Structural Optimization Study Leading to Orally Bioavailable Cyclic Peptides Inhibiting an Intracellular Tough Target

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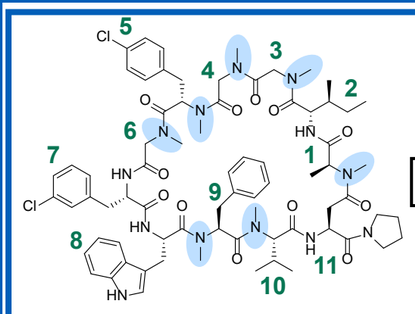
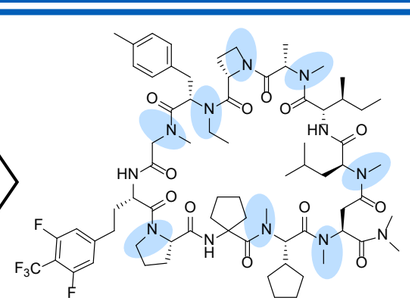
CHUGAI
Roche Group

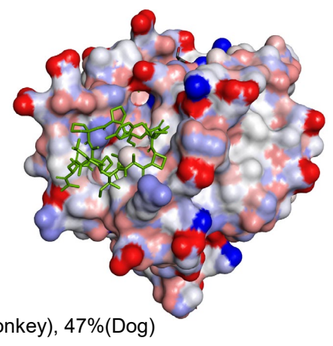


AP8784 (mRNA display Hit)
- 7 *N*-alkyl amino acids, cLogP = 12.7
- PPI KRAS^{G12D}-SOS1 IC_{50} = 54 nM
- Cellular IC_{50} > 20,000 nM (AsPC-1)

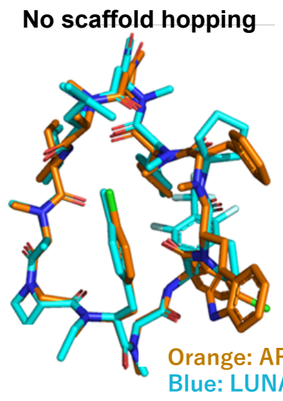


LUNA18 (Clinical compound)
- 8 *N*-alkyl amino acids, cLogP = 14.5
- PPI KRAS^{G12D}-SOS1 IC_{50} < 2 nM
- Cellular IC_{50} = 1.4 nM (AsPC-1)
- Oral BA = 21%(Mouse), 22%(Rat), 26%(Monkey), 47%(Dog)

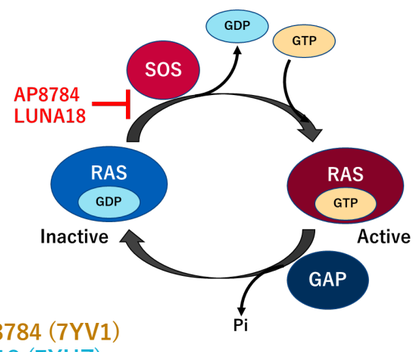
LUNA18-bound KRAS^{G12D} (X-ray, 7YV1)



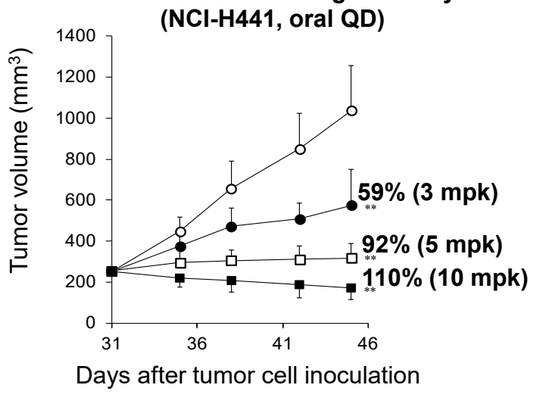
No scaffold hopping



Orange: AP8784 (7YV1)
Blue: LUNA18 (7YUZ)

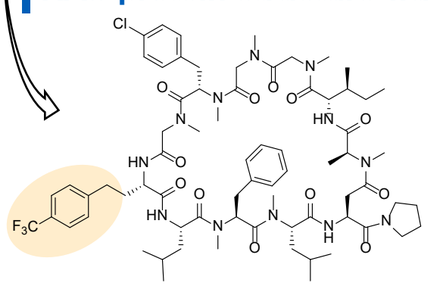


LUNA18 mouse xenograft study (NCI-H441, oral QD)

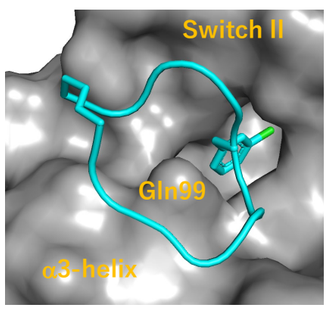


(1) *J. Am. Chem. Soc.* 2023, *145,* 16610, (2) *Bioorg. Med. Chem.* Accepted (10.1016/j.bmc.2024.117830)

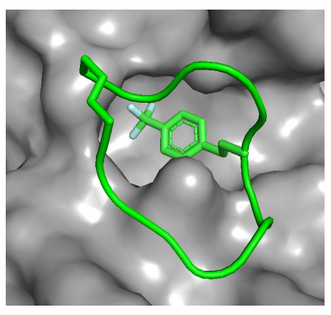
RAS protein structure was dramatically changed through induced-fit to our peptide side chain.



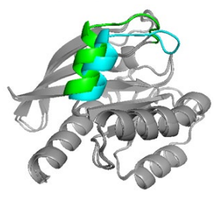
Compound 18
PPI IC_{50} = 28 nM

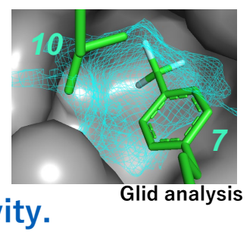


X-ray, AP8784 (7YUZ)



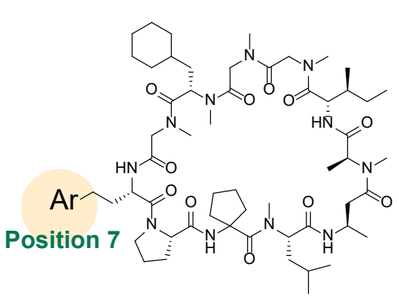
X-ray, compound 18 (8JJS)

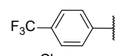
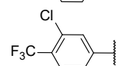
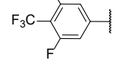




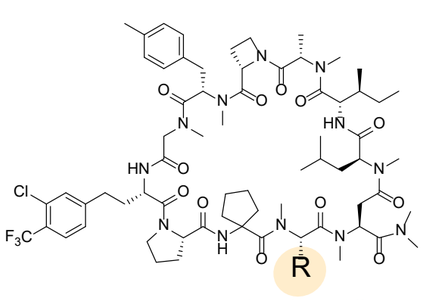
Glid analysis

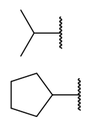
SBDD of side-chains located in the pocket was effective to improve bioactivity.



Ar	PPI IC_{50} (nM)
	110
	16
	10
	>5000

KRAS^{G12D}-SOS1



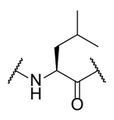
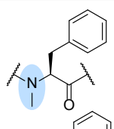
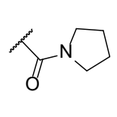
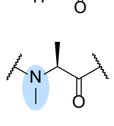
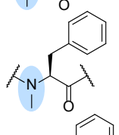
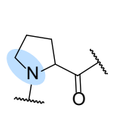
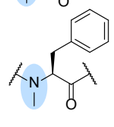
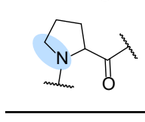
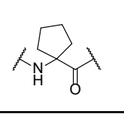
R	K_D(nM)
	0.73
	0.057

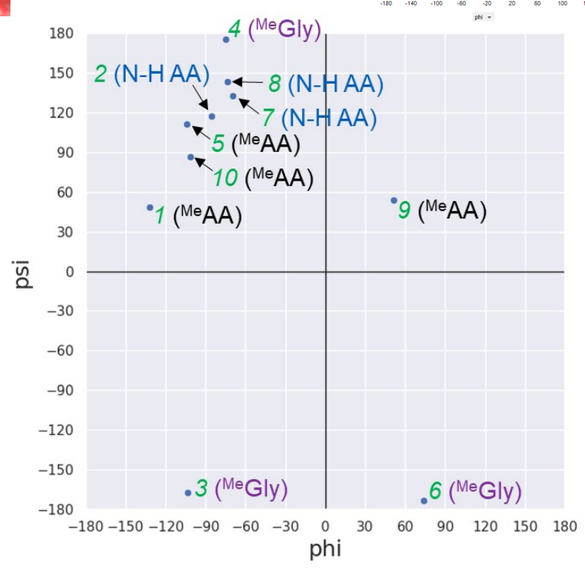
KRAS^{G12D}

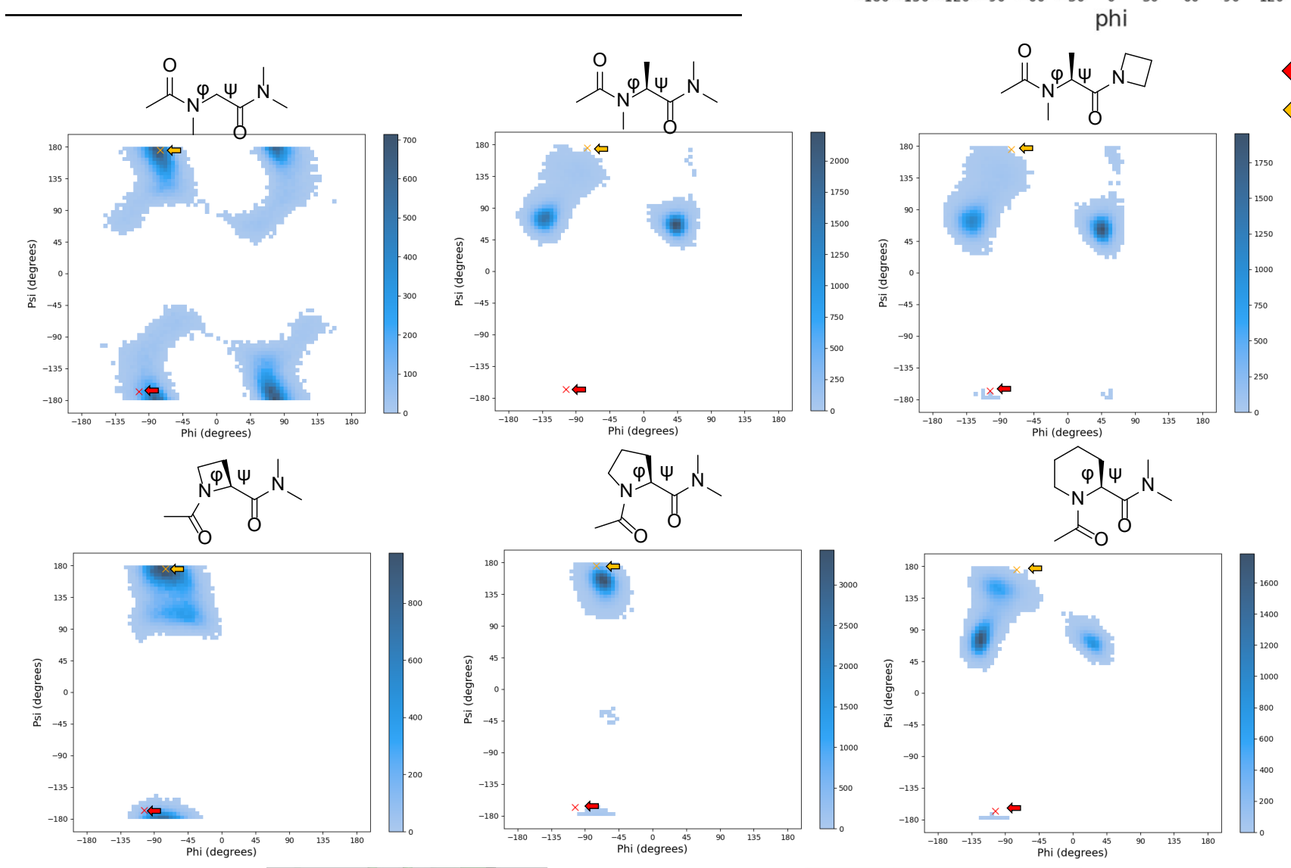
Semi-quantitative understanding of peptide structure by φ/ψ plot enabled rational restriction of cyclic peptide conformation resulting in improved membrane permeability.

X-ray, 8JJS

CSD (Aib)

		R	cLogP	PPI IC_{50} (nM)	Caco-2 P_{app} x 10^{-6} (cm/s)
			14.0	28	0.11
		Me	13.2	170	0.05
		Me	13.1	25	0.16
		Me	13.4	36	0.39





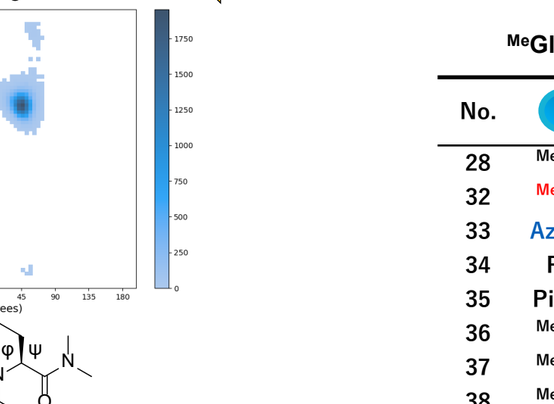
^{Me}Ala-Aze(2) model structure (based on 8JJS)

X-ray structure of LUNA18 (7YV1)

After identification of the membrane-permeable backbone, we were able to obtain LUNA18 by the combination of SAR.

Compound 40

LUNA18



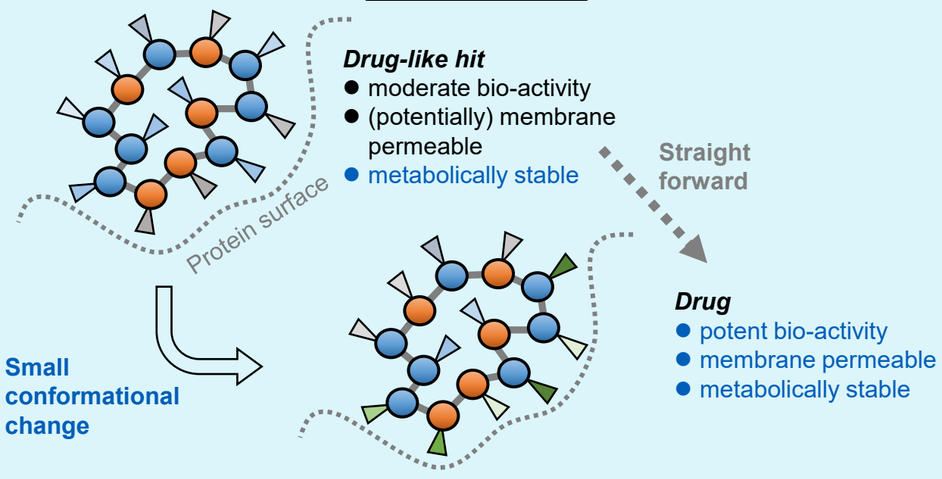
Compound 41 (X = O)
Compound 42 (X = C)

No.	species	route	dose (mg/kg)	$T_{1/2}$ (h)	AUC_{inf} (ng h/mL)	CL (mL/min/kg)	V_{ss} (L/kg)	F (%)
41	Mouse	iv	1	5.98	1350	12.4	3.0	-
		po	20	4.16	3880	-	-	14
	Rat	iv	1	5.57	2140	7.81	0.85	-
		po	10	3.96	3520	-	-	16
	Monkey	iv	0.167	8.14	673	4.30	0.82	-
		po	15	7.71	12500	-	-	21
42	Mouse	iv	1	8.62	2860	5.90	2.6	-
		po	20	6.46	3270	-	-	5.7
	Rat	iv	1	6.23	7970	2.13	0.37	-
		po	10	3.95	5770	-	-	7.2
	Monkey	iv	0.168	7.30	1590	1.81	0.49	-
		po	10	8.65	10700	-	-	11

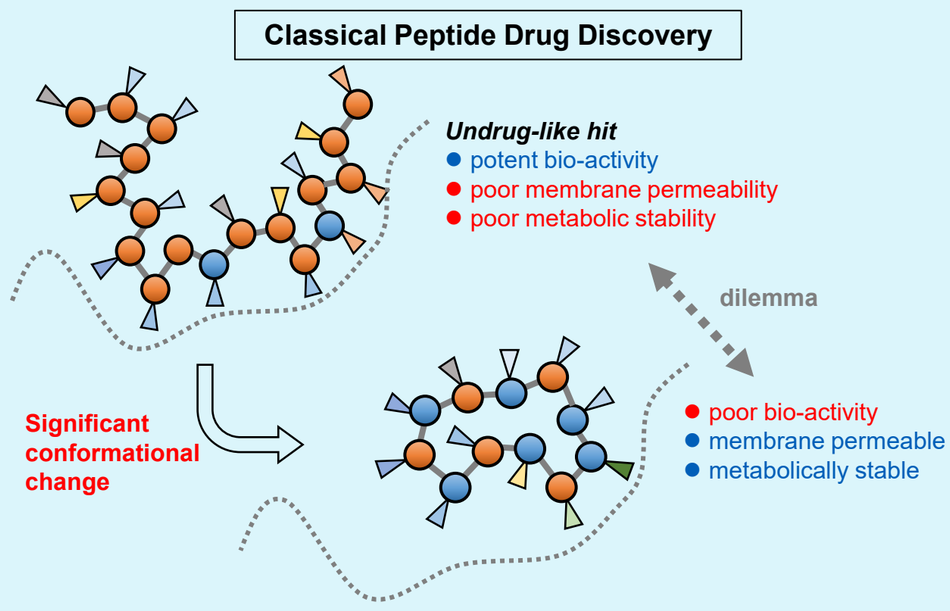
Through the overall structural optimization from Hit to LUNA18…

- Strong Affinity (K_D=35 pM) was achieved without using polar side chains such as amino groups and carboxylic acids.
- Main exploration range of cLogP was as narrow as 13–15, which was sufficient to acquire membrane permeability.

Our Approach



Classical Peptide Drug Discovery



Desired values to acquire membrane permeable and metabolically stable cyclic peptide

Number of residues	Number of N-alkyl amino acids	cLogP	Amino acids
9 to 11	≧6	≧12.9	No polar amino acids such as Lys, Asp, Glu etc.

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X-ray, 8JJS

^{Me}Gly | ^{Me}Ala | Aze(2) | Pro | Pic(2)

No.			cLogP	KRAS^{G12D}-SOS PPI IC_{50} (nM)	AsPC-1 IC_{50} (nM)	Caco-2 P_{app} x 10^{-6} (cm/s)
28	^{Me}Gly	^{Me}Gly	13.9	11	230	0.73
32	^{Me}Ala	^{Me}Gly	14.4	4900	>3000	N.T.
33	Aze(2)	^{Me}Gly	13.7	51	1100	1.38
34	Pro	^{Me}Gly	14.2	170	1200	1.75
35	Pic(2)	^{Me}Gly	14.8	3800	>3000	N.T.
36	^{Me}Gly	^{Me}Ala	14.4	2500	>3000	N.T.
37	^{Me}Gly	Aze(2)	13.7	22	230	0.99
38	^{Me}Gly	Pro	14.2	61	720	1.57
39	^{Me}Gly	Pic(2)	14.8	990	>3000	N.T.
40	^{Me}Ala	Aze(2)	14.2	24	110	1.62

Mouse SDPK (*compound 40*)

route	dose (mg/kg)	$T_{1/2}$ (h)	AUC_{inf} (ng h/mL)	CL (mL min^{-1} kg^{-1})	V_{ss} (L/kg)	F (%)
iv	5	3.16	65200	1.29	0.2	-
po	50	3.27	49700	-	-	7.6