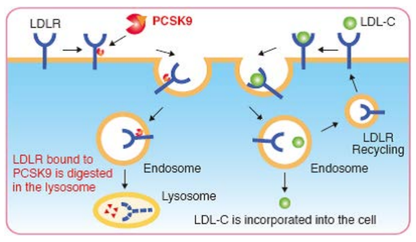
https://doi.org/10.17952/38EPS.2026.P327

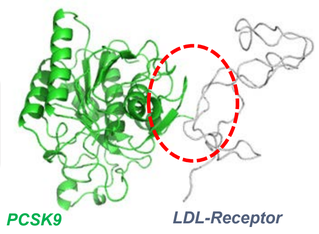
A PCSK9 Vignette: From mRNA Display to Passively Permeable Macrocycles

Yuhua Huang
on behalf of the PCSK9 team
Merck & Co., Inc., Rahway, NJ, USA

Proprotein Convertase Subtilisin-Kexin type 9 (PCSK9) as a therapeutic target



Blocking PCSK9:LDL-R increases clearance of LDL particles (↓LDL-C)



- **PCSK9 binds to LDL-R: blockade of PCSK9 relieves inhibition of LDL-R, permitting LDL particle clearance**
- **Large and flat interface of PCSK9:LDL-R amenable to mAb and peptide modalities. No SM leads**
- **Repatha® and Praluent® approved mABs PCSK9 inhibitors, sub-Q, 75-300 mg/2-4 weeks, costly**
- **Patients and prescribers prefer oral delivery; oral agents remain a significant unmet need**

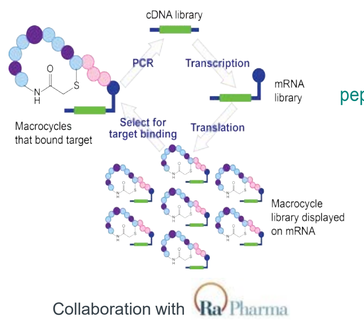
Formulation enabled oral (FEO)

Intrinsic passive permeability (IPP)

mRNA Display libraries as "hit" finding platform

Macrocyclic peptides can bind large, flat surfaces and can be more stable than linear peptides

mRNA Display screen

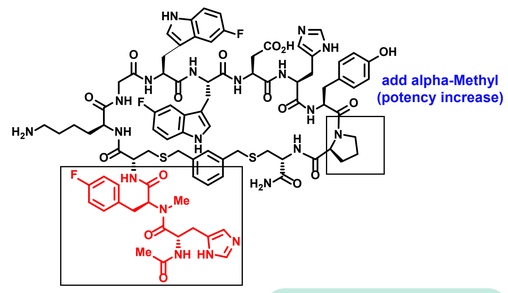


Collaboration with Ra Pharma

A trillion barcoded peptides in an eppendorf tube



Massive diversity

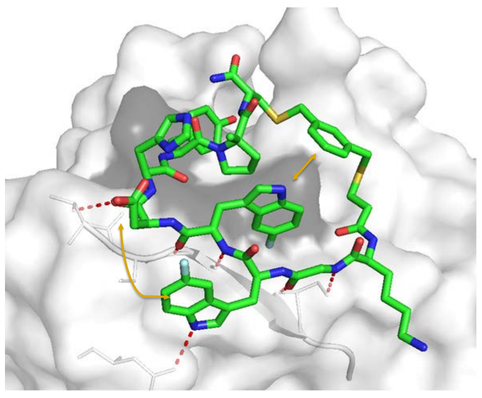


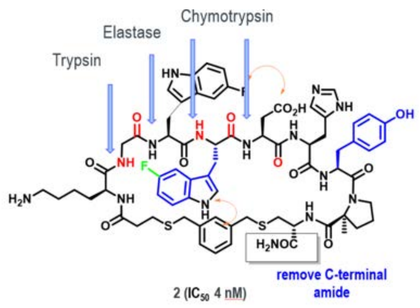
Lead A / compound 1
MW = 1889; 10-13 AA
TR-FRET IC_{50} = 483 nM

- Good potency
- Poor metabolic stability
- Poor physical properties
- No structural enablement
- No cellular permeability

J. Med. Chem. **2020**, 63, 13796; *J. Med. Chem.* **2021**, 64, 16770; *J. Biol. Chem.* **2013**, 289, 942; *Nat. Struct. Mol. Biol.* **2017**, 24, 848; Angew. *Chem. Int. Ed.* **2015**, 54, 8896.

Structure based drug design drives optimization of SAR



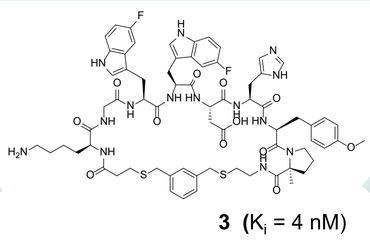


- Doughnut-like, flat contact surface (~190 Å²)
- 5 H-bonds with beta-sheet
- Key hydrophobic interactions
- 5F-TRP in center is a key interaction
- Potential areas for cross linking
- Sites prone to protease cleavage identified early

J. Med. Chem. **2020**, 63, 13796.

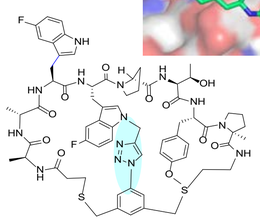
Cross-linking results in improvement in potency and protease stability

Top cross-link

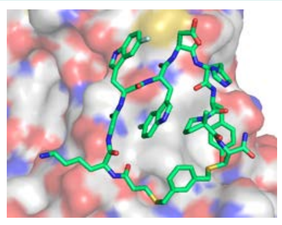


3 (K_i = 4 nM)

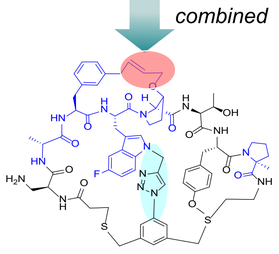
Middle cross-link



5 (K_i = 1.36 nM)



combined



4 (K_i = 0.3 nM)

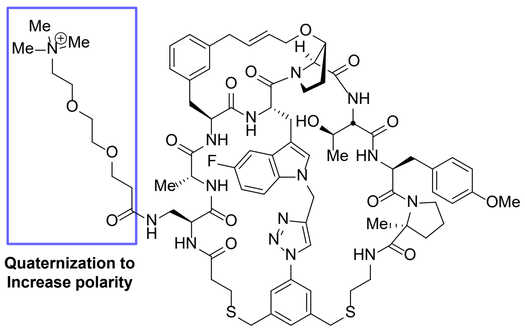
6 (K_i = 0.006 nM, protease stable PK improvement needed)

- 5 of the 8 amino acids are unnatural
- 3 of the unnatural amino acids are not commercially available

J. Med. Chem. **2020**, 63, 13796; *J. Med. Chem.,* **2021**, 64, 16770.

Optimize lead cyclic peptide

Chemistry tactics improved PK and stability



7 (K_i = 0.002 nM)

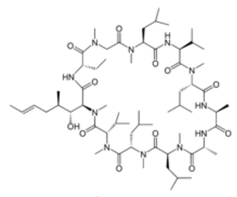
Pharmacokinetic Profile		
7	**Cyno**	**Rat**
CL (mL/min/kg)	**1.4**	**4**
$t_{1/2}$ (hr)	**7.6**	**4.6**
PPB (% bound)	**74**	**75**
Clu (mL/min/kg)	**6**	**15**
%F (30% Labrasol/PBS)	**2.9***	**1.4***

- ✓ Clean – MK499, CYP, ion channels
- ✓ PanLabs – no hits
- ✓ Oral delivery (enabled formulation)

*Dosed at 1 mpk PO gavage in cyno; 5 mpk ID (catheter) in rats.

Fully protease stable and not an OATP substrate.
Further Developed into MK-0616 (Elicitide) and approved as Lipfendra.

Inspired by precedence for bRo5, bioavailable macrocycles

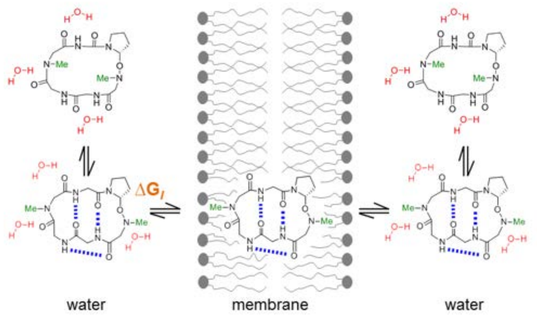


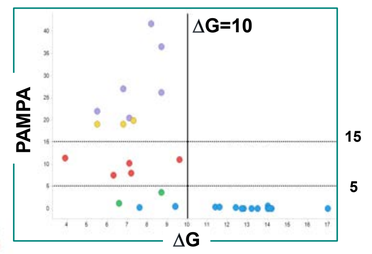
Cyclosporin A



HBD = 4
HBA =12
EPSA = 70
MW = 1215
Aromatic ring count = 0
ΔG = 5
F%: 10-89%

Build in chameleon-like properties driven by ΔG - incorporation of IMHBs





For PCSK9: *Predicted ΔG of <10 correlates well with passive permeability*

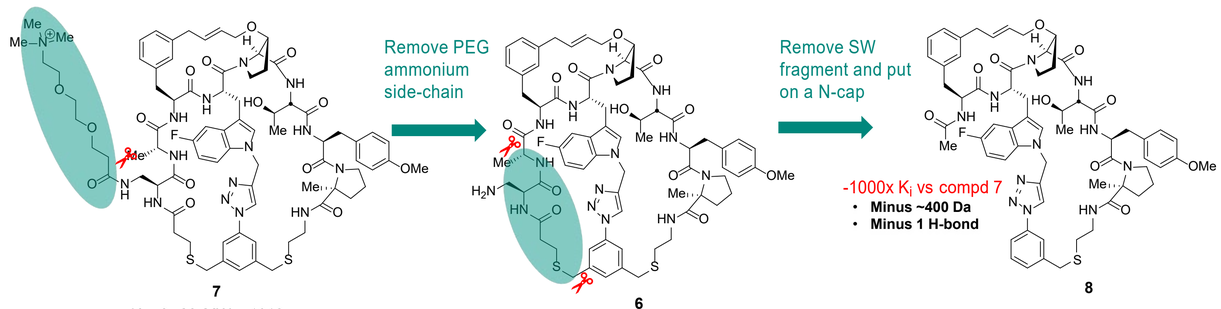
Guidelines for passive permeability:
EPSA <80; ΔG <15; HBD <4; MW <1000
Pampa >15; Papp >10;
Stable to stomach/gut enzymes

Passive transport

- *ΔG_I the free energy for transferring the LDC (low dielectric conformer) from water to the low-dielectric environment;* ***most informative for predicting passive permeability***
- Peptides in H_2O - adopt multiple, rapidly inter-converting conformations.
- The low dielectric of the membrane promotes the formation of intramolecular H-bonds (IMHBs)

Identification of minimum pharmacophore(s) is crucial in path to optimization

Reducing MW by removing PEG Ammonium side-chain and SW fragment



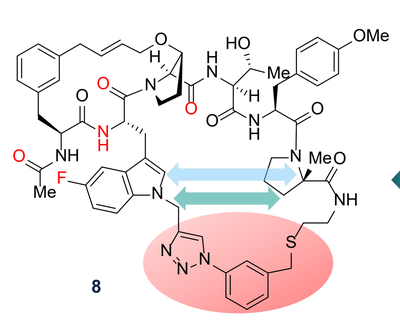
7
K_i = 2 pM; MW = 1612

6
K_i <6 pM; MW = 1408

8
K_i = 2.1 nM; MW = 1194
EPSA = 132; HBD = 6; ΔG = 13
Pe = **0**; Papp = 0.59
Rat Cl = 56 (OATP substrate)

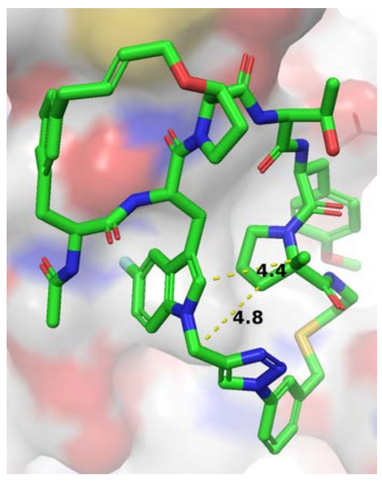
- Potent (single digit nM); 4 critical HBD retained with MW reduced by one third
- Few compds have Pe = 5-10; Papp – poor (compd stickiness)
- Poor physicochemical properties (very poor solubility)
- No oral BA
- ΔG = 13

Insight from structure suggests opportunity for new design



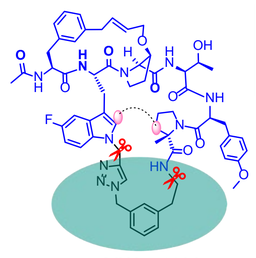
8

Structure guided truncation cross-link



As designed the truncated molecule ***retains the critical C=O/N-H*** that make contacts with the protein. The distances highlighted by the arrows are within ***4-5 Å, ideal for new crosslinks*** comprising of around 4 atoms. These new crosslinks can then replace the portion of the molecule highlighted in teal and still maintain the overall rigidity of the peptide.

Assess C2 connectivity

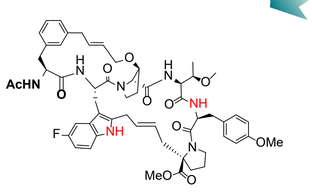


9 (K_i = 0.4 nM)

MW = 1162
Pe = 0
EPSA= 132

- **Permeability enhancement through N-methylation**
- Capping of N-terminal H-bond donor led to additional ***gains in permeability***

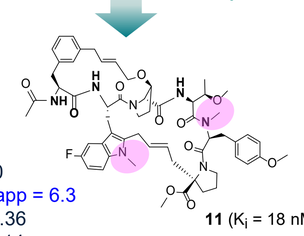
Truncate southern linker
Connect at C-2



10 (K_i = 35 nM)

MW = 1032
Pe = 16 , Papp = 2.0
EPSA = 102.5
PSA = 210.0

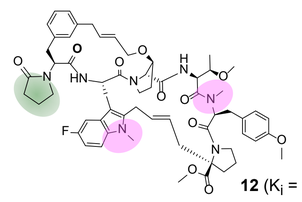
N-Methylation



11 (K_i = 18 nM)

MW = 1060
Pe = 38, Papp = 6.3
EPSA = 95.36
PSA = 177.14
ΔG = 8.3, F% = 0.3 (Rat PK)

Capping of N-terminal



12 (K_i = 164 nM)

MW = 1086
Pe = 21.85, Papp = 9.7
EPSA = 96.23
PSA = 183.4
ΔG = 6.8; F% = 2 (Rat PO)

Rat IV PK
CL = 32 mL/min/kg
Vss = 3.34 L/kg
MRT = 1.7 hr

Rat PO PK
%F = 2.2%
%F = 9.7% + pgp inhibitor
%F = 28.0% + pgp & CYP inhibitor

Conclusions

Translating permeability into oral absorption — balancing the potency trade-off

✓ C-2 linked series SAR validates medicinal chemistry strategy to maintain PCSK9 binding affinity and achieve moderate passive permeability in MDCKII cells

✓ **First moderately potent IPP molecule to have F >1% without any permeation enhancer**

- Additional in vivo studies suggest significant first pass metabolism and transporter activity
- Met ID studies suggest benzylic, olefin and N-methyl oxidative metabolism
- What is the impact of metabolism and transporter activity on bioavailability?

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