Table 11.1 SARs for anti-HCV activity and immunosuppressive potential for representative derivatives of CsA.

	EC_{50} (μM)			EC_{50} (μM)	
Structure	*HCV*	*IL-2*	*Structure*	*HCV*	*IL-2*
[*O*-Acetyl-MeBmt]^{1}CsA[72]	>10	>2xCsA	[MeIle]^{4}CsA (NIM811)	0.1	>2
[Thr]^2[5′-HOMeLeu]^{9}CsA[158]	<3	ND	[4′-AcOMeLeu]^{4}CsA (**13**)	0.25	0.03
[D-Sar-OMe]^{3}CsA[136]	0.06	<0.1	[MeVal]^{5}CsA	0.77	0.05
[D-Sar-SMe]^3[4′-HOMeLeu]^{4}CsA	0.04	0.06	[BnVal]^{5}CsA	0.5	>10
[D-Sar-$SCH_2CH_2NMe_2$]^3[4′-HOMeLeu]^{4}CsA	0.1	>2	[Me-D-Ala]^3[EtVal]^{4}CsA (alisporivir)	0.03	>2
[D-Sar-SCH_2(3-pyridyl)]^3[4′-HOMeLeu]^{4}CsA (**10**)	0.06	>10	[D-Lys]^{8}CsA	2.0	0.1
[D-Sar-SCH_2(4-thiazolyl)]^3[4′-HOMeLeu]^{4}CsA (**11**)	0.1	24	[3-CN-D-Ala]^{8}CsA	0.04	0.005
[D-Sar-$CH_2SCH_2CH_2NMe_2$]^3[4′-HOMeLeu]^{4}CsA (**12**)[139]	0.05	ND	[*N*-ε-Me_2-D-Lys]^{8}CsA	0.14	0.4
[MeVal]^{4}CsA[72]	0.15	>10	[MeAla]^{10}CsA[72]	8.48	>15xCsA

HCV IC_{50} refers to activity in Con-1a sub-genomic replicon systems using Huh-7 hepatoma cells; IL-2 secretion assays utilized Jurkat cells stimulated with phorbol 12-myristate 13-acetate and phytohemagglutinin; IL-2 levels in the medium were determined by ELISA 24 h after stimulation. Calcineurin is required for stimulation of IL-2 production, and inhibition of IL-2 secretion is a surrogate measure of calcineurin inhibition and immunosuppression.

The [MeLeu]^4 modification is of importance for controlling immunosuppression, and several reports have appeared that describe modification of this residue alone to achieve potent anti-HCV activity. Scynexis has explored [4′-ROMeLeu]^{4}CsA analogs where R = ester[160] (*e.g.* **13**) or ether,[161] from which work [4′-BnOMeLeu]^{4}CsA was found to have comparable activity to CsA itself. Enanta, in a series of patent applications, described various [MeThrOR]^{4}CsA derivatives in which the threonine hydroxyl is functionalized with alkyl chains of varying length, often bearing a substituted heteroatom (N, O).[150–155]

Alkylation of the nitrogen of [Val]^5 to attenuate immunosuppressive potential without significantly impacting CypA binding was outlined earlier and introduction of simple benzyl or allyl groups was found to retain anti-HCV activity, albeit with a loss of potency.[162] Combination of such [Val]^5 *N*-substitution, including fully saturated heteroatom-substituted alkyl chains, with optimal [Sar]^3 functionality such as D-alkyl ethers and D-alkyl thioethers,[163] and also D-alkyl groups,[164] led to several compounds exhibiting potent anti-HCV activity (<200 nM).

Of the remaining positions, 6–11, only for [D-Ala]^8 has any detailed description of anti-HCV activity been described. Replacing [D-Ala]^8 with D-Lys derivatives of varying length to control immunosuppression yielded compounds with greater anti-HCV potency than CsA itself, including [3′-CN-D-Ala]^{8}CsA (40 nM) and the less immunosuppressive [3′-$CH_2CH_2CH_2NMe_2$-D-Ala]^{8}CsA (140 nM).[122,165] Peptolides in which an ester bond containing [Hiv]^8 in place of [D-Ala]^8 were described by the DebioPharm group.[166] A number of other amino acid changes were incorporated in addition to the changes at position 8 that led