Bioorganic & Medicinal Chemistry Letters 20 (2010) 6542–6546

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and biological evaluation of [D-lysine]^{8}cyclosporin A analogs as potential anti-HCV agents

Andrew Scribner [a,*], David Houck [a], Zhuhui Huang [b], Sarah Mosier [a], Michael Peel [a], Bernard Scorneaux [a]

[a] SCYNEXIS, Inc., PO Box 12878, Research Triangle Park, NC 27709-2878, USA
[b] Southern Research Institute, 431 Aviation Way, Frederick, MD 21701, USA

ARTICLE INFO

Article history:
Received 7 August 2010
Revised 3 September 2010
Accepted 8 September 2010
Available online 21 September 2010

Keywords:
Cyclosporin
Cyclophilin
Hepatitis C

ABSTRACT

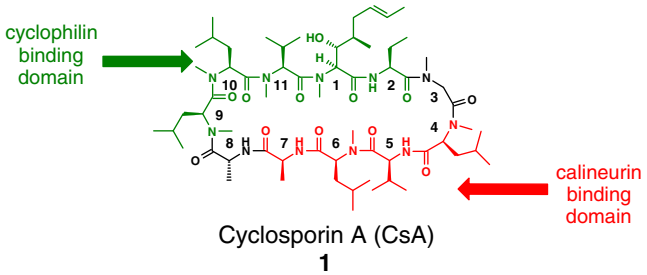
An efficient synthesis of [D-lysine]^{8}cyclosporin A has been developed. Several analogs of [D-lysine]^{8}cyclosporin A have been synthesized and show promising anti-HCV activity, particularly compounds **39** and **43**, which each exhibit an anti-HCV EC_{50} <200 nM, and are each ≥50-fold less immunosuppressive than cyclosporin A.

Nearly 200 million people worldwide are infected with hepatitis C, a disease of the liver caused by the hepatitis C virus (HCV), which can lead to liver fibrosis, liver cirrhosis, liver cancer, and ultimately death.[1] There is presently no vaccine for HCV, and current interferon-based therapeutics are successful in only 40–50% of patients treated,[1] prompting the need for new drugs with alternative modes of action. While there are several drugs in development that target key HCV proteins, most notably NS3-4A serine protease and NS5B RNA-dependent RNA polymerase,[1,2] there remains a need to find additional drug targets to increase options for drug cocktails that would combat HCV drug resistance.

Cyclosporins are cyclic undecapeptides that exhibit a broad spectrum of biological activities.[3] Cyclosporin A (**1**, CsA, shown in Fig. 1), isolated from the fungus *Tolypocladium inflatum*, is a powerful immunosuppressant of T-cells, and is the active ingredient of Sandimmune® and Neoral® for preventing organ transplant rejection, as well as Restatis® for treating the autoimmune disease dry eye.[3] CsA exerts its immunosuppressive activity by binding to two proteins sequentially to form a ternary complex.[4] The first of these is a cyclophilin (Cyp), which is a *cis–trans* proline isomerase, the most predominant in humans being cyclophilin A (CypA).[5] The binary CsA–CypA complex is a potent inhibitor of the phosphatase activity expressed by calcineurin (CN), a calcium-dependent serine/threonine phosphatase that promotes the synthesis of T-cell lymphokines such as interleukin-2 (IL-2).[6] Thus, CN inhibition ultimately suppresses immune response.[6]

X-ray crystallography has revealed which residues of CsA bind to CypA and CN, respectively.[4] Figure 1 shows that residues 9, 10, 11, 1, and 2 form the 'cyclophilin binding domain' that binds to CypA, while residues 4–7 comprise the 'calcineurin binding domain' that binds to CN. Residues 3 and 8 are at the interfaces between these two binding domains, and can potentially have an impact on both CypA and CN binding. Figure 2 depicts how CsA binds to CypA and CN from X-ray crystallographic data, with key CypA and CN residues that form hydrogen bonds shown.

In addition to the immune diseases discussed above that can be addressed by CN inhibition, there are a variety of diseases that are treated by Cyp inhibition alone, most notably the infectious diseases caused by human immunodeficiency virus 1 (HIV-1)[7] and HCV.[8] At the moment, there is some debate regarding what role Cyp plays in HCV inhibition, and in fact which Cyp (CypA or CypB) is the operative agent, although recently a consensus has emerged indicating that CypA is the predominant if not exclusive



Cyclosporin A (CsA)
1

Figure 1. The binding domains of CsA.

* Corresponding author. Tel.: +1 919 544 8610.
E-mail address: andrew.scribner@scynexis.com (A. Scribner).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.09.036

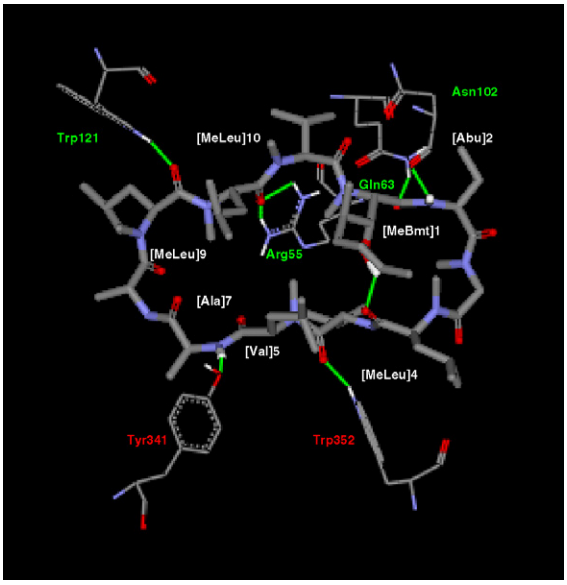


Figure 2. CsA in its active conformation with hydrogen bonds to both CypA and CN. CsA residues are labeled in white; CypA residues are labeled in green; CN residues are labeled in red.

Cyp that supports HCV-specific RNA replication and protein expression in human cells.[9] In any event, a drug that inhibited a host Cyp protein would clearly complement a drug that targeted a viral protein[10]; however, a CsA-based drug used to treat such an infectious disease must be devoid of immunosuppressive activity, as this would antagonize host immune mechanisms for clearing the virus.

Cyclosporins that have been developed for treating HIV and/or HCV are shown in Figure 3. Each of these compounds possess a modification at the 4-position that interferes with CN binding, but not Cyp binding. NIM-811[11] (**2**) has an *N*-methylisoleucine at the 4-position instead of an *N*-methylleucine; Debio-025[12] (**3**) has a D-alanine at the 3-position and an *N*-ethylvaline at the 4-position; our clinical candidate SCY-635[13] (**4**) has a dimethylaminoethyl-thioether at the 3-position and a 4′-hydroxymethylleucine at the 4-position.

In our drug discovery program focused on expanding SAR around SCY-635, we were curious if a water-solubilizing amine-bearing tether at the 8-position would have a similar anti-HCV effect as it does at the 3-position of SCY-635. Furthermore, we wondered if lengthening an amine sidechain at position 8 would attenuate CN binding without reducing Cyp binding, as is reported for 8-position modified cyclosporins,[14] particularly [D-Ser]^{8}CsA analogs.[14a,b] In this study, we report a synthesis of [D-lysine]^{8}cyclosporin A ([D-Lys]^{8}CsA), a known precursor to a reagent used in an ELISA assay to measure a compound's binding affinity for Cyp.[15] We then present the synthesis of several [D-Lys]^{8}CsA analogs and evaluate their anti-HCV activity along with their CypA binding, CypB binding, and immunosuppressive activity.

Although there is a published total synthesis of [D-Lys]^{8}CsA[16] in 20 steps in an overall yield of 7.0–11.4%, it does not include the synthesis of the tripeptide composing residues 9–11, nor the total synthesis of the (4*R*)-4-[(*E*)-2-butenyl]-4-methyl-L-threonine (MeBmt) residue at the 1-position. The former requires six steps in 68% yield,[17] while the highest yield reported to date for the latter is 22% in 12 steps,[18] bringing the overall yield of [D-Lys]^{8}CsA to 1.0–1.6% in a prohibitive 38 steps. In the years since these publications, technology has allowed for the industrial-scale biosynthesis of CsA, which can now serve as a viable starting material. Our synthesis (Scheme 1) starts from CsA and combines regioselective ring-opening methodology first reported by Seebach[19] and further exemplified by Eberle,[20] Edman degradation and amino acid coupling chemistry to replace D-alanine with *N*-Boc-D-lysine at the 8-position,[20,16] and then ring closure as reported by Rich.[16]

CsA (**1**) was first treated with acetic anhydride to protect the hydroxyl group of MeBmt.[21] The resulting acetate (**5**) was then treated with 1.0 equiv of Lawesson's reagent, which regioselectively reacted with the amide carbonyl groups of residues 4 and 7 to give a statistical mixture of 7-thioamide (**6**), 4-thioamide (**7**), 4,7-bisthioamide (**8**), and unreacted **5**, all of which were partially separated by chromatography, and identified by reported proton NMR spectra.[19,20] A mixture of **6** and **8** was then treated with benzyl bromide and DBU to give the corresponding benzyl thioimidates.[17] The 4,7-bisbenzyl thioimidate (**10**) was considerably less polar than the desired 7-benzyl thioimidate (**9**), and the components of the resulting mixture were more readily separated by chromatography than then the corresponding mixture of thioamides **6** and **8**. Hydrolysis of **9** with HCl afforded 7,8-secocyclosporins **11**, which was then subjected to Edman degradation conditions to first prepare thiourea **12** and then decapeptide **13**.[20] Installation of Fmoc-protected D-Lys and related D-amino acids afforded undecapeptides **14**–**19**.[16] Treatment with base hydrolyzed the acetate at [MeBmt]^1, the thioester at [Ala]^7, and the Fmoc group of the 8-position to give 7,8-secocyclosporin **20**–**25**.[16] Cyclization with *n*-propylphosphonic anhydride under dilute conditions gave cyclic undecapeptides **26**–**31**[16]; in the case of amides **24** and **25**, cyclization occurred with concomitant amide dehydration to give nitriles **30** and **31**. Subsequent treatment of Boc-protected amino acids **26**–**29** with CF_3CO_2H yielded primary amines **32**–**35**,[16] including [D-Lys]^{8}CsA (**35**), which was prepared in 6.3% overall yield in 10 steps, and compares favorably to the previously reported 1.0–1.6% overall yield in 38 steps. We then treated primary amines **32**–**35** with formaldehyde or acetaldehyde under reductive amination conditions to ultimately afford dimethylamines **36**–**39** and diethylamines **40**–**43**, respectively.

Biological activity of [D-Lys]^{8}CsA analogs is presented in Table 1. Compound binding affinity to CypA and CypB was measured using an ELISA assay, as described by Quesniaux.[15] In most cases, binding to CypA was within fivefold of that of CsA and SCY-635 themselves, although there were exceptions (**26**, **32**, **33**, **34**, **37**, **40**, **41**) which showed weaker Cyp binding affinity. For most compounds, CypB binding was within twofold of that of CypA binding; however, two of the four compounds with a lengthy 4-carbon

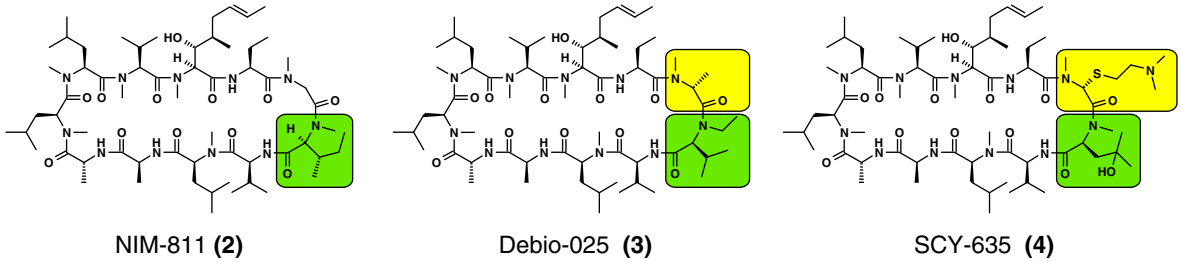


Figure 3. Non-immunosuppressive cyclosporins.