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Harnessing GPCRs Biased Signaling: Novel Macrocyclic Analogs for Enhanced Cardiovascular Treatment with Reduced Side Effects

Kien Tran,[1,2] Xavier Sainsily,[1] Jérôme Côté,[1] David Coquerel,[1] Pierre Couvineau,[2] Sabrina Saibi,[1] Lounès Haroune,[1] Élie Besserer-Offroy,[1] Joël Flynn-Robitaille,[1] Martin Resua Rojas,[1] Alexandre Murza,[1] Jean-Michel Longpré,[1] Mannix Auger-Messier,[1] Michel Bouvier,[2] Éric Marsault, Philippe Sarret and Pierre-Luc Boudreault[1]

1. Institut de Pharmacologie de Sherbrooke, Faculté de Médecine et des Sciences de la Santé, Université de Sherbrooke
2. 2. Institut de Recherche en Immunologie et en Cancérologie, Université de Montréal

INTRODUCTION

The discovery of signaling bias and the development of biased ligands represents a recent and significant advancement in drug discovery, offering the potential for more selective and effective therapies with reduced side effects. Biased ligands can preferentially activate or inhibit specific signaling pathways downstream of a receptor, thus providing a novel approach to modulating biological functions with greater precision.1 The innovation of biased signaling is particularly relevant in the context of cardiovascular diseases, where the precise modulation of receptor pathways can have significant implications for heart function and blood pressure regulation. [Pyr1]-apelin-13, an isoform of the apelin peptide acting on the APJ receptor, exemplifies this potential. It plays a pivotal role in cardiovascular physiology, mediating effects such as vasodilation and cardiac contractility enhancement, which are beneficial in the treatment of heart failure and hypertension. However, the therapeutic application of [Pyr1]-apelin-13 and its analogs has been limited by factors such as rapid degradation in vivo and the activation of multiple signaling pathways, which could lead to side effects.

HYPOTHESIS

Stable and Biased ligands targeting the APJ receptor, designed to selectively modulate specific signaling pathways, will enhance therapeutic outcomes in cardiovascular diseases by improving efficacy and reducing side effects compared to non-biased ligands.

OBJECTIVES

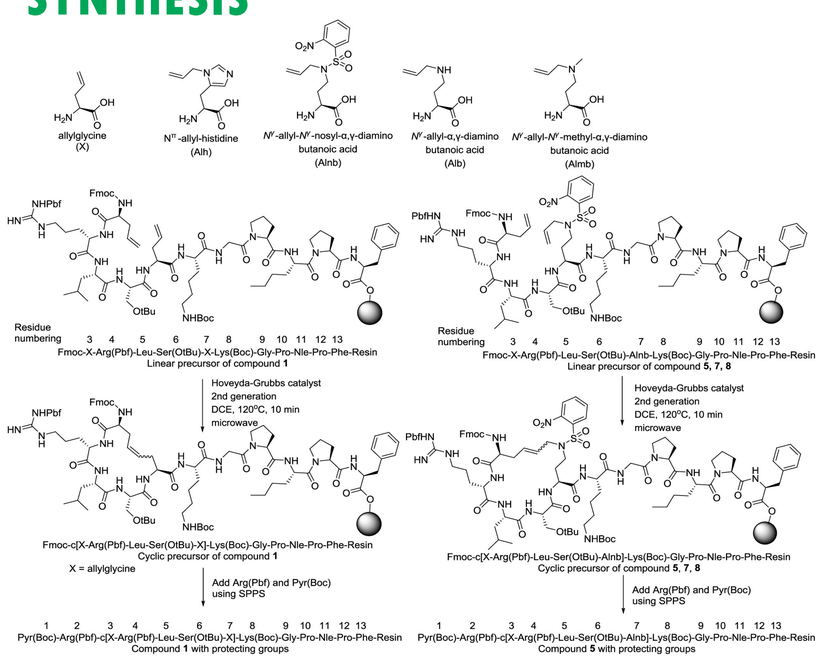
Develop stable, biased, and potent macrocyclic analogs of Pyr-apelin-13 (Ape13).

Pyr Arg Pro Arg Leu Ser His Lys Gly Pro Met Pro Phe

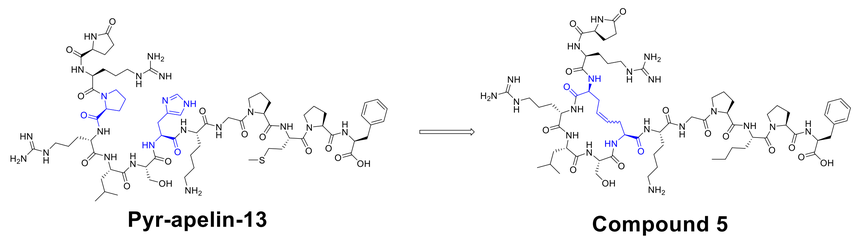
N-terminal pharmacophores
- Affinity (essential)

C-terminal pharmacophores
- Affinity (important)
- Functional activation

SYNTHESIS



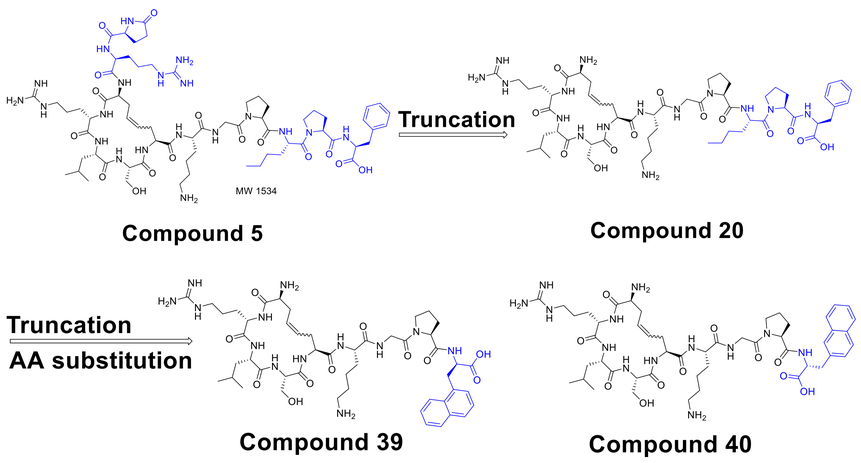
RESULTS



Macrocyclization of Ape13: Scan for macrocycle position

No	Macrocyclic analogues	Binding K_i (nM)	$G\alpha_i$ EC_{50} (nM)	β-arr2 EC_{50} (nM)	$T_{1/2}$ in vitro
Ape13	Pyr-R-P-R-L-S-H-K-G-P-M-P-F	0.7 ± 0.1	1.1 ± 0.2	60 ± 4	0.4 ± 0.1
2	c[X-R-P-R-X]c-S-H-K-G-P-Nle-P-F	5.7 ± 3.5	23 ± 6	857 ± 195	0.8 ± 0.2
3	Pyr-c[Rx-P-R-L-X]c-H-K-G-P-Nle-P-F	4.5 ± 1.4	6.0 ± 1.4	179 ± 21	2.2 ± 0.3
4	Pyr-c[X-P-R-L-X]c-H-K-G-P-Nle-P-F	509 ± 146	--	--	0.7 ± 0.1
5	Pyr-R-c[X-R-L-S-X]c-K-G-P-Nle-P-F	3.0 ± 0.1	3.7 ± 0.9	126 ± 26	3.9 ± 0.6
6	Pyr-R-P-c[Rx-L-S-H-X]c-G-P-Nle-P-F	9.1 ± 1.4	19 ± 5	512 ± 131	7.7 ± 0.3
7	Pyr-R-P-c[X-L-S-H-X]c-G-P-Nle-P-F	6074 ± 1168	--	--	9.9 ± 1.2
8	Pyr-R-P-R-c[X-S-H-K-X]c-P-Nle-P-F	41 ± 5	--	--	3.5 ± 0.8
9	Pyr-R-P-R-L-c[Sx-H-K-G-X]c-Nle-P-F	188 ± 84	--	--	> 24h
10	Pyr-R-P-R-L-c[X-H-K-G-X]c-Nle-P-F	268 ± 125	--	--	4.1 ± 0.8
11	Pyr-R-P-R-L-S-c[X-K-G-P-X]c-P-F	1.4 ± 0.4	20 ± 2	400 ± 35	6.6 ± 0.4
12	Pyr-R-P-R-L-S-H-c[X-G-P-Nle-X]c-F	14 ± 7	30 ± 7	825 ± 113	4.6 ± 0.7
13	Pyr-R-P-R-L-S-H-K-c[X-P-Nle-P-X]c	1.1 ± 0.1	19 ± 4	272 ± 7	8.6 ± 0.6
14	Pyr-R-P-R-L-S-H-K-G-c[X-Nle-P-F-X]c	310 ± 59	--	--	7.8 ± 1.1

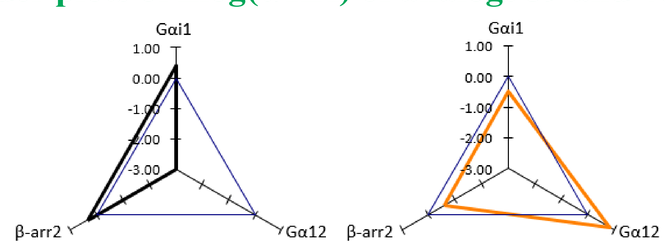
N-terminus and C-terminus truncation



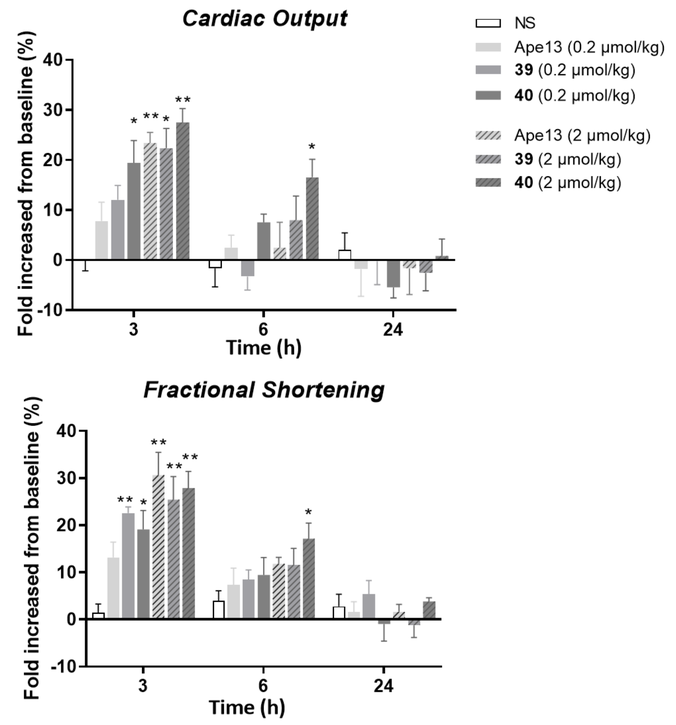
Functional activation

No	EC_{50} $G\alpha_{i1}$ (nM)	EC_{50} $G\alpha_{12}$ (nM)	EC_{50} β-arr2 (nM)
Ape13	0.8 ± 0.2 (100%)	49 ± 1 (100%)	37 ± 8 (100%)
5	3.7 ± 0.9 (101%)	22 ± 2 (98%)	126 ± 26 (98%)
20	6.7 ± 1.2 (100%)	45 ± 2 (98%)	143 ± 33 (98%)
39	0.8 ± 0.1 (103%)	> 10000 (3%)	31 ± 7 (70%)
40	5.0 ± 0.6 (105%)	2.4 ± 0.6 (40%)	232 ± 4 (55%)

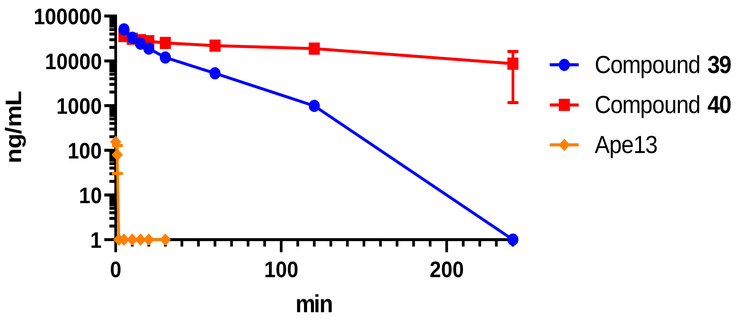
Spider plots of Δlog(τ/KA) of analogs 39 and 40



Long-lasting *in vivo* cardiac effect



Pharmacokinetic *in vivo* in rat



	Ape13	39	40
$T_{1/2}$ *in vivo*	< 1 min	24 min	220 min
AUC (mg/mL.min)	0.0002 ± 0.0001	1.33 ± 0.18	8.80 ± 0.41
Clearance (Cl) mL/(min.kg)	Very high	2.3	0.3

CONCLUSION

- The macrocyclization is a powerful method to improve the stability and affinity of the apelin peptide.
- Macrocyclic analogue **39** and **40** has significant lower MW (33%) than **apelin-13** but display similar potency.
- Compounds **39** is biased agonists of APJ receptor on the $G\alpha_{12}$ pathway.
- Analogue **39** and **40** display a longer half-life and long-lasting cardiac effect *in vivo* up to 6h after administration.

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