Drug-likeness of cyclic peptides beyond Rule of 5

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Abstract

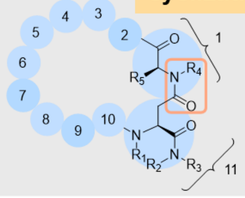
Drug discovery by small molecules accelerated after the emergence of the Rule of 5, a guideline defined by factors governing drug-likeness, such as membrane permeability and metabolic stability. To expand the drug discovery field to tough targets such as intracellular protein-protein interactions, it is critical to utilize compounds of molecular weight larger than 500 g/mol. One possible approach is to utilize cyclic peptides. However, outside the scope of the Rule of 5, general guidelines akin to the Rule of 5 have not been elucidated. In this study, we identified several governing factors needed for cyclic peptides to acquire drug-likeness—here defined as the compatibility of membrane permeability and metabolic stability—outside the scope of the Rule of 5.

To find a drug-likeness area and to determine the particular structural features that grant peptides drug-likeness, we evaluated the membrane permeability and metabolic stability of more than 700 cyclic peptides. The results suggest that a drug-likeness area can be defined by cyclic peptides, having about 11 residues, more than half of *N*-alkyl amino acids, and calculated logP (ClogP) of 12.9 or more. It is noteworthy that 11-residue peptides showed better possibility to be drug-like than peptides with fewer residues. This observation could be explained by the following hypothesis. Because 11-residue peptides like Cyclosporin A are structurally more flexible, a more lipophilic conformation could be feasible in the lipophilic membrane, while oxidative metabolism could be suppressed by a more hydrophilic conformation in water. Furthermore, an effect caused by differences in N-alkyl patterns on membrane permeability could be recognizable. The effect can be due to the ease of taking the lipophilic structure in the membrane. Moreover, we confirmed that it is preferable not to contain ionic functional groups. The number and types of acceptable polar functional groups would be more restricted compared to those of small molecules. Finally, the oral absorption of our drug-like cyclic peptides was validated by pharmacokinetic studies in several animal species.

The proposed criteria for drug-like cyclic peptides could be utilized both to design libraries for obtaining drug-like hits against intracellular targets and to facilitate optimization of them to a clinical compound.

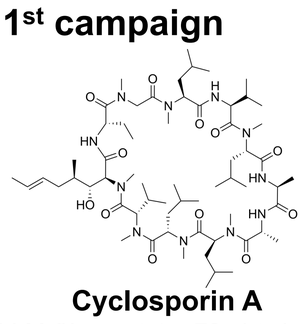
[1] Ohta, A. *et al. J. Am. Chem. Soc.* 2023, 145, 24035-24051. [2] Tanada, M. *et al. J. Am. Chem. Soc.* 2023, 145, 16610-16620.

Proposed criteria for drug-like cyclic peptides

Cyclization	Number of residues	Number of N-alkyl amino acids	ClogP	Amino acids
Amide cyclization 	9 to 11	≥ 6	≥ 12.9	Membrane permeable/metabolically stable amino acids (No polar amino acids such as Lys, Asp, etc.)

Peptide Design for Drug-likeness Study as 1st campaign

Properties	553 peptides		Cyclosporin A
	min.	max.	
Number of amino acid residues	8	12	11
Number of *N*-alkyls	0	8	7
ClogP	4.4	15.2	14.4
Number of OH groups	0	1	1
MW (g/mol)	845	1,499	1,203

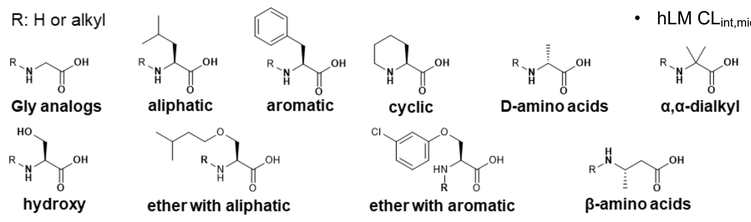


Cyclosporin A

hLM $CL_{int,mic}$ (+) = 72 μL/min/mg protein
Caco-2 P_{app} = 6.3 x 10^{-6} cm/s

53 incorporated amino acids

R: H or alkyl



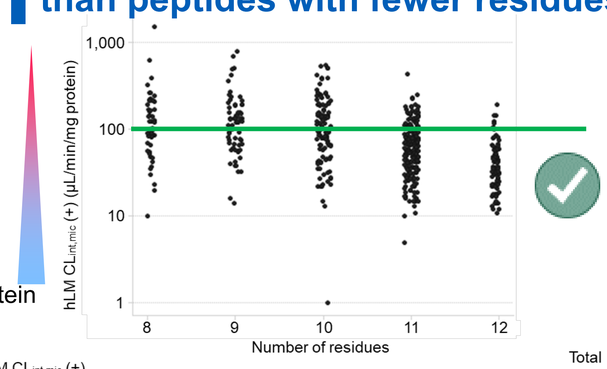
- hLM $CL_{int,mic}$ (+), human LM $CL_{int,mic}$ with NADPH

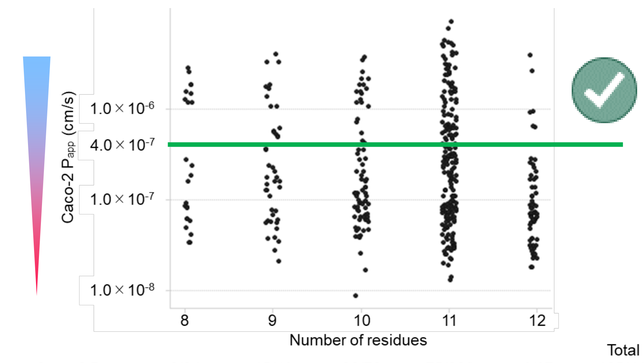
etc…

✓ Various types of amino acids

✓ Parameters of Cyclosporin A were referred
✓ Positions of *N*-alkyls and an OH group were randomly arranged to give a variety of structures

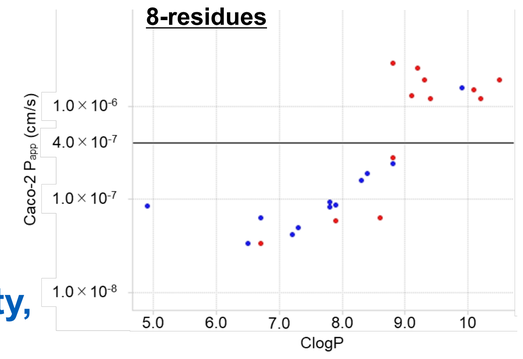
Part I. 11-residue peptides showed better possibility to be drug-like than peptides with fewer residues

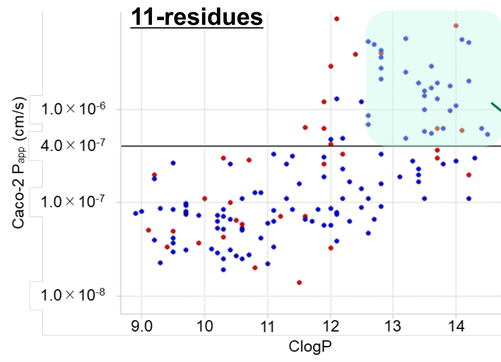




hLM $CL_{int,mic}$ (+) ≤100 (μL/min/mg protein)	8	9	10	11	12	Total
	25/51 49%	27/65 42%	56/106 53%	153/196 78%	75/82 91%	336/500 67%

Caco-2 P_{app} ≥4.0×10^{-7} (cm/s)	8	9	10	11	12	Total
	9/24 38%	15/43 35%	20/76 26%	75/200 38%	6/67 9%	125/410 30%
drug-like (hLM-Caco-2)	1/24 4%	4/43 9%	8/74 11%	34/151 23%	4/65 6%	51/357 14%



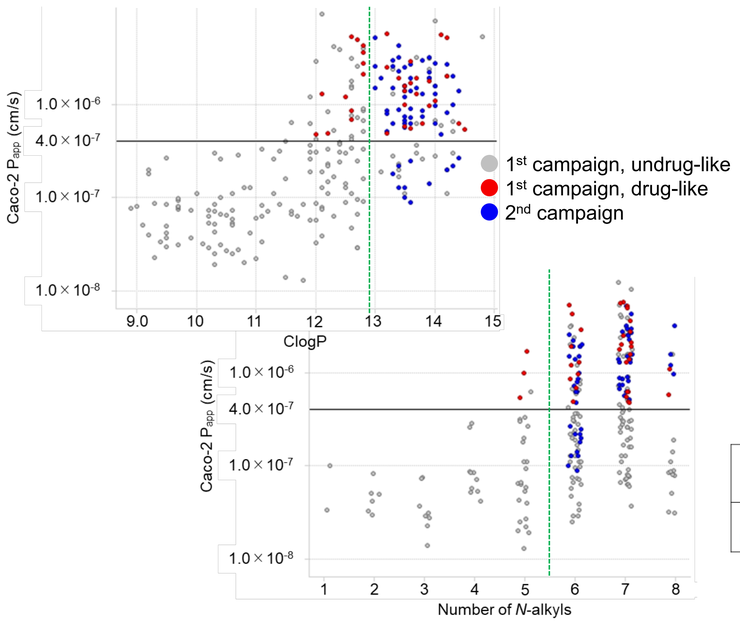


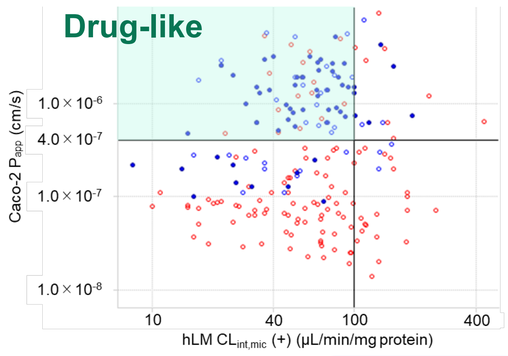
hLM $CL_{int,mic}$ (+) (μL/min/mg protein)
● ≤ 100
● > 100

Permeable
Stable

Part II. Drug-likeness area can be defined by approximately 11-residue cyclic peptides with higher *N*-alkylation, higher lipophilicity, and fewer polar functional groups

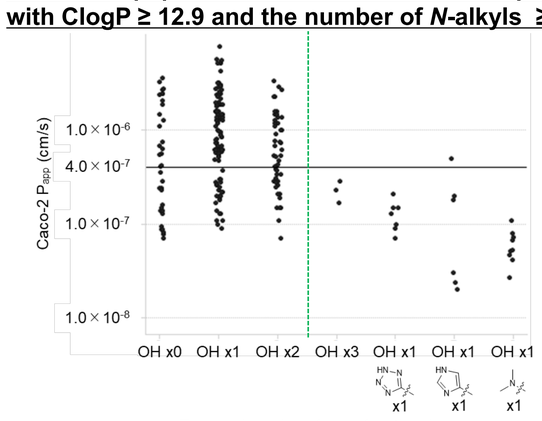
11-residue peptides with 0~1 OH group from 1st and 2nd campaign





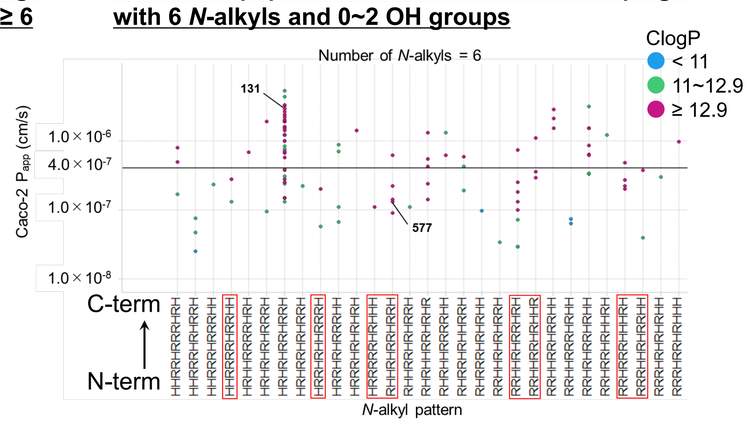
	1st campaign ClogP < 12.9 or *N*-alkyl < 6 ○	1st + 2nd campaign ClogP ≥ 12.9 & *N*-alkyl ≥ 6 ○ + ●
drug-like (hLM-Caco-2)	15/116 13%	63/95 66%

11-residue peptides from 1st, 2nd and 3rd campaign with ClogP ≥ 12.9 and the number of *N*-alkyls ≥ 6



Caco-2 P_{app} ≥4.0×10^{-7} (cm/s)	OH x0	OH x1	OH x2	OH x3	OH x1	OH x1	OH x1
	19/35 54%	81/105 77%	30/49 61%	0/3 0%	0/7 0%	1/6 17%	0/9 0%

11-residue peptides from 1st, 2nd and 3rd campaign with 6 *N*-alkyls and 0~2 OH groups

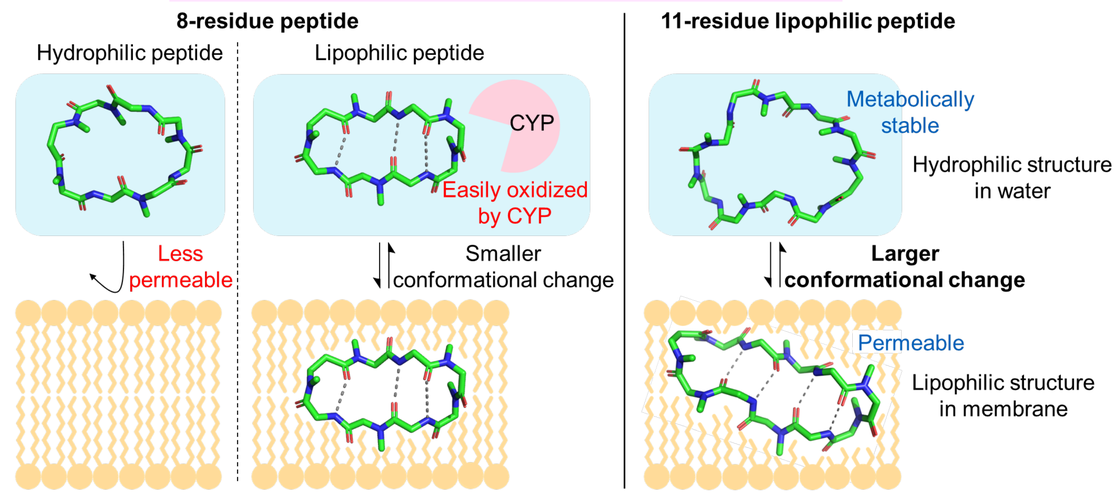


peptide No.	Sequence	ClogP	*N*-alkyl pattern	Caco-2 P_{app} ($\times 10^{-6}$ cm/s)	hLM $CL_{int,mic}$ (+) (μL/min/mg protein)
131	D-Val **MePhe** Leu **MeLeu** Thr **MeGly MeLeu** Ser(tBu) **MePhe MeVal** Asp-pip	13.6	HRHRHRRHRRH	2.9	22
577	**D-Pro** Phe(3-Cl) Ser(iPen) **MeVal** Thr **MeGly MePhe** Ser(iPen) **MePhe MeVal** Asp-pip	13.5	RHHRHRRHRRH	0.13	31

- 2nd campaign: 60 peptides with 11-residues, *N*-alkyl ≥ 6, ClogP ≥ 12.9, and 0~1 OH group
- 3rd campaign: 106 peptides with 11-residues, *N*-alkyl ≥ 6, ClogP ≥ 12.9, 0~3 OH groups, and 0~1 ionic functional group

✓ There is a clear difference in permeability of each *N*-alkyl pattern
✓ Permeability difference was observed between similar sequences with only one part difference in *N*-alkyl patterns
✓ hLM $CL_{int,mic}$ were acceptable for almost all *N*-alkyl patterns (*data not shown*)

Our hypothesis to explain the observation



Schematic representation of the conformational flexibility of 8- and 11-residue cyclic peptides

Part III. Peptides satisfying our drug-like criteria showed acceptable oral bioavailability and *in vivo* exposure

***in vitro* properties of drug-like 11-residue peptides**

Peptide No.	ClogP	Number of *N*-alkyl	Caco-2 P_{app} ($\times 10^{-6}$ cm/s)	LM $CL_{int,mic}$ (+) (μL/min/mg protein)	
				Human	Mouse
131	13.6	6	2.9	22	31
147	12.2	7	0.49	42	50
553	13.6	5	1.7	63	77
554	13.6	6	1.4	38	39
561	13.3	7	2.6	22	27
588	13.7	6	1.5	33	49
601	13.4	7	2.3	67	54
720	14.1	7	1.5	48	58
722	13.3	5	0.61	63	45

Pharmacokinetic parameters of these peptides in mice

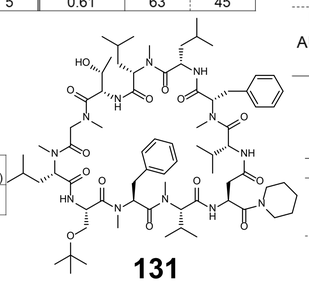
Peptide No.	131	147	553	554	561
Dose (iv, mg/kg)	5	5	3	2	5
CL (mL/min/kg)	3.1 ± 0.3	5.3 ± 0.7	6.5 ± 0.8	1.3 ± 0.4	1.5 ± 0.1
V_{ss} (L/kg)	1.3 ± 0.2	0.20 ± 0.03	0.50 ± 0.04	0.35 ± 0.03	0.51 ± 0.06
Dose (po, mg/kg)	20	20	20	20	20
AUC_{inf} (po, ng·h/mL)	53,000 ± 1,600	4,400 ± 2,000	12,000 ± 1,700	46,000 ± 5,400	66,000 ± 20,000
F (%)	49 ± 1	6.8 ± 3.0	22 ± 3	18 ± 3	31 ± 10

Peptide No.	588	601	720	722
Dose (iv, mg/kg)	3	3	5	3
CL (mL/min/kg)	1.7 ± 0.2	3.9 ± 0.8	2.0 ± 0.2	1.3 ± 0.1
V_{ss} (L/kg)	0.67 ± 0.08	0.56 ± 0.09	0.34 ± 0.06	0.37 ± 0.07
Dose (po, mg/kg)	20	20	20	20
AUC_{inf} (po, ng·h/mL)	52,000 ± 17,000	11,000 ± 3,200	12,000 ± 5,900	34,000 ± 10,000
F (%)	27 ± 9	12 ± 4	7.1 ± 3.4	14 ± 4

✓ 5/9 peptides showed F of ≥15%

Pharmacokinetic parameters of 131 in various species

Species	Mouse	Rat	Dog	Monkey
Dose (iv, mg/kg)	5	1	0.25	0.5
CL (mL/min/kg)	3.1 ± 0.3	9.3 ± 1.5*	2.7 ± 0.6*	1.7 ± 0.3
V_{ss} (L/kg)	1.3 ± 0.2	3.0 ± 0.6*	1.9 ± 0.3*	0.70 ± 0.05
Dose (po, mg/kg)	20	20	3	1
AUC_{inf} (po, ng·h/mL)	53,000 ± 1,600	9,100 ± 3,800*	2,600 ± 960*	2,000 ± 940
F (%)	49 ± 1	25 ± 10*	13 ± 5*	20 ± 10



131

✓ 131 showed F of around 20% or more regardless of animal species

- Data of CL, V_{ss}, AUC_{inf}, and F are expressed as the mean ± SD of three animals
- * indicates that the data are expressed as the mean of four animals

iv, intravenous administration; po, oral administration; CL, *in vivo* plasma clearance; V_{ss}, volume of distribution at the steady state; AUC_{inf}, area under the curve from the time of dosing to the last measurable concentration and extrapolated to infinity; F, bioavailability

Conclusion

By comprehensively investigating the individual membrane permeability or metabolic stability of more than 700 cyclic peptides, this study revealed several key tendencies and proposed criteria for drug-like cyclic peptides

✓ around 11-residues, the number of *N*-alkyls ≥ 6, ClogP ≥ 12.9, and restricted polar functional groups
✓ The effect on the permeability by specific differences in *N*-alkyl patterns