs the functional expression of Vpr, which is needed for translocation of virus to the nucleus, and induction of cell cycle arrest and apoptosis in infected T cells.

In summary, there are several stages in the HIV-1 life cycle that involve CypA, some understood more clearly than others. Clearly, a CypA inhibitor that bound to CypA alone and not CN would potentially be an effective anti-HIV therapeutic. Such compounds have been developed, and will be discussed in Section C.

Hepatitis C virus: The first report of HCV inhibition by CsA emerged in 1988, even predating HCV being named as such. Clinical studies in HCV infected patients undergoing liver transplantation showed a superior virologic response to CsA in combination with interferon α2b versus interferon α2b alone, implicating Cyp inhibition as an approach to HCV therapy. It was not until 2003 that CsA was found to inhibit HCV in a cell-culture-based replicon system, however, the immunosuppressive activity of CsA still obscured the role of Cyp in HCV replication.

Initial studies identified CypB and/or CypC as being crucial for HCV replication; however, subsequent reports have indicated that CypA alone is crucial for replication of HCV genotypes 1a, 1b, and 2a. To complicate matters, a recent study suggests HCV replication occurs at different stages by several Cyps, including CypA (regulation of transcription and translation), CypB and CypC (protein conformation and transport), CypE and CypH (regulation of mRNA splicing, generation of host proteins necessary for HCV), Cyp40 (regulation of translation, non-vascular transport of cholesterol, co-chaperone of Hsp90). Further support for CypA being the primary Cyp involved in HCV RNA replication include a demonstration that the catalytic PPIase activity of CypA is required for efficient HCV RNA replication, and the finding that a specific binding between CypA and NS5A is dissociable by addition of CsA-based Cyp inhibitors. While a recent consensus has emerged that CypA is likely the principal and perhaps exclusive Cyp involved in the HCV replication complex, roles for other Cyp in different aspects of HCV propagation should be considered.

Evidence to support the binding of CypA to several HCV proteins, including NS5A, NS5B, and NS2, has been published, which is consistent with the finding that in vitro resistance to Cyp inhibitors, while slow to emerge, is associated with mutations in both NS5A and NS5B. The demonstration that knockdown of CypA inhibited replication of a full length HCV genome significantly more effectively than a sub-genomic replicon system (NS3–NS5B) supports the idea that CypA is involved in interactions with HCV proteins other than NS5A and NS5B.

Details of the specific role of CypA in the HCV replication complex have recently appeared; however, a consensus mechanism has not emerged. While it is clear that CypA is an essential component of the HCV replication complex, different models have been proposed to explain this requirement, including a role in actively incorporating NS5B into the replication complex versus a model whereby CypA, pre-residing in crude replication complex membrane fractions, serves to associate NS5A–NS5B into an assembling replication complex. Clues toward an understanding of the role that CypA PPIase activity might play in HCV RNA replication were presented by Bartenschlager in a study of the rate of HCV polyprotein processing. A resistance mutation to the Cyp inhibitor Alisporivir, occurring near the cleavage site of the NS5A–NS5B polyprotein, resulted in delayed processing of the protein and impaired replication activity. This suggests that CypA may be required to allow the NS5A–NS5B protein to achieve the required conformation to allow effective cleavage. The virus may escape the need for CypA at a specific location by mutation of residues close to the cleavage site that introduce more conformational flexibility, however this comes at a cost to the virus of impaired replication capacity. The high barrier to resistance to a Cyp inhibitor may result from the need for multiple virus mutations to appear to escape the need for all CypA mediated reorganizations, with each mutation incurring a replication ‘fitness’ cost.

Most recently, Giese and co-workers explored genetic variants of CypA via nonsynonymous single nucleotide polymorphisms (SNPs), and how this impacted HCV replication. These studies showed that several SNPs present in human populations that cause depletion of intracellular CypA also reduce HCV infectivity.

In summary, there is ample evidence that the HCV life cycle is dependent on host cell Cyp for perhaps multiple processes, as was the case with HIV-1. A Cyp inhibitor, and in particular a CypA inhibitor that is non-immunosuppressive, would be an effective anti-HCV therapy. Such compounds have been developed, and will be discussed later in this review.
double knockdown of CypA and CypB, and the PPIase activity of CypA was shown to be essential for effective replication of Dengue and WNV. Tang further showed that CypA directly interacts with the NS5 protein of WNV; however, this interaction did not affect the enzymatic activity of NS5 in vitro.41 Consistent with these findings, CsA was shown to block the interaction of CypA with NS5 of WNV, suppress viral RNA synthesis, and inhibit a broad spectrum of RNA viruses at non-toxic concentrations.

A role for CypB in the replication of Japanese Encephalitis Virus (JEV) was demonstrated by the rescue of JEV replication by expression of wild type CypB, but not PPIase-deficient CypB, in various mammalian cells in which endogenous CypB was suppressed.42 This finding was again supported by the finding that CsA potently inhibits the replication of JEV in vitro.42

Non-flaviviruses: While Cyp inhibition has been demonstrated in restricting flavivirus replication, the role of Cyps in other viruses is less clear. A convincing argument for Cyp involvement in the replication of Vaccinia Virus (VV) was provided by the demonstration that CsA analogs with strong binding affinity for CypA were potent inhibitors of VV replication, while analogs with weak affinity for CypA lost antiviral activity.43 Subsequent studies revealed an incorporation of CypA into VV virions as an essential event for successful maturation.44 A similar incorporation of CypA into viral particles has been reported for Vesicular Stomatitis virus45 and SARS coronavirus,46 however, in each case additional roles for CypA have been proposed.

A novel function of a Cyp in viral entry was described for human papillomavirus type 16 involving a cell-surface fraction of CypB which, following virus attachment, serves to effect a conformational change in capsid proteins L1 and L2, leading to efficient infection.47 Incorporation of CypB into virions of Measles Virus (MV) has been shown to be an important requirement for expanding the tropism of the virus to include epithelial and neuronal cells.48 Neutralizing antibodies directed at CD-147/EMMPRIN (receptor for CypA and CypB) served to limit infection of epithelial cells by MV.48 Inhibition of MV infection was also demonstrated by treatment with CsA, and has led to the proposal that MV incorporates CypB, but not CypA, into mature virions, and that this CypB serves to allow effective infection of CD-147/EMMPRIN expressing epithelial cells.48

A role for Cyps in the replication of herpes simplex virus has been suggested by the finding that CsA inhibits virus production in vitro;49 however, the immunosuppressive properties of CsA need to be taken into consideration when assessing such results. While a supportive function of Cyps in the replication of numerous viruses has been identified, some evidence of Cyps being restrictive toward viral replication has been reported. CypA was shown to bind to the M1 protein of influenza A and to restrict viral replication has been reported. CypA resulted in a decrease in interferon-β production and an increase in viral replication, with both effects being reported to be independent of the PPIase activity of CypA.46

A role for Cyps in signaling pathways that are activated following viral infection was first suggested by the demonstration that CypB is involved in regulation of IRF-3.50 Knockdown of CypB was found to suppress Newcastle Disease Virus-induced phosphorylation of IRF-3, which blocked IRF-3 dimerization and inhibited the production of interferon-β. A recent report from Hopkins51 describes a novel induction of type I and III interferons in HCV-infected patients following treatment with the non-immunosuppressive Cyp inhibitor SCY-635. These results point to a possible role for Cyps in virus-induced blocks on innate immune responses that would otherwise serve to clear the infection and place Cyp inhibition as a potentially important immunotherapy approach to virology.

Factors affecting cyclophilin and calcineurin binding: The Cyp binding domain of CsA comprises of residues 9, 10, 11, 1 and 2; hence, altering these residues tends to have a significant effect on Cyp binding. Modifications to residues in the CN binding domain (4, 5, 6, and 7), on the other hand, influence Cyp binding indirectly through effects on conformation, but often exert a more profound affect on CN binding. The (4R)-4-[(E)-2-butenyl]-4-methyl-α-threonine (MeBmt) group at position 1 is unique in that it resides in the Cyp binding domain, yet its sidechain drapes across the CsA scaffold and ultimately into the CN binding domain (Fig. 4). In general, modifications to MeBmt tend to decrease Cyp binding. Removing or altering the stereochemistry of the 2-methyl group reduces activity, as does introducing a second methyl group at this position.44 Likewise, removing, replacing, or capping the 3'-hydroxy as an ester also reduces potency.45 One case in which Cyp binding is actually improved is when the C=C double bond is replaced with a bioisosteric sulfur atom (‘MeThiaBmt’), which was found to bind to Cyp with 178% of the affinity of CsA itself.46

The [Sar]1 position lies on the periphery on the Cyp binding domain, and consequently structural changes at [Sar]1 can directly, or indirectly through conformational bias, impact Cyp binding. Substitution at the 2'-, or α-carbon of [Sar]1 is extremely well-tolerated, provided that it resides on the β-face to give the Re, or α-configuration; substitution on the α-face that gives the Si, or γ-configuration, results in steric clashing between this substitutent and the N-methyl group of [MeLeu]4, and hence an altered conformation of the CsA scaffold that does not bind Cyp as well.52 Both [α-MeAla]3CsA and [α-MePhe]3CsA bind to Cyp with affinity comparable to that of CsA,53 while [α-MeSer]3CsA binds to Cyp with over threefold greater affinity than CsA.52 This is an important attribute that has been exploited in the design of CsA-based therapeutics.

An essential feature of Cyp inhibitors designed to be used as antiviral agents is that the immunosuppressive activity due to CN inhibition is reduced or removed. This has been most effectively achieved by modification of the side chain of the [MeLeu]4 residue, which is involved in a tight ‘aromatic sandwich’ with CN residues Trp352 and Phe356 (Fig. 4). Surprisingly, moving one methyl group at the [MeLeu]4 residue to give [Melle]4CsA (NIM-811), was found to remove immunosuppressive activity, while retaining the
same affinity for CypA as CsA itself. This ability to reduce immunosuppressive activity by making small chemical changes at [MeLeu][MeVal]59 and [4’-HOMeLeu]4CsA59,60 which show >2500-fold and >100-fold loss of immunosuppressive activity, respectively.

A summary of both CypA and CN binding SAR of CsA derivatives is shown in Figure 5.

Antiviral structure–activity relationships—HIV, HCV: Despite the large volume of work describing the effects of CsA modification on immunosuppressive activity, antiviral activity for relatively close.

| Compound | Anti-HIV-1 EC50 (nM) | Ref. |
|----------|----------------------|-----|
| CsA (1)  | 450                  |     |
| [8’-HOMeBmt][CsA (2)  | 460                  | 61a |
| [Sar-o-SCH2CH2NEt2][4’-HOMeLeu]4CsA (SCY-635, 4) | 2.5     | 61a |
| [Sar-o-SCH2CH2NMes][4’-HOMeLeu]4CsA (NIP843, 5) | 45  | 64 |
| [Sar-o-OMe][4-HOMeLeu]4CsA (6) | 94.9 | 65 |
| [o-Ala][EtVal]4CsA (Alisporivir, 7) | 60 | 66 |
| [MeVal]4CsA (8) | 54 | 61a |
| [MeLeu]4CsA (NIM811, 9) | 54 | 61a |
| [4’-HOMeLeu]4CsA (10) | 279 | 61a |
| [4’-HOMeLeu]4CsA (11) | 255 | 61a |
few of these compounds have been published. Representative published examples are shown in Tables 1 and 2.

The [MeBmt] group is perhaps the most synthetically approachable position for modification. Hydroxylation of the terminal methyl group to give [8'-HOMeBmt]CsA (2) shows comparable anti-HIV activity as CsA (1).\(^6\) and a MeBmt-reduced derivative, [dihydroMeBmt][4'-HOMeLeu] CsA (not shown in the table), shows ~fourfold greater anti-HIV activity than CsA.\(^6\)

Interestingly, capping the MeBmt 3'-OH as an acetate does not appear to impede anti-HIV activity, as both [3'-AcOMeBmt][Sar-OAc]CsA and [SarOAc]CsA have anti-HIV IC\(_{50}\)S of 115 and 119 nM, respectively (not shown in table).\(^6\) In view of the well-described loss of CypA binding potency as a result of esterification of the MeBmt-hydroxyl, this points to the importance of an appropriate substituent at [Sar] in antiviral activity. No HCV data is yet available for these derivatives.

Table 2

| Compound |
|----------|
| CsA (1)  |
| [Sar-o-SCH\(_2\)CH\(_2\)NMe\(_2\)][4'-HOMeLeu]CsA (SCY-635, 4) |
| [Sar-o-SMe][4'-HOMeLeu]CsA (12) |
| [Sar-o-SCH\(_2\)CH\(_2\)-N-morpholino][4'-HOMeLeu]CsA (13) |
| [Sar-o-C\(_2\)H\(_4\)S\(_2\)O][4'-HOMeLeu]CsA (14) |
| [o-Ala][EtVal]CsA (Alisporivir, 7) |
| [4'-BnOMeLeu]CsA (15) |
| [o-Ala][N-(E)-CH\(_2\)CH=CH\(_2\)OH-Val]CsA (16) |
| [N,N-dimethyl-o-Lys]CsA (17) |
| [Me-o-Ala][EtVal][Leu][5'-HOMeLeu][[Leu]CSA (18) |
| [Thr][5'-HOMeLeu][CSA (19) |

| Anti-HCV EC\(_{50}\) (nM) | Ref. |
|--------------------------|-----|
| 230–410                  | 65  |
| 122                      | 65  |
| 40                       | 67  |
| 20                       | 64  |
| 25                       | 68  |
| 410                      | 69  |
| 50                       | 70  |
| 140                      | 71  |
| 37                       | 72  |
| <3000                    | 73  |

Figure 6. Clinically advanced anti-HCV drugs: Alisporivir (7) and SCY-635 (4).
Both the 3- and 4-positions have been explored extensively in the design of antiviral drugs. The [Sar]$^3$ residue is ripe for efficient synthetic modification without the need for a lengthy synthesis. A polar functional group appended to [Sar]$^3$ can augment drug-like properties such as water solubility, and compounds bearing an $\alpha$-alkylamino thioether or ether sidechain at [Sar]$^3$ have been described with very potent antiviral activity, such as 3.$^{63}$ Similarly, strong antiviral activity is seen in compounds bearing functionality at the 4-position designed to remove immunosuppressive potential, such as [4'-HOMeLeu]$^4$ (10), [MeVal]$^4$ or [EtVal]$^4$ (8), or [Melle]$^4$ (9). Compounds of high interest that have arrived from these include those possessing both optimal [Sar]$^3$ substitution for Cyp binding and/or drugability with [MeLeu]$^4$ functionality to attenuate immunosuppressive potential, such as 4–7 and 12–14.

Both the 5- and 8-positions have also been modified to both improve drug-like properties and diminish immunosuppressive potential, resulting in potent antivirals such as 16 and 17. Other scaffold positions that have been successfully modified to yield antivirals include position 2 (19), 9 (11, 19), and 10 (18).

From these, Alisporivir (7) and SCY-635 (4) have proceeded the furthest into clinical development. Building on data showing that replacement of the 4-position leucine residue with a valine resulted in a loss of immunosuppressive activity, along with metabolism data from CsA indicating that demethylation of the amide of [MeLeu]$^4$ is a major clearance mechanism, Debiopharm identified [MeAla]$^3$[EtVal]$^4$CsA (Alisporivir, 7) as a very potent CypA binder with good anti-HIV activity.$^{64}$ This drug has demonstrated good Cyp activity in combination with Peg-IFN$\alpha_2a$ and ribavirin, and was active as monotherapy against HCV genotypes 2 and 3.

Combination of the [Sar]$^3$ modifications that result in potent anti-HIV activity with a [4'-HOMeLeu]$^4$ residue results in SCY-635 (4) that has potent anti-HIV and anti-HCV activity, and is non-immunosuppressive.$^{75}$ SCY-635 was shown to be active against multiple HCV genotypes, to have a high barrier to resistance, and to be less inhibitory than CsA or Alisporivir toward key transporters (Mrp-2) involved in bilirubin clearance.$^{76}$ The finding that SCY-635 has lower in vitro CYP450 and transporter interactions than CsA or Alisporivir is likely to be important for diseases such as HCV, for which a polypharmacy approach is likely. While Alisporivir and SCY-635 represent the most clinically advanced non-immunosuppressive Cyp inhibitors, a number of comparable structures beyond those shown in Tables 1 and 2 have appeared in the patent literature, many of which involve modification of the [MeLeu]$^4$ residue. Figure 6 shows synthetic pathways towards Alisporivir (7) and SCY-635 (4).$^{63a}$

Efforts over the last 10 years to identify non-CsA-based inhibitors of Cyps have met with some success. Sanglifehrin A (Fig. 7) is a natural product that was first described in 1999 and shown to be a potent inhibitor of CypA.$^{78}$ Derivatives of Sanglifehrin A have been described with good anti-HCV activity; however, these compounds have not yet progressed to clinical studies.$^{79}$

In conclusion, Cyp inhibition has emerged, over the last 10 years, from a supporting role required for immunosuppression via CN inhibition, into a viable approach towards treating a wide variety of disease states of which virology is the most mature. Host Cyps are clearly hijacked by several viruses to enable effective replication, and Cyp inhibition offers a promising new approach to antiviral therapy. The additional finding that Cyp inhibition plays a role in uncoupling a virus (HCV) to immune surveillance is potentially exciting; however, discovery efforts in this field are likely to be challenging, as many in vitro virus infection screening systems are successful due to an immunological defect that renders the host cell permissive to viral infection.

The discovery of non-immunosuppressive Cyp inhibitors, such as Alisporivir, allows Cyp inhibition to be considered in disease states wherein immunosuppression would be prohibitive. Furthermore, the demonstration of SCY-635 to have good oral bioavailability and DDI potential clearly indicates that relatively large, macrocyclic peptides could, in some cases, be considered as ‘drug-like’. As the biology of Cyps continues to unfold, the challenges to medicinal chemistry will be to discover compounds that can selectively inhibit members of the Cyp family, and to use these tools to validate the role of Cyps in disease states and as leads for drug discovery efforts. While CsA has served admirably as the starting point for Cyp research, little selectivity within the Cyp family has been described for derivatives of this compound. New inhibitory templates are beginning to appear that may deliver improved selectivity; however, the application of fragment-based screening, with the aid of available structural data to support the location of binding, should allow inhibitors to be designed that exploit regions of the Cyp protein that differ among family members.

References and notes

1. Calne, R. Y.; White, D. J.; Thrus, S.; Evans, D. B.; McMaster, P.; Dunn, D. C.; Graddock, G. N.; Pentlow, B. D.; Rolles, K. Lancet 1978, 2, 1323.
2. Kallen, J.; Mikol, V.; Quereaux, V. F. J.; Walkinshaw, M. D.; Schneider-Scherzer, E.; Schorgendorfer, K.; Weber, G.; Fluri, H. G. In Biotechnology, 2nd Ed., VCH, Weinheim, Germany, 1997; Vol. 7, pp. 535.
3. Halsestrup, A. P. J. Bioenerg. Biomembr. 2009, 41, 113.
4. (a) Vollter, J.; Wray, V.; Shubert, U. Future Virol. 2007, 2, 65; (b) Tang, H. Viruses 2010, 2, 1621.
5. (a) Fischer, G. Angew. Chem. 1994, 106, 1479; (b) Fischer, G. Angew. Chem., Int. Ed. Engl. 1995, 34, 1415; (c) Kiefhaber, T.; Quaes, R.; Hahn, U.; Schmid, F. X. Biochemistry 1990, 29, 3081.
6. Daum, S.; Schumann, M.; Mathes, A.; Aumueller, T.; Balsley, M. A.; Constant, S.; Leaux de Lacroix, B.; Kruisza, F.; Braun, M.; Schiene-Fischer, C. Biochemistry 2009, 48, 6268.
7. Galat, A. Proteins: Struct. Funct. Biol. 2004, 56, 808.
8. (a) Kang, C. B.; Hong, Y.; Dhe-Paganan, S.; Yoon, H. S. Neurosurgery 2008, 66, 1318; (b) Schiene, C.; Fischer, G. Curr. Opin. Struct. Biol. 2005, 15, 40.
9. Schreiber, S. L. Science 1991, 251, 283.
10. Ryo, A.; Liu, Y.-C.; Lu, K. P.; Wulf, G. J. Cell Sci. 2003, 116, 773.
11. (a) Wildemann, D.; Erdmann, F.; Hernandez Alvarez, B.; Stoller, G.; Zhou, X. Z.; Fanghanel, J.; Schutzkowsky, M.; Lu, K. P.; Fischer, G. J. Med. Chem. 2006, 49, 2147; (b) Daum, S.; Erdmann, F.; Fischer, G.; Feuge de Lacroix, B.; Hassiemian-Alinejad, A.; Houben, S.; Frank, W.; Braun, M. Angew. Chem. Int. Ed. 2006, 45, 7454.
12. Harrison, R. K.; Stein, R. L. Biochemistry 1990, 29, 1684.
13. Wang, H. C.; Kim, K.; Bakhtiar, R.; Germans, J. P. J. Med. Chem. 2004, 47, 2593.
14. Zhao, Y.; Ke, H. Biochemistry 1996, 35, 7356.
15. Zyadowsky, D. L.; Ezekkom, F. A.; Chang, H. Y.; Ferguson, S. B.; Stolz, L. A.; Ho, S.; Walsh, C. T. Protein Sci. 1992, 1, 1092.
16. Ke, H.; Hua, Q. Front. Biosci. Landmark Ed. 2004, 9, 2285.
17. Yan, L.; Zucker, S.; Tooie, B. P. Thromb. Haemost. 2005, 93, 119.
18. Stemmy, E. J.; Balsley, M. A.; Jurjus, R. A.; Dansker, J. M.; Bukrinsky, M. I.; Constant, S. L. Am. J. Respir. Cell Mol. Biol. 2011, 45, 991.
19. Kato, N.; Kosugi, T.; Sato, W.; Ishimoto, T.; Kojima, H.; Sato, Y.; Sakamoto, K.; Maruyama, S.; Yuzawa, Y.; Matsu, S.; Kadomatsu, K. Am. J. Pathol. 2011, 178, 572.
20. Seizer, P.; Ochmann, C.; Schoenberger, T.; Zach, S.; Rose, M.; Borst, O.; Klingel, K.; Rando, M.; MacDonald, H. R.; Nowak, R. A.; Engelhardt, S.; Lang, F.; Gawaz, M.; May, A. E. Arterioscler. Thromb. Vasc. Biol. 2011, 31, 1377.
21. Cour, M.; Loufoat, J.; Paillard, M.; Augeul, L.; Goudable, J.; Ovize, M.; Argaud, L. Eur. Heart J. 2011, 32, 226.
22. Liu, J.; Farber, J. D.; Jr.; Lane, W. S.; Friedman, J.; Weissman, I.; Schreiber, S. L. Cell 1991, 66, 807.
23. Billich, A.; Hammerschmidt, F.; Peichl, P.; Wengler, R.; Zenke, G.; Quereaux, V.; Rosenworth, B. J. Virol. 1995, 69, 2451.
24. (a) Yang, F.; Robert, J. M.; Nelson, H. B.; Irissler, A.; Kenworthy, R.; Tang, H. J. Virol. 2008, 82, 5260; (b) Kaul, A.; Stauffer, S.; Berger, C.; Pertel, T.; Schmitt, J.;
