Repurposing of cyclophilin A inhibitors as broad-spectrum antiviral agents

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Cyclophilin A (CypA) is linked to diverse human diseases including viral infections. With the worldwide emergence of severe acute respiratory coronavirus 2 (SARS-CoV-2), drug repurposing has been highlighted as a strategy with the potential to speed up antiviral development. Because CypA acts as a proviral component in hepatitis C virus, coronavirus and HIV, its inhibitors have been suggested as potential treatments for these infections. Here, we review the structure of cyclosporin A and sanglifehrin A analogs as well as synthetic micromolecules inhibiting CypA; and we discuss their broad-spectrum antiviral efficacy in the context of the virus lifecycle.

Keywords: Cyclophilin A; Antiviral; Drug repurposing; Cyclosporin A; Debio-025

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Introduction

Drug repurposing is an approach used to discover new medicinal uses for authorized or experimental medications that are not currently prescribed for such uses.[1] This approach has several benefits in drug development. First, the toxicity of repurposed drugs is likely to be minimal when compared with newly developed drugs, because they have previously been proven to be sufficiently safe in preclinical or clinical Phase I studies. Thus, most tolerability research, such as safety evaluations, dosage optimization and route determination, can be bypassed or minimized.[2–3] Second, drug redevelopment investment can be lowered by using already established large-scale production processes and quality specifications or test methods for manufacturing processes. The absence of approved vaccines or virus-specific antivirals at the beginning of the currently circulating severe acute respiratory syndrome virus 2 (SARS-CoV-2) pandemic has focused attention on the potential value of successful drug repurposing strategies.

An example of the most recently developed, drug-repurposed antiviral agent is molnupiravir (also named MK-4482 or EIDD-2801) – a viral RNA-dependent RNA polymerase inhibitor for oral treatment of SARS-CoV-2 infection. The repurposing of molnupiravir was initiated by synthesizing an isopropylester prodrug of the ribonucleoside analog N4-hydroxycytidine (EIDD-1931) for development as a direct-acting, antiviral agent against influenza virus.[4–5] Being distinctive from the direct-acting antivirals, an alternative drug repurposing approach utilizes host-targeted antiviral agents. These include inhibitors of cyclophilin A (CypA), which is a ubiquitous, cytosolic protein with peptidyl–prolyl cis–trans isomerase (PPIase) activity first described in 1989, belonging to the immunophilin family.[6] There is abundant experimental evidence suggesting that CypA is crucially involved in human diseases including not only cardiovascular diseases and cancers but also viral infections.[7–15] However, in viral infections, CypA has converse roles: it supports viral replication in cells infected with hepatitis C virus (HCV), coronavirus (CoV), HIV or hepatitis B virus (HBV); whereas it destabilizes a viral protein or triggers host defense mechanisms in cells infected with influenza virus or rotavirus. To understand these contradictory biological functions (proviral and antiviral), this review focuses on the molecular interactions between CypA and different viral proteins or other cellular factors and the accompanying signaling pathways. It also summarizes progress in the development of CypA-targeting antiviral agents, based on the chemical structural diversity. We provide an insight into drug development strategies for broad-spectrum antivirals that target CypA and for the chemical modifications with improved antiviral efficacy but reduced immunosuppressive activity or undesirable side effects, such as nephrotoxicity, hypertension and dyslipidemia.[16–18]

CypA as a proviral host factor

CypA has been identified as a promising target for antiviral development because it works as a cofactor in viral genome replication, controls innate immune responses and stabilizes or structurally modulates viral proteins. Here, the proviral roles of CypA and its interaction with viral proteins or other cellular factors will be discussed by scrutinizing the lifecycles of different viruses, such as HCV, CoV, HIV and HBV.

HCV

HCV is a member of the family *Flaviviridae* and has a positive-sense ssRNA genome that encodes a single polyprotein of ~ 3010 amino acids in length.[19] It actively facilitates intracellular membrane rearrangement to maintain its cytoplasmic replication and exploits or modifies cellular proteins involved in cellular lipid metabolic pathways or immune responses.[20–21] Before the identification of CypA being one of the host proviral factors for HCV replication, cyclosporin A (also called ciclosporin A; CsA) was characterized as having antiviral activity against HCV in a cultured human hepatocyte cell line infected with HCV as well as in a viral replicon system.[22–23] At least three viral proteins: nonstructural protein 5B (NS5B; RNA-dependent RNA polymerase), NS5A and NS2, are known to bind directly to CypA.[24–26] Another report suggested that NS5A–NS5B cleavage is controlled by CypA.[12] The interactions of CypA with these proteins rely on the PPIase activity of CypA.[27–28] Indeed, when CypA is mutated at either arginine at position 55 or histidine at position 126, within the hydrophobic active pocket of the enzyme, viral replication is drastically impaired.[24] This enzymatic activity of CypA is required for NS5B-mediated formation of double-membrane vesicles (DMVs), in which HCV RNA replication occurs (Fig. 1, left panel). These intracellular compartments are also thought to contribute to preventing cytosolic pattern recognition receptors from sensing viral RNAs as nonself.[29–30] CsA, a natural cyclic polypeptide immunosuppressant, was reported to inhibit DMV formation efficiently in HCV-infected cells (Fig. 1, right panel).[31]

Other studies demonstrated that the interaction of CypA with NS5A downregulates the activation of protein kinase R (PKR), which is the primary antiviral effector, and that this subsequently inhibits interferon regulatory factor-1 (IRF1) responses (Fig. 1, left panel).[26,32–33] In detail, to promote CypA binding to NS5A, interferon (IFN)-stimulated gene product 15 (ISG15) is covalently attached at the CypA-binding site within NS5A (lysine residue 305) by the HERC E3-ubiquitin ligase.[34] Like NS5B, NS5A is also involved in the maintenance of the membranous replication compartment by binding to CypA. Thus, CsA inhibits the binding of NS5A to CypA and eventually dissociates each component from the NS5A-NS5B-CypA complex (Fig. 1, right panel). Intrinsically, CsA is immunosuppressive by blocking enzymatic activity of calcineurin phosphatase, a key enzyme for interleukin (IL)-2 production.[35–36] Paradoxically, it participated in type I IFN stimulation by inhibiting CypA–NS5A interaction in HCV-infected cells.[26] With understanding of these two contrasting functions of CsA in HCV infection, clinical trials of three CypA inhibitors [Debio-025 (alisporivir), SCY-635 and NIM811] have been scheduled.[37] When compared with CsA, they are designed not to inhibit calcineurin activity and thus are not immunosuppressive, enhancing the pharmacological safety margin.[38–40]

CoV

CoVs are enveloped and positive-strand RNA members of the *Coronaviridae* family. The CoV genome encodes four structural proteins: spike (S), envelope (E), membrane (M) and nucleocap-
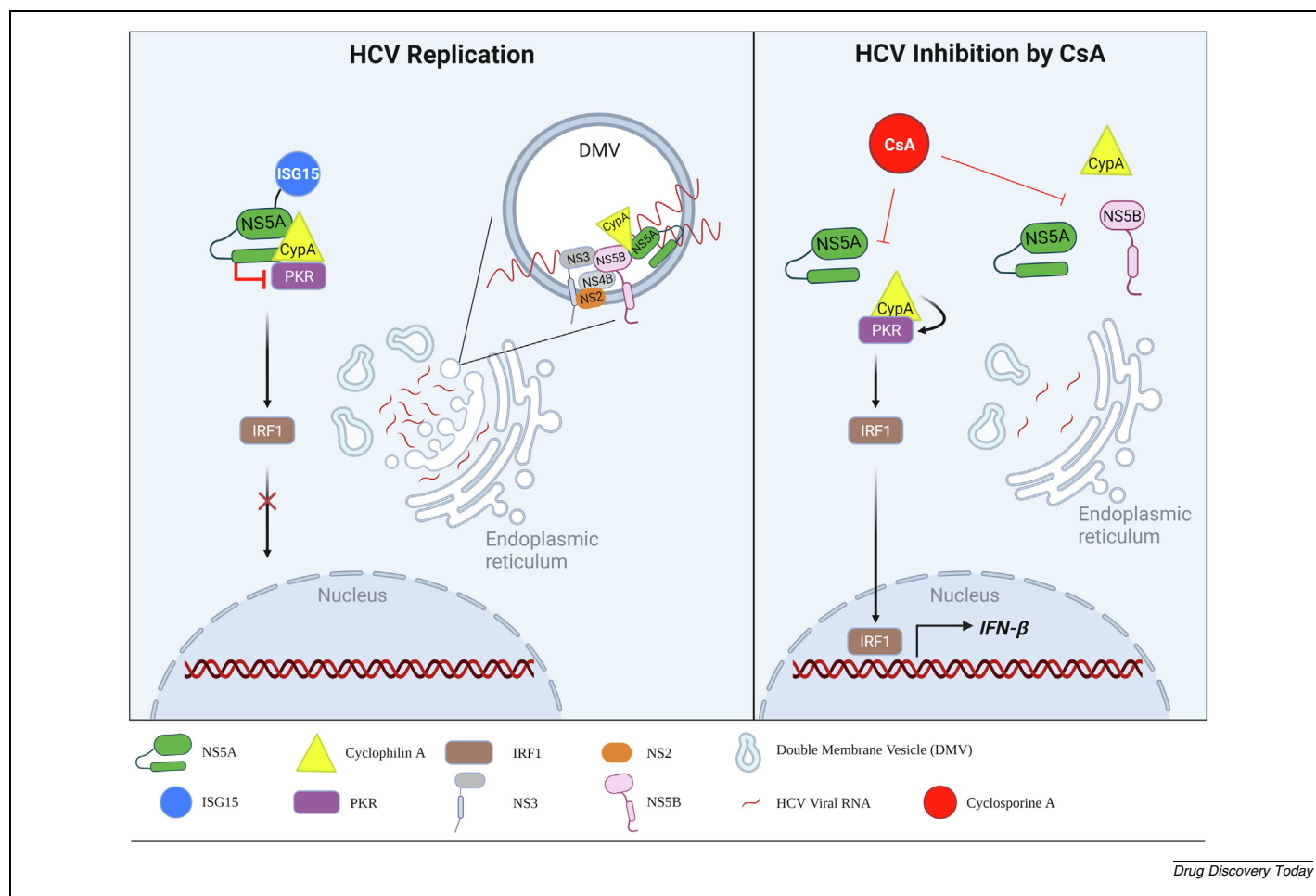
FIGURE 1

Proviral functions of CypA in HCV replication. (Left panel) CypA roles in HCV replication. CypA recruits ISGylated NS5A to PKR, inhibiting the PKR signaling pathway and nuclear migration of IRF1. CypA participates in the genomic replication of HCV by interacting with viral proteins: NS5B and NS5A, within the double-membrane vesicles (DMVs) that provide an isolated compartment for HCV RNA-dependent RNA replication. (Right panel) Two ways of antiviral machinery of CsA – a CypA inhibitor. It interferes with CypA–NS5A interaction, resulting in activation of PKR-mediated antiviral response. Alternatively, CsA blocks CypA binding to NS5A and NS5B, the main components of the RNA-dependent RNA polymerase complex, finally resulting in disruption of DMV formation. Abbreviations: CypA, cyclophilin A; NS, nonstructural viral protein; ISG15, interferon-stimulated gene product 15; PKR, protein kinase R; IRF1, interferon regulatory factor 1; CsA, cyclosporin A.

sid (N), and at least 16 nonstructural proteins (Nsps).[41] CypA has been shown to support SARS-CoV and HCoV-NL63 infection by interacting with viral N protein and Nsp1.[8,14] SARS-CoV infection or Nsp1 overexpression stimulated the calcineurin/nuclear factor of activated T cells (NFAT) pathway and induced IL-2 expression through the activation of immune cells. It means that SARS-CoV activates the NFAT pathway via CypA-interacting Nsp1 for the cytokine dysregulation and immune-dependent pathogenesis. In the same context, single nucleotide polymorphisms near the active site of CypA or inhibition of its PPIase activity negatively affected HCoV-229E and MERS-CoV infections, emphasizing the importance of CypA enzymatic activity during CoV replication.[42–43] Based on these findings, it is not surprising that CsA inhibits a large population of coronaviruses, including HCoV-NL63, SARS-CoV, feline CoV and porcine CoV.[14] As a pan-coronavirus antiviral agent, CsA inhibited SARS-CoV-2 infection of Vero E6 cells with an EC_{50} of

3.5 μM,[44] whereas Debio-025 inhibited SARS-CoV-2 infection with an EC_{50} of 0.46 μM.[45]

Understanding the molecular basis of the cytokine storms induced by acute SARS-CoV-2 infection is of crucial importance for developing antivirals. Extracellular CypA has been suggested to interact with CD147 (basigin), which was also identified as a putative SARS-CoV-2 S-binding receptor together with angiotensin converting enzyme 2 (ACE2).[46–47] CD147 causes the activation of the mitogen-activated protein kinase (MAPK) pathway, resulting in the upregulation of cytokines and chemokines and promotion of a cytokine storm upon infection with SARS-CoV-2 and its variants.[48] Moreover, the expression levels of CypA and CD147 are higher in SARS-CoV-2-infected patients than in uninfected people, and this might contribute to the prominent lymphocytopenia associated with COVID-19.[49] However, it remains to be clearly addressed why both molecules, CsA and Debio-025, are effective against SARS-CoV-2 irrespective of immunosuppressive activity[45] and whether antiviral activity

and immunological safety of CsA can be guaranteed in SARS-CoV-2-infected patients undergoing a cytokine storm. Intuitively, immunosuppressive CypA inhibitors developed in the early generation, than nonimmunosuppressive ones (discussed below), could be more potent and clinically favorable against acute viruses, such as SARS-CoVs, that cause hyperinflammatory immune responses, but the community is awaiting the final results from clinical trials.

HIV

There are several reports exploring the multifunctions of CypA on retroviral infection or assembly.[50–52] However, they exhibited converse experimental results in a cell-type- or viral-strain-dependent manner, in terms of favorable roles of CypA in viral infectivity. Because we have an interest in CypA as an antiviral target and, importantly, many studies agree with the fact that its genetic knockout suppresses most of retroviral infections,[53] we will discuss its functions focusing on wild-type HIV type I (HIV-1) infection in human cells. HIV-1 is a member of the *Retroviridae* family with a positive-strand RNA genome. Its primary target cells are cluster of differentiation 4 (CD4)-positive T cells expressing C–C chemokine receptor 5 (CCR5) and/or C-X-C chemokine receptor type 4 (CXCR4) as a co-receptor and macrophages (Fig. 2).[54] It was believed that HIV-1 uncoating occurs in the cytoplasm before reverse transcription.[55] By contrast, recent studies suggest that nuclear import of the viral core proceeds reverse transcription and uncoating.[56] After translation of viral mRNA in producer cells, the Gag polyprotein is cleaved by a viral protease to generate mature matrix (MA), capsid (CA) and nucleocapsid (NC) proteins. The host protein CypA selectively and directly binds to the N-terminal domain of CA and is packaged into the viral particle during virus assembly (Fig. 2, left).[10–11,57] It catalyzes the isomerization of CA by binding to a single exposed loop containing Gly89 and Pro90 within CA.[58–59] These findings have raised an expectation that CypA incorporated within the viral particles could modulate uncoating and viral infectivity.[11,60] However, CypA-deficient virus produced from *CypA* null cells comparably infected Jurkat T cells as CypA-bearing HIV-1 particles did.[53] Irrespective of CypA incorporation, their infectivity was reduced in *CypA* null target cells, indicating that the presence of CypA is required for incoming HIV infection only in target cells rather than in producer cells.
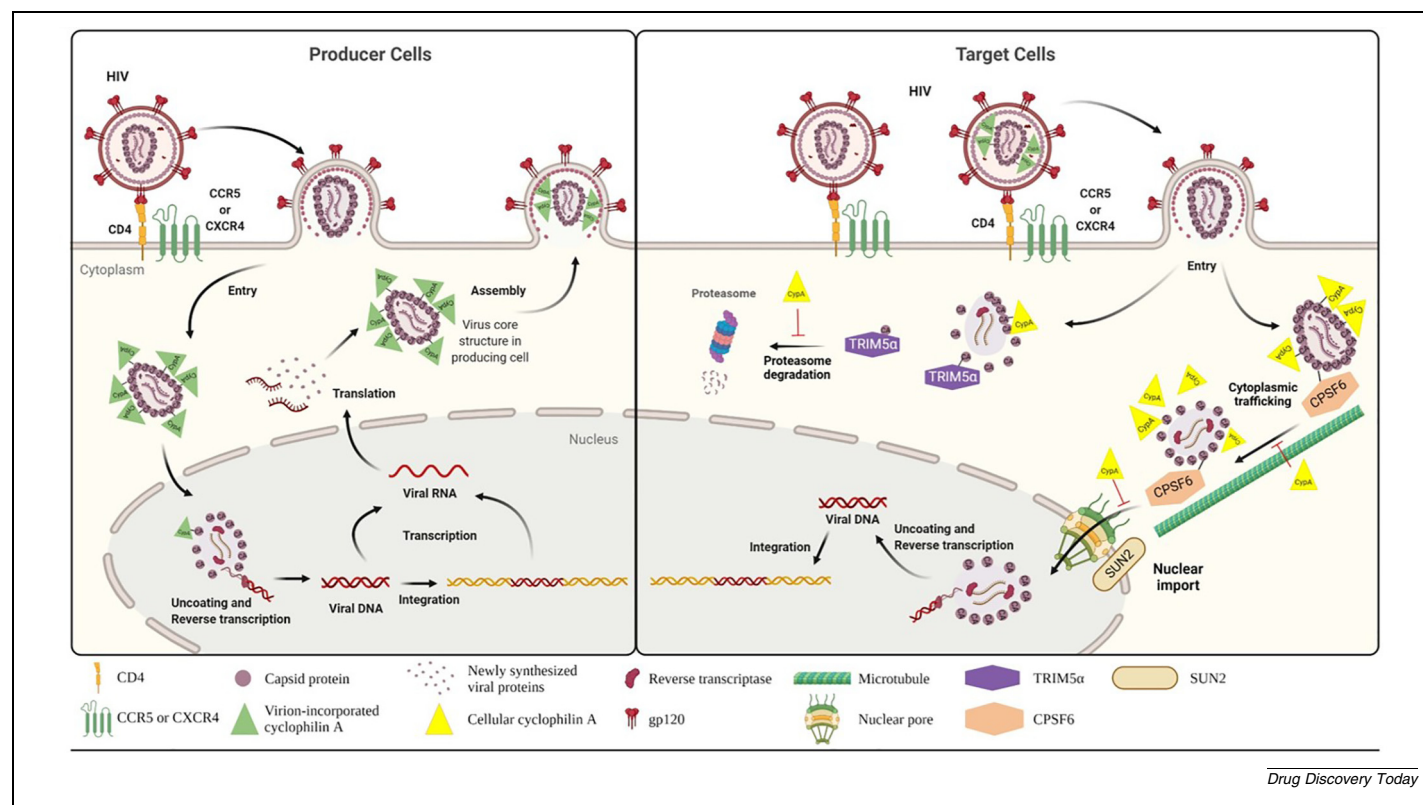
FIGURE 2

Proviral functions of CypA in HIV-1 infection. (Left panel) CypA association with HIV-1 CA in producer cells. HIV-1 enters T cells by sensing CD4 as a receptor and either CCR5 or CXCR4 as a co-receptor. Nuclear viral mRNA is exported to the cytoplasm to express viral proteins. These viral proteins are self-assembled and complexed with CypA, for which the viral RNA genome is packaged inside. CypA-bearing progeny virions are secreted from the producer cells. (Right panel) Host factors affected by CA–CypA interaction in target cells. CypA null and CypA-bearing HIV-1 particles can enter CD4-expressing target cells. Host restriction factor TRIM5α facilitates degradation of CA. CypA inhibits TRIM5α binding to CA in a competitive manner. CPSF6 interacts with CA and traffics the viral core on microtubules, to accomplish HIV-1 capsid uncoating. CPSF6 recruits HIV-1 core to SC35 nuclear speckles for reverse transcription with help of inner nuclear envelope protein SUN2. CypA blocks these two steps by competing with CPSF6 for binding to CA. Abbreviations: CD4, cluster of differentiation 4; CCR5, C–C chemokine receptor 5; CXCR4, C-X-C chemokine receptor type 4; gp120, envelope glycoprotein 120; TRIM5α, tripartite-containing motif 5α; CPSF6, cleavage and polyadenylation specific factor 6; SUN2, inner nuclear membrane protein.

In the same context, the inhibition of the CypA–CA interaction by CsA treatment suppressed HIV-1 infection in target cells, such as T cells, primary lymphocytes and macrophages.[53,61–66].

For modulation of HIV-1 infectivity, diverse intracellular proteins interact with CA in a CypA-dependent manner particularly at the early stage of virus infection in target cells. For example, antiviral tripartite-containing motif 5α (TRIM5α) competes with CypA for binding to HIV-1 CA, whereas cleavage and polyadenylation-specific factor 6 (CPSF6), SUN2 (UNC84B) and nucleoporins are recruited to CA depending on the presence of CypA for viral fusion, uncoating or nuclear localization and even reverse transcription.[50,56,67–69] Of these, functions of TRIM5α and CPSF6 in CA–CypA interactions have been most actively investigated to address questions about how they regulate HIV-1 infection (Fig. 2, right). A retrovirus resistance factor in humans, named restriction factor 1 (Ref1), has been identified as TRIM5α.[70] It blocks HIV-1 infection by accelerating degradation of cytosolic CA.[71–72] As a counter molecule, CypA protects HIV-1 CA from this CA-specific restriction factor TRIM5α.[68,73] Meanwhile, among the essential factors for HIV-1 infection, CPSF6 is also complexed with the CA protein. CPSF6 traffics HIV-1 core on microtubules for viral uncoating and recruits it to SC35 nuclear speckles for reverse transcription.[56,69] Disruption of CypA–CA binding after treatment with CsA increases the CA–CPSF6 interaction and prominently guides capsid trafficking to microtubules in HeLa cells and primary peripheral blood mononuclear cells (PBMCs). These findings inform that CypA interacts with CA to prevent CPSF6–CA interaction and affects cytoplasmic trafficking and uncoating of the viral nucleocapsid at the post-entry step.[69] However, there is little evidence explaining whether stimulation of this proviral role of CPSF6 by blocking CypA could be directly induced by CsA in HIV-1-infected cells.

HBV

HBV is a member of the *Hepadnaviridae* family and is a small enveloped virus with a partially double-stranded circular DNA genome. HBV has abundant hepatitis B surface antigen (HBsAg), which affects pathogenesis during viral infection. HBsAg is composed of large HBV (LHB), middle HBV (MHB) and small HBV (SHB) surface antigens.[74] Induction of CypA is accompanied by increased secretion of SHB into the sera of HBV-expressing mice.[75] The secretion of SHB is reduced by treatment with CypA inhibitors CRV431 and Debio-025.[76–77] CypA acts as a proviral factor for HBV infection during virus entry, assembly and release. Interestingly, CsA inhibits the entry of HBV by directly interacting with its entry receptor sodium taurocholate co-transporting polypeptide (NTCP) receptor, potentially independently of CypA.[78–80]

CypA as an antiviral host factor

In contrast to the proviral effects discussed above, CypA inhibits virus replication either by inducing the degradation of a viral protein that is crucial for nuclear export of the viral genome during the virus assembly step (e.g., in influenza virus-infected cells) or by stimulating cellular antiviral responses (e.g., in rotavirus-, influenza- or Sendai-virus-infected cells).[81–82] In these cases, CypA inhibitors should be applied more carefully, because suppression of CypA can result in minimal antiviral effect or even undesirable virus amplification.
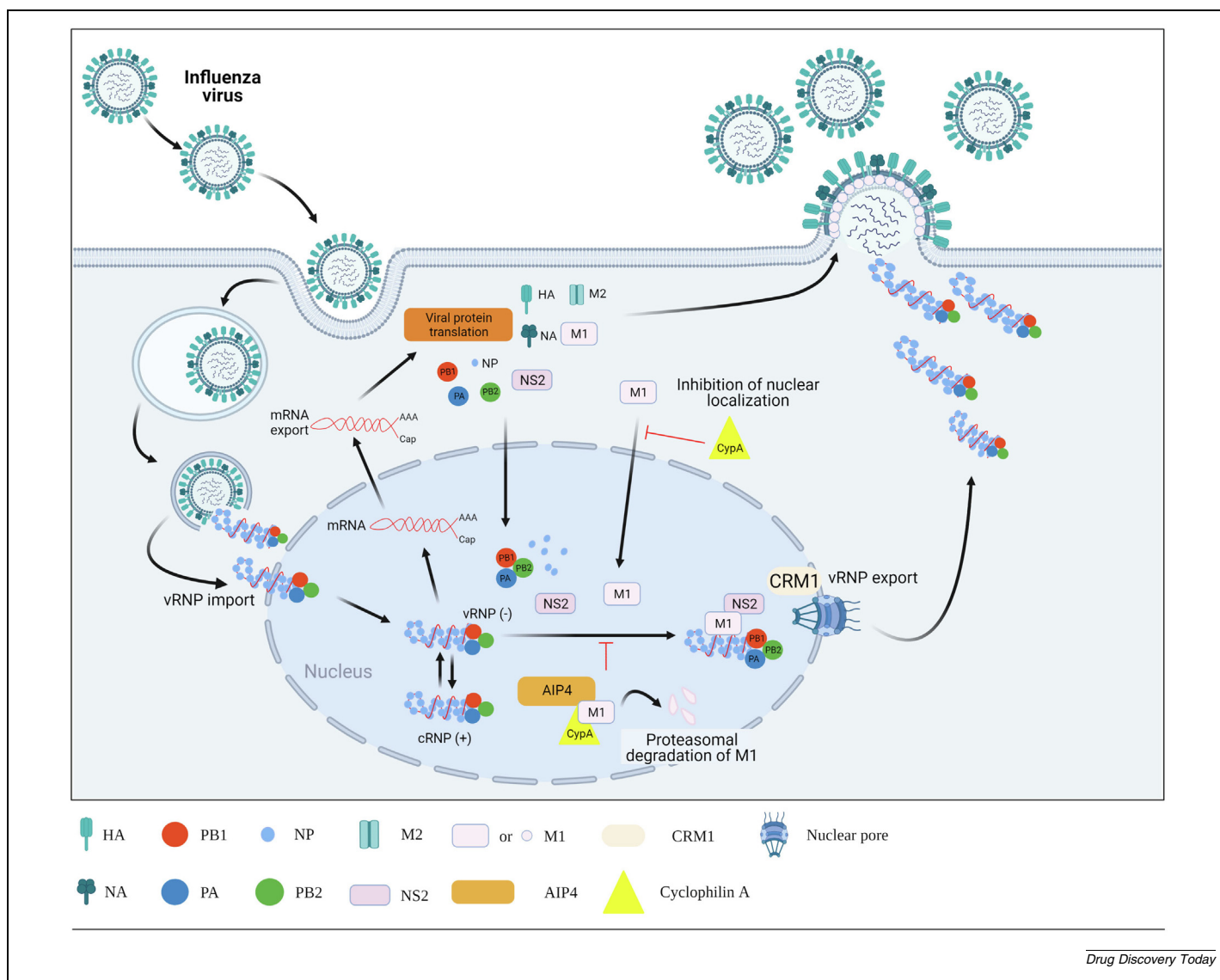
Influenza virus

Influenza viruses are negative-sense single-stranded enveloped RNA viruses comprising eight segments, belonging to the *Orthomyxoviridae* family. They are among the major human respiratory pathogens and encode at least ten distinct viral proteins.[83–84] At the entry step of the virus life cycle, low endosomal pH not only stimulates membrane fusion, mediated by hemagglutinin 2 protein (HA2), but also opens the proton channel of matrix protein 2 (M2), driving cytoplasmic release of viral ribonucleoproteins (vRNP) from the inner shell composed of the viral matrix protein 1 (M1).[85–86] In the nucleus, where RNA-dependent RNA replication occurs, newly synthesized vRNP again interacts with M1, recruiting nonstructural protein 2 (NS2; also named nuclear export protein, NEP), which can bind to the nuclear export receptor: chromosomal maintenance 1 (CRM1) (Fig. 3).[87–91] This series of interactions is essential for nuclear export of vRNPs as well as their migration to the plasma membrane for production of viral progeny.

As described for HIV-1 above, CypA is incorporated into influenza virus particles.[92] Yeast two-hybrid assays and mammalian cell-based assays have shown that CypA directly interacts with the M1 protein, which negatively affects virus replication at two steps: the localization of M1 protein to the nucleus and M1-mediated nuclear export of vRNP complexes (Fig. 3).[13] With regard to the latter mechanism, CypA accelerates M1 degradation by summoning the E3 ubiquitin ligase atrophin-interacting protein 4 (AIP4) to M1, thereby blocking nuclear escape of vRNP.[81,93] Crucially, the findings, showing increased influenza viral infectivity in the absence of CypA using a CypA-knockout cell line but strong disease resistance to viral infection in human CypA-expressing transgenic mice, prove that CypA is an antiviral factor restricting influenza viral infection *in vitro* and *in vivo*.[81,94] Liu and colleagues suggested that CsA inhibits influenza virus replication with reduction of the viral protein level but failed to exhibit inhibition of influenza viral genome replication or transcription.[95] Mysteriously, CsA impairs the nuclear export of viral mRNA in CypA-depleted cells, which was independent of calcineurin signaling, meanwhile it enhanced CypA–M1 interaction in CypA-expressing cells. The isomerase activity of CypA is not even necessary for influenza viral replication in cells.[13] As they reviewed, this incomprehensible antiviral mechanism of CsA might be induced via complicated, CypA-dependent and -independent pathways.[95] A limited number of reports proposed that CsA and its analogs block influenza viral infection, thus further accumulating evidence is required for ensuring feasibility of CypA as an anti-influenza viral target and for understanding its CypA-dependent or -independent modes of antiviral action.

Rotavirus

Human rotaviruses are members of the *Reoviridae* family and are non-enveloped viruses. Rotavirus genomes consist of 10–12 segmented, double-stranded DNA molecules that encode the λ, μ and σ proteins. Rotaviruses are gastrointestinal pathogens that cause acute diarrheal disease.[96] CypA is induced in the early stage of rotavirus infection and inhibits rotavirus replication.

FIGURE 3

Antiviral roles of CypA in influenza virus infection. After receptor-mediated endocytosis, eight-segmented influenza vRNP complexes migrate into the nucleus for triggering RNA-dependent RNA replication. M1 plays a crucial part in nuclear export of newly synthesized vRNPs by recruiting NS2 (also named NEP), which has an ability to bind the nuclear export receptor CRM1. CypA blocks nuclear import of M1 and induces M1 degradation by recruiting AIP4 – an E3 ubiquitin ligase. Abbreviations: HA, hemagglutinin; NA, neuraminidase; PB1, polymerase basic protein 1; PB2, polymerase basic protein 2; PA, polymerase acidic protein; NP, nucleoprotein; M1, matrix protein 1; M2, matrix protein 2; NS2, nonstructural protein 2; CRM1, chromosomal maintenance 1; AIP4, atrophin-interacting protein 4; CypA, cyclophilin A.

Although the Nsp1 of rotavirus inhibits IRF3, IRF5, IRF7 and nuclear factor (NF)-κB to sequentially downregulate the type I IFN signaling pathway, the production of IFN-β is still induced after rotavirus infection via activation of CypA. Interestingly, this event occurs depending on c-JUN N-terminal kinase (JNK) activation rather than the PPIase activity of CypA.[97] Consistent with this, CsA treatment marginally affected the growth of rotavirus.[98] In addition, the elevated expression of CypA in enterocytes prevented acute dehydrating diarrhea, following rotavirus infection, in BALB/c mice.[99] Furthermore, CypA is recruited to within rotavirus particles via its interaction with the structural protein VP2, which reduces rotavirus infectivity. It is unclear why the virus allows incorporation of the antiviral CypA protein into progeny virus particles at the assembly step.

CypA inhibitors

Among the CypA inhibitors, CsA and sanglifehrin A are naturally existing peptidic macromolecules and historically presentative ones. Both molecules are immunosuppressive and can inhibit PPIase activity of a class of cyclophilins.[100–101] These PPIase enzymes catalyze the interconversion of the *cis–trans* peptide bond of proline residues. Because the *cis* conformation of peptidyl–prolyl bonds is found in > 30% of newly synthesized proteins in cells and needs to be converted to the *trans* conformation for those proteins to have biological activity, it follows that cyclophilins are essential in the post-translational process.[102] In this section, the chemical structures, antiviral efficacy and immunosuppressive effect of CypA inhibitors will be discussed.

CsA analogs

CsA, a commonly used immunosuppressant medication, binds to CypA and this complex then interacts with a third partner: calcineurin. Binding of CsA–CypA to calcineurin inhibits its calcium-dependent phosphatase activity, which plays a vital part in the nuclear translocation of NFAT.[103] Natural CsA (structure **1**, Fig. 4) is a cyclic peptide with multiple methylated amide bonds, of which molecular weight is > 1200 Da.[104] The high molecular weight, cyclic structure and conformational flexibility of CsA contribute to its cellular permeability and oral bioavailability.[105] The numbering pattern of CsA is shown in Fig. 4. The binding affinity of CsA for CypA mainly relates to the side chains P1, P2, P9 and P10.[106] The rest of the CsA molecule has relatively little impact on CypA binding, based on the structural analysis of CsA–CypA co-crystals. The immunosuppressive effect of CsA is due to its ability to bind to calcineurin, mainly through the P4, P5 and P6 side chains of CsA (see Figure S1 in supplementary material online).[107] Consequently, to generate a CsA derivative with more-potent CypA inhibition but a lesser immunosuppressive effect, a modified CsA has been designed with substitutions in the side chains P4, P5 and P6 and a change to the P3 side chain to increase its binding to CypA.

In addition to its potential CypA inhibition activity and the related immunosuppressive effect, CsA was also found to remarkably inhibit several endogenous transporters, including organic anion transporting receptors (OATP1B1 and OATP1B3),[108] bile salt export pump, multidrug resistance protein 1 (MDR1; also named ABCB1 or P-gp)[109] and MDR2.[110] From 1984 to 1997, CsA derivatives were chemically synthesized and evaluated for their ability to inhibit CypA, and were subjected to detailed SAR analyses by several research groups. As shown in Fig. 4, introducing a benzyl group as a P3 side chain into CsA to obtain derivative **2** does not affect the ability of CsA to inhibit CypA but dramatically decreases its immunosuppressive effect.[111] Intro-
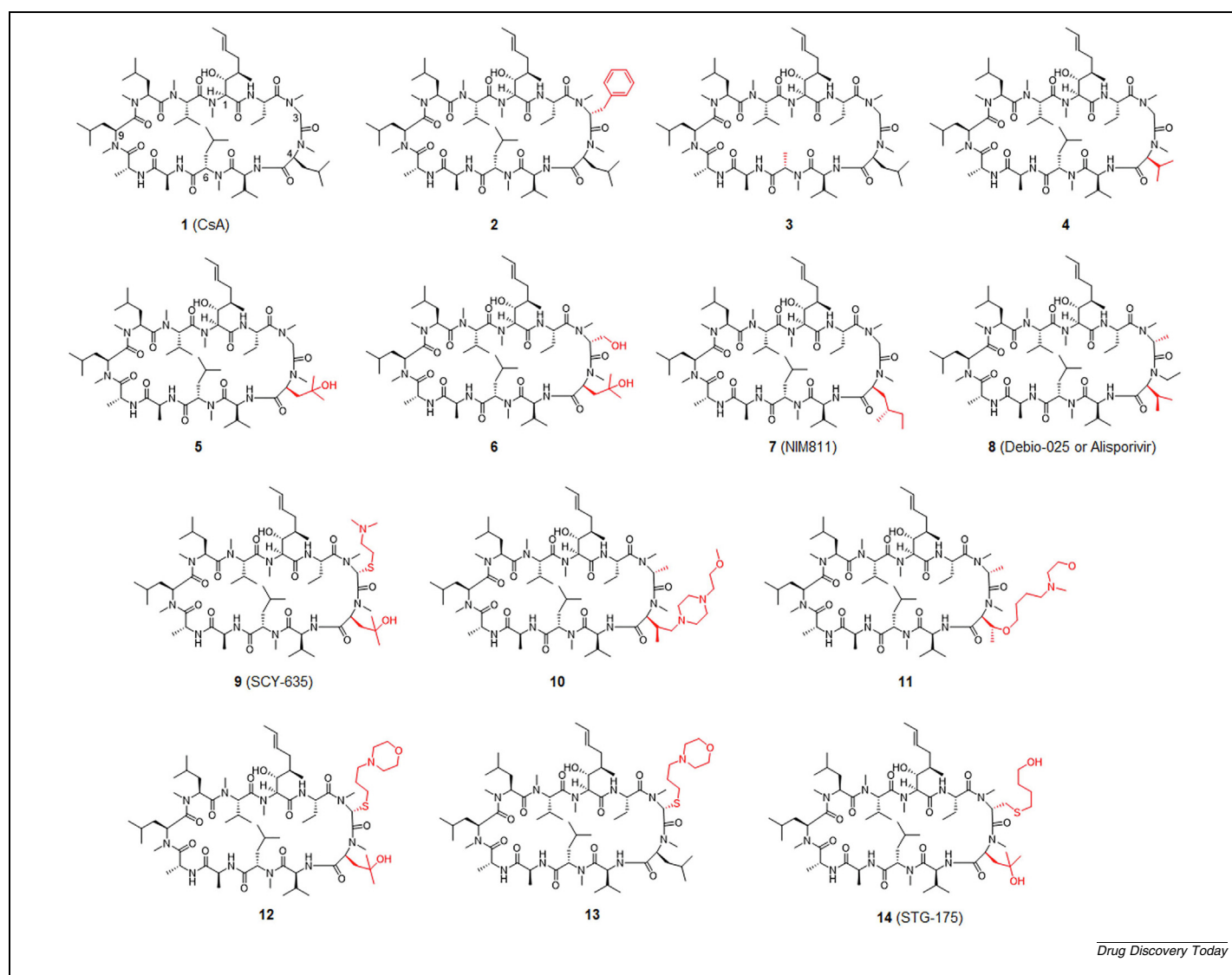

FIGURE 4

Chemical structure of CsA (compound **1**) and its derivatives as CypA inhibitors. The analogs, compounds **2** and **3** are modified at positions 3 (P3) and 6 (P6), respectively. The analogs **4** to **14** are modified at position 3 (P3) and/or 4 (P4) of CsA. Modification sites are highlighted in red.

ducing a bulky group into P3 resulted in the first identified, non-immunosuppressive CypA inhibitor. By switching an isopropyl to a methyl group as the P6 side chain the resulting CsA derivative (**3**) has ~ 50% of the CypA binding affinity of natural CsA. Surprisingly, it also reduces immunosuppression to 0.4% of that of CsA.[112]

Replacement of the P4 side chain in **1** to produce derivatives **4** or **5** can preserve or improve CypA binding while sharply reducing its activity in immunosuppression assays. Based on these results, the authors concluded that calcineurin has a unique pocket for P4. This hypothesis was later confirmed by crystallization experiments.[113] Derivatives **6** and **7** (NIM811) with changes in the P4 side chain inhibited replication of HIV in cellular assays while significantly impairing the immunosuppressive effect. Intriguingly, derivative **6** was 4-times more effective than derivative **7** in binding assays but, in the anti-HIV cellular assay, **6** was less potent than **7**.[114–115] In these preliminary tests, derivative **7** exhibited a stronger affinity for CypA than natural CsA.[116] This inhibitor has been tested in animal and cell models of numerous diseases, as well as in clinical trials as an HCV treatment.[117] Following the finding that it inhibits HCV replication, further research involving the derivative **7** was carried out to examine whether it could be employed as a therapy for HCV infection.[118] Non-immunosuppressive CypA inhibitors have substantially high genetic barriers to the evolution of viral resistance, according to this research, which is also predicted to be the case for host-targeted therapies.

CsA derivative **8** (Debio-025 or alisporivir) has a potency of 5–10-times that of CsA in a cellular subgenomic HCV replication system.[119] This inhibitor was more effective than derivative **7** in cell-culture-based antiviral assays.[120] The antiviral activity of derivative **8** was higher than that of earlier CsA-like inhibitors, which might be attributable to its altered P3 groups, conferring higher affinity for CypA. The progression of HCV resistance to derivative **8** is a protracted one. Cells swiftly remove the replicon when this molecule is coupled with pegylated (peg)IFN-α and ribavirin.[120] Moreover, this CypA inhibitor has been reported to be very effective for the treatment of genotype 2 and 3 infections of HCV.[121] Originally, the modified CsA derivative **8** was developed as a therapy against HIV infection. The anti-HIV action of derivative **8** stems from its ability to prevent CypA from attaching to the HIV-p24 Gag protein.[122] Once-daily treatment resulted in a moderate decrease in HIV-1 plasma RNA in an initial 10-day investigation in infected individuals.

Derivative **9** was found in a pharmaceutical investigation aimed at developing a novel type of CsA inhibitor for the treatment of HIV infections. It improved anti-HIV efficiency while simultaneously lowering *in vitro* immunosuppressive activity.[123] This chemical inhibited CypA isomerase activity more effectively than native CsA **1**.[124] The decrease in HCV replication *in vitro* was time-dependent, with total replication suppression not occurring until 72 h.[125] In comparison with other CypA inhibitors, derivative **9** was a less effective transporter protein inhibitor. P-glycoprotein efflux activity was totally inhibited at 15 μM, although multidrug resistance 2 (MRP2)-mediated transport was only moderately affected 150 μM.

A series of novel CsA analogs with a variety of P3 and P4 residues have been further synthesized and analyzed. The most representative compound is derivative **10**, an analog with a morpholine moiety at the P4 residue. Surface plasmon resonance (SPR) assays revealed that it had a binding affinity of 1.1 nM. It inhibited HCV replication with an EC_{50} of 36 nM and was eight-fold less effective in inhibiting the OATP1B1 transporter.[126]

EDP-546 (a structurally unknown CypA inhibitor) is similar to derivative **11**. In cellular passaging assays with EDP-546 using HCV genotype 1a and 1b replicon systems, selection for the D320E mutation within the NS5A protein resulted in moderately (two-to-fivefold) higher resistance. The antiviral activity of combinations of HCV protease inhibitors and HCV NS5A inhibitors was synergistic, and co-incubation of EDP-546 with these antiviral agents reduced viral resistance to these agents. The cellular activity of the inhibitor was unaffected by the addition of 40% human serum to the growth medium, and EDP-546 was more stable in human microsomes than derivative **8**. EDP-546 exhibited limited blood clearance and substantial distribution in the liver in preclinical pharmacokinetic investigations. In aqueous buffer solutions, good solubility was reported, especially at pH values less than 7. Furthermore, EDP-546 is a significantly weaker inhibitor of bilirubin and drug transporters MRP2 (about 100-fold less) and OATP1B1 (roughly threefold less) than derivative **8**.[127]

Derivative **12**, an example as a 3- and 4-modified CsA analog, showed potent anti-HCV activity in a cellular replicon assay. Derivative **13** differs from derivative **12** in having a hydroxyl group at the P4 chain and differs from CsA in having a bulky thioether group at P3 (**1**). Both derivatives exhibited similar CypA inhibition in an *in vitro* enzyme assay but lower inhibition of immune responses, with 50-fold lower immunosuppression than that of CsA in a mixed lymphocyte reaction.[128]

Derivative **14** (STG-175) is a CsA analog with a thioether and a hydroxyl butyl group in the P3 side chain. It inhibits CypA isomerase activity with an IC_{50} of 0.6 nM. Its immunosuppressive effect was ~ 1000-fold less than that of CsA in an IL-2 release assay with the Jurkat cell line. The anti-HCV activity EC_{50} values of derivative **14** on different HCV replicon genotypes, including GT1a, GT1b, GT2a, GT3a and GT4a, ranged from 11.5 nM to 38.9 nM. It could greatly enforce the HCV resistance barrier when combined with direct-acting antivirals without any cross-resistance.[129]

A series of modifications at P1 has been challenged (Fig. 5). Derivative **15** differs from CsA in having an extended P1 and an additional methyl group on P3. It was 15-fold more potent in inhibiting CypA PPIase activity than CsA but dramatically decreased immunosuppression.[130]

Derivative **16** (CPI-431–32 or CRV-431) efficiently inhibited HIV-1 and HCV growth individually and during co-infection. Derivative **16** inhibited CypA–HCV-NS5A and CypA–HIV-1-CA interactions with IC_{50} values of 0.18 μM and 0.21 μM, respectively. In addition, it inhibited CypA isomerase activity with an IC_{50} of 1.8 nM, indicating that it was almost ninefold more potent than CsA. Derivative **16** suppressed HIV-1 reverse transcription and nuclear import.[131–132]

Derivative **17**, with a benzimidazole group in P1, was developed as an extracellular CypA inhibitor to suppress leukocyte trafficking. The binding affinity of derivative **17** was similar to that of CsA in a PPIase binding assay. Because cellular CypA is
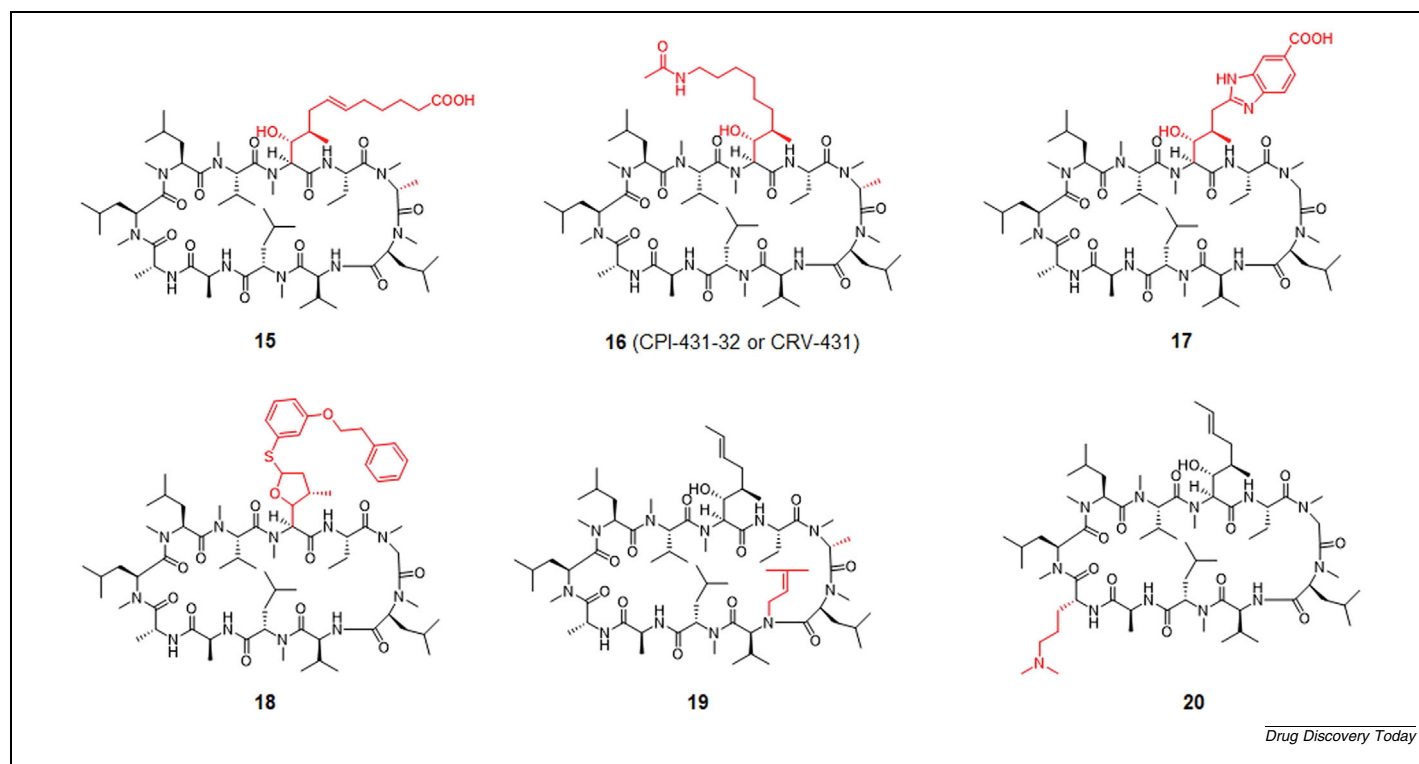
FIGURE 5

Chemical structure of CsA derivatives, including compounds **15** to **20**. In compounds **15** and **16** P1 and P3 are modified; in compounds **17** and **18** P1 is modified; in compound **19** P3 and P5 are modified; and in compound **20** P8 is modified compared with CsA (**1**) (Fig. 4). Modification sites are highlighted in red.

crucial, analysis using fluorescently labeled CsA derivatives showed that derivative **15** was at least 50-fold less permeable than CsA. Despite its reduced cell permeability, derivative **15** hindered leukocyte migration to CypA. In a mouse model of allergic contact hypersensitivity, this inhibitor also decreased leukocyte activation.[115]

A recent study showed that a P5-modified CsA analog acts as an NTCP inhibitor and reduces bile acid uptake. The markedly potent and specific inhibitor, derivative **18**, exhibited broad antiviral activity against genomic HBV and hepatitis D virus infection *in vitro* with excellent pharmacokinetics and oral bioavailability. However, it had lower immunosuppressive potency than CsA.[133]

Through another approach, the P3- and P5-modified CsA derivative, **19**, was identified as a CypA and CypD inhibitor with a dissociation constant (K_d) of 60 nM and an $EC_{50} < 200$ nM in an HCV replicon assay. Derivative **19** was ~ 40-fold more potent in inhibiting IL-2 release in a stimulated T cell assay than CsA.[134] Changing the side chain at the P8 position of CsA, resulting in derivative **20**, improved its anti-HCV activity but reduced the immunosuppressive activity.[135]

Sanglifehrin A derivatives

Sanglifehrin A (compound **21**; Fig. 6) is the most extensively studied natural product of its family and binds to CypA 60-times more strongly than CsA. The immunosuppressive effect of sanglifehrin A is not attributable to a reduction in its calcium-regulated phosphatase activity.[101] The 3D co-

crystalized structure with CypA revealed detailed information regarding this interaction (see Figure S2 in supplementary material online).[136] Modifications to the spatial domain substitution pattern (regions C13–C26 on sanglifehrin A) consistently reduced potency. For example, in a procycling binding experiment, synthetic macrolide derivative **23** was ~ 600-times less effective than derivative **22**.[101]

As shown in Fig. 6, the degradation product of sanglifehrin A, derivative **24** (AAE931), is a potent anti-HCV inhibitor with an EC_{50} of 130 nM in a replicon assay. The bioengineered product of sanglifehrin A, derivative **25** (NVP018), is a potential orally available CypA inhibitor for the treatment of HCV, HBV and HIV-1 infections. Derivative **26** (BC544) was developed through a combination of bioengineering and semi-synthesis. BC544 inhibits PPIase with an IC_{50} of 2.7 nM and the NS5A–CypA interaction with an IC_{50} of 0.36 µM, whereas it suppresses replication and replicons of the HCV genotype 1b in Huh5-2 cells with an EC_{50} of 125 nM, without exhibiting cytotoxicity at the maximum tested concentration of 100 µM.[137–138]

With a pilot screen of a chemical library of ~ 19,000 chemical compounds, hit compounds were not identified in a time-resolved fluorescence resonance energy transfer (FRET)-based binding assay.[137] Chemical synthesis of sanglifehrin A simplified its structure and resulted in the synthesis of derivative **27** (corresponding to compound 3 from the original paper). Retention of the diene moiety and the reduction of the complexity of the molecule resulted in a CypA binding affinity (K_d) of 25 nM and a K_i value of 16 nM in a PPIase functional assay. It inhibited
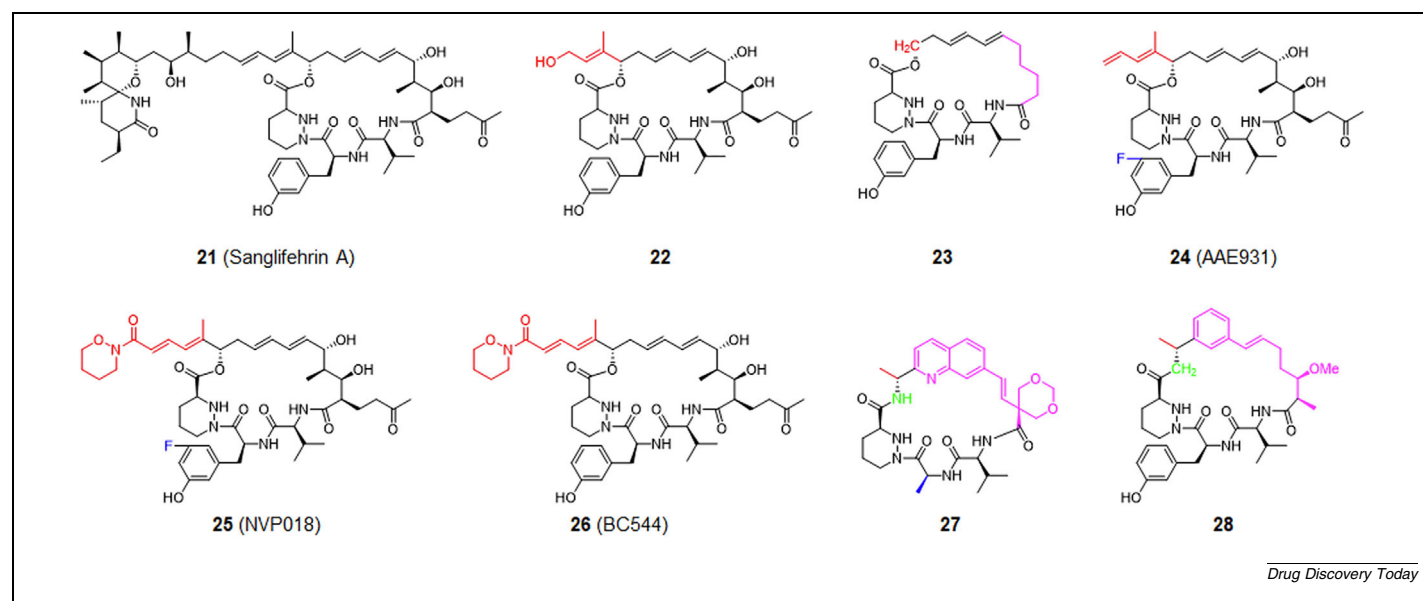
FIGURE 6

Chemical structure of sanglifehrin A (**21**) and its simplified derivatives, compounds **22 to 28**, as CypA inhibitors. Modification sites are highlighted in different colors depending on their positions.

HCV genotype 1b replication with an EC$_{50}$ of 600 nM.[139] Switching the diene for a phenyl moiety led to a novel skeleton of sanglifehrin A, derivative **28**, which had almost 17-fold more potency than **27** in inhibiting HCV genotype 1b replication and replicons. Determining the X-ray crystal structure of these new simplified sanglifehrin-A-based CypA inhibitors would be of significant value for the SAR analyses of related inhibitors and development of novel CypA inhibitors. The most representative analog was derivative **27** which possessed a quinolone unit. This derivative has a very high oral absorption rate in rats and dogs and is less effective in generating drug–drug interactions. The strong binding affinity (K_d = 5 nM) and the availability of a systematic synthetic route establishes it as a candidate for HCV treatment.[137]

Micromolecular CypA inhibitors

Owing to the difficulty of synthesizing large molecules, and the possible side effects caused by their peptide skeletons, the synthesis of micromolecular CypA inhibitors has become the focus of extensive research efforts during the past two decades. Furthermore, the development of computer-aided drug design and virtual screening has nurtured robust growth in this area. Starting with a linear tetrapeptide: Suc-Ala-Gly-Pro-Phe-pNA, the macrocyclization of the template has led to the generation of a novel class of CypA inhibitors, such as derivative **29**, that have a moderate K_d of 10.5 μM for CypA, good aqueous solubility and a low rate of hepatic clearance (Table 1).[140] This study provided the template for the subsequent development of diverse micromolecular CypA inhibitors.

By structure-based screening a chemical library of > 200,000 compounds to identify hits with potential binding affinity for the CsA–CypA complex, compound **30** with a bisamide skeleton was identified as a promising hit compound. Compared with the

synthetic schemes for CsA and sanglifehrin A, this bisamide was synthesized easily through a one-step Ugi reaction, which significantly reduced the difficulty in the preparation of macromolecular CypA inhibitors. Without causing cytotoxicity or immunosuppression, compound **30**, when combined with IFN-α, ribavirin and telaprevir, showed excellent synergistic properties for the treatment of HCV infections.[141] It had little or no immunosuppressive effect but, notably, inhibited HCV replication *in vitro* and *in vivo*. Its efficacy for the treatment of HCV infection in mice also increased synergistically when it was co-administered with other direct-acting antivirals.[141–143] SAR analysis of bisamides for antiviral activity has been extensively discussed in these reports. The more potent, optimized bisamide analogs as CypA inhibitors are compound **31** and **32**, which inhibited HCV replicons with an EC$_{50}$ of 5.3 μM and 4.1 μM, respectively, both with no cytotoxicity at a maximum concentration of 100 μM.
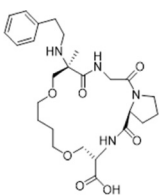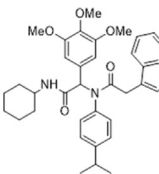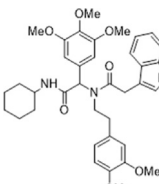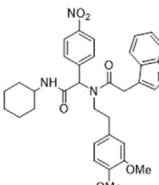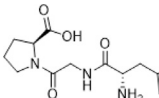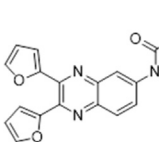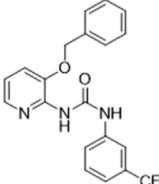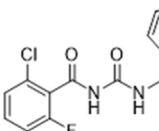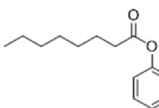
By dissection of the binding pocket of CsA on CypA, Pang and colleagues proposed that a peptide with three or four residues would be an effective CypA inhibitor. Virtual screening of a peptide library was utilized to identify compound **33** (WGP), which inhibited the PPIase activity of CypA with an EC$_{50}$ of 33.11 nM. A SPR assay indicated that compound **33** has a K_d value of 3.41 μM and inhibited HIV-1 replication by 75.5% at a concentration of 1 mM.[144]

One novel quinoxaline derivative, compound **34** (DC838), was designed and synthesized as a CypA inhibitor with a K_d value of 7.1 μM for CypA, as measured by the SPR assay. Moreover, it suppressed PPIase activity with an IC$_{50}$ of 0.41 μM and inhibited the replication of the HIV-1 cycle.[145]

Compound **35** (corresponding to compound 2 in the original paper), a CypA inhibitor with a molecular weight of < 400 Da, was identified using a computer-aided drug design approach with virtual screening and pharmacophore construction. This com-

TABLE 1

Micromolecular CypA inhibitors.

Compound no.	Chemical formula	Background and biological properties	Publication date
29		SPR analysis binding affinity, K_D 7.09 μM; PPIase inhibition, IC_{50} = 0.41 μM; inhibition on the proliferation of spleen cells, IC_{50} = 4.32 μM; no obvious effect on cell viability at 10 μM concentration	2016[140]
30		Identified by virtual screening and chemical modification, CC_{50} > 100 μM, EC_{50} = 5.2 μM; no effect on immune system; reducing the expression of NS5A and NS5B without cytotoxicity; K_d = 570 nM; synergistic effect when combined with IFN-α, ribavirin or telaprevir for HCV infection in mice	2015[141]
31		Anti-HCV activity, EC_{50} = 5.3 μM and CC_{50} > 100 μM; SPR analysis, K_D = 3.66 μM	2020[143]
32		Anti-HCV activity, EC_{50} = 4.1 μM; inhibiting the expression of HCV core protein, K_D = 4.6 μM determined by SPR	2021[142]
33		Identified by virtual screening focusing on peptide library; inhibition on the PPIase activity of CypA, IC_{50} = 33.11 nM; SPR assay, K_D = 3.41 μM; exhibiting 75.5% inhibition of HIV-1 IIIB infection at 1 mM concentration	2011[144]
34		SPR assay binding affinity, K_D = 7.09 μM; PPIase inhibition, IC_{50} = 0.41 μM; inhibition on the proliferation of spleen cells, IC_{50} = 4.32 μM; no obvious effect on cell viability at 10 μM concentration	2006[145]
35		Virtual screening and pharmacophore-based drug design; PPIase isomerase inhibition, IC_{50} = 303 nM; inhibition on HIV-1 replication cycle on immortalized cells or human peripheral blood mononuclear cells	2006[146]
36		De novo drug design using the crystal structure of CypA with sanglifehrin A macrolide and pharmacophore-based design; CypA inhibition, IC_{50} = 1.52 nM	2009[147]
37		Chemical modification of existing hit compounds; inhibition of HCV activity replicon assay (isolate H77, genotype 1a), EC_{50} = 0.85 μM; inhibition JFH1 (genotype 2a), EC50 = 0.45 μM; inhibition of Con1 (genotype 1b), EC50 = 0.81 μM; CC_{50} > 16 μM; stable in pH 7.4	2015[148]

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TABLE 1 (*CONTINUED*)
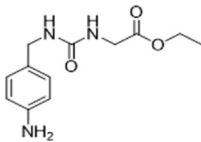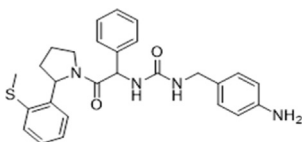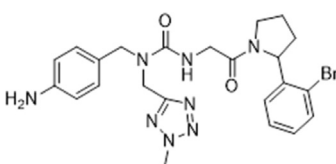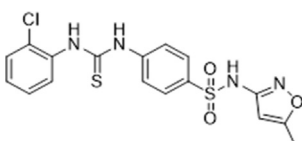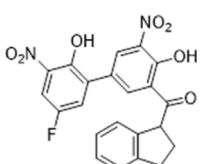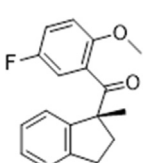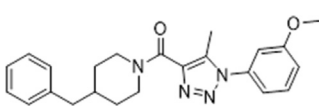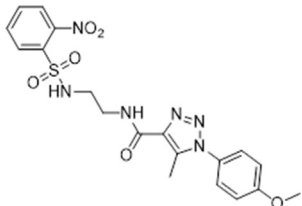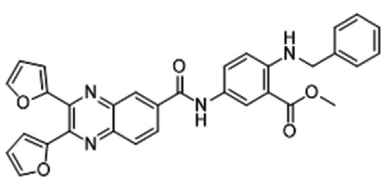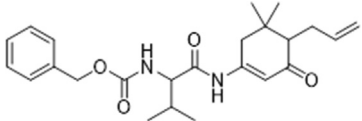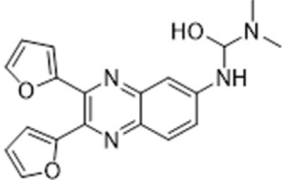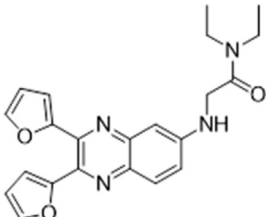
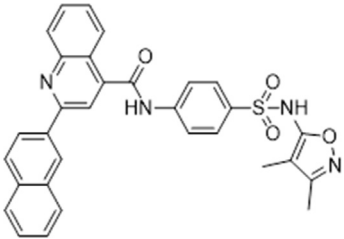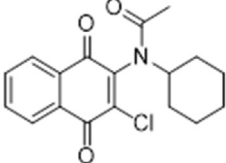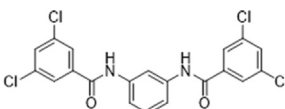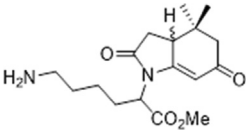| Compound no.	Chemical formula	Background and biological properties	Publication date
38		Fragment-based drug design using NMR and X-ray crystallography; CypA inhibition, $IC_{50} = 13.1\ \mu M$	2016[149]
39		Inhibition of HCV genotype 1b replicon in Huh7 cells, $EC_{50} = 0.4\ \mu M$, $CC_{50} > 100\ \mu M$; inhibition of HCoV-229E in MRC5 cells, $EC_{50} = 44.7\ \mu M$, $CC_{50} > 100\ \mu M$; no calcineurin inhibition when treated with CypA; no effect on IL-2 production	2016[149]
40		Through molecular dynamics simulation on CypA; SPR analysis, $K_d = 0.6\ \mu M$; X-ray structure determined; dose-dependent growth inhibition at low micromolar concentrations (ranging from 10 nM to 10 μM) resulting in GI_{50} values twofold better than those for CsA; less toxic than CsA in MDA-MB-231 cells	2019[150]
41		Identified through lead modification; PPIase activity assay, $IC_{50} = 0.65\ \mu M$; dual inhibitor against HIV-1 capsid and human CypA	2009[151]
42		CypA-specific inhibitor, $K_i = 0.52\ \mu M$; isothermal titration calorimetry, $K_D = 22.6\ \mu M$, inhibition on the chemotactic activity of CypA but not CypB	2009[152], 2011[153]
43		CypA-specific inhibitor, $K_i = 7.5\ \mu M$	2009[152], 2011[153]
44		Identified by virtual screening; >30% inhibition of HIV-1 replication in MT4 and U87 cells at the concentration of 10 μM	2013[154]
45		Identified by virtual screening; <30% inhibition of HIV-1 replication in MT4 and U87 cells at the concentration of 9 μM	2013[154]
46		Identified by virtual screening; SPR analysis, $K_d = 8.19\ \mu M$; PPIase inhibition, $IC_{50} = 0.25\ \mu M$	2006[155]
47		Identified by virtual screening; $K_d = 22\ \mu M$; inhibition of fecundity and growth of *Caenorhabditis elegans*, $IC_{50} = 190\ \mu M$	2007[156]

TABLE 1 (CONTINUED)

Compound no.	Chemical formula	Background and biological properties	Publication date
48		Identified by virtual screening; SPR assay, K_d = 0.218 μM; PPIase inhibition, IC_{50} = 3.47 μM; inhibition of HIV-1 replication by 65.2% at 10 μM; CC_{50} > 69.73 μM	2007[157]
49		Identified by virtual screening; SPR assay, K_d = 5.12 μM; PPIase activity on human CypA; IC_{50} = 0.312 μM	2006[158]
50		Identified by virtual screening; SPR assay, K_d = 10 μM; PPIase assay, IC_{50} = 2.84 μM; inhibition of the proliferation of mouse spleen cells at 10 μM	2006[159]
51		Identified by HTS; CypA PPIase inhibition, K_d = 2 μM determined by SUPREX (stability of unpurified proteins from rates of H/D exchange) analysis	2010[160]
52		Identified by pharmacophore-based virtual screening; IC_{50} = 930 nM, promoting neurite outgrowth with EC_{50} around 100 to 1000 nM	2003[161]
53		Native electron ionization mass spectrometry, K_d = 72.6 μM; direct stoichiometric analysis, K_d = 15.9 μM; PPIase assay, K_i = 6.8 μM; inhibition of normal growth and reproduction of C. elegans	2011[162]

pound exhibited an IC_{50} value of ∼ 0.3 μM and substantially inhibited HIV infectivity.[146]

Two papers describing the identification of a small molecular CypA inhibitor were published by the same research group in China in 2009 and 2015.[147–148] The mother skeleton of the derivative series was carbonyl urea, which was discovered by combining virtual screening and pharmacophore-based drug design. Further lead optimization of these carbonyl urea CypA inhibitors was undertaken. Compound 36 (corresponding to analog 3i in the original paper) has an IC_{50} value of 1.52 nM. Compound 37 (corresponding to compound 25 in the original paper) inhibited different HCV genotypes with IC_{50} values ranging from 0.19 to 0.85 nM, including H77 (genotype 1a), JFH (genotype 2a) and Con1 (genotype 1b) in replicon assays and virus assays without causing toxicity at a maximum concentration of 16 μM.

Fragment-based drug discovery (FBDD) is based on the identification of small organic compounds (or fragments) that are then extended or combined to yield therapeutically beneficial drug lead compounds. A novel CypA inhibitor, which was structurally different from CsA and sanglifehrin A, was identified by using a combination of the FBDD method, NMR and X-ray crystallography. Compound 38 (hit compound 22 in the original paper) showed very potent inhibition of CypA with an IC_{50} of 13.1 μM. Structure-based lead optimization of this hit resulted in compound 39 (analog 33 in the original paper) being selected as a lead compound. It inhibited CypA, CypB and CypD more potently than compound 38 and showed broad-spectrum antiviral activities against HCV genotype 1b and human coronavirus HCoV-229E, without causing cytotoxicity or immunosuppressive effects.[149]

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The CsA–CypA co-crystal X-ray structure identified Pro, Abu and three o'clock pockets, indicating that it could be used to develop a tri-vector CypA inhibitor that targets three pockets simultaneously. Molecular dynamics simulations designed for chemical optimization led to the design and synthesis of a series of linear CypA inhibitors with urea as the backbone. The most promising compound was **40** which bound to CypA, B and D with binding affinities of 0.07 to 0.6 μM. Furthermore, improved inhibition and reduced cytotoxicity were observed in cell assays, indicating that the FEB-based generation of this novel CypA inhibitor could provide a new approach for other structure-based drug designs.[150]

A CypA and HIV-1 CA dual inhibitor with thiourea was first described in 2009. The representative inhibitor, compound **41** (named D23 in the original paper), inhibited PPIase activity with an IC_{50} of 0.65 μM.[151] The activities of CypA inhibitors with the aryl 1-indanylketone structure (compounds **42** and **43**) have been discussed in many previous reports.[152–153]

From 2003 to 2013, many reports described the use of virtual screening of different compound databases to identify potential CypA inhibitors. This resulted in the identification of compounds **44** to **50**), the binding potentials of which were analyzed using SPR assays.[154–159] The promising CypA inhibitor was compound **48** (named FD-12), which had a quinoxaline-based backbone and a K_d value of 0.218 μM. In addition, compound **51**, a CypA inhibitor with a naphthalene-1,4-dione backbone and a K_d value of 2 μM, determined by HTS, was described in a paper published in 2010.[160]

Similarly, CypA inhibitors with a N,N'-(1,3-phenylene)dibenzamide structure, such as compound **52**, had IC_{50} values of ~ 930 nM. Because such compounds can protect motor neurons and promote neurite growth at a concentration of 10 μM they have the potential to treat CNS diseases.[161] Native-ESI-MS for high-throughput sampling and direct stoichiometric analysis was described by Dunsmore et al. and identified potent CypA inhibitors (including compound **53**). Such an approach might provide an alternative route for the development of other anti-HCV agents.[162]

Concluding remarks

CypA, a ubiquitous, cellular protein with PPIase activity, is involved in the replication of chronic and acute viruses, such as HCV, CoV, HIV, HBV and influenza virus, with multiple roles in regulating uncoating, intracellular trafficking, viral RNA replication, and the stability of viral proteins and even in affecting host innate immune responses. In this review, we have discussed the opposing functions of CypA (i.e., its proviral and antiviral roles) and its modes of action in different viral infections. Clinical trials to evaluate antiviral potency and safety of CsA in humans have been challenged mainly against HCV and HIV (at https://www.clinicaltrials.gov). In 2008, a clinical trial to examine CsA in HCV clearance following liver transplantation finished (Phase IV; NCT00821587). However, the immunosuppressive property of CsA still raises concerns for its application as an antiviral.[163] Long-term follow-up studies with a single treatment of non-immunosuppressive Debio-025 (Phase III; NCT02753699) or its combination with approved antivirals such as pegIFN-α2a and ribavirin (Phase II; NCT00537404) have completed against HCV infections. In parallel, Phase II studies to assess antiviral efficacy of NIM811 (NCT00983060) and SCY-6235 (NCT01265511) have been finished. To date, there is little progress report on clinical studies with Debio-25 or any other non-immunosuppressive CypA inhibitors against HIV.

The ongoing SARS-CoV-2 pandemic has generated significant interest in the feasibility of employing CypA inhibitors to combat infections caused by this highly variable RNA virus. The current urgent need for clinically efficacious antivirals has also focused attention on drug-repurposing strategies. To our knowledge, there are at least ten clinical trials of CsA, either alone or in combination with other drugs (NCT04412785, NCT04392531, NCT04540926, NCT04492891, NCT04979884, NCT04451239 and NCT04488081), and of Debio-025 (NCT04608214) and NIM-811 (NCT00983060) for the treatment of SARS-CoV-2 infection, as well as SARS-CoV-2-mediated pneumonia (at https://www.clinicaltrials.gov). In the case of SARS-CoV-2, immunosuppressive CypA inhibitors, such as CsA, could be employed additively to control severe hyperimmune responses acutely caused by this virus. By contrast, depending on host immune status, CypA inhibitors with no or weaker immunosuppression might be safer. Most of all, responding to these different clinical cases with the same virus, it is necessary to build a diverse antiviral candidate pool. For long-term development, the structural simplification is important not only for rendering them druggable by satisfying antiviral efficacy and pharmacokinetic parameters, such as immunotoxicity, oral bioavailability, membrane permeability, solubility and stability, but also for facilitating large-scale production. In line with this approach, minimizing the size of sanglifehrin-A-based derivatives has provided an important direction for the development of CypA inhibitors in recent years. Moreover, several small molecules, with excellent CypA inhibition in the nM range and reduced immunosuppression, have been discovered by HTS or computer-aided drug design methods. Basically, with understanding of biological roles of CypA, newly discovered CypA inhibitors should be reassessed in terms of their antiviral selectivity and broad genotypic or serotypic coverage.

Acknowledgements

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.drudis.2022.05.016.

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