In vitro selection of highly N-alkylated cyclic peptides for targeting intracellular protein-protein interactions

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Abstract

Peptides present a promising therapeutic approach to inhibiting protein-protein interactions (PPIs), a difficult goal often pursued by small molecule drugs. Techniques such as phage display and mRNA display have been employed to discover novel PPI inhibitor peptides. However, peptides derived from in vitro display selections, primarily composed of proteinogenic amino acids (AA), are not orally available, metabolically stable and lack cell membrane permeability. To achieve "drug-like" properties, including sufficient oral availability, metabolic stability and membrane permeability, significant chemical derivatization and optimization with substantial structural changes are necessary. Conversely, cyclosporine, a natural product derivative, shows the capability to inhibit intracellular proteins and oral availability. Two remarkable structural features of cyclosporine—backbone cyclization and N-alkylation of amide bonds—contribute to its "drug-like" properties.

In this study, we report "drug-like" cyclic peptides against KRAS using mRNA display. We employed amide cyclization and a genetic code rich in N-alkyl AA. The combination of Native Chemical Ligation (NCL) and radical-based desulfurization allows us to generate amide cyclic peptide library in an mRNA display. The genetic code was extensively engineered by using aminoacyl pCpA and mutant aminoacyl tRNA synthetases (mutARS), resulting in a codon table containing 10 N-alkyl AA and 4 other non-proteinogenic AA. We applied our "drug-like" library to the intracellular protein KRAS. The derived cyclic peptide, AP8784, contains 7 N-alkyl AA out of a total of 11 AA, similar to cyclosporine. AP8784 inhibited the KRAS-SOS1 interaction with an IC50 of 180 nM in an Alpha-Screen assay. X-ray structure analysis of the AP8784-KRAS complex revealed multiple interaction points between the cyclic backbone of AP8784 and the Gln99 residue of KRAS.

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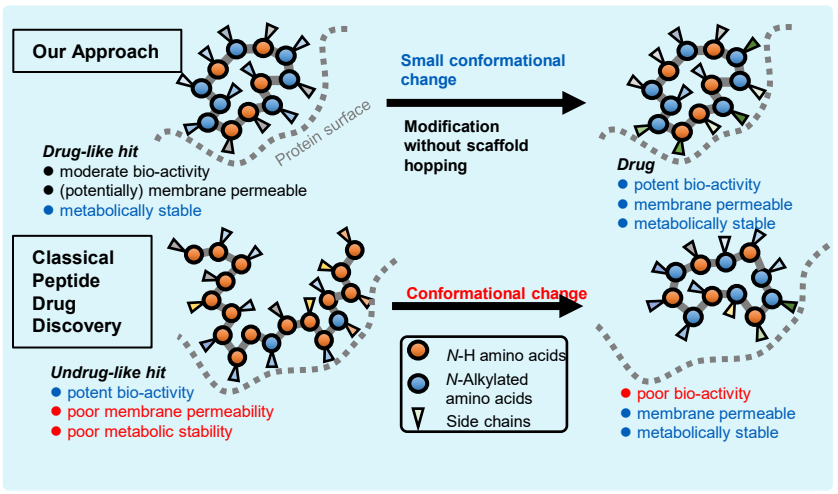
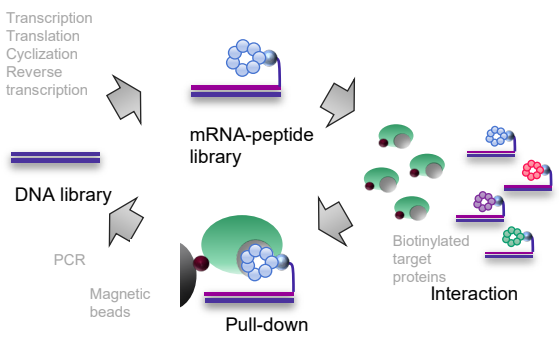
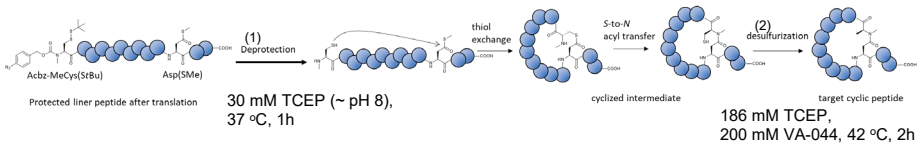


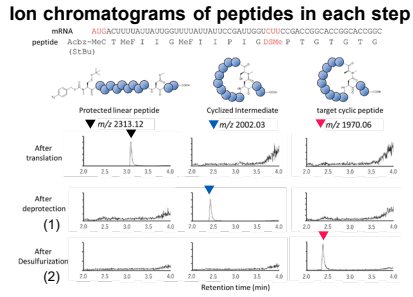
Binder enrichment cycle of cyclic peptides By mRNA display



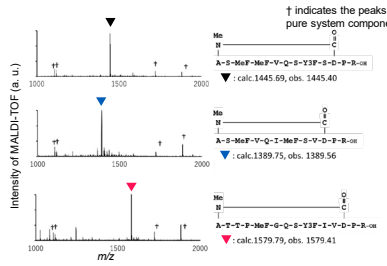
Amide cyclization method using native chemical ligation and desulfurization was established



Ion chromatograms of peptides in each step

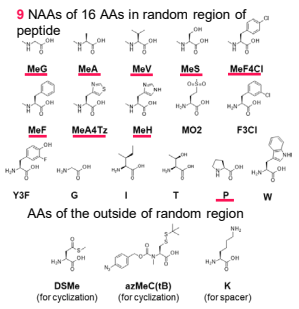


MALDI-TOF analysis of three different cyclic peptides



Peptide library oriented to our criteria was constructed

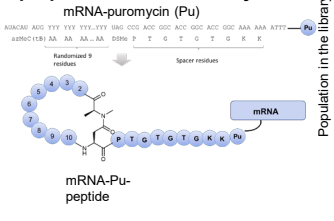
Amino acids and the codon table used in the panning

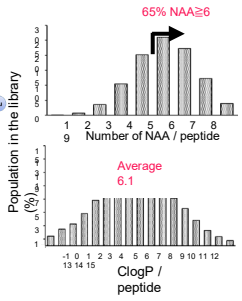


1st	2nd U	2nd C	2nd A	2nd G	3rd
U	MeF†				U
			Y3F†		C
					A
	F3Cl‡		DSMe‡	W†	G
C			MeH†		U
					C
	MeG‡				A
		P†	MeA4Tz‡	MeF4Cl‡	G
A	I†	T†		MeS†	U
					C
			K†		A
	azMeC(tB)‡			MO2‡	G
G	MeV†	MeA†		G†	U
					C
					A
					G

† and ‡ indicates the AA incorporated by ARS and pCpA, respectively.

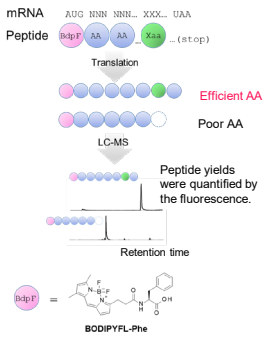
Library design and properties of initial library



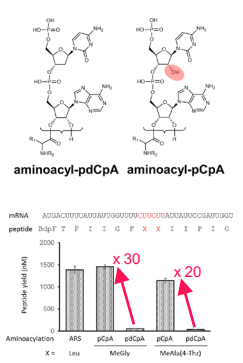


Consecutive NAA incorporations were improved by the pCpA and mutant ARS methods

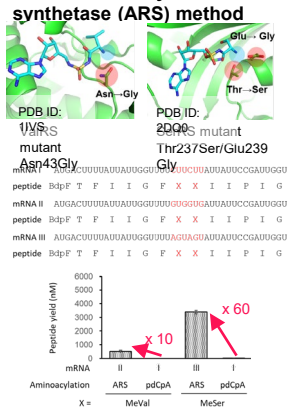
The evaluation method of translation products



pCpA method



Mutant aminoacyl-tRNA synthetase (ARS) method



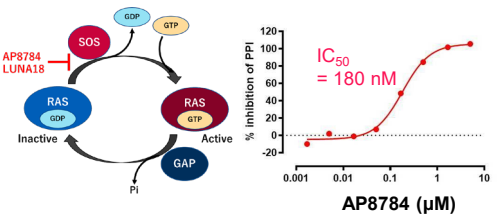
KRAS Inhibitors Were Acquired by mRNA Display

Top 10 enriched sequences in the panning and their properties

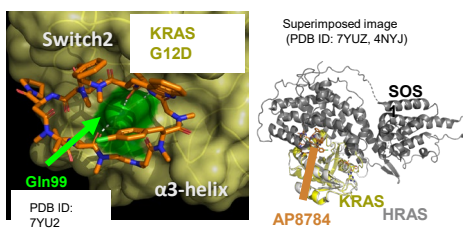
Compound No.	Frequency at round 5 (%)	X1	X2	X3	X4	X5	X6	X7	X8	X9	No. of NAA	ClogP	K_D‡ (μM)
1	36	W	MeG	Y3F	MeF	Y3F	MeF4Cl	I	P	T	5	10.5	2.1± 0.5
2	19	W	MeG	Y3F	MeF	Y3F	MeF4Cl	MeV	P	I	6	12.7	No binding response
3	7.8	MeG	MeF	MeG	MeF	T	MeH	T	F3Cl	F3Cl	6	7.8	52± 6
4	5.5	W	MeG	Y3F	MeF	Y3F	MeF4Cl	I	P	I	5	12.6	N.D.
5 (AP8784)	4.9	I	MeG	MeG	MeF4Cl	MeG	F3Cl	W	MeF	MeV	7	12.7	0.34± 0.04
6	4.9	W	P	Y3F	F3Cl	Y3F	MeF4Cl	T	P	MeV	5	11.1	No binding response
7	2.9	Y3F	MeA4Tz	MeA4Tz	P	Y3F	MeV	Y3F	MeH	Y3F	6	7.6	N.D.
8	1.8	W	MeG	I	G	MeF	I	F3Cl	MeF	MeA4Tz	5	11.5	2.9± 0.4
9	1.7	W	MeF	MeF4Cl	P	Y3F	Y3F	G	MeH	F3Cl	5	10.9	1.2± 0.3
10	1.5	Y3F	MeA4Tz	MeA4Tz	P	F3Cl	MeV	Y3F	MeF4Cl	F3Cl	6	12.8	No binding response
										Max. No.	7	12.8	

Pyt indicate pyrrolidine at C-term.
‡ indicate KD determination for GDP-KRAS-WT at 30 °C

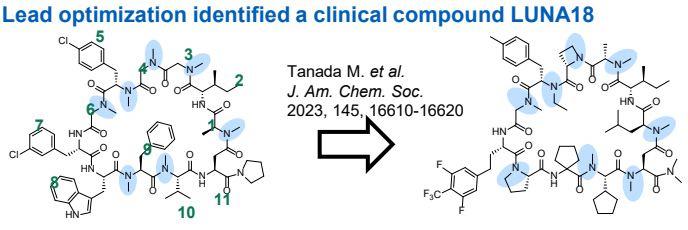
KRAS as the tough target and identified PPI inhibitors



Structural analysis of KRAS-AP8784 complex



Lead optimization identified a clinical compound LUNA18

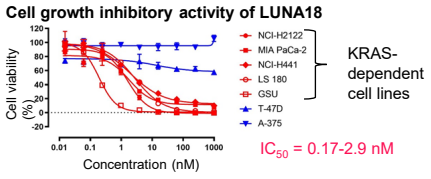


AP8784 (mRNA display Hit)
- 7 NAAs, ClogP = 12.7
- PPI $KRAS^{G12D}$-SOS1 IC_{50} = 54 nM
- Caco-2 Papp = 0.02 x 10^{-6} cm/s

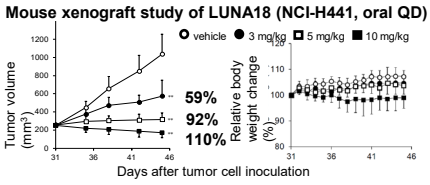
LUNA18 (Clinical compound)
- 8 NAA, ClogP = 14.5
- PPI $KRAS^{G12D}$-SOS1 IC_{50} < 2 nM
- Caco-2 Papp = 2.3 x 10^{-6} cm/s

LUNA18 gave 21, 22, 47, and 26% bioavailability in mice (10 mg/kg), rats (10 mg/kg), dogs (0.3 mg/kg), and monkeys (3 mg/kg) after oral administration.

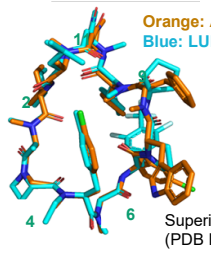
Cell growth inhibitory activity of LUNA18



Mouse xenograft study of LUNA18 (NCI-H441, oral QD)



The main chains of AP8784 and LUNA18 in the KRAS binding form overlap well (no scaffold hopping)



Conclusion

- Cyclic peptides were generated from our designated library contained 5–7 NAAs in 11-residue residues for KRAS.
- AP8784 from our library afforded orally available LUNA18 without scaffold hopping, which supports the validity of our concept.