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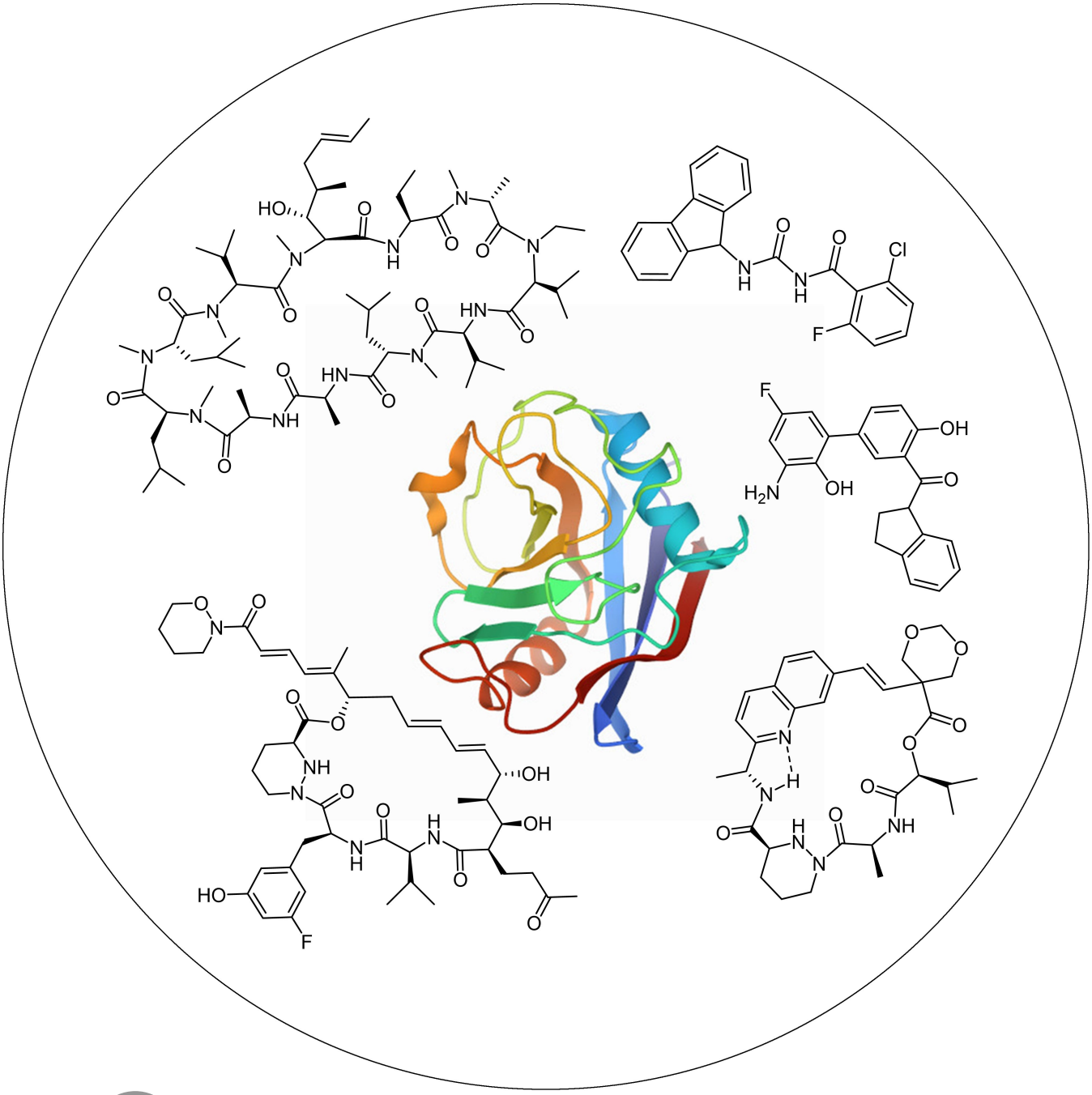
Medicinal Chemistry

Non-Immunosuppressive Cyclophilin Inhibitors

Cordelia Schiene-Fischer, Gunter Fischer,* and Manfred Braun**



Abstract: Cyclophilins, enzymes with peptidyl-prolyl cis/trans isomerase activity, are relevant to a large variety of biological processes. The most abundant member of this enzyme family, cyclophilin A, is the cellular receptor of the immunosuppressive drug cyclosporine A (CsA). As a consequence of the pathophysiological role of cyclophilins, particularly in viral infections, there is a broad interest in cyclophilin inhibition devoid of immunosuppressive activity. This Review first gives an introduction into the physiological and pathophysiological roles of cyclophilins. The presentation of non-immunosuppressive cyclophilin inhibitors will commence with drugs based on chemical modifications of CsA. The naturally occurring macrocyclic sanglifehrins have become other lead structures for cyclophilin-inhibiting drugs. Finally, de novo designed compounds, whose structures are not derived from or inspired by natural products, will be presented. Relevant synthetic concepts will be discussed, but the focus will also be on biochemical studies, structure–activity relationships, and clinical studies.

1. Introduction

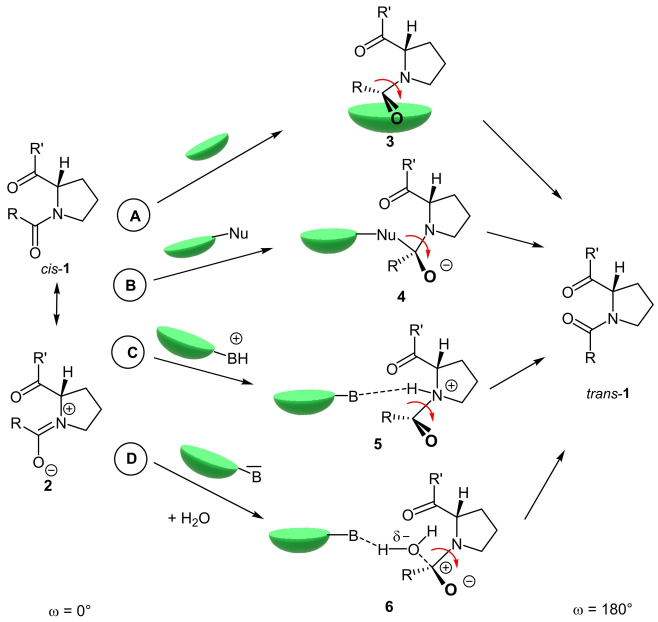
The cyclophilins belong to the family of folding helpers that have peptidyl-prolyl *cis*/*trans* isomerase (PPIase) activity in common.[1] PPIases are enzymes that catalytically accelerate the interconversion of the isomers *cis*-**1** and *trans*-**1** (Scheme 1) by rotation around the imidic peptide bond preceding a proline residue, namely the prolyl bond. In addition to cyclophilins, PPIases include the FK506-binding proteins and the parvulins.[2]

The rotation around the prolyl bonds is characterized by an activation barrier of 75 to 100 kJ mol^{-1} originating from the amide resonance that, expressed by the mesomeric formula **2**, provides a rationale for the partial double-bound character of the amide bond. In the course of this process, the torsion angle ω changes from 0 to 180°. In peptides and unfolded proteins, the isomer *trans*-**1** predominates; however, native proteins frequently contain a specific prolyl bond in the *cis* conformation. Additionally, the *cis*/*trans* isomerization of specific prolyl bonds contributes to the conformational heterogeneity of native structured proteins.

Several molecular mechanisms, shown in Scheme 1, were proposed to rationalize the manner in which PPIases catalytically lower the energy barrier of the *cis*/*trans* isomerization.[3] The molecular basis of cyclophilin catalysis is not yet fully understood. Experimental support from structural data, site-directed mutagenesis, and kinetic experiments has been obtained for different molecular mechanisms, including catalysis by distortion (path A) and hydrogen bond formation between a cyclophilin active site group and the proline nitrogen atom (path C).

The importance of cyclophilins for living systems is manifested by their presence across all kingdoms of life in all kinds of cells. Usually, several cyclophilin isoforms are expressed in the same species.[4]

The prototypic cyclophilin A (CypA) is the most abundant member of the cyclophilin family in human tissue and the major player in the cellular PPIase activity.[5] CypA is the cellular receptor of the immunosuppressive drug cyclosporin A (CsA, **7**),[6] a cyclic undecapeptide (Figure 1, top) that was discovered and developed at Sandoz in the 1970s and, since then, has proven to be indispensable in organ transplantation.[7] It is also used for the treatment of



Scheme 1. Possible catalytic mechanisms for the peptidyl-prolyl *cis*/*trans* isomerization. A) Stabilization of the twisted amide transition state **3** by a hydrophobic environment. B) Formation of the tetragonal intermediate **4** by nucleophilic catalysis. C) Protonation of the imide nitrogen atom (**5**). D) Stabilization of transition state **6** by water. R,R′: polypeptide chains.

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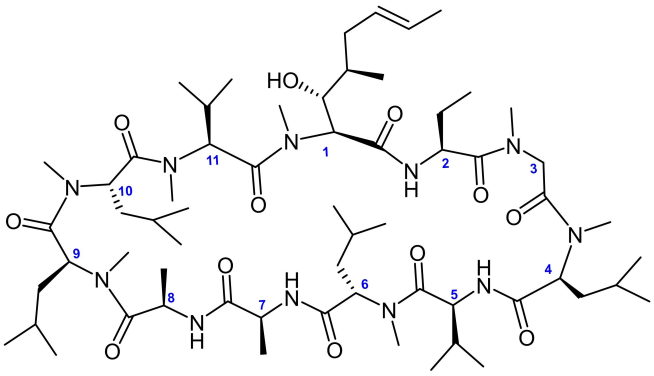

Cyclosporin A (CsA) (7)

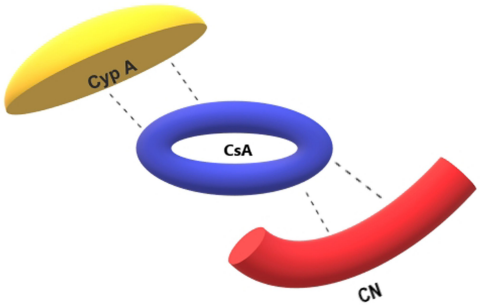

Figure 1. Structure of CsA (**7**; top). Schematic representation for visualizing the binding in the ternary complex CypA-CsA-CN (bottom).

rheumatoid arthritis, psoriasis, nephrotic syndrome, and dry eye syndrome.[8]

CsA binds to CypA through the formation of a binary complex with a dissociation constant in the lower nanomolar range, thereby inhibiting the PPIase activity of CypA. CsA-mediated immunosuppression is achieved by a so-called *gain-of-function* mechanism characterized by the formation of a ternary complex between the drug CsA, the PPIase CypA, and the Ca^{2+}-dependent phosphatase calcineurin (CN), in which the phosphatase activity of CN is blocked and thus dephosphorylation and nuclear shuttling of the nuclear factor of activated T-cells (NFAT) is inhibited.[9] Structure determination of the CypA-CsA-CN ternary complex reveals that the active site of CypA binds to the "north-western" region encompassing amino acid residues 1, 2, 9, 10, and 11, whereas CN occupies the "south-eastern" region of the cyclo-undecapeptide CsA including the amino acid residues 4 to 7 (Figure 1, bottom).

As a consequence of the functional importance of cyclophilins for a large variety of biological processes, there is considerable interest in the development of PPIase inhibitors as mechanistic tools and potential drugs for various diseases.[4c,10] In particular, compounds that solely inhibit cyclophilins without side activities are highly desirable. As cyclophilins were found to function as host proteins that a variety of different viruses need to replicate, it was an intriguing idea to develop inhibitors of cyclophilins as antiviral drugs.[11] However, if an antiviral cyclophilin-binding drug is aimed at, any immunosuppressive activity is, quite obviously, highly contraproductive as it would lock-down the immune system. The development of cyclophilin

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Gunter Fischer studied chemistry at the University of Halle and completed his PhD under the guidance of Professor Alfred Schellenberger in 1971. He completed his habilitation there in 1978. In 1992 he became the group leader of the research group "Enzymology of the Peptide Bond" of the Max Planck Society in Halle. In 1992, he became a professor of molecular biochemistry at the University of Halle. In 1997 he became director of the Max-Planck Research Unit for Enzymology of Protein Folding in Halle. His research focused on the structure and activity of protein-folding helper enzymes and the effect of imino acids on protein conformations. He retired in 2012.

Manfred Braun studied chemistry at the University of Karlsruhe and completed his PhD with Professor Dieter Seebach in Gießen in 1975. After postdoctoral research with Professor George H. Büchi at the Massachusetts Institute of Technology, he joined the group of Professor Hans Musso at the University of Karlsruhe and completed his Habilitation there in 1981. In 1985, he became a professor of organic chemistry at the Heinrich-Heine-University Düsseldorf. His areas of research included the development of new synthetic methods (especially for asymmetric synthesis) and syntheses of biologically active compounds. He retired in 2014.

inhibitors that function as antiviral agents, therefore, requires the immunosuppressive activity arising from CN inhibition to be reduced or removed, in other words: the binding of either a derivative of CsA or any other de novo designed cyclophilin inhibitor to CN has to be prohibited.

2. Cyclophilins

2.1. Cyclophilins and their Physiological Roles

Cyclophilins are highly conserved through evolution, with their typical three-dimensional structure consisting of an eight-stranded antiparallel β barrel capped by α helices.

Among the eighteen different cyclophilin isoenzymes in humans, there are eight single-domain cyclophilins consisting only of the cyclophilin domain itself of about 18 kDa and in some cases with additional localization signals, and ten multidomain proteins which are formed by the prototypical cyclophilin domain supplemented by N- or C-terminally attached domains or modules with different regulatory or signaling functions. The most prominent member of the single-domain cyclophilins, the cytoplasmic CypA, was the first PPIase discovered.[1a] It reaches high levels in many mammalian cells; for example, it represents 0.4 % of the total cytosolic protein fraction of T cells.[12] Other different cyclophilin isoenzymes can be found specifically in distinct subcellular compartments of cells. Human cyclophilin B (CypB) mainly differs from CypA by the presence of a cleavable N-terminal signal sequence that directs the protein to the endoplasmic reticulum (ER), whereas human cyclophilin D (CypD) is a single-domain cyclophilin that targets the mitochondrial matrix, and eight cyclophilins are mainly found in the nucleus. Interestingly, several nuclear cyclophilins have been identified in specific spliceosomal complexes.[13] In the larger cyclophilins, the cyclophilin domain is accompanied by different functional domains which are known to be implicated in intracellular targeting, RNA recognition, and protein–protein interactions.[4b]

To the conserved signature amino acid residues involved in the formation of the active site and essential for the PPIase activity of cyclophilins belong a phenylalanine residue and an arginine residue, which are in position 60 and position 55 of the prototypic human CypA, respectively (Figure 2). For CypA, it was shown that the side chain of Phe60, as part of the binding pocket of the prolyl ring, mediates correct substrate positioning, and the Arg55 guanidinium group probably promotes catalysis by stabilizing sp^3 hybridization of the proline nitrogen atom in the transition state (Scheme 1, path C).

For the evaluation of the PPIase activity of cyclophilins, processes can be used whereby the *cis*/*trans* isomerization of a prolyl bond is coupled to a fast irreversible process with high specificity for one of the isomers. Conveniently, the proteolysis of model substrates of the form succinyl-Ala-Xaa-Pro-Yaa-*p*-nitroanilide by isomer-specific proteases, such as chymotrypsin or trypsin cleaving the Yaa-*p*-nitroanilide bond only if the Xaa–Pro bond is in the *trans* conformation, is applied to monitor the *cis*/*trans* isomerization of the Xaa–Pro bond and its acceleration by cyclophilins or other PPIases.[1a] Modifications of the assay system were applied to improve the signal amplitude and even allow protease-free detection of the PPIase activity.[14]

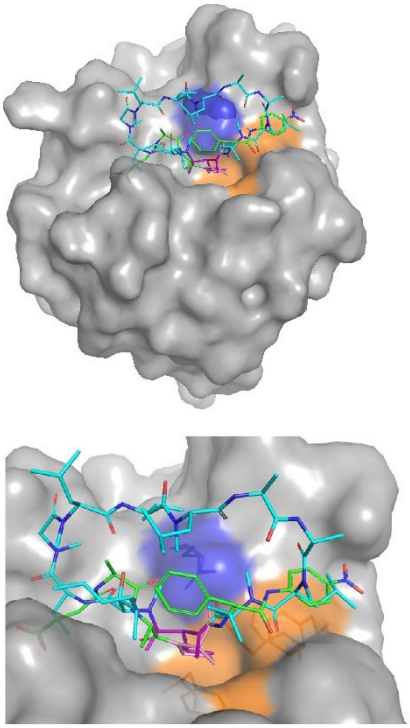


Figure 2. Superposition of CypA complexes (top) with the substrate succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide (green, PDB ID: 1RMH)[35] with the Pro residue shown in pink and CsA (cyan, PDB ID: 1CWA)[36] with [MeVal]^{11} in purple (top). Enlargement of the PPIase active site (bottom). The surface of Phe60, Met61, Phe113, and Leu122 is highlighted in orange and that of Arg55 in blue.

In general, cyclophilins exhibit a relatively broad specificity for the amino acid residue Xaa.[15] Several of the single-domain cyclophilins display a high catalytic power, with catalytic efficiencies in the diffusion-controlled range, whereas multidomain cyclophilins usually exhibit specificity constants significantly lower than that of CypA.[3]

Cyclophilins are involved in a wide variety of cellular processes such as folding, posttranslational modifications and transport of proteins, assembly of essential cellular protein complexes, and cell signaling. Numerous proteins have been shown to interact with, and be functionally controlled by, specific cyclophilins.[16] Three mammalian single-domain cyclophilin isoenzymes, CypA, CypB, and cyclophilin C (CypC), which are found not only inside cells but also in the extracellular space, are implied in the control of cell–cell communication.[4b,17]

Cyclophilins are targets of posttranslational modifications which may influence the PPIase activity as well as the susceptibility of these enzymes to inhibition. CypA, for example, is found to be acetylated inside cells and in the extracellular space. It was shown that the acetylation of CypA at Lys128 reduces the PPIase activity of the enzyme

and decreases the binding to CsA, although the affinity of acetylated CypA to CsA is still in the nanomolar range.[18] However, to date, the effects of posttranslational modifications on the enzymatic properties of cyclophilins have not been studied in detail for the different cyclophilin isoenzymes.

The availability of specific inhibitors of a distinct isoform of the cyclophilin would be beneficial to elucidate the role of the different cyclophilin isoenzymes as well as for low-dose application in the cellular context. On the other hand, the existence of pan-cyclophilin inhibitors offers the opportunity to suppress processes where there is a redundancy of different cyclophilins.

2.2. Pathophysiological Significance of Cyclophilins

The physiological functions of distinct cyclophilins are often of pathophysiological relevance and cyclophilins are implicated in various diseases.[4b,19]

2.2.1. Cyclophilins in Viral Infection

CypA as a host cell protein interacts with viral proteins and can thus promote or inhibit viral replication and infection of a variety of RNA viruses, most prominently HIV-1 and HCV. The CypA-dependent viruses also include the influenza A virus, flaviviruses such as the West Nile virus, dengue virus, tick-borne encephalitis virus, yellow fever virus, and Zika virus, as well as nidoviruses such as NL63, MERS- and SARS-coronavirus.[19f,g,20] For some viruses, an interaction between viral proteins and cyclophilin isoforms other than CypA—mostly with CypB—have been described.

In HIV-1, CypA binds to the capsid p24 protein at the sequence around Gly89 and Pro90 and is incorporated into nascent virions. CypA seems to be involved in several steps during HIV-1 replication and infection. It is considered to promote the uncoating of the virus particle, reverse transcription, and nuclear import, and it has been shown to protect HIV-1 to restriction by the restriction factor tripartite-containing motif (TRIM) 5α before reverse transcription.[19f,21] In HCV, CypB was initially found to interact with the viral RNA polymerase NS5B. Later on, the direct interaction between CypA and domains II and III of the NS5A protein was found to be important for HCV replication and assembly. NS5A and CypA play a role in the formation of membranous replication organelles that are thought to shield viral replication intermediates from cytosolic pattern recognition receptors.[19g,22]

The inhibition of CypA by CsA and CsA analogues was reported to be clinically effective against HCV and HIV-1.[11a,b] Such host-directed antiviral compounds are considered to be beneficial because of the low probability of resistance emerging and the broad specificity for different subtypes of a virus.

CsA was also shown to suppress the replication of various kinds of coronaviruses such as HCoV-229E and HCoV-NL63 as well as MERS- and SARS-coronavirus (CoV) in human cells, which implicates the involvement of cyclophilins.[23] Consistently, a depletion of CypA in human cells suppressed HCoV-229E, HCoV-NL63, and MERS-CoV replication.[20c,23b] For SARS-CoV, the role of CypA remains unclear, since the depletion of CypA in human cells did not result in a reduction in SARS-CoV replication.[24]

The evaluation of different drugs with known antiviral activity against the novel SARS-CoV2 showed an inhibitory potency of CsA with a low micromolar IC_{50} value against virus production and the CypA/nucleocapsid (N) protein interaction.[25] Most interestingly, the application of CsA in addition to steroid treatment was found to lead to a decrease in mortality of COVID-19 patients.[26] This outcome may result from the CypA/CsA-mediated inhibition of CN reducing hyperinflammation in COVID-19; nevertheless, direct inhibition of CypA could also be involved in the effectiveness of CsA because of the involvement of CypA in both viral replication as well as inflammation.

2.2.2. Cyclophilins in Inflammation

Cyclophilins secreted into the extracellular space are considered, in particular, to play an important role in human diseases associated with acute or chronic inflammation such as rheumatoid arthritis, sepsis, asthma, and cardiovascular diseases.[4b,19e,27,28] In these cases, CypA is involved in abdominal aortic aneurysm formation, atherosclerosis, myocardial ischaemia and reperfusion, inflammatory and hypertrophic cardiomyopathies, and thrombosis. Hallmark events of these diseases were found to be reduced in CypA knockout mouse models and by CypA inhibition.[18b,28]

Extracellular cyclophilins initiate signaling cascades of inflammatory processes by binding to the transmembrane protein CD147.[17c,19e]

2.2.3. Cyclophilins in Cell Death Related Processes

The mitochondrial cyclophilin CypD as a regulator of the opening of the mPTP—a hallmark of mitochondrial dysfunction, which leads to cell death—is implicated in pathophysiological processes associated with necrosis and apoptosis, such as ischemia/reperfusion injury in in the brain, heart, and kidney, in neurodegenerative disorders, muscular dystrophies, and non-alcoholic steatohepatitis (NASH).[29] In Alzheimer's disease, CypD is involved in Aβ-induced mitochondrial perturbation and associated neuronal cell death, for which the direct interaction of CypD with mitochondrial Aβ was found to be important.[30]

2.2.4. Cyclophilins in Cancer

The expression of members of the cyclophilin family is upregulated in several human malignancies. For example, the overexpression of CypA is associated with hepatocellular, gastric, nasopharyngeal, and squamous cell carcinoma as well as pancreatic cancer.[31] The increased CypA expression

in tumor cells appears to be causally connected with malignant cell transformation: the reduction of CypA expression by RNAi led to the inhibition of tumor growth.[32] Possibly, CypA promotes cellular survival under the stressful conditions of cancer and may be important to maintain the active conformation of oncogenic proteins.

3. Non-Immunosuppressive Cyclooligopeptides Derived from Cyclosporine A

3.1. General Aspects of Inhibition by CsA and Strategies for CypA Modification

As a consequence of its nanomolar affinity to CypA, the cyclic undecapeptide CsA isolated from the fungus *Tolypocladium inflatum* can be considered as the gold standard of cyclophilin inhibition. However, because CsA does not only inhibit the PPIase activity of cyclophilins but also in complex with CypA the phosphatase activity of CN and the activity of several cellular transporters,[33] the biological consequences of the application of CsA should be considered to originate from either i) direct inhibition of the PPIase activity of one or more members of the cyclophilin family, ii) *gain-of-function*-mediated inhibition of the phosphatase activity of CN, or iii) the inhibition of a transporter.

A double strategy was applied to develop non-immunosuppressive derivatives of CsA: maintain or even enhance the binding to cyclophilin and reduce, as much as possible, the affinity towards CN. For effective CypA binding, the residues of the "north-western" region CsA are required. In the CypA-CsA complex, the central $[MeVal]^{11}$ moiety of CsA resides in the proline binding pocket formed by residues Phe60, Met61, Phe113, and Leu122 in CypA (Figure 2). In the cyclophilin-bound state, the amide bond between $[MeLeu]^{9}$ and $[MeLeu]^{10}$ adopts a *trans* conformation, and the amide bond between $[MeBmt]^{1}$ and $[Abu]^{2}$ is exposed and, thus, available for intermolecular hydrogen bonding to CypA residues Glu63 and Asn102.[34]

The conformation adopted by CsA when bound to the active site of cyclophilins is very similar to the one in water and other polar solvents,[37a] and differs from the crystalline state by its 9,10-*cis*-amide bond.[37b] By binding to the cyclophilin active site, CsA acts as a competitive tight binding inhibitor of the PPIase activity of cyclophilins, with a K_i value of 1.6 nM for CypA. The tight binding inhibition by CsA is dependent on the presence of a tryptophan residue (Trp121 in CypA) in the cyclophilin active site, which is present in nine of the human cyclophilins.[14a,38]

Various approaches have been taken to obtain non-immunosuppressive derivatives of CsA: firstly, by fermentation, which is not only the method used for the industrial production of the parent compound, but also used for several derivatives. Secondly, total syntheses have also been performed. They rely on established methods of peptide synthesis to build the linear undecapeptide that is subsequently cyclized.[39] Total syntheses were performed to obtain special derivatives or metabolites, but rarely for synthesizing drugs. In the last case, partial synthesis is the method of choice, which starts from the parent compound or a biotechnologically produced derivative and modifies them by chemical or enzymatic conversions.

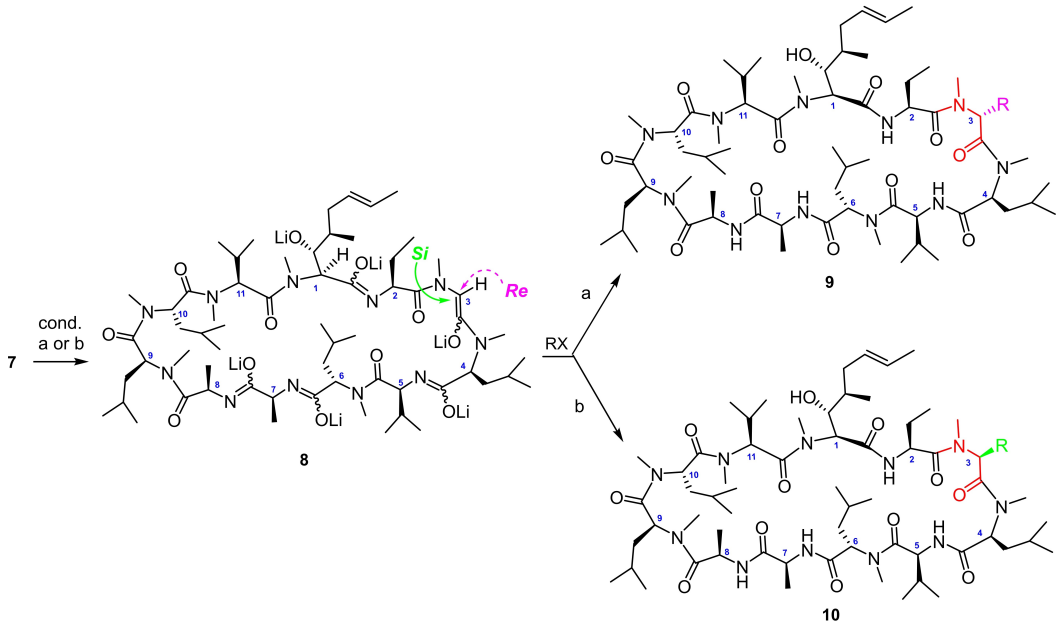
3.2. Non-Immunosuppressive CsA Derivatives Modified in Positions 3 and 4

3.2.1. Modifications of Position 3 ($[Sar]^3$)

Interestingly, the modification of residue 3 of CsA may affect CN binding and also impact Cyp binding either directly or through conformational changes. $[Sar]^3$ is located at the interface of the two proteins in the Cyp-CsA-CN complex. A seminal and imaginative method for CsA derivatization at this position was developed by the Seebach group in collaboration with Sandoz in the 1990s.[40] They studied CsA metalation with lithium diisopropylamide (LDA) and/or *n*-butyllithium (BuLi), both used in high excess. Although numerous side reactions and decomposition were envisaged, the metalation experiments—clearly performed in an optimistic manner—cleanly led to the hexalithiated intermediate **8**, which consists of a lithium alkoxide, four lithium azaenolates, and one lithium enolate moiety derived from sarcosine (Scheme 2). The subsequent alkylation is highly selective and occurs exclusively at the sarcosine enolate in position 3.

Furthermore, control over the diastereoselectivity is achieved: depending on the reaction conditions (LDA versus LiCl/LDA/BuLi), either diastereomer **9** is formed by a predominant *Re*-face attack to the sarcosine-enolate moiety, or diastereomers **10** are obtained selectively by an *Si*-face attack. Although the diastereoselectivity is not complete, the major isomer could in most cases be easily isolated by column chromatography. At first glance, this divergent stereochemical outcome is surprising, as the "same" intermediate **8** serves as the nucleophile. However, one has to be aware of the different solvation of the lithium enolate under the different conditions.[41]

Remarkably, two sarcosine-alkylated products **9**, namely $[D\text{-}MePhe]^3$- and $[D\text{-}(3\text{-hydroxy})\text{–}MePhe]^3$-CsA, [**9**, $R = CH_2Ph$ and $R = CH_2(3\text{-}HO)C_6H_4$], prepared according to this procedure, turned out to be the first compounds that were non-immunosuppressive, while binding to CypA with an affinity similar to CsA.[42] The overall metalation of CsA and various derivatives thereof followed by treatment of the enolate with different carbon and hetero-electrophiles enabled the introduction of a large variety of side chains. When these chains contain functional groups, the possibility for further derivatization is opened.[43] For example, the benzoate substitution in sarcosine derivative $[(4\text{-carboxy})\text{-}MePhe]^3$-CsA [**9**/**10**, $R = CH_2(4\text{-}CO_2H)C_6H_4$], which inhibits the PPIase activity of CypA and CypD similar to CsA itself, was used to conjugate, through a spacer, the lipophilic alkyltriphenylphosphonium cation to CsA for mitochondrial targeting.[44]



Scheme 2. Modification of [Sar]^3 in CsA by alkylation of the enolate **8**. Conditions: a) 6–14 equiv LDA, electrophile RX, ca. −78 °C, THF; b) up to 30 equiv LiCl, 6.5 equiv LDA, 6 equiv BuLi, electrophile RX, ca. −78′ in THF.

3.2.2. Modifications of Position 4 ([MeLeu]^4)

A very efficient modification for the removal of immunosuppression is the substitution of the [MeLeu]^4 residue of CsA. Early studies on CsA derivatives performed at Sandoz led to the conclusion that CN binds tightly with the side chain in position 4.[45] An explanation for this effect was provided by the structure elucidation of the Cyp-CsA-CN complex, which revealed that the [MeLeu]^4 residue is involved in a tight "aromatic sandwich" with the Trp352 and Phe356 moieties of the catalytic subunit of CN, which constitutes the major binding force to this protein (Figure 3).

This is in accordance with the previous observation that a seemingly small alteration, the replacement of *N*-methylleucine by *N*-methylisoleucine, led to the derivative [MeIle]^4-CsA, which became known as NIM-811 (**11**). This compound was produced by fermentation of a strain of *Tolypocladium inflatum* (syn. *T. niveum*) in the presence of D-threonine in the culture medium and isolated by repeated chromatography from a complex mixture of cyclosporins, with CsA as the major component.[46] Another early observation indicating that the methylene group of the MeLeu residue of CsA is crucial for the interaction with CN and that α-branched residues in position 4 of CsA are beneficial to reduce the immunosuppressive properties of the compound was provided by the derivative [MeVal]^4-CsA (**12**): It binds with a similar affinity as CsA to CypA but exhibits a 2500-fold loss of immunosuppressive activity.[47] A similar decrease in immunosuppressive activity was observed for the respective *N*-ethyl derivatives [EtIle]^4-CsA and [EtVal]^4-CsA.[48] Furthermore, a hydroxylated [MeLeu]^4 residue found in [4′HO-MeLeu]^4-CsA results in the reduction of the immunosuppressive properties of the

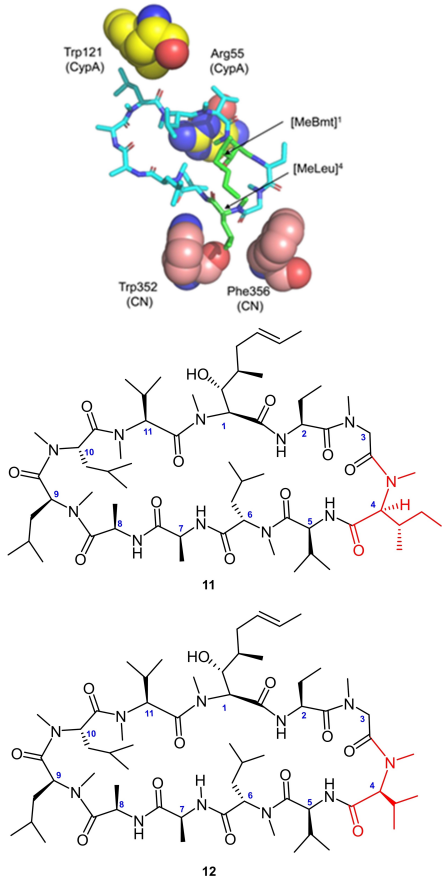


Figure 3. Location of amino acid residues of CypA and CN relevant for CsA binding or catalysis relative to CsA in the CypA-CsA-CN complex (PDB ID: 1M63)[9b] (top); structures of non-immunosuppressive CsA derivatives [MeIle]^4-CsA (NIM-811; **11**) and [MeVal]^4-CsA (**12**; bottom).

compound.[49] Several relatively small modifications at position 4 result only in minor changes of the immunosuppressive potential of the compound compared to CsA.[45]

NIM-811 (**11**) exhibits a strongly reduced immunosuppressive activity that is 1700 times less than that of CsA but retains full capacity to inhibit CypA PPIase activity with a K_i value of 2.1 nM.[50] The CsA derivative exhibits potent antiviral activity against HIV-1 and HCV.[46,51] The application of NIM-811 (**11**) also results in anti-inflammatory effects. It reduces the number of leukocytes in lung tissue and the airways of allergic mice and inhibits platelet activation during platelet-dependent thrombus formation.[52]

Similar to CsA, NIM-811 (**11**) is able to inhibit the mitochondrial cyclophilin CypD which controls the opening of the mitochondrial permeability transition pore.[53] This inhibition results in suppression of the mitochondrial permeability transition, a key event in cell death. Thus, this inhibition is considered to form the basis for the protecting effects of NIM-811 (**11**) against ischemia/reperfusion injury of heart, liver, and skeletal muscle. Furthermore, in contrast to CsA itself, NIM-811 (**11**) is able to penetrate the intact blood–brain barrier, which allows mitochondrial permeability transition to be prevented and thus to protect against cerebral ischemia/reperfusion injury.[54] Additionally, NIM-811 (**11**) has a beneficial effect in models of collagen VI congenital muscular dystrophy, in which mitochondrial permeability transition pore opening forms a key factor of the pathogenesis.[55]

Modification of position 4 appears not to influence the inhibition of the PPIase activity of CypD: [MeVal]^4-CsA (**12**) exhibits only a slightly decreased inhibition of CypD PPIase activity compared to CsA, but still inhibits the mitochondrial permeability transition pore opening efficiently.[56] The antiangiogenetic property of [MeVal]^4-CsA (**12**) provided proof of a CN-independent mechanism of CsA-mediated inhibition of angiogenesis.[57]

3.2.3. Modifications of Positions 3 and 4: Alisporivir (13), SCY-635 (14), STG-175 (15), and NIM-258 (16)

Based on these beneficial effects exhibited by structural variation of the amino acids at positions 3 or 4, further non-immunosuppressive derivatives were developed through the combination of modifications at positions 3 and 4 in CsA. This concept turned out to be fruitful and led to the drug alisporivir (Debio025; **13**) as the most advanced drug in this series as well as SCY-635 (**14**; Figure 4). Besides the exchange of [Sar]^3 by [MeAla]^3, the *N*-methylleucine residue in position 4 of CsA is replaced by *N*-ethylvaline in alisporivir (**13**), whereas SCY-635 (**14**) features an *N*-methylhydroxyleucine residue in position 4 and the sarcosine moiety is replaced by dimethylaminoethylthiosarcosine. Additional non-immunosuppressive derivatives of CsA subsequently developed with modified amino acids in positions 3 and 4 are STG-175 (**15**) and NIM-258 (**16**). The former compound has in common with SCY-635 the hydroxylated *N*-methylleucine, but differs by the (hydroxybutylthio)methylsarcosine moiety in position 3. The CsA derivative NIM-258 (**16**) is substituted by a (2-methoxyethyl)piperazine unit in the side chain at position 4 in addition to the [MeAla]^3 modification. All four compounds were obtained from the parent compound CsA by partial syntheses.

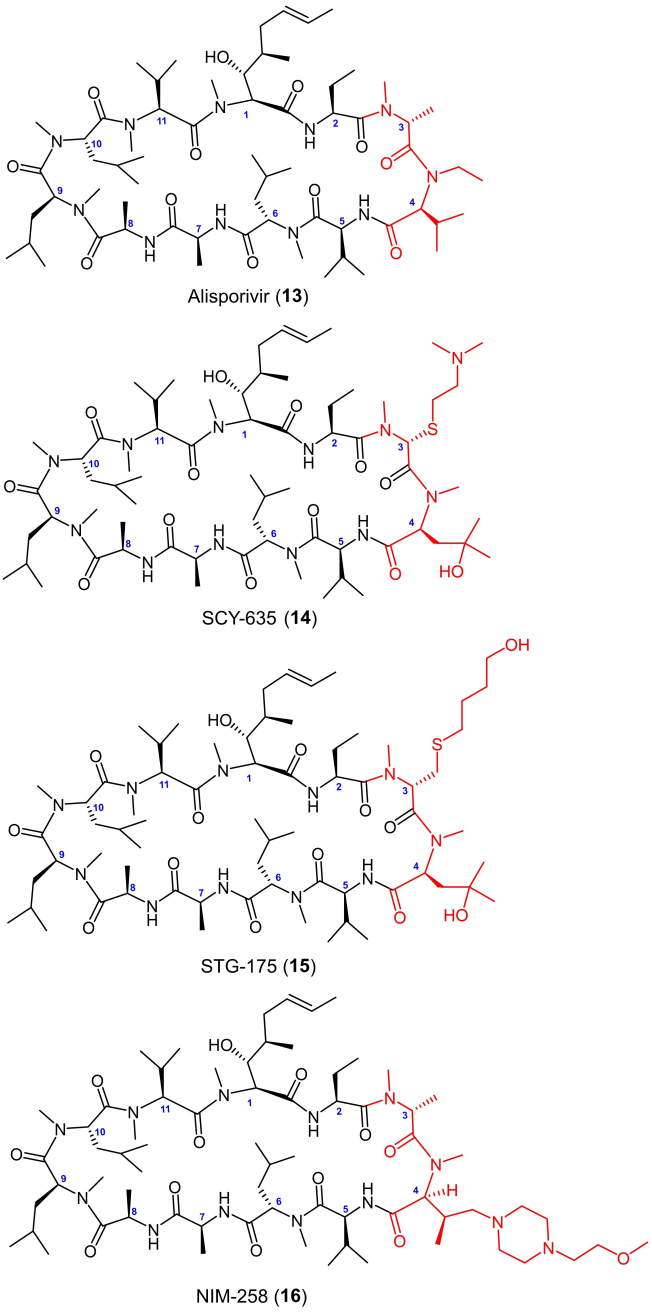
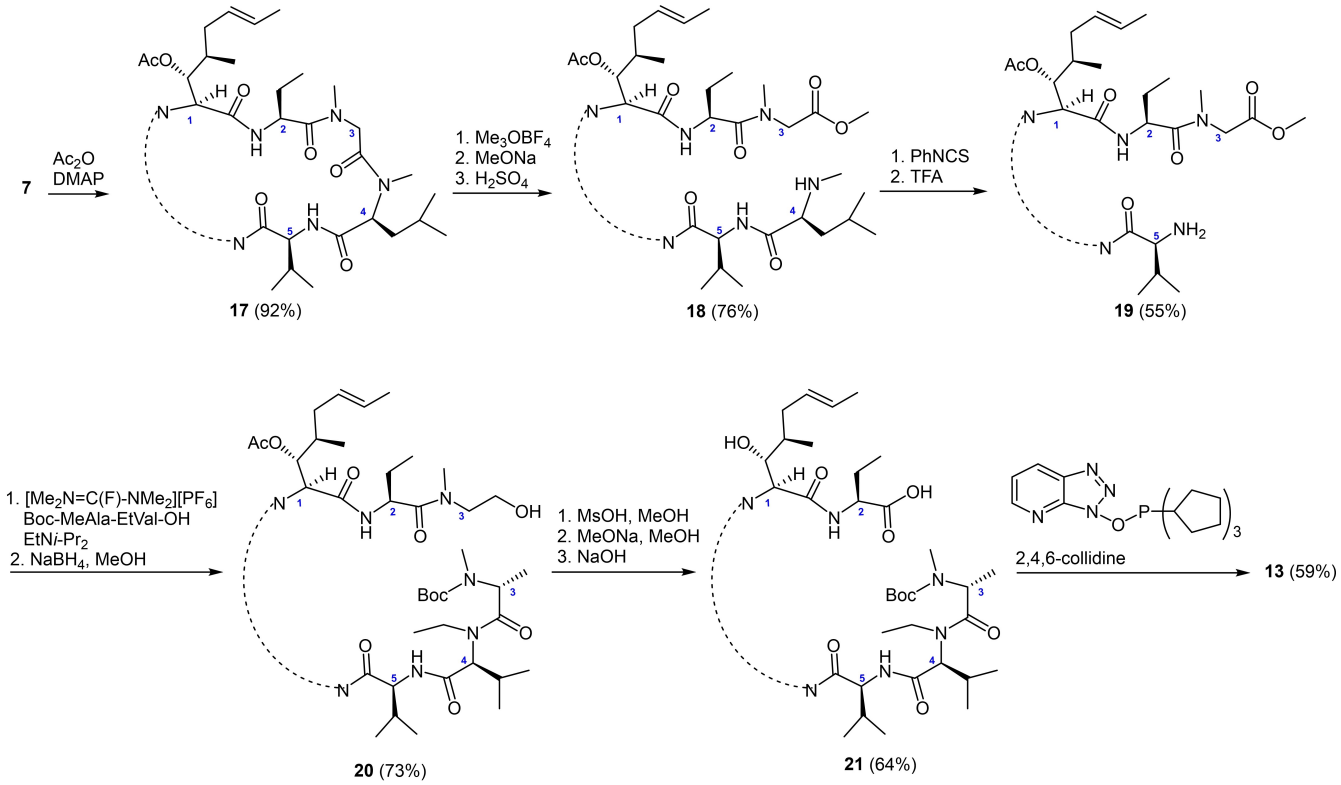


Figure 4. Structures of non-immunosuppressive CsA-Derivatives **13**–**16**, modified at positions 3 and 4.

The synthetic route to alisporivir (**13**), the drug developed by Novartis/Debiopharm,[58] is displayed in Scheme 3. It involves a sequence of classical methods with an elegant key step: the selective cleavage of the peptide bond at the "predetermined breaking point" between amino acid moieties 3 and 4. This was accomplished, after protection of the secondary alcohol in the alkenyl side chain, by treatment of compound **17** with Meerwein's salt, which selectively reacts



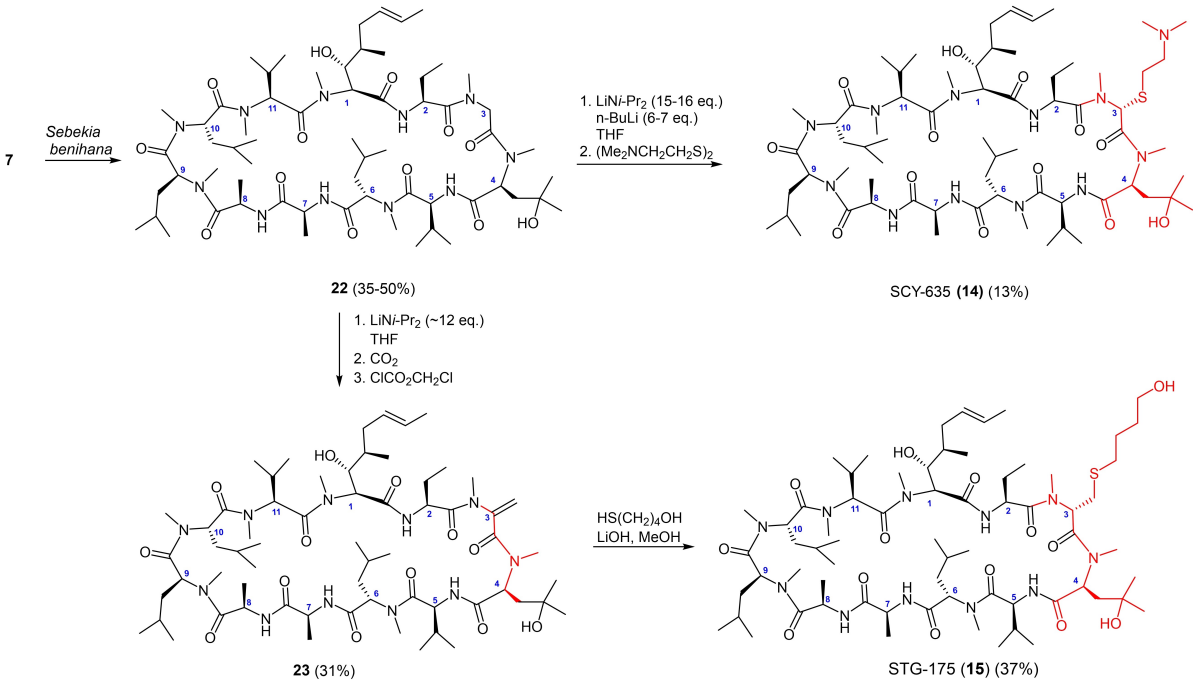
Scheme 3. Synthesis of alisporivir (**13**) from CsA (**7**).

with the only α-unsubstituted amino acid, sarcosine, in position 3. The imino ether thus formed was converted in situ to the methyl ester **18** through ring opening of the cyclopeptide skeleton. A subsequent Edman degradation removed [MeLeu]^4 with formation of compound **19**. For the planned modification of CsA, the dipeptide Boc-MeAla-Et-Val-OH was introduced by coupling through the acid fluoride. Then, reduction of the methyl ester delivered compound **20**. Subsequent acidic and basic hydrolyses liberated the aminobutyric acid in position 2 and cleaved the acetate ester and Boc protecting group to give amino acid **21**. Finally intramolecular peptide coupling generated alisporivir (**13**).[58a]

This synthetic concept was applied with the appropriate modification also for the partial synthesis of NIM-811 (**11**)[43] and provided an alternative route to the fermentation process outlined above. The methodology applied in the synthesis of alisporivir according to Scheme 3 is based upon earlier studies of the systematic exchange of amino acid moieties though seco-CsA, the open-chained undecapeptide. The subsequent building of the modified peptide was also performed by solid-phase syntheses.[39]

A two-step synthesis was developed for SCY-635 (**14**; Scheme 4). Firstly, a hydroxylation of the leucine side chain was performed using a previously disclosed biotransformation of CsA by the microorganism *Sebekia benihana* to yield compound **22**.[59] Subsequently, Seebach's polylithiation method was applied to the cyclopeptide **22**, including a deprotonation of the [(4′-OH)MeLeu]^4 hydroxy group. The enolate position of [MeSar]^3 is the most reactive nucleophile, so that treatment with the disulfide $(Me_2NCH_2CH_2S)_2$ resulted in the formation of the drug SCY-635 (**14**), as disclosed by Aventis Pharma and further developed by Aventis spinoff SCYNEXIS.[60] Despite the poor yield, the straightforwardness of the synthesis is attractive. The CsA derivative **22** with the hydroxylated leucine residue in position 4 is also used as an intermediate in a straightforward synthesis of STG-175 (**15**), as shown in Scheme 4.[61] Here the reactive enolate generated by polylithiation enabled the introduction of a methylene group into the [Sar]^3 residue. This was accomplished by carboxylation of the enolate followed by treatment with chloromethyl chloroformate in a one-pot reaction. The thus-generated compound **23** underwent a diastereoselective vinylogous addition of 4-mercapto-1-butanol to yield STG-175 (**15**).

Both alisporivir (**13**) and SCY-635 (**14**) are potent inhibitors of CypA PPIase activity, with K_i values of 0.34 nM and 1.8 nM, respectively.[50b,62] They exhibit potential for a wide range of therapeutic applications. Special focus has been placed on their prominent antiviral properties against HCV and HIV, thus making them promising candidates for the treatment of viral infections. Alisporivir (**13**) and SCY-635 (**14**) were shown to display potent in vitro anti-HIV-1 activity with EC_{50} values in the nanomolar range;[50b,60] however, further evaluation of alisporivir in HIV patients showed only a limited efficacy of the compound in contrast to a considerable anti-HCV activity.[63] In the case of HCV, clinical trials have shown that treatment with alisporivir (**13**) as well as with SCY-635 (**14**) results in a decrease in the viral RNA in the plasma of infected



Scheme 4. Synthesis of CsA derivatives SCY-635 (**14**) and STG-175 (**15**).

patients.[64] Both compounds perturb the interaction of CypA with the viral NS5 A protein and prevent viral RNA production. Alisporivir (**13**) and SCY-635 (**14**) inhibit HCV replication in cell cultures with EC_{50} values of 0.03 μM and 0.1 μM, respectively.[62,65]

Alisporivir (**13**) was also found to inhibit the replication of the flavivirus tick-borne encephalitis virus in different infected cell lines.[66] Information about the effectiveness of CsA derivatives for other flaviviruses shown to be susceptible to CsA is lacking.

Alisporivir was also shown to have antiviral activity against coronaviruses in cell cultures. Alisporivir (**13**) inhibited the virus-induced cytopathic effect observed in MERS- and SARS -infected cells as well as HCoV-NL63 and HCoV-229E replication with EC_{50} values in the lower micromolar range depending on the strain and cell line used.[23b,d,67] The relatively high concentrations of alisporivir required for SARS-CoV inhibition in cell culture compared to the inhibition of HCV may account for the ineffectiveness of the compound in a mouse model of SARS-CoV infection. Most interestingly, alisporivir (**13**) reduced SARS-CoV-2 infection in a dose-dependent manner in a Vero E6 cell model with an EC_{50} value of 0.46 μM. Therefore, it was suggested to test alisporivir (**13**) in patients with or at risk of severe forms of SARS-CoV-2 infection.[68] Other diseases where cyclophilins play key roles have also been investigated for potential treatment by alisporivir (**13**) and SCY-635 (**14**). Both drugs inhibit the PPIase activity of the mitochondrial cyclophilin CypD nearly as efficiently as that of CypA.[61a] Thus, beneficial effects were observed in the alisporivir-mediated inhibition of CypD, which is involved in the regulation of cell death by control of the mitochondrial permeability transition, in animal models of muscular dystrophies, nonsteroidal anti-inflammatory drug induced small intestinal ulceration, and ischemia/reperfusion injury.[69] Interestingly, SCY-635 (**14**, renamed as WS635) was reported to reduce anesthesia/surgery-induced cognitive impairment in mice.[70] Similar to alisporivir (**13**) and SCY-635 (**14**), compound STG-175 (**15**) exhibits a very high efficiency in inhibiting the PPIase activity of CypA, strongly reduces the immunosuppressive potential, and has a high anti-HCV activity in luciferase reporter replicon cell lines with EC50 values, depending on the HCV genotype, between about 11 and 39 nM.[61a]

However, CsA itself as well as alisporivir (**13**) and other CsA derivatives have been found to exhibit an undesired inhibition of various drug transporters such as the P-glycoprotein, the multidrug resistance-associated protein MRP2 and the organic anion transporting polypeptide OATP.[71] On the other hand, SCY-635 (**14**) with the rather hydrophilic *N*-methylhydroxyleucine residue in position 4 exhibited reduced inhibition of P-glycoprotein mediated transport.[62]

The enhancement of the hydrophilicity of the side chain at position 4 generally was consistently found to be suitable for overcoming the problem by reducing transporter inhibition. These observations led to CsA derivative **16** (NIM-258), which was developed by researchers at Novartis.[72] The synthetic strategy for obtaining **16** is similar to that outlined above for alisporivir. The compound shows a 1.2 nM inhibition of CypA and a highly efficient anti-HCV activity in cell culture. Remarkably, it has a lower transporter inhibition than the preceding non-immunosuppressive derivatives.[72]

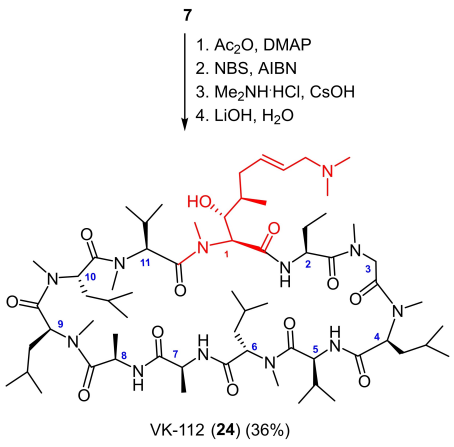
3.3. Non-Immunosuppressive CsA Derivatives with Modifications in Position 1 [MeBmt]^1

The side chain in position 1 with its unusual amino acid (4*R*)-4-([*E*]-2-butenyl)-4-methyl-*N*-methyl-(L)-threonine (MeBmt) in position 1 is the most accessible position for synthesizing derivatives of CsA. It offers just several positions for attack: the hydroxy group may be protected as an ester or substituted, allylic oxidation permits introduction of hetero-substituents in the ω-position, and the carbon–carbon double bond can be modified by olefin metathesis or cleaved oxidatively, and the resulting aldehyde may be submitted to carbonyl olefination. Many derivatives were synthesized based on these methods, with the aim of improving the bioavailability.[61b,73] Early approaches towards CsA derivatives with a modified MeBmt side chain were undertaken by Rich et al. in collaboration with Evans and researchers at Merck Sharp and Dhome.[11b,74]

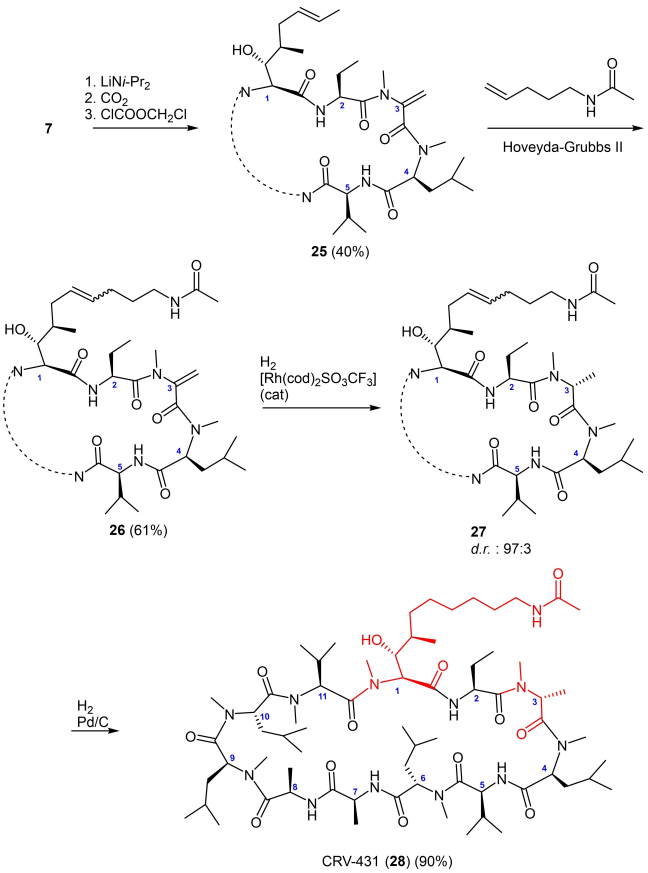
Although the MeBmt side chain is located in the "northwestern" part of CsA and involved in cyclophilin binding, CsA derivatives with modifications at residue 1 have been shown to exhibit decreased immunosuppressive activity without loss of cyclophilin affinity. This behavior probably results from the location of the MeBmt side chain, which protrudes away from the cyclophilin binding site into the CN binding region (Figure 2, top) of CsA in its cyclophilin-bound conformation. Typical for this conformation, which is also adopted in polar solvents, is a hydrogen bridge between the hydroxy group in the MeBmt side chain to the carbonyl group of the [MeLeu]^4 unit.[37]

Here, we will focus on two recently disclosed CsA derivatives with reduced immunosuppressive action and retained CypA inhibition: For a "fine-tuning" of the inhibition profile, a series of compounds with relatively simple modifications in the side chain were synthesized and biochemically characterized, not only with regard to their ability to suppress, in complex with CypA, the CN phosphatase activity, but also their preference to localize extra- or intracellularly.[75] To illustrate the principal, compound **24**, (VK-112) is highlighted. It is readily available from CsA, as outlined in Scheme 5, by acylation of the secondary alcohol, radical bromination of the terminal methyl group in the MeBmt side chain, substitution of bromine by dimethylamine, followed by deprotection of the hydroxy group. The CsA derivative **24** was also proved to be a non-immunosuppressive, cell-penetrating drug with a 10 nM Cyp-A inhibition. Similar to alisporivir (**13**), compound **24** is effective against infections with the human corona viruses HCoV-229E and HCoV-NL63.

A further promising drug candidate is CRV-431 (previously named CPI-431-32; **28**), which contains a [MeAla]^3 unit as well as a modified MeBmt residue. The compound was accessed by multistep syntheses using conventional methods.[61b,76] Recently, a more straightforward route was disclosed (Scheme 6). It starts with a methylenation of [Sar]^3



Scheme 5. Synthesis of VK-112 (**24**).



Scheme 6. Synthesis of CRV-431 (**28**).

by polylithiated CsA, analogous to that described in Scheme 4, to afford compound **25**. Modification of the MeBmt side chain was accomplished by olefin metathesis to give the *E*/*Z* mixture of **26**. The methylene group in the sarcosine moiety was submitted to a rhodium-catalyzed hydrogenation that yielded the desired configuration of [MeAla]^3 in a diastereomeric ratio of 97:3 in compound **27**. Finally, the carbon–carbon bond in the side chain was saturated conventionally to give CRV-431 (**28**) in 90 % combined yield for the two steps. The olefination step from CsA to methylene sarcosine intermediate **25** could be

performed in a more efficient way by application of flow chemistry, with an enhancement of the yield to 65 %.[77]

CRV-431 (**28**) was found to potently inhibit several cyclophilin isoforms besides CypA, namely CypB, CypD, and CypG. It has shown very potent antiviral activity against hepatitis C and HIV-1 in preclinical studies, which correlates with the ability of the compound to inhibit the PPIase activity of CsA. CRV-431 (**28**) is considered to be effective against chronic liver diseases. It was found to be efficient in mouse models of liver fibrosis and non-alcoholic steatohepatitis (NASH).[78] Interestingly, CRV-431 (**28**) inhibits hepatitis B virus replication by an as yet unknown mechanism; however, this activity is suggested to be independent of cyclophilin inhibition.[79]

3.4. Other CsA Derivatives with Reduced Immunosuppression

3.4.1. Modification at [Val]^5

CsA can be regiospecifically alkylated at the NH group of [Val]^5 using the phosphazene base P4-*t*-Bu and reactive bromides. Replacement of the amide proton by a benzyl or allyl group resulted in strongly reduced immunosuppression while retaining the affinity of the compounds to CypA. The reduced ability of the derivatives to form a ternary complex with CypA and CN may result from the induction of a conformation of [Val]^5 detrimental for the formation of the hydrogen bond between its backbone carbonyl oxygen atom and the $N^{\varepsilon 2}$ position of Trp352 in the catalytic subunit of CN. Furthermore, a [MeLeu]^4 side chain conformation unfavorable for CN binding could be induced by the modification.[80]

3.4.2. Modification of Position 6

In the CypA-CsA-CN complex, the side chain of [MeLeu]^6 makes extensive contacts with three hydrophobic residues of CN and, thus, is very important for CN binding. [MeAla]^6-CsA, in which the isopropyl group of the leucyl group is removed, and [MeAbu]^6-CsA have a CypA binding affinity similar to CsA itself, but exhibit a decrease in immunosuppressive activity. [MeAla]^6-CsA was shown to promote neurite outgrowth.[74c,81]

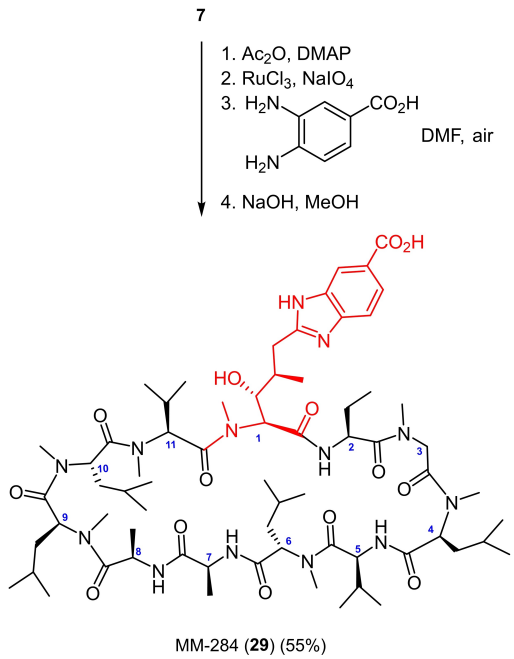
3.4.3. Modification of Position 8

The CsA amino acid [Ala]^8 was replaced by (D)-*N*,*N*-dimethyl- and *N*,*N*-diethyllysine in a multistep route that involved as a key step a nonregioselective amide to thioamide conversion in position 7 and, induced thereby cleavage between amino acids [L-Ala]^7 and [D-Ala]^8. The [D-Lys]^8-CsA derivatives exhibit promising anti-HCV activity with EC_{50} values lower than 200 nM; they are >50 times less immunosuppressive than CsA.[82]

3.4.4. CsA Derivatives with Targeted Localization

A CsA derivative restricted to the extracellular space was initially obtained by attachment of a $(D\text{-}Glu)_6$-Gly-OH moiety and a 5(6)-carboxytetramethylrhodamine as a fluorescence probe to the carboxy group of [*O*-carboxymethyl-D-Ser]^8-CsA.[83] It was later shown that the modification of CsA in position 1 by several different substituents also results in the inability of the derivative to enter cells. The extracellular CsA derivative MM-284 (**29**) with a benzimidazole modification in position 1 efficiently inhibits CypA PPIase activity with a K_i value of 10.7 nM.[84] It efficiently reduces myocardial inflammation as well as endothelial cell apoptosis in pulmonary arterial hypertension, thus indicating involvement of extracellular CypA in these processes and suggesting inhibition of extracellular CypA as a novel therapeutic approach in inflammatory diseases.[85]

MM-284 (**29**) is readily available by the straightforward partial synthesis shown in Scheme 7. Following a method



Scheme 7. Synthesis of MM-284 (**29**).

developed by Park and Meier,[86] the carbon–carbon double bond was cleaved to the aldehyde after the hydroxy group had been protected. Condensation of the aldehyde with 3,4-diaminobenzoic acid in air and a final cleavage of the acetate leads to MM-284 (**29**) in 55 % overall yield.

Mitochondria targeting by CsA was achieved by conjugation of the alkyl-linked triphenylphosphonium cation to the CsA derivative with a 4-methylbenzoate substitution in position 3, as described in Section 3.2.1.[44] The resulting compound inhibits CypA PPIase activity with a K_i value of 8 nM.[87]

CypA complexes of both MM-284 (**29**) as well as the mitochondria-targeted CsA show strongly reduced inhibition of CN in a cell-free system, which indicates that their non-immunosuppressive character is independent of their intracellular localization. Nevertheless, CsA derivatives with defined cellular localization other than the cytoplasm can be generally considered to be per se devoid of immunosuppressive activity even if they have the potential to inhibit CN, because the immunosuppressive activity of CsA is based on the inhibition of cytoplasmatic CN, which leads to the prevention of NFAT shuttling from the cytosol to the nucleus.[88]

3.4.5. Cyclic Peptoid Library

Although not derived from CsA, attention should also be paid to de novo synthesized cyclic peptoids that were generated in a one-bead-one-compound (OBOC) library **30** (Figure 5). It was composed of two aliphatic, two cyclic, and six aromatic amines serving as monomers.

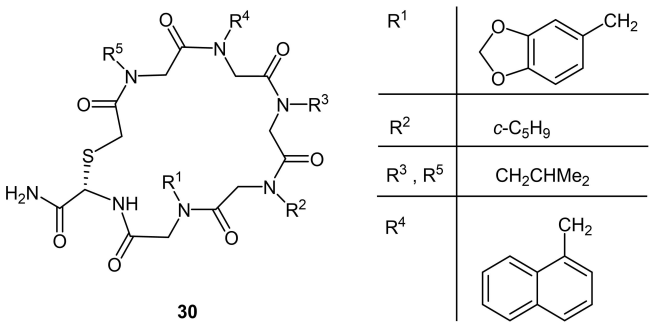


Figure 5. Cyclic peptoid library **30** and substitution pattern of the hit compound.

The study afforded several hits, with the most promising one having the substitution pattern shown in Figure 5. This compound inhibits the CypD-mediated opening of the mPTP, as shown by the recovery of the mitochondrial membrane potential recovery in neuroblastoma cells.[89]

4. Cyclophilin Inhibitors Based on Structural Simplifications of the Macrocycle Sanglifehrin As Lead Structure

4.1. Structure of Sanglifehrin A and Mode of Cyclophilin Inhibition

A broad microbiological screening, performed at Novartis, of actinomycetes for cyclophilin-binding metabolites led to the discovery of a novel class of natural products featuring a 22-membered macrocycle that originates from a mixed peptide/polyketide biosynthesis. They are produced from *Streptomyces* sp. A92-308110 and were named sanglifehrins after the discoverers Sanglier and Fehr. The most abundant member in the sanglifehrin family, whose members have in common the macrocyclic core unit, is sanglifehrin A (**31**).[90] So far, three total syntheses of the natural product sanglifehrin A have been described.[91,92] In addition, valuable syntheses of building blocks and methodological studies on key reactions have been published.[93] Sanglifehrin A (**31**) forms complexes with different cyclophilin isoforms such as CypA, CypB, and CypD.[94]

Similar to CsA, sanglifehrin A (**31**) is immunosuppressive, however, by a different, but as yet unknown, molecular mechanism. In contrast to CsA, its immunosuppressive activity was shown to be independent from complex formation with CypA because competitive displacement of the compound did not affect immunosuppression, and it does not involve inhibition of the phosphatase activity of CN.[95] On the other hand, the formation of a ternary complex between CypA, sanglifehrin A (**31**), and inosine-5′-monophosphate dehydrogenase 2 (IMPDH2) was observed, which suggests the involvement of this complex in immunosuppression.[96]

Sanglifehrin A (**31**) inhibits the PPIase activity of CypA with an IC_{50} value of 12.8 nM.[95a] Similar to CsA, sanglifehrin A (**34**) binds to the cyclophilin active site, as shown by a crystal structure of the complex formed between CypA and sanglifehrin A.[97] The direct interaction between sanglifehrin A and CypA is based on six intermolecular hydrogen bonds directed at a relatively small fragment of sanglifehrin, the tripeptide moiety (Val-*meta*-Tyr-Pip) in the macrocyclic part of the molecule, as shown in Figure 6. The superposition of CypA complexes with the substrate succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide and sanglifehrin A in the PPIase active site shows that the piperazic acid moiety of sanglifehrin A occupies the hydrophobic pocket of CsA formed by Phe60, Met61, Phe113, and Leu122, where the proline ring of the substrate and the [MeVal][11] moiety of CsA reside.

Low oral bioavailability and low solubility have hampered the application of the compound in biological systems.

Not surprisingly, efforts directed at generating non-immunosuppressive analogues of sanglifehrin focused on that peptidic sequence embedded in a macrocycle, while modifying, simplifying, or omitting the periphery of the natural product, which is considered less important for the interaction with cyclophilins. A proof of this hypothesis was provided by an early degradation study of sanglifehrin A: aldehyde **33** (see Scheme 8) lacking the complex spirobicyclic moiety—the "western region" of the natural product—exhibits inhibition of CypA with an IC_{50} value of approximately 30 nM.[95b] Based on these observations, two synthetic strategies were pursued for obtaining non-immunosuppressive drugs: firstly, modification of fermentation products, and secondly, de novo syntheses of targets whose structures are inspired by, but not derived from, the natural product sanglifehrin A (**31**).

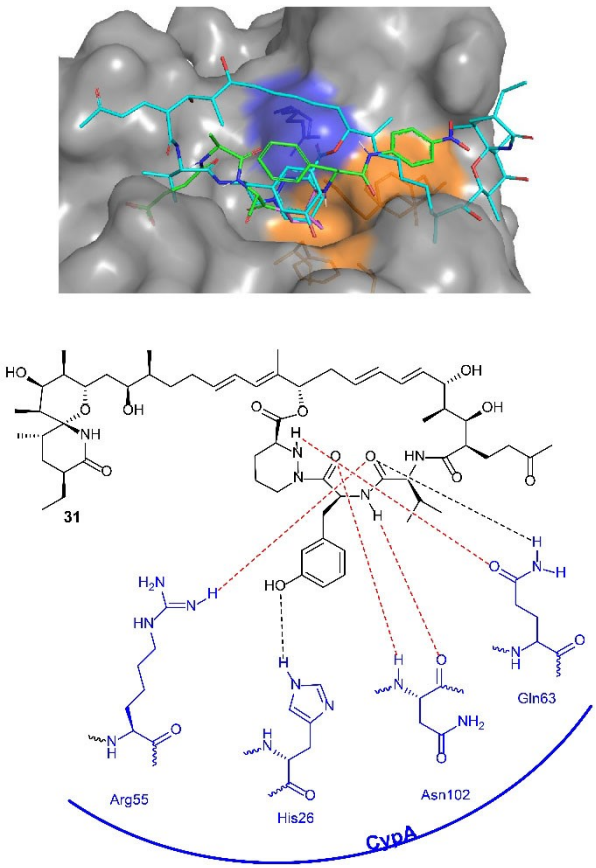
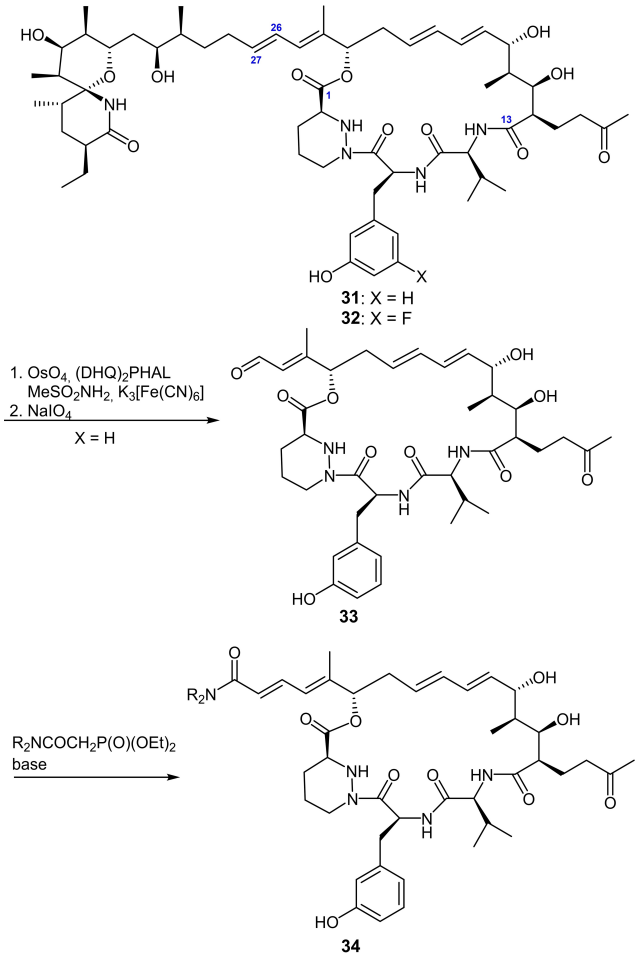


Figure 6. Superposition of CypA complexes with the substrate succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide (green, PDB ID: 1RMH)[35] with the Pro residue in pink and sanglifehrin A (**31**; cyan, PDB ID: 1YND)[97] in the PPIase active site (top). The surface of Arg55 is highlighted in blue and that of Phe60, Met61, Phe113, and Leu122 in orange. Sanglifehrin A (**31**; black) and hydrogen bonds (red) to CypA (blue) are shown according to Ref. [97] (bottom).



Scheme 8. Synthesis of sangamides **34** derived from sanglifehrin **31**.

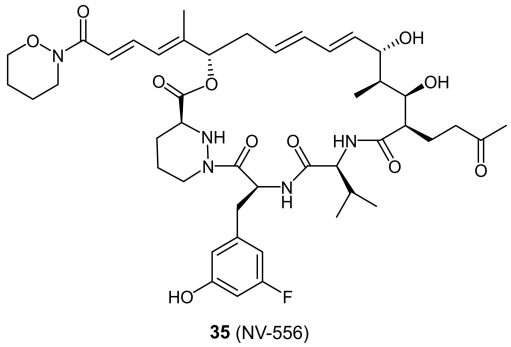


Figure 7. Structure of non-immunosuppressive sangamide NV-556 (**35**).

4.2. Non-Immunosuppressive Drugs Prepared from Sanglifehrins by Partial Syntheses

Side-chain degradation and subsequent modification opened a route to the first non-immunosuppressive derivatives of sanglifehrin, the so-called sangamides, which were developed by Biotica Technology and cooperating research groups. The straightforward partial synthesis is illustrated in Scheme 8.[98] The application of Sharpless' asymmetric dihydroxylation procedure in the first step occurred in a completely selective manner at the C26–C27 double bond. Subsequent glycol cleavage delivered the aldehyde **33** in 63 % overall yield.[99] A subsequent Horner–Wadsworth–Emmons olefination delivered dienamides **34** with various substitution patterns at the amide nitrogen atom in the side chain.

A promising drug candidate in the sangamide family is NV-556, formerly known as NVP018 and BC556 (**35**; Figure 7), which was promoted by NeuroVive Pharmaceutical AB, when this company acquired the cyclophilin inhibitor program from Biotica.[100] Here also, the western region of sanglifehrin was cut off and replaced by a 1-(tetrahydro-2*H*-1,2-oxazin-2-yl) end group, with an additional fluorine substitution in the aromatic amino acid group. The synthesis of NV-556 starts with fluorinated ("mutasynthetic") sanglifehrin **32**, as shown in Scheme 8.[101] This compound is readily available by fermentation of the bioengineered strain *Streptomyces* sp. BIOT4585.[102] Feeding with methyl (*S*)-2-amino-3-(3-fluoro-5-hydroxyphenyl) propanoate and *rac*-piperazic acid is required to produce the

fluorinated sanglifehrin **32**. Then, the application of the three-step procedure described in Scheme 8 leads to **35** (NV-556).

The antiviral effects of NV-556 (**35**) against HIV-1 and HCV were proven in vitro and in vivo.[102,103] Furthermore, NV-556 (**35**) was evaluated as a potential antifibrotic compound for liver fibrosis[104] and found to reduce fibrosis and hepatocellular carcinoma development in mice with NASH.[105] A further advantage of NV-556 (**35**) is that it shows minimal inhibition of drug transporters.[102]

4.3. Non-Immunosuppressive Synthetic Sanglifehrin-Inspired drugs

In recent years, researchers at Gilead, Selcia, and Cypralis have pursued the goal of developing small-molecule cyclophilin inhibitors, whose structures were inspired by sanglifehrin.[106] A rigorous simplification of the natural product should enable the drugs to be obtained solely through chemical total synthesis. The research program aimed at developing orally bioavailable, antiviral drugs. The researchers maintained the "southern part" with the peptidic skeleton, including the piperazic amide moiety, thereby giving them a relatively large degree of freedom in the construction of the "northern part". In a first approach,[106b] the C18–C21-diene of sanglifehrin was replaced by a styryl unit, among other simplifications, to provide derivative **36**. Crystal-structure analyses in complex with CypA revealed a π-stacking interaction of this styrene moiety with the guanidinium group of Arg55 in the PPIase active site of CypA as a new binding mode, as shown in Figure 8. As the

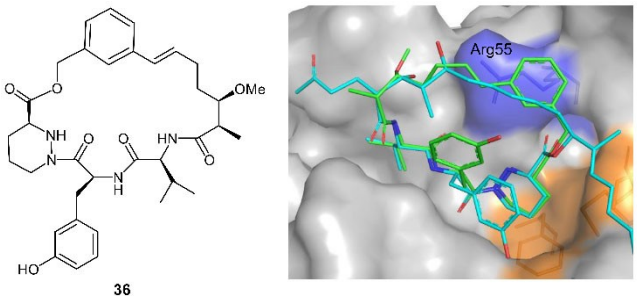


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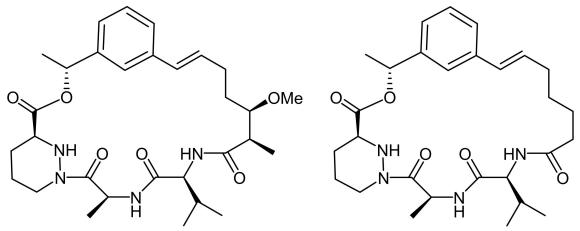
Figure 8. Structure of "simplified sanglifehrin" **36** (left) and the stacking of the styrene group of **36** (green) with the guanidinium group of Arg55 in CypA (right). Superposition of CypA complexes with **36** (green, PDB ID: 5T9Z)[106b] and sanglifehrin A (cyan, PDB ID: 1YND)[97] in the PPIase active site. The surfaces of Arg55 is highlighted and Phe60, Met61, Phe113, and Leu122 in orange.

m-tyrosine residue was found to be displaced in the solvent, this amino acid, considered as non-essential for CypA inhibition, was substituted by alanine, with the benefit of lowering the molecular weight.

These results led to the novel synthetic 22-membered macrocycles **37** and **38** as new lead compounds (Figure 9). A time-resolved fluorescence resonance energy transfer bio-

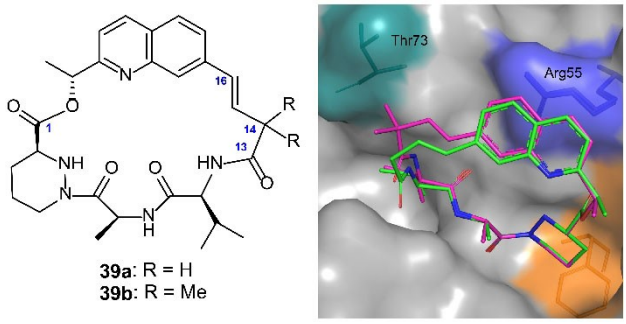


37 38

Figure 9. Structures of CypA inhibitors **37** and **38** featuring further simplification compared to sanglifehrin, with a styrene moiety in the "northern part".

chemical assay showed they display an affinity to CypA of $K_d = 24$ nM and 48 nM, respectively.[106b]

Despite this potent CypA inhibition and modest anti-HCV activity, the pharmacokinetics were evaluated as poor. Therefore, the collaborating research groups undertook a second approach that led to compound **40** by a step-to-step alteration and modification of the lead structure **36**. In a first phase of development, the styrene moiety was replaced by an isoquinoline, then a quinoline block, and the size of the macrocycle was reduced from 22 to 21 members. Compounds **39a** and **39b**, shown in Figure 10, may serve as



39a: R = H
39b: R = Me

Figure 10. Structures of compounds **39** (left) and overlay of compound **39a** (green) and homologue **39b** (purple) in complexes with CypA,[106c] illustrating that the positioning of the C13–C16 atoms closer to the protein surface and the C14 pro-*R* methyl group forming a lipophilic interaction with Thr73. The surface of Arg55 is highlighted in blue, of Phe60, Met61, Phe113, and Leu122 in orange, and of Thr73 in cyan (right). Data kindly provided by T. C. Appleby.

representative examples with an improved binding affinity. Their crystal structures in complex with CypA show that the quinoline moiety maintains the binding to Arg55. In addition, the pro-*R* diastereotopic methyl group in the C14 geminal-disubstituted derivative **39b** forms a lipophilic interaction with the Thr73 residue.[106c]

In a second optimization effort, the functional groups at C-1 and C-13, lactone and lactam, respectively, were switched, with the consequence of an intramolecular hydrogen bridge between the lactam at C-1 to the quinoline nitrogen atom in compound **40**. (Figure 11). This feature led to a substantially improved membrane permeability—an important step to the desired oral application. Finally

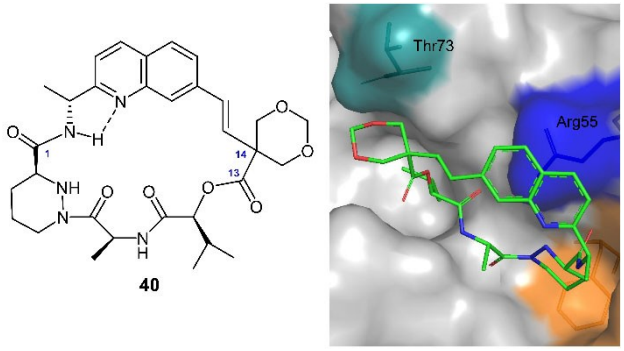
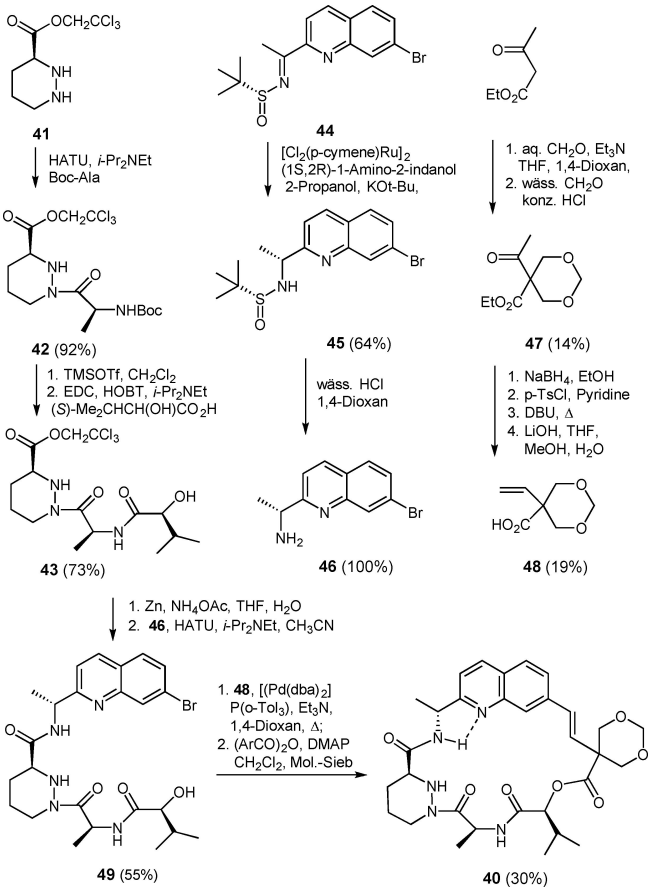


Figure 11. Structure of optimized non-immunnosuppressive inhibitor **40** (left) and view of compound **40** bound to CypA.[106c] The surface of Arg55 is highlighted in blue, of Phe60, Met61, Phe113, and Leu122 in orange, and of Thr73 in cyan (right). Data kindly provided by T. C. Appleby.

position 14 was transformed into a spiro center by introducing a 1,3-dioxane moiety. This modification caused a lower distribution coefficient and reduced the oxidative metabolism and pregnane-X(PXR) activation, which is considered a measure of drug–drug interaction.

Compounds **36**–**40** and a variety of analogues and derivatives were obtained by total syntheses. Here, we highlight the highly convergent route to compound **40**, which was synthesized in a diastereomerically and enantiomerically pure form, as outlined in Scheme 9, with the three building blocks **43**, **46**, and **48** as key intermediates.[106c] The first of the three parallel branches started with the trichloroacetate of (*S*)-piperazic acid **41**.[93a] An asymmetric synthesis of the acid itself had been developed previously, based on a diastereoselective hydrazination of an Evans oxazolidinone.[107] More recently, a biotechnological route to enantiomerically pure piperazic acid was opened through the use of transgenic microorganisms.[108]

The ester **41** was regioselectively coupled with Boc-protected alanine to give the amide **42**, as published previously.[106b] Cleavage of the Boc-protecting group and standard peptide coupling with (*S*)-2-hydroxy-3-methylbutanoic acid led to the first building block **43** as a pure stereoisomer. To obtain the second key intermediate, amine **46**, a double stereodifferentiation method[109] was applied based, on the one hand, on the chiral substrate **44** and, on the other hand, on a chiral ligand in the hydrogenation catalyst. Thus, by following a procedure described by Guijarro, Pablo, and Yus,[110] the Davis–Ellman-type[111] sulfinimine (*R*)-**44**, generated from 7-bromo-2-acetylquinoline and (*R*)-*t*-butylsulfinamide was submitted to a rhodium-catalyzed transfer hydrogenation with (1*S*,2*R*)-1-amino-2-indanol. The combination of this enantiomer of the ligand at the noble metal center with (*R*)-sulfinimine **44** created a matched pair situation leading to sulfonamide **45** with optimal diastereoselectivity. Acidic cleavage of the nitrogen–sulfur bond delivered quinoline **46**, the second building block. The third one, the achiral dioxane **48**, was obtained by standard procedures from acetoacetate via dioxanyl-keto ester **47**. The poor yield in this sequence can be tolerated



Scheme 9. Synthesis of cyclophilin inhibitor **40**. HATU = *O*-(7-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; EDC = 1-ethyl-3-(3-dimethylamino)propyl)carbodiimide; HOBT = 1-hydroxybenzotriazole; $(ArCO)_2O$ = 2-methyl-6-nitrobenzoic anhydride.

when one considers that all the substances required are bulk chemicals.

The three building blocks were combined in a four-step sequence, starting with the cleavage of the trichloroethyl ester in the intermediate **43**. The carboxylic acid thus liberated was coupled with the second building block, 2-aminoethyl quinoline **46**, to give tripeptide **49**. Subsequently, dioxane **48** was constructed by means of a Heck reaction. The final step, the closure of the macrocycle, was achieved by macrolactonization under application of Shiina's conditions[112] under high dilution. In summary, the combination of the building blocks with a non-optimized yield of 17 % starting from compound **41** gave the final product **40** in gram quantities; an up-scaling to 100 to 1000 g has been announced.

Sanglifehrin-inspired, but substantially simplified compound **40** exhibits strong CypA inhibition (K_d = 5 nM), remarkable anti-HCV 2a activity (EC_{50} = 98 nM), and high oral bioavailability in rats (100 %) and dogs (55 %).[106c] Recently, compound **39b** (Figure 10) was used in a study that revealed that HCV uses CypA to evade the effector protein kinase R (PKR).[113]

In view of the tremendous efforts that are required for synthesizing a large multitude of compounds that exhibit

cyclophilin inhibition, the question arises whether this process can be facilitated and simplified by prediction of the appropriate structures based upon theoretical calculations. Indeed, this approach has been pursued in collaboration by researchers at Gilead and Schrödinger by using the free-energy perturbation (FEP) method (FEP+).[114] The binding affinities of more than two dozen compounds of the series **36**–**40** and analogues thereof to CypA were calculated and compared with ten compounds that were synthetically available and whose binding affinities had been determined by time-resolved fluorescence resonance energy transfer (TR-FRET) competitive binding assays. The agreement of the calculated with the—previously determined—experimental data was found to be fair in this "retrospective study",[114] and the calculations may help in developing improved non-immunosuppressive CypA inhibitors of macrocyclic structures related to compound **40**.

5. De Novo Designed Small Molecules as Non-Immunosuppressive Cyclophilin Inhibitors

5.1. Aryl Indanyl and Biaryl Indanyl Ketones

A third approach towards non-immunosuppressive cyclophilin inhibitors is based upon de novo designed and accordingly synthesized small molecules. Their structures are not inspired by or derived from natural products, but rather designed intuitively, rationally, or virtually by taking into account hypothetical or computationally modeled binding modes at the enzyme's active site.

In our research groups, we developed aryl indanyl ketones, originally as inhibitors of the PPIase activity of the human parvulin Pin1,[115] which represents an anticancer drug target.[116] We were guided by the idea that aryl indanyl ketones and biaryl indanyl ketones will mimic the assumed twisted amide transition state **3** (Figure 12) in the peptidyl-

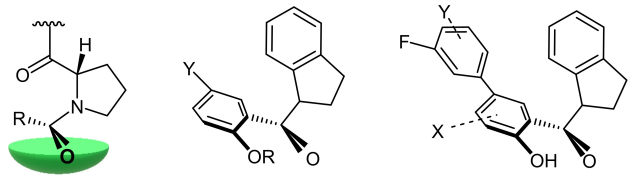


Figure 12. Aryl indanyl and biaryl indanyl ketones mimicking the hypothetical enzymatic PPIase transition state **3** (cf. Scheme 1).

prolyl *cis*/*trans* isomerization with a concomitant change of hybridization (cf. Scheme 1).[115a]

Straightforward syntheses enabled us[115a,117] to obtain a series of aryl indanyl and biaryl indanyl ketones, a selection of which are shown in Figure 13: enantiomerically pure aryl indanyl ketones (*R*)- and (*S*)-**50**, prepared from the corresponding enantiomer of 1-methyl-1-indanecarboxylic acid,[118] and racemic biaryl indanyl ketones. By using a

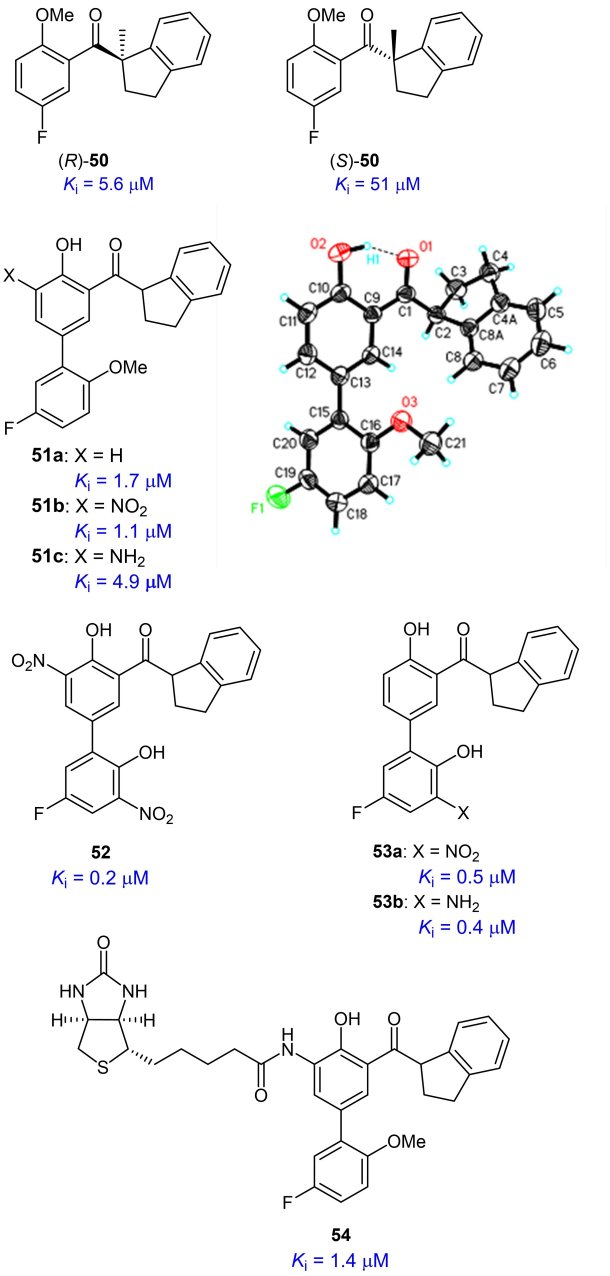
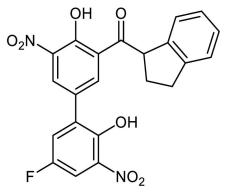
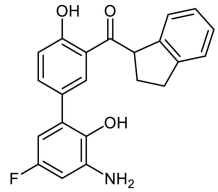
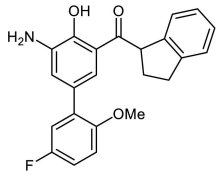
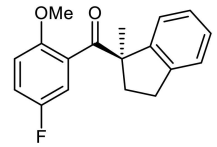
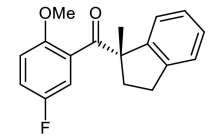


Figure 13. Selected aryl indanyl and biaryl indanyl ketones **50**–**54** with their K_i values of Pin1 inhibition. The crystal structure of compound **51a** is shown.

dynamic kinetic resolution procedure based upon of indanecarboxylic acid[119] we obtained ketone (*R*)-**51a** (OMe instead of OH) in 94% *ee*.[118] At first glance it would seem reasonable to submit this substance as well as a racemic sample to in vitro inhibition tests. We noticed, however, that, under those conditions, a ready racemization of the enantiomerically enriched ketone occurs through enolization.[120] A crystal-structure analysis of derivative **51a** reveals that the biaryl-carbonyl and the indanyl plane are oriented nearly perpendicular, so that, in a sense, compounds of this series fulfill the requirements of a mimic of the hypothetical twisted amide transition state **3**.

Table 1: Selective inhibition of CypA versus CypB by ketones **50**, **52**, and **53**.

Entry	Compound	K_i [μM] CypA	K_i [μM] CypB
1	**52** 	0.52 ± 0.15	> 100
2	**53 b** 	0.3 ± 0.1	12 ± 5
3	**53 c** 	1.7 ± 0.5	8.6 ± 0.9
4	(*R*)-**50** 	7.5 ± 1.5	40 ± 10
5	(*S*)-**50** 	> 100	> 100

The inhibition of human Pin1 by aryl and biaryl indanyl ketones was determined in a protease-free PPIase assay with succinyl-Ala-Glu-Pro-Phe-*p*-nitroanilide as the substrate.[14b] Selected results are shown in Figure 13 along with the structures. Ketones **51** and **54** are active inhibitors with K_i values in the single-digit micromolar range. Substitution of the phenolic ketones by nitro or amino residues led to enhancement to sub-micromolar activity with compounds **52** and **53**. The biotin derivative **54** provided proof of reversible inhibition in a suitable strepavidin test.[115a]

Aryl indanyl ketones (*R*)- and (*S*)-**50** with a configurationally stable stereogenic center were chosen to study the influence of enantiomerism on the PPIase activity. The K_i values, shown together with the chemical structures in Figure 13, reveal that the inhibition activity of the enantiomers differs by an order of magnitude, with the *R* enantiomer of **50** being the more active compound. The different activity of the enantiomeric ketones (*R*)- and (*S*)-**50** was furthermore also proved to exist in cells by two experiments, the first performed using a luciferase reporter gene assay with a p53 response element in MCF-7 cells (breast cancer cell line), the second by studying the effect on the oncogenic transcriptional activator β-catenin. Here again, the *R* enantiomer of ketone **50** was more active than (*S*)- and *rac*-**50**.[115a]

After having demonstrated that aryl indanyl and biaryl indanyl ketones are able to inhibit the PPIase activity of human Pin1, we started studying whether inhibition occurs in cyclophilins as well. Indeed, a reasonably good binding to CypA was observed, as shown in Table 1 for selected compounds, with inhibition constants in the single-digit- to sub-micromolar scale (column 3). Even more remarkably, these small molecules exhibit a distinct selectivity towards the different cyclophilin isoforms, as evidenced by the results that show a clear discrimination in favor of CypA over CypB (columns 3 vs. 4). This holds in particular for biaryl indanyl ketones (entries 1–3), but, to a lower extent, also for the *R* entantiomer of ketone **50**, whereas (*S*)-**50** is inactive towards both isoforms (entries 4 and 5). The highest ability to discriminate between CypA and CypB is exhibited by dinitro-substituted bisphenol **52**, with a factor of > 200 difference. In view of the simplicity of its molecular

structure on the one hand and the high extent of homology between CypA und CypB (63 % of identical amino acids) in the PPIase domain on the other hand, this degree of selectivity appears surprising. A proof of the discrimination in vivo was provided by inhibition of CypA- but not CypB-mediated chemotaxis of mouse CD4 + T cells by **52**.[121]

To obtain a computational insight into the inhibition of cyclophilin by small molecules, Monte Carlo statistical sampling coupled to free-energy perturbation theory (MC/FEP) calculations were performed by Sambasivarao and Acevedo, who put a particular emphasis on elucidating the origin of the above-mentioned isoform-specific inhibition by aryl indanyl ketones.[122] The computed binding energies of compounds **51c**, **52**, **53b**, and (*R*)- and (*S*)-**50** were in a good agreement with those determined by us experimentally.[121] For the highly selective compound **52**, the calculations identified the most favorable binding mode in CypA and CypB, which turned out to be distinctly different in the orientation of the indanyl ring. The binding modes are shown in Figure 14 (top) with an overlay of the CypA- and CypB binding sites. In the favorable CypA complex, the indanyl residue points into a polar region of the enzyme with a noncovalent bonding of the carbonyl group to His54. A further weak electrostatic interaction occurs between the guanidinium group of Arg55 of CypA and the oxygen atom of the nitro substituent (*meta* to the carbonyl group) of ketone **52** (Figure 14, bottom). The calculated total energy of this combined Coulomb and van der Waals interaction amounts to $-16.3\ \mathrm{kcal\,mol^{-1}}$.

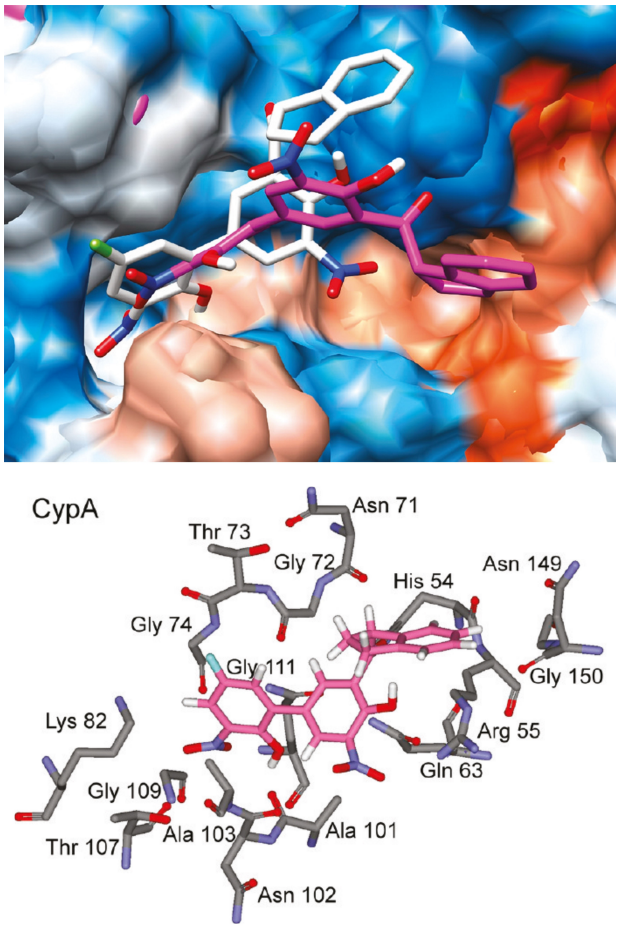


Figure 14. Overlaid CypA (gray) and CypB (pink) active sites with biaryl indanyl ketone **52** bound at the active sites (top). Compound **52** bound to the active site of CypA with key residues indicated. Nearby water molecules are removed for clarity (bottom; copied from Ref. [122]).

The highly different CypA inhibition potency of the enantiomeric compounds (*R*)- and (*S*)-**50** was also confirmed by the calculated binding energies in this computational study. The authors concluded that the steric demand of the methyl group at the stereogenic center causes an unfavorable docking of the *S*- compared to the *R* enantiomer in the active site. Figure 15 shows an overlay of (*R*)- and (*S*)-**50** in CypA.

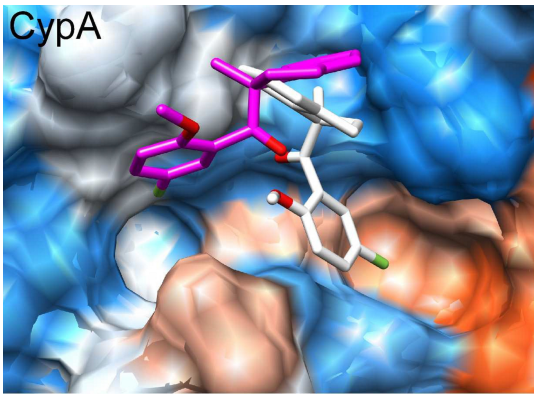


Figure 15. Overlay of (*R*)-**50** (gray) and (*S*)-**50** (pink) in the Cyp active site. Nearby water molecules are removed for clarity (copied from Ref. [122]).

As expected for biaryl indanyl ketones, compound **53b** was proven to be a non-immunosuppressive inhibitor of CypA with no activity against CN. Similar to non-immunosuppressive CsA derivatives, it is able to inhibit human umbilical vein endothelial cell (HUVEC) proliferation, which forms a key step of angiogenesis, with an IC_{50} value of 5.4 μM. The well-known antiangiogenetic property of CsA was originally ascribed to the inhibition of CN; however, the effectiveness of cyclophilin inhibitors devoid of the ability to inhibit CN suggests that the inhibition of cyclophilins is sufficient to prevent HUVEC proliferation and angiogenesis.[57]

5.2. *N,N′*-Disubstituted Urea Derivatives

N,N′-Disubstituted urea is another structural motif that forms the core unit of cyclophilin-inhibiting small molecules resulting from de novo drug design. The simply structured compounds used for this purpose are either commercially available or accessible by facile conventional transformations. By means of a virtual screening, Guichou et al.

identified and optimized aryl-pyridyl ureas as "structure-based designed" CypA isomerase in vitro inhibitors. Starting with the lead structures **55a**,**b**, the optimized compounds **56a**,**b** showed IC_{50} values of 14 and 20 nM, respectively. Compound **55a** was found to inhibit HIV-1 replication (Figure 16).[123]

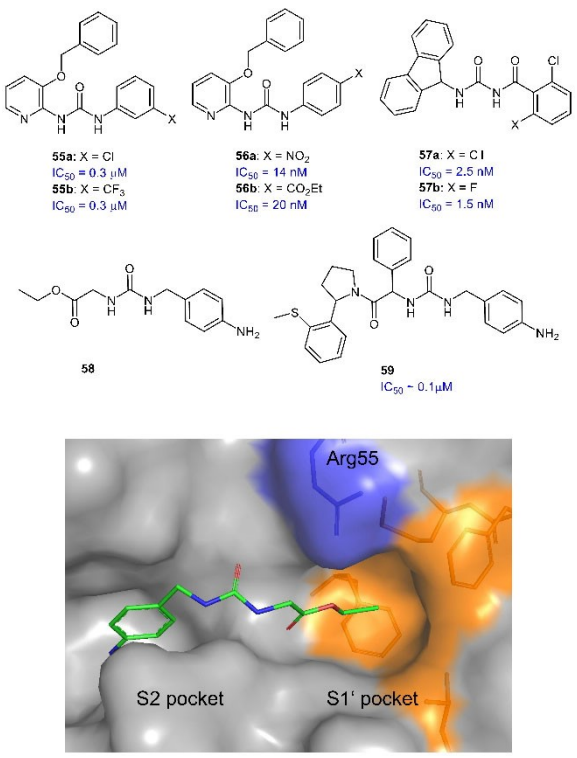


Figure 16. CypA inhibitors with a urea scaffold. Pyridyl-aryl ureas **55** and **56** and benzoyl ureas **57** together with the IC_{50} values for the inhibition of CypA PPIase activity (top).[126] Lead fragment **58** and optimized CypA, CypB, and CypD inhibitor **59** (middle); the crystal structure of lead compound **58** is shown in complex with CypA (PDB ID: 3RDD).[124] The S1′ pocket is defined as the proline binding pocket, formed by Phe60, Met61, Phe113, and Leu122 (represented in orange). The S2 pocket is defined as the pocket for binding amino acid residue P2 of the substrate (bottom).

Inspired by these results, Li and co-workers used a computer- assisted drug design approach to develop improved inhibitors based on the urea scaffold. The most efficient compounds resulting from this approach are benzoylureas **57a** and **57b** with IC_{50} values in the single-digit nanomolar region for the inhibition of the PPIase activity of CypA. A docking model of compound **57b** into the active site of CypA suggests a hydrogen bond between Arg55 and the benzoyl oxygen atom of the compound.[125,126]

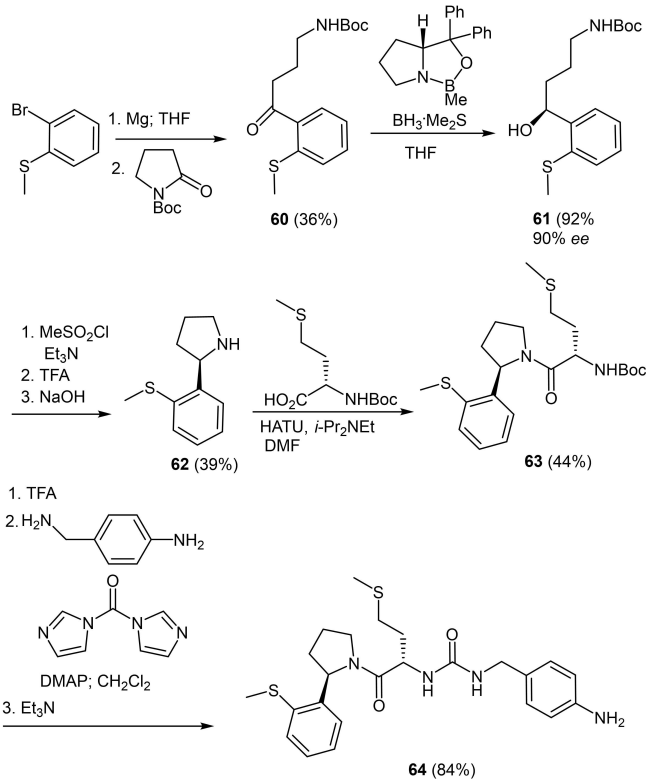
In the search for cyclophilin inhibitors, the concept of "fragment-based drug discovery" was also applied. For this purpose, numerous fragments are computationally docked into the active site of the target. From these results, a small number of fragments are selected and combined in a chemically reasonable concept. Finally, selected designed compounds are synthesized and evaluated with respect to their activity. This strategy was applied by Guichou et al. and led to compounds in which the selected fragments are connected by a urea moiety. The substituents occupy the S2 and the S1′ pockets of CypA as well as CypD, as shown in the crystal structures of the cyclophilin complexes with compound **58** resulting from the initial combination of the fragments (Figure 16). The structure of the CypA-**58** complex shows that the urea moiety forms a bridge over the saddle between the two pockets, and the ester function forms a hydrogen bond to the cyclophilin active site arginine residue Arg55. During optimization, a phenyl-pyrrolidine moiety was introduced to occupy the S1′ pocket. This results in a shift of the arginine hydrogen bond towards the urea carbonyl group in the cyclophilin–inhibitor complex. In this way, retained enthalpically driven binding is combined with a less unfavorable entropic term.

N,*N*′-Disubstituted urea **59** was described to give the optimum result (Figure 16). The compound, synthesized as a racemic mixture of two diastereomers, inhibits CypA, B, and D with IC_{50} values of 0.1, 0.08, and 0.2 μM, respectively, and exhibits antiviral activity against HCV, HIV, coronaviruses, and flaviviruses in vitro. The complex of CypA and compound **59** did not show inhibition of CN.[124,127]

Based on the high affinity of 2-aryl pyrrolidine analogues to CypD demonstrated by the Guichou group,[127b] O'Neil and co-workers analyzed *N*,*N*′-disubstituted urea derivatives and their stereochemical requirements as CypD inhibitors. The most active compound, **64**, was synthesized in a stereoselective manner, as outlined in Scheme 10.[128] The Grignard reagent generated from *o*-bromothioanisol was reacted with Boc-protected pyrrolidinone to give ketone **60**. Then, the carbonyl group was reduced enantioselectively by means of CBS catalysis to yield carbinol **61** in 90 % *ee*. Subsequently, generation of the mesylate, cleavage of the Boc protecting group, and base-induced cyclization led to pyrrolidine **62**. A subsequent coupling with Boc-protected (*S*)-methionine yielded dipeptide **63**. The final steps consisted of N-deprotection and coupling with carbonyldiimidazole and *p*-aminobenzylamine to afford the target urea derivative **64** as a single, enantiomerically pure diastereomer,[129] but a 2:1 mixture of rotamers.

The binding and inhibition constants of compound **64** towards CypD were determined to be $K_d = 0.41$ μM and $K_i =$ 99 nM. The binding energies were measured to be $\Delta G = -8.71$, $\Delta H = -12.49$, and $T\Delta S = -3.78$ kcal mol^{-1}. The unfavorable entropy term that leads to a reduction of the ΔG value compared to the ΔH value has been attributed to the conformational flexibility of the enzyme. The crystal structure of compound **64** bound to CypD (Figure 17) features a variety of noncovalent cooperative interactions, including hydrogen bonds to Arg55, Thr107, and Asn102 (CypA numbering). The (*S*)-methionine side chain of **64** is positioned near Asn102, Ala103, and Gly104 in a similar way to the [MeBmt]^1 residue of CsA, which is known to be in van der Waals contact with Ala103.

By further use of the fragment-based approach in the search for CypD inhibitors, a bicyclic fragment was found which matched the S2 pocket of CypD. This fragment was combined with the S1′ binding part of compound **59**, thereby



Scheme 10. Stereoselective synthesis of urea derivative **64**. HATU = *O*-(7-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate.

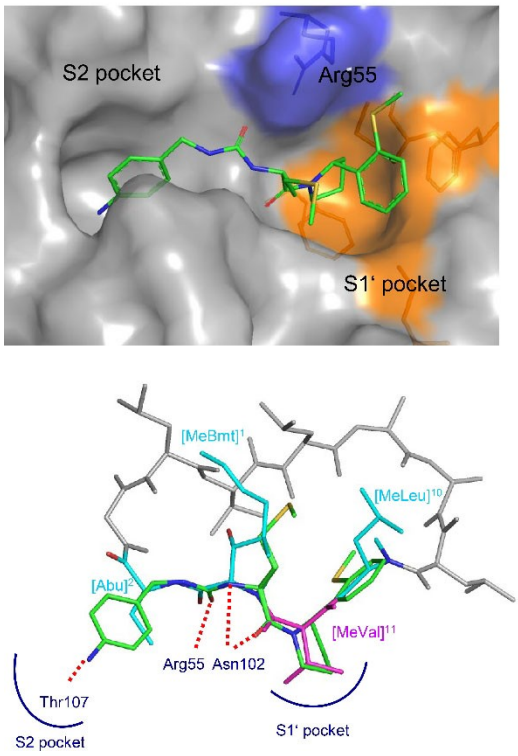


Figure 17. Crystal structure of compound **64** bound to CypD (PDB ID: 4J5C).[124] The surface of Arg55 is highlighted in blue and the surface of Phe60, Met61, Phe113, and Leu122 in orange, with numbering adapted to correspond to CypA. (Numbering in CypD: Arg96, Phe101, Met102, Phe154, and Leu163; top.) Overlay of compound **64** (green, PDB ID: 4J5C)[124] and CsA (cyan/grey, PDB ID: 2Z6W)[130] bound to CypD. The protein structure is omitted for clarity. Hydrogen bonds of compound **64** to active site residues of CypD are depicted schematically. CsA [MeVal]^{11} (pink) resides in the S1′ pocket of the cyclophilin (bottom).

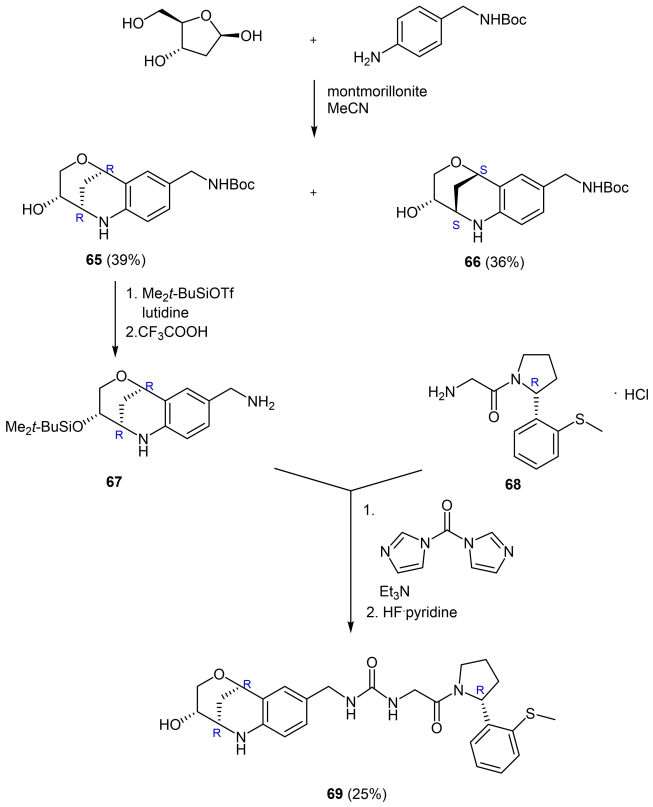
resulting in the most potent urea derivative **69** with a K_d of 6 nM determined by surface plasmon resonance (SPR) and an IC_{50} value for inhibition of the PPIase activity of CypD of 4 nM.[131] The use of an oxalyl or amide moiety instead of the urea linker to connect the fragments results in a reduced affinity to CypD. This holds also for other diastereomers of compound **69**—an observation that may underline the importance of the proper configuration in the oxazabicyclo-[3.3.1]nonane moiety for matching the S2 pocket.

The ex-chiral-pool synthesis of *N*,*N*′-disubstituted urea **69**, outlined in Scheme 11, starts with a montmorillonite-mediated condensation between 2-deoxy-D-ribose and Boc-protected 4-aminobenzylamine. The reaction leads to a nearly 1:1 mixture of the two diastereomers **65** and **66**. After chromatographic separation, product **65** with (*R*,*R*)-configured bridgehead centers was isolated in 39% yield and converted into intermediate **67** by protection of the hydroxy group and cleavage of the Boc groups. The intermediate **67** was subsequently condensed with (*R*)-pyrrolidine **68** in the presence of carbonyldiimidazole to effect the urea formation. Final cleavage of the silyl protecting group leads to the product **69**, which is obtained as a pure diastereomer and enantiomer.

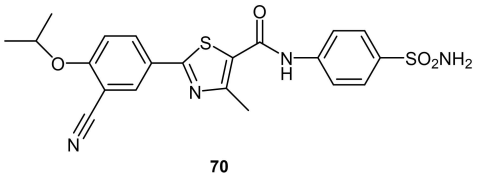
Interestingly, further alkylation of the urea nitrogen atom directed into the S2 binding pocket in compounds derived from lead compound **58** appended an additional contact site between the inhibitor and the cyclophilin active site, which in some cases improved the affinity. A K_d value of 70 nM for an optimized alkylated aryl-pyrrolidine urea was determined by SPR.[132]

As a consequence of the efficient inhibition of CypD by the *N*,*N*′-disubstituted urea derivatives, the influence of these inhibitors on mitochondrial permeability transition and associated signaling pathways are of great interest. Compound **59** was found to inhibit the opening of the mitochondrial permeability transition pore (mPTP) in heart and liver and protected against ischemia/reperfusion injury in mouse liver.[133] The protection of murine as well as human pancreatic acinar cells against toxin-induced loss of mitochondrial membrane potential and necrosis, which are critical events in the pathogenesis of acute pancreatitis by compound **59**, can also be considered as a result of the inhibition of CypD-mediated mPTP opening.[127]

"Fragment-based drug discovery" also identified a variety of simple, commercially available, mostly heterocyclic compounds as binders to the different isoforms of cyclophilins. Typical representatives are 3,4-diaminobenzamide and 2-amino-3-methylpyridine. The tools used in this study were X-ray diffraction, SPR, and molecular dynamics simulations. Free energy values of binding were calculated, with binding constants estimated to be millimolar. The



Scheme 11. Synthesis of compound **69**.



70

Figure 18. Structure of sulfonamide **70**.

biochemical effects of these simple compounds have not yet been disclosed.[134]

4.6. Sulfonamides

A third structural unit, the classical sulfonamide pharmacophore, has recently seen a renaissance as a small-molecule cyclophilin inhibitor. Structure-based design, pharmacophore modeling, virtual screening, and docking studies identified sulfonamide **70** as an inhibitor of CypD with a K_i value of 1.3 μM[135] (Figure 18).

Sulfonamide **70**, synthesized by standard methods, was found to exhibit activity against Aβ-induced mitochondrial dysfunction and cell death, and was recommended as a candidate for the treatment of Alzheimer's disease.[135]

5. Conclusion

The search for cyclophilin inhibitors devoid of immunosuppressive activity has been recognized as a worthwhile effort. Multiple modifications of CsA and sanglifehrin by partial syntheses, but also total the synthesis of de novo designed small molecules have provided a large variety of non-immunosuppressive cyclophilin inhibitors. Many of these compounds have proved themselves to be antiviral agents in vivo and in vitro, and several have been evaluated in clinical trials. This tour d'horizon may encourage further efforts to develop approved drugs based upon the concept of cyclophilin inhibition by non-immunosuppressive compounds.

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Conflict of Interest

The authors declare no conflict of interest.

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[1] a) G. Fischer, H. Bang, C. Mech, *Biomed. Biochim. Acta* **1984**, *43*, 1101–1111; b) G. Fischer, B. Wittmann-Liebold, K. Lang, T. Kiefhaber, F. X. Schmid, *Nature* **1989**, *337*, 476–478; c) N. Takahashi, T. Hayano, M. Suzuki, *Nature* **1989**, *337*, 473–475.
[2] a) G. Fischer, *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1415–1436; *Angew. Chem.* **1994**, *106*, 1479–1501; b) G. Fischer, *Chem. Soc. Rev.* **2000**, *29*, 119–127; c) J. Fanghänel, *Angew. Chem. Int. Ed.* **2003**, *42*, 490–492; *Angew. Chem.* **2003**, *115*, 506–508.
[3] J. Fanghänel, G. Fischer, *Front. Biosci.* **2004**, *9*, 3453–3478.
[4] a) A. Galat, J. Bua, *Cell. Mol. Life Sci.* **2010**, *67*, 3467–3488; b) H. Hoffmann, C. Schiene-Fischer, *Biol. Chem.* **2014**, *395*, 721–735; c) C. Schiene-Fischer, T. Aumüller, G. Fischer, *Top. Curr. Chem.* **2013**, *328*, 35–67.
[5] Review: P. Wang, J. Heitman, *Genome Biol.* **2005**, *6*, 226.
[6] R. E. Handschumacher, M. W. Harding, J. Rice, R. J. Drugge, D. W. Speicher, *Science* **1984**, *226*, 544–547.
[7] For controversial opinions on the immunosuppressive effect of cyclosporin, see a) J. F. Borel, Z. L. Kis, *Transplant. Proc.* **1991**, *23*, 1867–1874; b) H. F. Stähelin, *Experientia* **1996**, *52*, 5–13; c) K. Heusler, A. Pletscher, *Swiss Med. Wkly.* **2001**, *131*, 299–302.
[8] C. Tapia, T. A. Nessel, P. M. Zito, *Cyclosporine*, StatPearls, Treasure Island, **2021**.
[9] a) J. Liu, J. D. Farmer, Jr., W. S. Lane, J. Friedman, I. Weissman, S. L. Schreiber, *Cell* **1991**, *66*, 807–815; b) Q. Huai, H. Y. Kim, Y. Liu, Y. Zhao, A. Mondragon, J. O. Liu, H. Ke,

Proc. Natl. Acad. Sci. USA **2002**, *99*, 12037–12042; c) L. Jin, S. C. Harrison, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 13522–13526.

[10] a) B. M. Dunyak, J. E. Gestwicki, *J. Med. Chem.* **2016**, *59*, 9622–9644; b) P. T. Flaherty, P. Jain, *Annu. Rep. Med. Chem.* **2011**, *46*, 337–349.

[11] For reviews describing the development of this field up to the beginning of the 2010s, see a) P. A. Gallay, *Immunol. Res.* **2012**, *52*, 200–210; b) M. Peel, A. Scribner, *Bioorg. Med. Chem. Lett.* **2013**, *23*, 4485–4492; c) N. V. Naoumov, *J. Hepatol.* **2014**, *61*, 1166–1174.

[12] A. J. Koletsky, M. W. Harding, R. E. Handschumacher, *J. Immunol.* **1986**, *137*, 1054–1059.

[13] C. Rajiv, T. L. Davis, *Biomol. Eng.* **2018**, *8*, 161.

[14] a) J. L. Kofron, P. Kuzmic, V. Kishore, E. Colon-Bonilla, D. H. Rich, *Biochemistry* **1991**, *30*, 6127–6134; b) B. Janowski, S. Wollner, M. Schutkowski, G. Fischer, *Anal. Biochem.* **1997**, *252*, 299–307; c) G. Zoldák, T. Aumüller, C. Lücke, J. Hritz, C. Oostenbrink, G. Fischer, F. X. Schmid, *Biochemistry* **2009**, *48*, 10423–10436.

[15] R. K. Harrison, R. L. Stein, *Biochemistry* **1990**, *29*, 3813–3816.

[16] a) N. Baum, C. Schiene-Fischer, M. Frost, M. Schumann, K. Sabapathy, O. Ohlenschläger, F. Grosse, B. Schlott, *Oncogene* **2009**, *28*, 3915–3925; b) K. Bauer, A. K. Kretzschmar, H. Cvijic, C. Blumert, D. Löffler, K. Brocke-Heidrich, C. Schiene-Fischer, G. Fischer, A. Sinz, C. V. Clevenger, F. Horn, *Oncogene* **2009**, *28*, 2784–2795; c) H. Pan, C. Luo, R. Li, A. Qiao, L. Zhang, M. Mines, A. M. Nyanda, J. Zhang, G.-H. Fan, *J. Biol. Chem.* **2008**, *283*, 623–637; d) C. Zhu, X. Wang, J. Deinum, Z. Huang, J. Gao, N. Modjtahedi, M. R. Neagu, M. Nilsson, P. S. Eriksson, H. Hagberg, J. Luban, G. Kroemer, K. Blomgren, *J. Exp. Med.* **2007**, *204*, 1741–1748; e) J. Colgan, M. Asmal, M. Neagu, B. Yu, J. Schneidkraut, Y. Lee, E. Sokolskaja, A. Andreotti, J. Luban, *Immunity* **2004**, *21*, 189–201; f) P. Sarkar, T. Saleh, S.-R. Tzeng, R. B. Birge, C. G. Kalodimos, *Nat. Chem. Biol.* **2011**, *7*, 51–57; g) J. C. Marini, W. A. Cabral, A. M. Barnes, W. Chang, *Cell Cycle* **2007**, *6*, 1675–1681; h) M. A. Rycyzyn, C. V. Clevenger, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 6790–6795; i) A. P. Halestrap, *Biochem. Soc. Symp.* **1999**, *66*, 181–203; j) C. P. Baines, M. Gutiérrez-Aguilar, *Cell Calcium* **2018**, *73*, 121–130; k) T. Ratajczak, B. K. Ward, R. F. Minchin, *Curr. Top. Med. Chem.* **2003**, *3*, 1348–1357.

[17] a) B. Sherry, N. Yarlett, A. Strupp, A. Cerami, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 3511–3515; b) E. R. Price, M. Jin, D. Lim, S. Pati, C. T. Walsh, F. D. McKeon, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 3931–3935; c) V. Yurchenko, G. Zybarth, M. O′Connor, W. W. Dai, G. Franchin, T. Hao, H. Guo, H.-C. Hung, B. Toole, P. Gallay, B. Sherry, M. Bukrinsky, *J. Biol. Chem.* **2002**, *277*, 22959–22965.

[18] a) M. Lammers, H. Neumann, J. W. Chin, L. C. James, *Nat. Chem. Biol.* **2010**, *6*, 331–337; b) N. N. Soe, M. Sowden, P. Baskaran, Y. Kim, P. Nigro, E. M. Smolock, B. C. Berk, *Cardiovasc. Res.* **2014**, *101*, 444–453; c) M. Schumann, C. H. Ihling, E. Prell, A. Schierhorn, A. Sinz, G. Fischer, C. Schiene-Fischer, M. Malešević, *Proteomics* **2016**, *16*, 2815–2826.

[19] a) S. D. Frausto, E. Lee, H. Tang, *Viruses* **2013**, *5*, 1684–1701; b) P. Nigro, G. Pompilio, M. C. Capogrossi, *Cell Death Dis.* **2013**, *4*, e888; c) J. Lee, S. S. Kim, *J. Int. Med. Res.* **2010**, *38*, 1561–1574; d) C. Xue, M. P. Sowden, B. C. Berk, *Arterioscler. Thromb. Vasc. Biol.* **2018**, *38*, 986–993; e) M. Bukrinsky, *Biochim. Biophys. Acta Gen. Subj.* **2015**, *1850*, 2087–2095; f) J. Luban, K. L. Bossolt, E. K. Franke, G. V. Kalpana, S. P. Goff, *Cell* **1993**, *73*, 1067–1078; g) K. Watashi, N. Ishii, M. Hijikata, D. Inoue, T. Murata, Y. Miyanari, K. Shimotohno, *Mol. Cell* **2005**, *19*, 111–122; h) F. S. van Dijk, I. M. Nesbitt, E. H. Zwikstra, P. G. Nikkels, S. R. Piersma, S. A. Fratantoni, C. R. Jimenez, M. Huizer, A. C. Morsman, J. M. Cobben, M. H. van Roij, M. W. Elting, J. I. Verbeke, L. C. Wijnaendts, N. J. Shaw, W. Hogler, C. McKeown, E. A. Sistermans, A. Dalton, H. Meijers-Heijboer, G. Pals, *Am. J. Hum. Genet.* **2009**, *85*, 521–527; i) G. Amanakis, E. Murphy, *Front. Plant Physiol.* **2020**, *11*, 595.

[20] a) X. Liu, Z. Zhao, W. Liu, *Viruses* **2013**, *5*, 182–191; b) M. Qing, F. Yang, B. Zhang, G. Zou, J. M. Robida, Z. Yuan, H. Tang, P. Y. Shi, *Antimicrob. Agents Chemother.* **2009**, *53*, 3226–3235; c) A. H. de Wilde, U. Pham, C. C. Posthuma, E. J. Snijder, *Virology* **2018**, *522*, 46–55.

[21] a) T. R. Gamble, F. F. Vajdos, S. Yoo, D. K. Worthylake, M. Houseweart, W. I. Sundquist, C. P. Hill, *Cell* **1996**, *87*, 1285–1294; b) A. De Iaco, J. Luban, *Retrovirology* **2014**, *11*, 11.

[22] a) F. Fernandes, I. U. Ansari, R. Striker, *PLoS One* **2010**, *5*, e9815; b) X. Hanoulle, A. Badillo, J. M. Wieruszeski, D. Verdegem, I. Landrieu, R. Bartenschlager, F. Penin, G. Lippens, *J. Biol. Chem.* **2009**, *284*, 13589–13601; c) D. Verdegem, A. Badillo, J. M. Wieruszeski, I. Landrieu, A. Leroy, R. Bartenschlager, F. Penin, G. Lippens, X. Hanoulle, *J. Biol. Chem.* **2011**, *286*, 20441–20454; d) U. Chatterji, M. Bobardt, A. Tai, M. Wood, P. A. Gallay, *Antimicrob. Agents Chemother.* **2015**, *59*, 2496–2507.

[23] a) Y. Ma-Lauer, Y. Zheng, M. Malesevic, B. von Brunn, G. Fischer, A. von Brunn, *Antiviral Res.* **2020**, *173*, 104620; b) J. Carbajo-Lozoya, Y. Ma-Lauer, M. Malesevic, M. Theuerkorn, V. Kahlert, E. Prell, B. von Brunn, D. Muth, T. F. Baumert, C. Drosten, G. Fischer, A. von Brunn, *Virus Res.* **2014**, *184*, 44–53; c) A. H. de Wilde, V. S. Raj, D. Oudshoorn, T. M. Bestebroer, S. van Nieuwkoop, R. Limpens, C. C. Posthuma, Y. van der Meer, M. Barcena, B. L. Haagmans, E. J. Snijder, B. G. van den Hoogen, *J. Gen. Virol.* **2013**, *94*, 1749–1760; d) A. H. de Wilde, D. Falzarano, J. C. Zevenhoven-Dobbe, C. Beugeling, C. Fett, C. Martellaro, C. C. Posthuma, H. Feldmann, S. Perlman, E. J. Snijder, *Virus Res.* **2017**, *228*, 7–13.

[24] A. H. de Wilde, J. C. Zevenhoven-Dobbe, Y. van der Meer, V. Thiel, K. Narayanan, S. Makino, E. J. Snijder, M. J. van Hemert, *J. Gen. Virol.* **2011**, *92*, 2542–2548.

[25] a) A. Pizzorno, B. Padey, J. Dubois, T. Julien, A. Traversier, V. Duliere, P. Brun, B. Lina, M. Rosa-Calatrava, O. Terrier, *Antiviral Res.* **2020**, *181*, 104878; b) M. Dittmar, J. S. Lee, K. Whig, E. Segrist, M. Li, B. Kamalia, L. Castellana, K. Ayyanathan, F. L. Cardenas-Diaz, E. E. Morrisey, R. Truitt, W. Yang, K. Jurado, K. Samby, H. Ramage, D. C. Schultz, S. Cherry, *Cell Rep.* **2021**, *35*, 108959; c) J. S. Redzic, E. Lee, A. Born, A. Issaian, M. A. Henen, P. J. Nichols, A. Blue, K. C. Hansen, A. D'Alessandro, B. Vogeli, E. Z. Eisenmesser, *J. Mol. Biol.* **2021**, *433*, 167108.

[26] J. L. Gálvez-Romero, O. Palmeros-Rojas, F. A. Real-Ramírez, S. Sánchez-Romero, R. Tome-Maxil, M. P. Ramírez-Sandoval, R. Olivos-Rodríguez, S. E. Flores-Encarnación, A. A. Cabrera-Estrada, J. Ávila-Morales, V. Cortés-Sánchez, G. Sarmiento-Padilla, S. E. Tezmol-Ramírez, D. Aparicio-Hernández, M. I. Urbina-Sánchez, M. A. Gómez-Pluma, S. Cisneros-Méndez, D. I. Rodríguez-Rivas, S. Reyes-Inurrigarro, G. Cortés-Diaz, C. Cruz-Delgado, J. Navarro-González, J. Deveaux-Homs, S. Pedraza-Sánchez, *J. Intern. Med.* **2021**, *289*, 906–920.

[27] a) A. Billich, G. Winkler, H. Aschauer, A. Rot, P. Peichl, *J. Exp. Med.* **1997**, *185*, 975–980; b) I. Tegeder, A. Schumacher, S. John, H. Geiger, G. Geisslinger, H. Bang, K. Brune, *J. Clin. Immunol.* **1997**, *17*, 380–386; c) E. J. Stemmy, A. S. Benton, J. Lerner, S. Alcala, S. L. Constant, R. J. Freishtat, *J. Asthma* **2011**, *48*, 986–993.

[28] a) P. Nigro, K. Satoh, M. R. O'Dell, N. N. Soe, Z. Cui, A. Mohan, J. Abe, J. D. Alexis, J. D. Sparks, B. C. Berk, *J. Exp. Med.* **2011**, *208*, 53–66; b) P. Seizer, C. Ochmann, T. Schonberger, S. Zach, M. Rose, O. Borst, K. Klingel, R. Kandolf, H. R. MacDonald, R. A. Nowak, S. Engelhardt, F. Lang, M. Gawaz, A. E. May, *Arterioscler. Thromb. Vasc. Biol.* **2011**, *31*, 1377–1386; c) S. N. I. von Ungern-Sternberg, S. Vogel, B. Walker-Allgaier, S. Geue, A. Maurer, A. M. Wild, P. Munzer, M. Chatterjee, D. Heinzmann, E. Kremmer, O. Borst, P. Loughran, A. Zernecke, M. D. Neal, T. R. Billiar, M. Gawaz, P. Seizer, *Thromb. Haemostasis* **2017**, *117*, 2063–2078.

[29] a) A. P. Halestrap, P. M. Kerr, S. Javadov, K. Y. Woodfield, *Biochim. Biophys. Acta Bioenerg.* **1998**, *1366*, 79–94; b) A. C. Schinzel, O. Takeuchi, Z. H. Huang, J. K. Fisher, Z. P. Zhou, J. Rubens, C. Hetz, N. N. Danial, M. A. Moskowitz, S. J. Korsmeyer, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 12005–12010; c) J. S. Park, R. Pasupulati, T. Feldkamp, N. F. Roeser, J. M. Weinberg, *Am. J. Physiol-Renal.* **2011**, *301*, F134–F150; d) J. Warne, G. Pryce, J. M. Hill, X. Shi, F. Lenneras, F. Puentes, M. Kip, L. Hilditch, P. Walker, M. I. Simone, A. W. Chan, G. J. Towers, A. R. Coker, M. R. Duchen, G. Szabadkai, D. Baker, D. L. Selwood, *J. Biol. Chem.* **2016**, *291*, 4356–4373; e) E. Palma, T. Tiepolo, A. Angelin, P. Sabatelli, N. M. Maraldi, E. Basso, M. A. Forte, P. Bernardi, P. Bonaldo, *Hum. Mol. Genet.* **2009**, *18*, 2024–2031; f) X. Wang, H. Du, S. Shao, T. Bo, C. Yu, W. Chen, L. Zhao, Q. Li, L. Wang, X. Liu, X. Su, M. Sun, Y. Song, L. Gao, J. Zhao, *Hepatology* **2018**, *68*, 62–77.

[30] H. Du, L. Guo, F. Fang, D. Chen, A. A. Sosunov, G. M. McKhann, Y. Yan, C. Wang, H. Zhang, J. D. Molkentin, F. J. Gunn-Moore, J. P. Vonsattel, O. Arancio, J. X. Chen, S. D. Yan, *Nat. Med.* **2008**, *14*, 1097–1105.

[31] a) Z. Gong, C. Chi, X. Huang, H. Chu, J. Wang, F. Du, L. Jiang, J. Chen, *Anticancer Res.* **2017**, *37*, 4443–4447; b) E. S. Grigoryeva, N. V. Cherdyntseva, M. S. Karbyshev, V. V. Volkomorov, I. V. Stepanov, M. V. Zavyalova, V. M. Perelmuter, M. A. Buldakov, S. G. Afanasjev, S. A. Tuzikov, Y. A. Bukurova, N. A. Lisitsyn, S. F. Beresten, *Pathol. Oncol. Res.* **2014**, *20*, 467–473; c) J. Yang, M. Zhou, R. Zhao, S. Peng, Z. Luo, X. Li, L. Cao, K. Tang, J. Ma, W. Xiong, S. Fan, D. C. Schmitt, M. Tan, X. Li, G. Li, *J. Proteomics* **2014**, *109*, 162–175; d) Y. Li, H. Guo, D. Dong, H. Wu, E. Li, *Diagn. Pathol.* **2013**, *8*, 207; e) M. Li, Q. Zhai, U. Bharadwaj, H. Wang, F. Li, W. E. Fisher, C. Chen, Q. Yao, *Cancer* **2006**, *106*, 2284–2294.

[32] Z. Li, X. Zhao, S. Bai, Z. Wang, L. Chen, Y. Wei, C. Huang, *Mol. Cell. Proteomics* **2008**, *7*, 1810–1823.

[33] a) R. Amundsen, H. Christensen, B. Zabihyan, A. Asberg, *Drug Metab. Dispos.* **2010**, *38*, 1499–1504; b) Y. Shitara, Y. Nagamatsu, S. Wada, Y. Sugiyama, T. Horie, *Drug Metab. Dispos.* **2009**, *37*, 1172–1178; c) A. A. K. El-Sheikh, R. Greupink, H. M. Wortelboer, J. J. van den Heuvel, M. Schreurs, J. B. Koenderink, R. Masereeuw, F. G. Russel, *Transl. Res.* **2013**, *162*, 398–409; d) P. R. Twentyman, *Biochem. Pharmacol.* **1992**, *43*, 109–117.

[34] H. Ke, D. Mayrose, P. J. Belshaw, D. G. Alberg, S. L. Schreiber, Z. Y. Chang, F. A. Etzkorn, S. Ho, C. T. Walsh, *Structure* **1994**, *2*, 33–44.

[35] Y. Zhao, H. Ke, *Biochemistry* **1996**, *35*, 7356–7361.

[36] V. Mikol, J. Kallen, G. Pflügl, M. D. Walkinshaw, *J. Mol. Biol.* **1993**, *234*, 1119–1130.

[37] a) R. M. Wenger, J. France, G. Bovermann, L. Walliser, A. Widmer, H. Widmer, *FEBS Lett.* **1994**, *340*, 255–259; b) S. K. J. Johnas, B. Dittrich, A. Meents, M. Messerschmidt, E. F. Weckert, *Acta Crystallogr. Sect. D* **2009**, *65*, 284–293. The drawings of CsA within this review are based thereupon.

[38] M. J. Bossard, P. L. Koser, M. Brandt, D. J. Bergsma, M. A. Levy, *Biochem. Biophys. Res. Commun.* **1991**, *176*, 1142–1148.

[39] S. Y. Ko, R. M. Wenger, *Helv. Chim. Acta* **1997**, *80*, 695–705.

[40] D. Seebach, A. K. Beck, H. G. Bossler, C. Gerber, S. Y. Ko, C. W. Murtiashaw, R. Naef, S. Shoda, A. Thaler, M. Krieger, R. Wenger, *Helv. Chim. Acta* **1993**, *76*, 1564–1590.

[41] D. Seebach, A. K. Beck, A. Studer in *Modern Synthetic Methods, Vol. 7* (Eds. B. Ernst, C. Leumann), Verlag Helvetica Chimica Acta, Basel/VCH, **1995**, pp. 1–178.

[42] V. F. J. Quesniaux, M. H. Schreier, R. M. Wenger, P. C. Hiestand, M. W. Harding, M. H. V. Van Regenmortel, *Eur. J. Immunol.* **1987**, *17*, 1359–1365.

[43] Z. Su, Z. Long, Z. Huang, S. Yang, US 9,890,198 B2, **2018**.

[44] S. Malouitre, H. Dube, D. Selwood, M. Crompton, *Biochem. J.* **2010**, *425*, 137–148.

[45] C. Papageorgiou, X. Borer, R. R. French, *Bioorg. Med. Chem. Lett.* **1994**, *4*, 267–272.

[46] R. Traber, H. Kobel, H. R. Loosli, H. Senn, B. Rosenwirth, A. Lawen, *Antiviral Chem. Chemother.* **1994**, *5*, 331–339.

[47] G. Zenke, G. Baumann, R. Wenger, P. Hiestand, V. Quesniaux, E. Andersen, M. H. Schreier, *Ann. N. Y. Acad. Sci.* **1993**, *685*, 330–335.

[48] F. Hubler, T. Ruckle, L. Patiny, T. Muamba, J. F. Guichou, M. Mutter, R. Wenger, *Tetrahedron Lett.* **2000**, *41*, 7193–7196.

[49] M. Kuhnt, F. Bitsch, J. France, H. Hofmann, J. J. Sanglier, R. Traber, *J. Antibiot.* **1996**, *49*, 781–787.

[50] a) A. Billich, F. Hammerschmid, P. Peichl, R. Wenger, G. Zenke, V. Quesniaux, B. Rosenwirth, *J. Virol.* **1995**, *69*, 2451–2461; b) R. G. Ptak, P. A. Gallay, D. Jochmans, A. P. Halestrap, U. T. Ruegg, L. A. Pallansch, M. D. Bobardt, M. P. de Béthune, J. Neyts, E. De Clercq, J. M. Dumont, P. Scalfaro, K. Besseghir, R. M. Wenger, B. Rosenwirth, *Antimicrob. Agents Chemother.* **2008**, *52*, 1302–1317.

[51] S. Ma, J. E. Boerner, C. TiongYip, B. Weidmann, N. S. Ryder, M. P. Cooreman, K. Lin, *Antimicrob. Agents Chemother.* **2006**, *50*, 2976–2982.

[52] a) E. J. Stemmy, M. A. Balsley, R. A. Jurjus, J. M. Damsker, M. I. Bukrinsky, S. L. Constant, *Am. J. Respir. Cell Mol. Biol.* **2011**, *45*, 991–998; b) P. Seizer, S. N. Ungern-Sternberg, T. Schönberger, O. Borst, P. Münzer, E. M. Schmidt, A. F. Mack, D. Heinzmann, M. Chatterjee, H. Langer, M. Malešević, F. Lang, M. Gawaz, G. Fischer, A. E. May, *Arterioscler. Thromb. Vasc. Biol.* **2015**, *35*, 655–663.

[53] K. Niimi, T. Yasui, A. Okada, Y. Hirose, Y. Kubota, Y. Umemoto, N. Kawai, K. Tozawa, K. Kohri, *Int. J. Urol.* **2014**, *21*, 707–713.

[54] A. S. Korde, P. Waldmeier, L. C. Pettigrew, S. D. Craddock, W. F. Maragos, *J. Cereb. Blood Flow Metab.* **2005**, *25*, S5–S5.

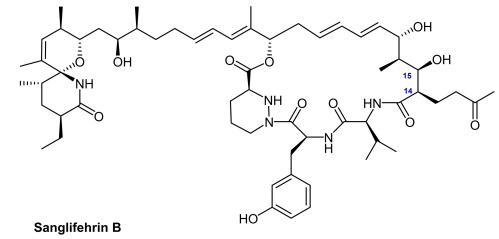
[55] A. Zulian, E. Rizzo, M. Schiavone, E. Palma, F. Tagliavini, B. Blaauw, L. Merlini, N. M. Maraldi, P. Sabatelli, P. Braghetta, P. Bonaldo, F. Argenton, P. Bernardi, *Hum. Mol. Genet.* **2014**, *23*, 5353–5363.

[56] a) A. Nicolli, E. Basso, V. Petronilli, R. M. Wenger, P. Bernardi, *J. Biol. Chem.* **1996**, *271*, 2185–2192; b) H. Fliri, G. Baumann, A. Enz, J. Kallen, M. Luyten, V. Mikol, R. Movva, V. Quesniaux, M. Schreier, M. Walkinshaw, R. Wenger, G. Zenke, M. Zurini, *Ann. N. Y. Acad. Sci.* **1993**, *696*, 47–53.

[57] B. A. Nacev, W. K. Low, Z. N. Huang, T. T. Su, Z. Su, H. Alkuraya, D. Kasuga, W. Sun, M. Träger, M. Braun, G. Fischer, K. Zhang, J. O. Liu, *J. Pharmacol. Exp. Ther.* **2011**, *338*, 466–475.

[58] a) P. Scalfaro, J.-M. Dumont, G. Vuagniaux, R.-Y. Mauvernay, WO 2006/038088, **2006**; b) for a crystal structure of alisporivir in complex with cyclophilin A, see M. Dujardin, J. Bouckaert, P. Rucktooa, X. Hanoulle, *Acta Crystallogr. Sect. F* **2018**, *74*, 583–592.

[59] S. Y. Ko, H. Kobel, B. Rosenwirth, D. Seebach, R. P. Traber, R. Wenger, P. Bollinger, EP 484281A2, **1992**.
[60] M. Evers, J. C. Barrière, G. Bashiardes, A. Bousseau, J. C. Carry, N. Dereu, B. Filoche, Y. Henin, S. Sable, M. Vuilhorgne, S. Mignani, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4415–4419.
[61] a) P. A. Gallay, U. Chatterji, M. D. Bobardt, Z. Long, S. Zhang, Z. Su, *PLoS One* **2016**, *11*, e0152036; b) Z. Su, S. Yang, Z. Long, WO 2017/200984A1, **2017**.
[62] S. Hopkins, B. Scorneaux, Z. Huang, M. G. Murray, S. Wring, C. Smitley, R. Harris, F. Erdmann, G. Fischer, Y. Ribeill, *Antimicrob. Agents Chemother.* **2010**, *54*, 660–672.
[63] R. Flisiak, A. Horban, P. Gallay, M. Bobardt, S. Selvarajah, A. Wiercinska-Drapalo, E. Siwak, I. Cielniak, J. Higersberger, J. Kierkus, C. Aeschlimann, P. Grosgurin, V. Nicolas-Métral, J. M. Dumont, H. Porchet, R. Crabbé, P. Scalfaro, *Hepatology* **2008**, *47*, 817–826.
[64] a) R. Flisiak, S. V. Feinman, M. Jablkowski, A. Horban, W. Kryczka, M. Pawlowska, J. E. Heathcote, G. Mazzella, C. Vandelli, V. Nicolas-Metral, P. Grosgurin, J. S. Liz, P. Scalfaro, H. Porchet, R. Crabbé, *Hepatology* **2009**, *49*, 1460–1468; b) S. Hopkins, B. DiMassimo, P. Rusnak, D. Heuman, J. Lalezari, A. Sluder, B. Scorneaux, S. Mosier, P. Kowalczyk, Y. Ribeill, J. Baugh, P. Gallay, *J. Hepatol.* **2012**, *57*, 47–54.
[65] a) L. Coelmont, X. Hanoulle, U. Chatterji, C. Berger, J. Snoeck, M. Bobardt, P. Lim, I. Vliegen, J. Paeshuyse, G. Vuagniaux, A. M. Vandamme, R. Bartenschlager, P. Gallay, G. Lippens, J. Neyts, *PLoS One* **2010**, *5*, e13687; b) S. Hopkins, M. Bobardt, U. Chatterji, J. A. Garcia-Rivera, P. Lim, P. A. Gallay, *Antimicrob. Agents Chemother.* **2012**, *56*, 3888–3897.
[66] A. I. Chiramel, L. Banadyga, J. D. Dougherty, D. Falzarano, C. Martellaro, D. Brees, R. T. Taylor, H. Ebihara, S. M. Best, *J. Infect. Dis.* **2016**, *214*, S355–S359.
[67] L. Sauerhering, A. Kupke, L. Meier, E. Dietzel, J. Hoppe, A. D. Gruber, S. Gattenloehner, B. Witte, L. Fink, N. Hofmann, T. Zimmermann, A. Goesmann, A. Nist, T. Stiewe, S. Becker, S. Herold, C. Peteranderl, *Eur. Respir. J.* **2020**, *56*, 1901826.
[68] L. Softic, R. Brillet, F. Berry, N. Ahnou, Q. Nevers, M. Morin-Dewaele, S. Hamadat, P. Bruscella, S. Fourati, J. M. Pawlotsky, A. Ahmed-Belkacem, *Antimicrob. Agents Chemother.* **2020**, *64*, e00876.
[69] a) M. Schiavone, A. Zulian, S. Menazza, V. Petronilli, F. Argenton, L. Merlini, P. Sabatelli, P. Bernardi, *Pharmacol. Res.* **2017**, *125*, 122–131; b) A. LoGuidice, V. Ramirez-Alcantara, A. Proli, B. Gavillet, U. A. Boelsterli, *Toxicol. Sci.* **2010**, *118*, 276–285; c) L. Gomez, H. Thibault, A. Gharib, J. M. Dumont, G. Vuagniaux, P. Scalfaro, G. Derumeaux, M. Ovize, *Am. J. Physiol. Heart Circ. Physiol.* **2007**, *293*, H1654–1661.
[70] J. Lin, F. Shen, J. Lu, F. Liang, Y. Zhang, Z. Xie, Y. Dong, *Front. Aging Neurosci.* **2021**, *13*, 688587.
[71] a) F. Loor, F. Tiberghien, T. Wenandy, A. Didier, R. Traber, *J. Med. Chem.* **2002**, *45*, 4598–4612; b) T. Kamisako, I. Leier, Y. Cui, J. König, U. Buchholz, J. Hummel-Eisenbeiss, D. Keppler, *Hepatology* **1999**, *30*, 485–490.
[72] J. Fu, M. Tjandra, C. Becker, D. Bednarczyk, M. Capparelli, R. Elling, I. Hanna, R. Fujimoto, M. Furegati, S. Karur, T. Kasprzyk, M. Knapp, K. Leung, X. Li, P. Lu, W. Mergo, C. Miault, S. Ng, D. Parker, Y. Peng, S. Roggo, A. Rivkin, R. L. Simmons, M. Wang, B. Wiedmann, A. H. Weiss, L. Xiao, L. Xie, W. Xu, A. Yifru, S. Yang, B. Zhou, Z. K. Sweeney, *J. Med. Chem.* **2014**, *57*, 8503–8516.
[73] T. Lazarova, Z. Weng, *Expert Opin. Ther. Pat.* **2003**, *13*, 1327–1332.
[74] a) D. H. Rich, C. Q. Sun, D. Guillaume, B. Dunlap, D. A. Evans, A. E. Weber, *J. Med. Chem.* **1989**, *32*, 1982–1987; b) J. D. Aebi, D. T. Deyo, C. Q. Sun, D. Guillaume, B. Dunlap, D. H. Rich, *J. Med. Chem.* **1990**, *33*, 999–1009; c) N. H. Sigal, F. Dumont, P. Durette, J. J. Siekierka, L. Peterson, D. H. Rich, B. E. Dunlap, M. J. Staruch, M. R. Melino, S. L. Koprak, D. Williams, B. Witzel, J. M. Pisano, *J. Exp. Med.* **1991**, *173*, 619–628.
[75] E. Prell, V. Kahlert, K. P. Rücknagel, M. Malešević, G. Fischer, *ChemBioChem* **2013**, *14*, 63–65.
[76] A. Hegmans, B. W. Fenske, D. J. Trepanier, M. D. Abel, S. Sugiyama, D. R. Ure, WO 2012079172A1, **2012**.
[77] F. X. Li, Q. Z. Zhang, S. J. Li, G. Lin, X. Y. Huo, Y. Lan, Z. Yang, *Org. Lett.* **2021**, *23*, 3421–3425.
[78] a) P. A. Gallay, M. D. Bobardt, U. Chatterji, D. J. Trepanier, D. Ure, C. Ordonez, R. Foster, *PLoS One* **2015**, *10*, e0134707; b) J. Kuo, M. Bobardt, U. Chatterji, P. R. Mayo, D. J. Trepanier, R. T. Foster, P. Gallay, D. R. Ure, *J. Pharmacol. Exp. Ther.* **2019**, *371*, 231–241.
[79] P. Gallay, D. Ure, M. Bobardt, U. Chatterji, J. Ou, D. Trepanier, R. Foster, *PLoS One* **2019**, *14*, e0217433.
[80] C. Papageorgiou, J. Kallen, J. France, R. French, *Bioorg. Med. Chem.* **1997**, *5*, 187–192.
[81] a) J. Liu, M. W. Albers, T. J. Wandless, S. Luan, D. G. Alberg, P. J. Belshaw, P. Cohen, C. MacKintosh, C. B. Klee, S. L. Schreiber, *Biochemistry* **1992**, *31*, 3896–3901; b) J. P. Steiner, M. A. Connolly, H. L. Valentine, G. S. Hamilton, T. M. Dawson, L. Hester, S. H. Snyder, *Nat. Med.* **1997**, *3*, 421–428.
[82] A. Scribner, D. Houck, Z. Huang, S. Mosier, M. Peel, B. Scorneaux, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6542–6546.
[83] M. Malešević, J. Kühling, F. Erdmann, M. A. Balsley, M. I. Bukrinsky, S. L. Constant, G. Fischer, *Angew. Chem. Int. Ed.* **2010**, *49*, 213–215; *Angew. Chem.* **2010**, *122*, 219–222.
[84] M. Malešević, D. Gutknecht, E. Prell, C. Klein, M. Schumann, R. A. Nowak, J. C. Simon, C. Schiene-Fischer, A. Saalbach, *J. Med. Chem.* **2013**, *56*, 7302–7311.
[85] a) D. Heinzmann, A. Bangert, A. M. Müller, S. N. von Ungern-Sternberg, F. Emschermann, T. Schönberger, M. Chatterjee, A. F. Mack, K. Klingel, R. Kandolf, M. Malešević, O. Borst, M. Gawaz, H. F. Langer, H. Katus, G. Fischer, A. E. May, Z. Kaya, P. Seizer, *PLoS One* **2015**, *10*, e0124606; b) C. Xue, M. Sowden, B. C. Berk, *Arterioscler. Thromb. Vasc. Biol.* **2017**, *37*, 1138–1146.
[86] S. B. Park, G. P. Meier, *Tetrahedron Lett.* **1989**, *30*, 4215–4218.
[87] H. Dube, D. Selwood, S. Malouitre, M. Capano, M. I. Simone, M. Crompton, *Biochem. J.* **2012**, *441*, 901–907.
[88] S. Ho, N. Clipstone, L. Timmermann, J. Northrop, I. Graef, D. Fiorentino, J. Nourse, G. R. Crabtree, *Clin. Immunol. Immunopathol.* **1996**, *80*, 40–45.
[89] S. Hyun, N. Park, S. H. Nam, D. H. Cheon, Y. Lee, H. S. Lim, J. Yu, *Chem. Commun.* **2021**, *57*, 2388–2391.
[90] a) J.-J. Sanglier, V. Quesniaux, T. Fehr, H. Hofmann, M. Mahnke, K. Memmert, W. Schuler, G. Zenke, L. Gschwind, C. Maurer, W. Schilling, *J. Antibiot.* **1999**, *52*, 466–473; b) T. Fehr, J. Kallen, L. Oberer, J.-J. Sanglier, W. Schilling, *J. Antibiot.* **1999**, *52*, 474–479. The structures od sanglifehrins B–D differ from that of sanglifehrin A in the spirocyclic moiety and/or acetal formation in the side chains at C-14 and C-15:



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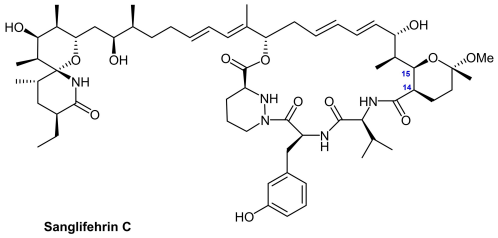

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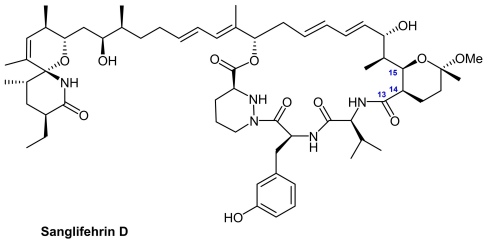


Sanglifehrin D

[91] a) K. C. Nicolaou, F. Murphy, S. Barluenga, T. Ohshima, H. Wei, J. Xu, D. L. F. Gray, O. Baudoin, *J. Am. Chem. Soc.* **2000**, *122*, 3830–3838; b) L. A. Paquette, M. Duan, I. Konetzki, C. Kempmann, *J. Am. Chem. Soc.* **2002**, *124*, 4257–4270; c) C.-F. Chang, H. A. Flaxman, C. M. Woo, *Angew. Chem. Int. Ed.* **2021**, *60*, 17045–17052; *Angew. Chem.* **2021**, *133*, 17182–17189.

[92] T. Lindel in *Organic Synthesis Highlights V* (Eds.: H.-G. Schmalz, T. Wirth), Wiley-VCH, Weinheim, **2003**, pp. 350–359.

[93] a) L. M. Martin Cabrejas, S. Rohrbach, D. Wagner, J. Kallen, G. Zenke, J. Wagner, *Angew. Chem. Int. Ed.* **1999**, *38*, 2443–2446; *Angew. Chem.* **1999**, *111*, 2595–2599; b) R. Bänteli, I. Brun, P. Hall, R. Metternich, *Tetrahedron Lett.* **1999**, *40*, 2109–2112; c) P. Hall, J. Brun, D. Denni, R. Metternich, *Synlett* **2000**, 315–318; d) J. Wagner, L. M. M. Cabrejas, C. E. Grossmith, C. Papageorgiou, F. Senia, D. Wagner, J. France, S. P. Nolan, *J. Org. Chem.* **2000**, *65*, 9255–9260; e) R. Bänteli, J. Wagner, G. Zenke, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1609–1612; f) M. K. Gurjar, A. R. Chaudhuri, *Tetrahedron Lett.* **2002**, *43*, 2435–2438; g) J. Wagner, H. Andres, S. Rohrbach, D. Wagner, L. Oberer, J. France, *J. Org. Chem.* **2005**, *70*, 9588–9590; h) J. S. Yadav, K. V. R. Rao, A. Kavita, D. K. Mohapatra, *Eur. J. Org. Chem.* **2013**, 2849–2858; i) L. Radhika, S. Chandrasekhar, *Synth. Commun.* **2014**, *44*, 3602–3609; j) K. Suttisintong, J. D. White, *J. Org. Chem.* **2015**, *80*, 2249–2262.

[94] M. A. Gregory, M. Bobardt, S. Obeid, U. Chatterji, N. J. Coates, T. Foster, P. Gallay, P. Leyssen, S. J. Moss, J. Neyts, M. Nur-e-Alam, J. Paeshuyse, M. Piraee, D. Suthar, T. Warneck, M. Q. Zhang, B. Wilkinson, *Antimicrob. Agents Chemother.* **2011**, *55*, 1975–1981.

[95] a) G. Zenke, U. Strittmatter, S. Fuchs, V. F. Quesniaux, V. Brinkmann, W. Schuler, M. Zurini, A. Enz, A. Billich, J. J. Sanglier, T. Fehr, *J. Immunol.* **2001**, *166*, 7165–7171; b) R. Sedrani, J. Kallen, L. M. Martin Cabrejas, C. D. Papageorgiou, F. Senia, S. Rohrbach, D. Wagner, B. Thai, A.-M. Jutzi Eme, J. France, L. Oberer, G. Rihs, G. Zenke, J. Wagner, *J. Am. Chem. Soc.* **2003**, *125*, 3849–3859.

[96] K. H. Pua, D. T. Stiles, M. E. Sowa, G. L. Verdine, *Cell Rep.* **2017**, *18*, 432–442.

[97] J. Kallen, R. Sedrani, G. Zenke, J. Wagner, *J. Biol. Chem.* **2005**, *280*, 21965–21971.

[98] S. J. Moss, M. Bobardt, P. Leyssen, N. Coates, U. Chatterji, D. J. Xie, T. Foster, J. L. Liu, M. Nur-e-Alam, D. Suthar, Y. S. Chen, T. Warneck, M. Q. Zhang, J. Neyts, P. Gallay, B. Wilkinson, M. A. Gregory, *MedChemComm* **2012**, *3*, 944–949.

[99] S. J. Moss, M. A. Gregory, B. Wilkinson, C. J. Martin, WO 2011/098805A1, **2011**.

[100] https://www.prnewswire.com/news-releases/neurovive-acquires-highly-potent-novel-cyclophilin-inhibitors-from-biotica-196827741.html.

[101] S. J. Moss, M. A. Gregory, B. Wilkinson, US 9,139,613 B2, **2015**.

[102] M. J. Hansson, S. J. Moss, M. Bobardt, U. Chatterji, N. Coates, J. A. Garcia-Rivera, E. Elmer, S. Kendrew, P. Leyssen, J. Neyts, E. A. M. Nur, T. Warneck, B. Wilkinson, P. Gallay, M. A. Gregory, *Chem. Biol.* **2015**, *22*, 285–292.

[103] M. Bobardt, M. J. Hansson, P. Mayo, D. Ure, R. Foster, P. Gallay, *PLoS One* **2020**, *15*, e0237236.

[104] S. Simón Serrano, A. Grönberg, L. Longato, K. Rombouts, J. Kuo, M. Gregory, S. Moss, E. Elmér, G. Mazza, P. Gallay, M. Pinzani, M. J. Hansson, R. Massoumi, *Cells* **2019**, *8*, 1409.

[105] J. Kuo, S. S. Serrano, A. Grönberg, R. Massoumi, M. J. Hansson, P. Gallay, *Front. Pharmacol.* **2019**, *10*, 1129.

[106] a) V. A. Steadman, K. G. Poullennec, L. Lazarides, C. Aciro, D. K. Dean, A. J. Keats, D. S. Siegel, A. J. Schrier, R. Mackman, P. Jansa, WO 2013/185093A1, **2013**; b) V. A. Steadman, S. B. Pettit, K. G. Poullennec, L. Lazarides, A. J. Keats, D. K. Dean, S. J. Stanway, C. A. Austin, J. A. Sanvoisin, G. M. Watt, H. G. Fliri, A. C. Liclican, D. Jin, M. H. Wong, S. A. Leavitt, Y. J. Lee, Y. Tian, C. R. Frey, T. C. Appleby, U. Schmitz, P. Jansa, R. L. Mackman, B. E. Schultz, *J. Med. Chem.* **2017**, *60*, 1000–1017; c) R. L. Mackman, V. A. Steadman, D. K. Dean, P. Jansa, K. G. Poullennec, T. Appleby, C. Austin, C. A. Blakemore, R. Cai, C. Cannizzaro, G. Chin, J. C. Chiva, N. A. Dunbar, H. Fliri, A. J. Highton, H. Hui, M. Ji, H. Jin, K. Karki, A. J. Keats, L. Lazarides, Y. J. Lee, A. Liclican, M. Mish, B. Murray, S. B. Pettit, P. Pyun, M. Sangi, R. Santos, J. Sanvoisin, U. Schmitz, A. Schrier, D. Siegel, D. Sperandio, G. Stepan, Y. Tian, G. M. Watt, H. Yang, B. E. Schultz, *J. Med. Chem.* **2018**, *61*, 9473–9499.

[107] K. J. Hale, J. Q. Cai, V. Delisser, S. Manaviazar, S. A. Peak, G. S. Bhatia, T. C. Collins, N. Jogiya, *Tetrahedron* **1996**, *52*, 1047–1068.

[108] J. Van Dyke-Blodgett, Y. Hu, US 2019/0002936A1, **2019**.

[109] a) C. H. Heathcock, C. T. White, J. J. Morrison, D. Vanderveer, *J. Org. Chem.* **1981**, *46*, 1296–1309; b) S. Masamune, W. Choy, J. S. Petersen, L. R. Sita, *Angew. Chem. Int. Ed. Engl.* **1985**, *24*, 1–30; *Angew. Chem.* **1985**, *97*, 1–31.

[110] D. Guijarro, O. Pablo, M. Yus, *J. Org. Chem.* **2010**, *75*, 5265–5270.

[111] a) F. A. Davis, P. Zhou, B. C. Chen, *Chem. Soc. Rev.* **1998**, *27*, 13–18; b) M. T. Robak, M. A. Herbage, J. A. Ellman, *Chem. Rev.* **2010**, *110*, 3600–3740.

[112] I. Shiina, M. Kubota, H. Oshiumi, M. Hashizume, *J. Org. Chem.* **2004**, *69*, 1822–1830.

[113] C. C. Colpitts, S. Ridewood, B. Schneiderman, J. Warne, K. Tabata, C. F. Ng, R. Bartenschlager, D. L. Selwood, G. J. Towers, *eLife* **2020**, *9*, e52237.

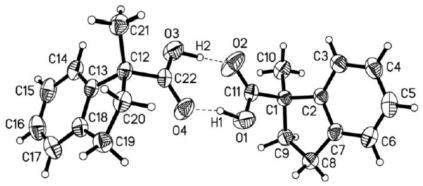
[114] J. L. Paulsen, H. S. Yu, D. Sindhikara, L. Wang, T. Appleby, A. G. Villasenor, U. Schmitz, D. Shivakumar, *J. Chem. Inf. Model.* **2020**, *60*, 3489–3498.

[115] a) S. Daum, F. Erdmann, G. Fischer, B. Féaux de Lacroix, A. Hessamian-Alinejad, S. Houben, W. Frank, M. Braun, *Angew. Chem. Int. Ed.* **2006**, *45*, 7454–7458; *Angew. Chem.* **2006**, *118*, 7615–7619; b) G. Fischer, C. Schiene-Fischer, S. Daum, G. Kuellertz, M. Braun, B. Féaux de Lacroix, WO2008019823, **2008**; c) B. Hernandez Alvarez, G. Fischer, M. Braun, A. Hessamian-Alinejad, H. Fliri, WO2003093258, **2003**; d) M. Braun, A. Hessamian-Alinejad, B. Féaux de Lacroix, B. Hernandez Alvarez, G. Fischer, *Molecules* **2008**, *13*, 995–1003.

[116] For reviews, see: a) G. G. Xu, F. A. Etzkorn, *Drug News Perspect.* **2009**, *22*, 399–407; b) R. Mathur, A. N. Bhatt, S. Gupta, B. S. Dwarakanath, *Front. Anti-Cancer Drug Discovery* **2013**, *2*, 226–263; c) J. D. Moore, A. Potter, *Bioorg. Med. Chem. Lett.* **2013**, *23*, 4283–4291.

[117] B. Féaux de Lacroix, Dissertation, University of Düsseldorf, **2006**.

[118] Assignment of the absolute configuration to (*R*)- and (*S*)-1-methyl-1-indanecarboxylic acid: H. des Abbayes, M. A. Boudeville, *Tetrahedron Lett.* **1976**, *17*, 2137–2140: for confirmation through a crystal structure of the dimeric *R* enantiomer, see F. Kruska, Dissertation, University of Düsseldorf, **2012**.



[119] J. Pietruszka, R. C. Simon, F. Kruska, M. Braun, *Eur. J. Org. Chem.* **2009**, 6217–6224.

[120] This observation guided us to develop ketones as Pin1 inhibitors lacking a stereogenic center in the α-carbonyl position but forming interconvertible atropisomers, cf. T. Hediger, W. Frank, M. Schumann, G. Fischer, M. Braun, *Chem. Biodiversity* **2012**, *9*, 2618–2634.

[121] S. Daum, M. Schuhmann, S. Mathea, T. Aumüller, M. A. Basley, S. L. Constant, B. Féaux de Lacroix, F. Kruska, M. Braun, C. Schiene-Fischer, *Biochemistry* **2009**, *48*, 6268–6277.

[122] S. V. Sambasivarao, O. Acevedo, *J. Chem. Inf. Model.* **2011**, *51*, 475–482.

[123] J.-F. Guichou, J. Viaud, C. Mettling, G. Subra, Y.-L. Lin, A. Chavanieu, *J. Med. Chem.* **2006**, *49*, 900–910.

[124] A. Ahmed-Belkacem, L. Colliandre, N. Ahnou, Q. Nevers, M. Gelin, Y. Bessin, R. Brillet, O. Cala, D. Douguet, W. Bourguet, I. Krimm, J.-M. Pawlotsky, J.-F. Guichou, *Nat. Commun.* **2016**, *7*, 12777.

[125] S. Ni, Y. Yuan, J. Huang, X. Mao, M. Lv, J. Zhu, X. Shen, J. Pei, L. Lai, H. Jiang, J. Li, *J. Med. Chem.* **2009**, *52*, 5295–5298.

[126] The representation of the structures of compounds such as **55**, **56**, and **57** in the *trans-trans* conformation corresponds to those published in Refs. [123125]. For compounds **57**, a docking study with CypA revealed the *trans-trans* confomation. Steric difficulties with the orientation of substituents may arise with alternative conformations.

[127] a) Q. Nevers, I. Ruiz, N. Ahnou, F. Donati, R. Brillet, L. Softic, M. Chazal, N. Jouvenet, S. Fourati, C. Baudesson, P. Bruscella, M. Gelin, J. F. Guichou, J. M. Pawlotsky, A. Ahmed-Belkacem, *Antimicrob. Agents Chemother.* **2018**, *62*; e00126; b) J. F. Guichou, L. Colliandre, H. Ahmed-Belkacem, J. M. Pawlotsky, WO2011076784, **2011**.

[128] E. R. Shore, M. Awais, N. M. Kershaw, R. R. Gibson, S. Pandalaneni, D. Latawiec, L. Wen, M. A. Javed, D. N. Criddle, N. Berry, P. M. O'Neill, L.-Y. Lian, R. Sutton, *J. Med. Chem.* **2016**, *59*, 2596–2611.

[129] The diastereomer of compound **73** with an *S* configuration at the stereogenic ceter in the pyrrolidine ring is distinctly lower in binding poteny: $K_d = 82$ μM.

[130] K. Kajitani, M. Fujihashi, Y. Kobayashi, S. Shimizu, Y. Tsujimoto, K. Miki, *Proteins Struct. Funct. Bioinf* **2008**, *70*, 1635–1639.

[131] U. Grädler, D. Schwarz, M. Blaesse, B. Leuthner, T. L. Johnson, F. Bernard, X. Jiang, A. Marx, M. Gilardone, H. Lemoine, D. Roche, C. Jorand-Lebrun, *Bioorg. Med. Chem. Lett.* **2019**, *29*, 126717.

[132] A. De Simone, C. Georgiou, H. Ioannidis, A. A. Gupta, J. Juárez-Jiménez, D. Doughty-Shenton, E. A. Blackburn, M. A. Wear, J. P. Richards, P. N. Barlow, N. Carragher, M. D. Walkinshaw, A. N. Hulme, J. Michel, *Chem. Sci.* **2019**, *10*, 542–547.

[133] a) M. Panel, A. Ahmed-Belkacem, I. Ruiz, J. F. Guichou, J. M. Pawlotsky, B. Ghaleb, D. Morin, *J. Pharmacol. Exp. Ther.* **2021**, *376*, 348–357; b) M. Panel, I. Ruiz, R. Brillet, F. Lafdil, F. Teixeira-Clerc, C. T. Nguyen, J. Calderaro, M. Gelin, F. Allemand, J. F. Guichou, B. Ghaleb, A. Ahmed-Belkacem, D. Morin, J. M. Pawlotsky, *Gastroenterology* **2019**, *157*, 1368–1382.

[134] C. Georgiou, I. McNae, M. Wear, H. Ioannidis, J. Michel, M. Walkinshaw, *J. Mol. Biol.* **2017**, *429*, 2556–2570.

[135] a) K. R. Valasani, Q. R. Sun, D. Fang, Z. H. Zhang, Q. Yu, Y. P. Guo, J. P. Li, A. Roy, S. S. D. Yan, *ACS Med. Chem. Lett.* **2016**, *7*, 294–299; b) K. R. Valasani, J. R. Vangavaragu, V. W. Day, S. S. Yan, *J. Chem. Inf. Model.* **2014**, *54*, 902–912.

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