Plant-derived peptides Rubiscolin-6, Soymorphin-6 and their *C*-terminal amide derivatives: pharmacokinetic properties and biological activity

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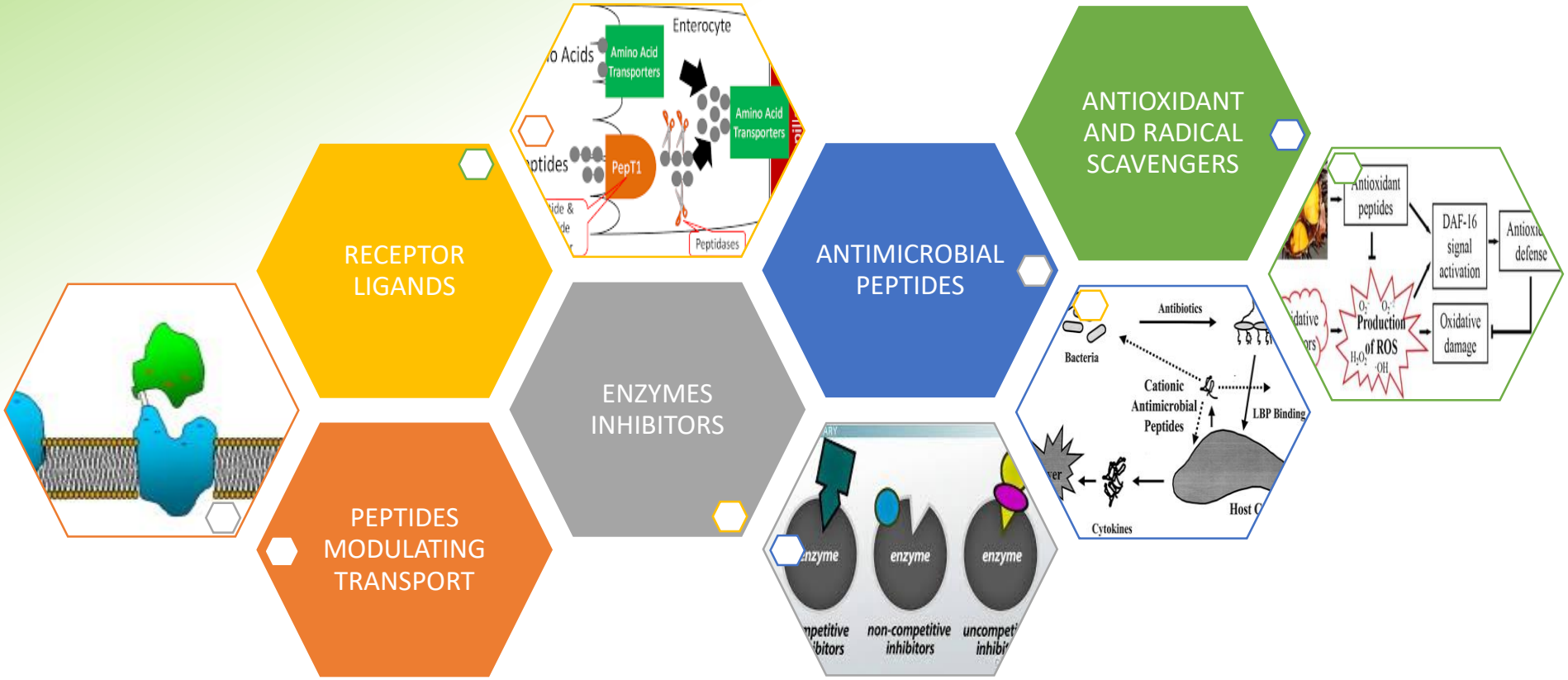
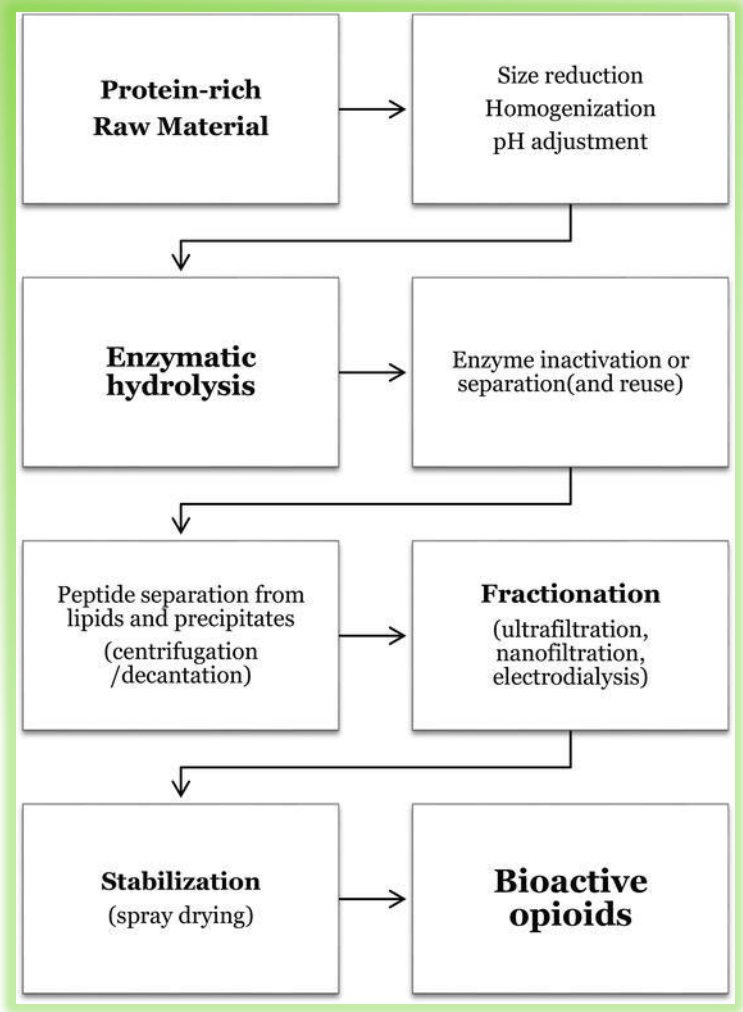
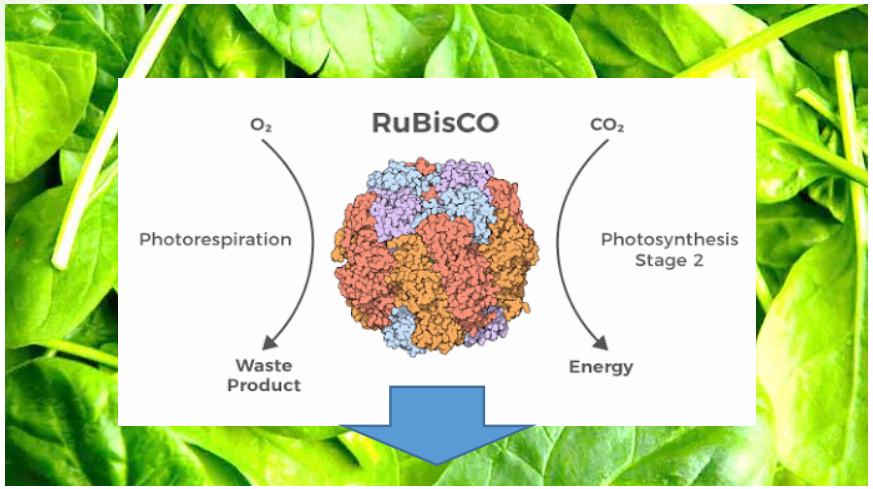
EXORPHINS


RECEPTOR LIGANDS
Enterocyte
Amino Acid Transporters
PepT1
Amino Acid Transporters
Peptidases
ANTIMICROBIAL PEPTIDES
Antibiotics
Bacteria
Cationic Antimicrobial Peptides
LBP Binding
Cytokines
ANTIOXIDANT AND RADICAL SCAVENGERS
Antioxidant peptides
DAF-16 signal activation
Production of ROS
Oxidative damage
ENZYMES INHIBITORS
enzyme
enzyme
enzyme
non-competitive inhibitors
PEPTIDES MODULATING TRANSPORT

THE ORIGIN OF SOYMORPHINS AND RUBISCOLINS





Rubiscolin-6: YPLDLF

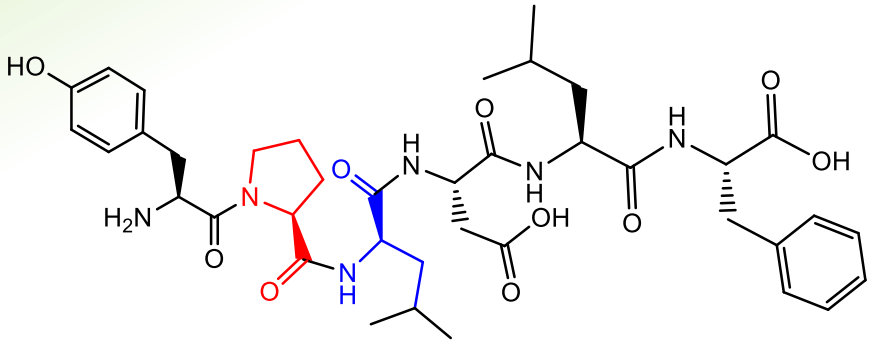
δ-opioid receptor ligand

Soymorphin-6: YPFVVN

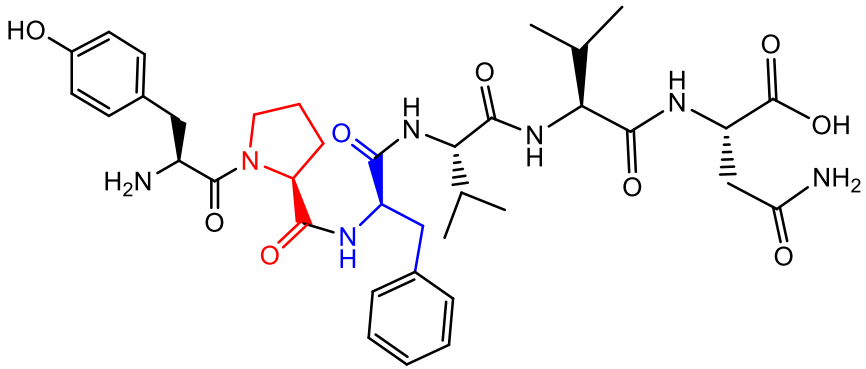
μ-opioid receptor ligand

BIOLOGICAL ACTIVITIES OF EXORPHINS

SOYMORPHIN-6	RUBISCOLIN-6
MOR agonist	DOR agonist
Anxiolytic activity	
Anti-nociceptive effect	
Anorexygenic effect	Orexygenic effect
	Memory-enhancing effect

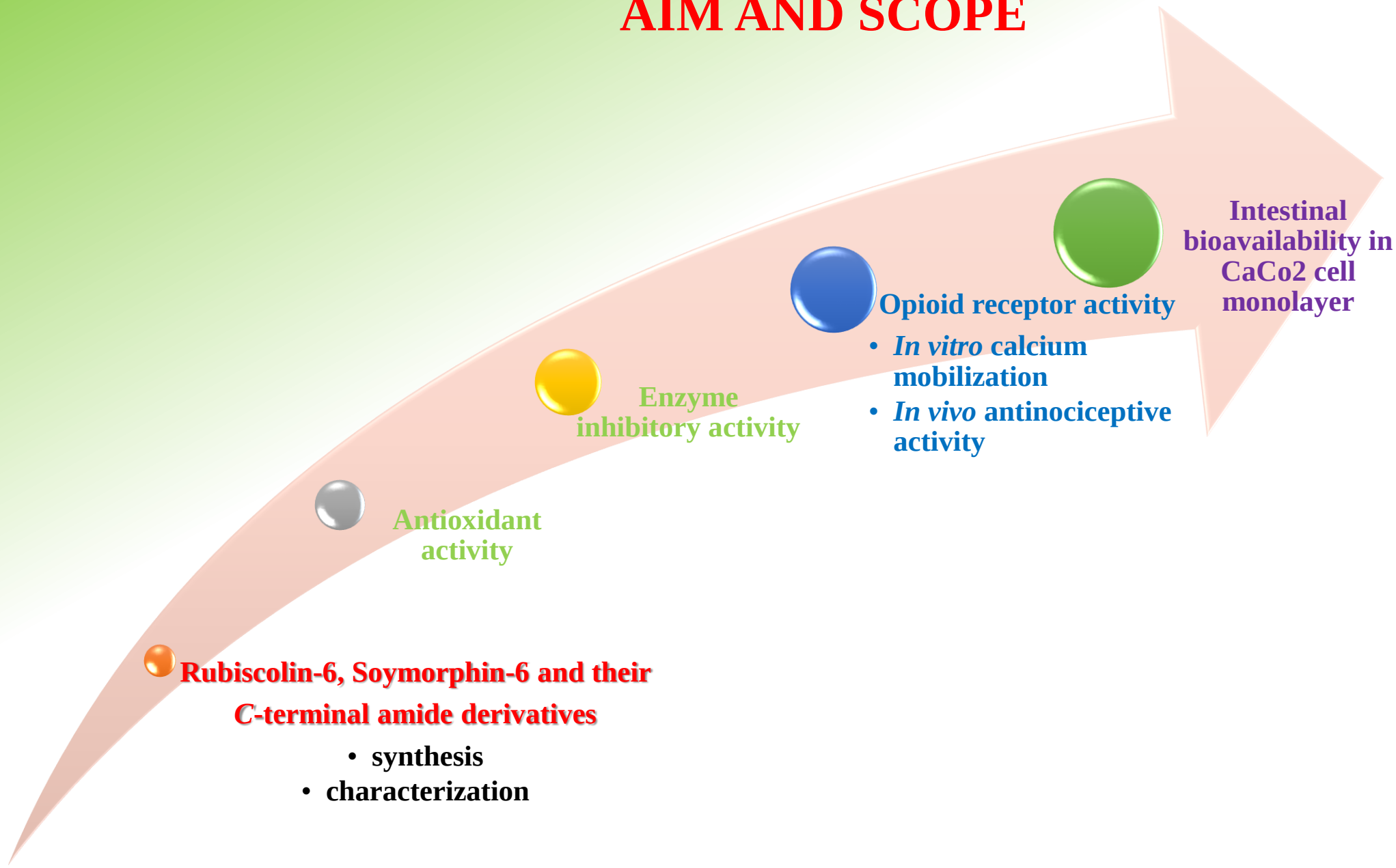


RUBISCOLIN-6



SOYMORPHIN-6

AIM AND SCOPE



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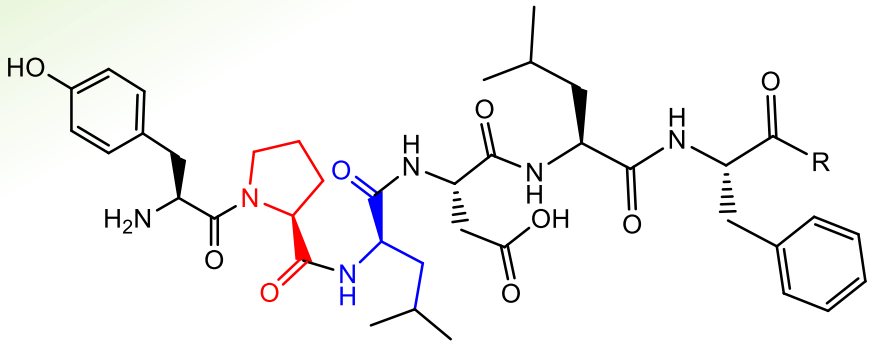
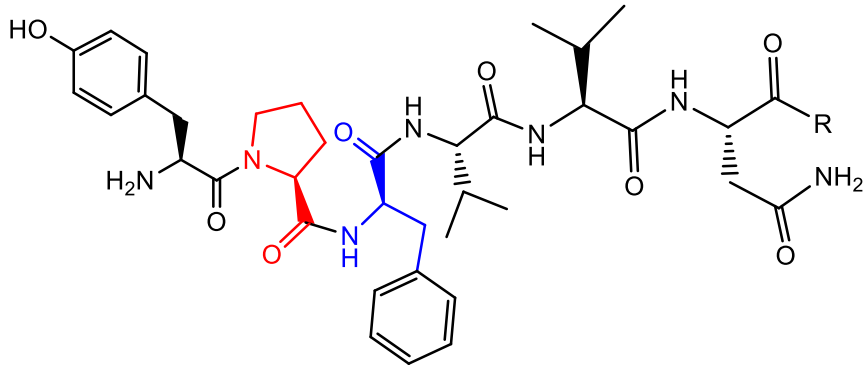
ANTIOXIDANT AND TYROSINASE INHIBITORY ACTIVITIES

Peptides	DPPH (mg TE/g)	ABTS (mg TE/g)	CUPRAC (mg TE/g)	FRAP (mg TE/g)	Tyrosinase inhibition (mg KAE/g)
Rubiscolin-6	2.41±0.33[a]	8.86±0.38[b]	10.09±0.02[a]	5.75±0.04[a]	24.51±1.99[a]
Soymorphin-6	2.65±0.70[a]	3.09±0.47[c]	9.10±0.08[b]	5.64±0.04[b]	na
Rubiscolin-6 *C*-amide	2.72±0.51[a]	0.77±0.01[d]	9.55±0.05[c]	5.79±0.03[a]	2.81±0.34[c]
Soymorphin-6 *C*-amide	1.38±0.21[b]	11.00±1.96[a]	9.35±0.13[c]	5.72±0.08[a]	9.19±1.43[b]

Table 1. Antioxidant properties and tyrosinase inhibitory effects of the tested peptides*
*Values are reported as mean ±S.D of three parallel experiments. TE: Trolox equivalents; KAE: Kojic acid equivalents; na: not active. a-d: Different letters indicate significant differences in the peptides (p <0.05).



R=OH: RUBISCOLIN-6
R=NH_2: RUBISCOLIN-6 *C*-AMIDE



R=OH: SOYMORPHIN-6
R=NH_2: SOYMORPHIN-6 *C*-AMIDE

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IN VITRO AND IN VIVO ACTIVITY ON OPIOID RECEPTORS

	CHO_μ		CHO_δ	
COMPOUNDS	pEC_{50} ($CL_{95\%}$)	E_{max} ± sem %	pEC_{50} ($CL_{95\%}$)	E_{max} ± sem %
Dermorphin	8.06 (7.65 – 8.46)	254 ± 18%	6.43 (5.95 – 6.91)[a]	78 ± 3% [a]
DPDPE	Inactive		7.51 (7.31 – 7.70)	223 ± 17%
EM-1	7.45 (7.08 – 7.82)	263 ± 30%	Crc incomplete, at 100µM 89 ± 15%	
Rubiscolin-6	Inactive		Crc incomplete, at 100µM 87 ± 19%	
Rubiscolin-6 *C*-amide	Crc incomplete, at 100µM 131 ± 29%		Crc incomplete, at 100µM 112 ± 29%	
Soymorphin-6	Crc incomplete, at 100µM 110 ± 13%		Crc incomplete, at 100µM 46 ± 14%	
Soymorphin-6 *C*-amide	Crc incomplete, at 100µM 81 ± 4%		Crc incomplete, at 100µM 79 ± 14%	

Table 2. Potencies (pEC_{50}) and maximal effects of standard and novel ligands at µ and δ-opioid receptors.
Data are mean of at least 5 separate experiments made in duplicate.

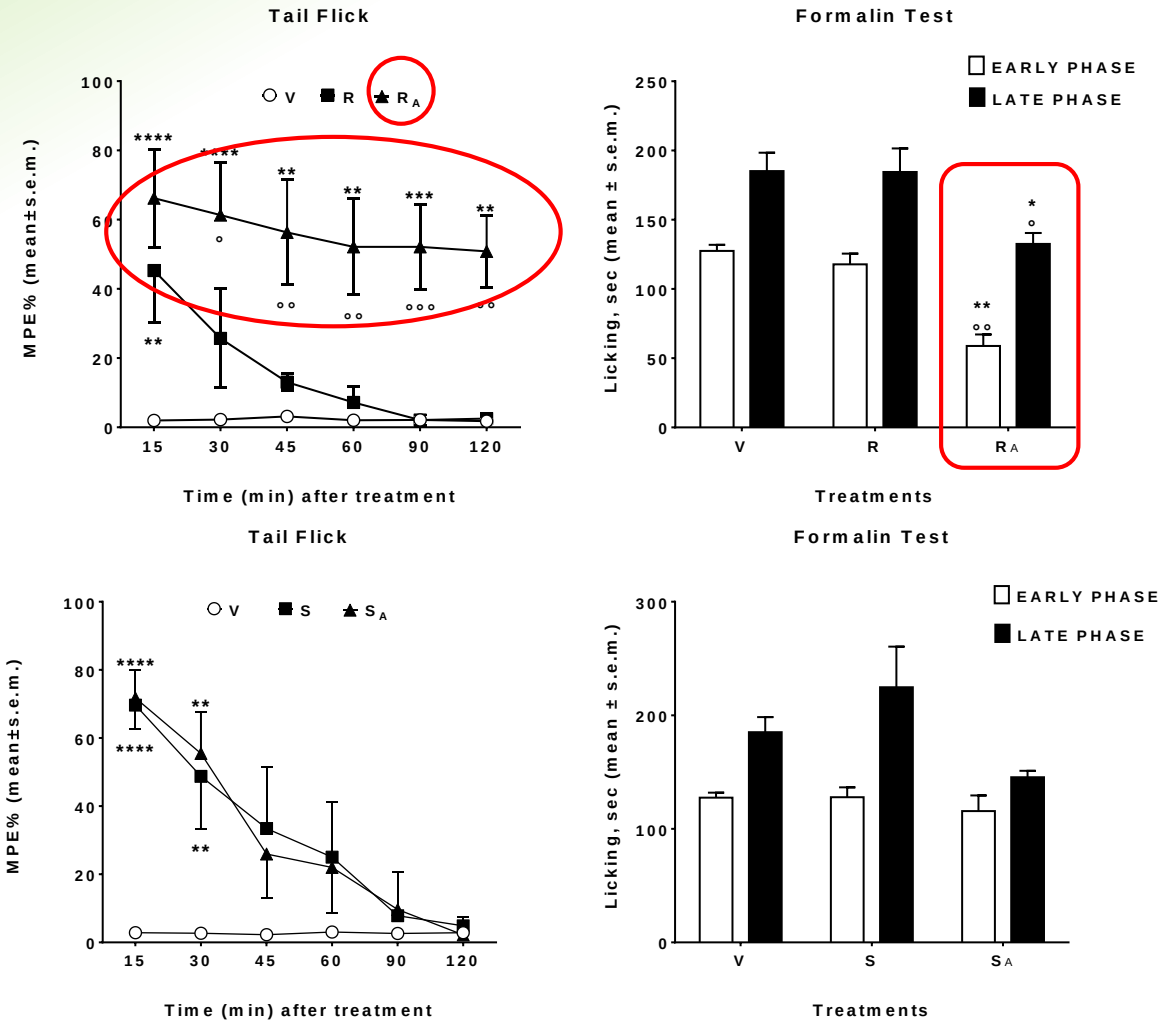

Figure 1. Effects induced by Rubiscolin-6 (R), Rubiscolin-6 *C*-amide (R_A), Soymorphin-6 (S), Soymorphin-6 *C*-amide (SA) in the tail flick test (left panel) and in the formalin test (right panel). In the tail flick test, compounds were administered i.c.v. at the dose of 10 µg/10 µL; in the formalin test, compounds were administered s.c., in the dorsal surface of the mouse hind paw, at the dose of 100 µg/20 µL, 15 min before formalin. V is for vehicle-treated animals. ** is for P<0.01, *** is for P<0.001, **** is for P<0.0001 *vs* V; ° is for P<0.05, °° is for P<0.01, °°° is for P<0.001 *vs* R. N=7.

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IN VITRO INTESTINAL BIOACCESSIBILITY

Peptide	%
Rubiscolin-6	10.2 ± 1.2
Soymorphin-6	7.3 ± 0.8
Rubiscolin-6 *C*-amide	5.4 ± 0.4
Soymorphin-6 *C*-amide	3.2 ± 0.3

Values are the means ± SD (n = 5; P < 0.01).

Table 3. *In vitro* intestinal bioaccessibility of peptides calculated as area under curve of chromatograms from HPLC-DAD analyses of intestinal digesta.

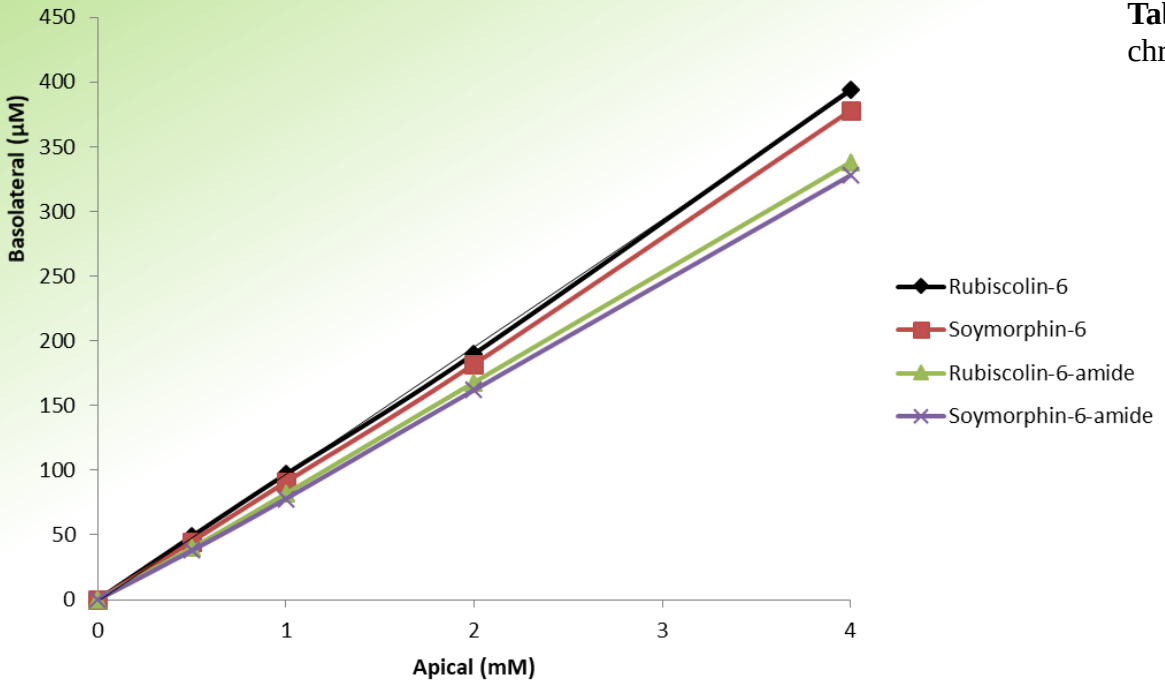

Figure 2. *In vitro* intestinal bioavailability of peptides. Quantification of peptides in CaCo2 cell monolayer apical and basolateral solutions using a five-point calibration curve of pure peptides as standard analysed by HPLC.

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DOCKING STUDY

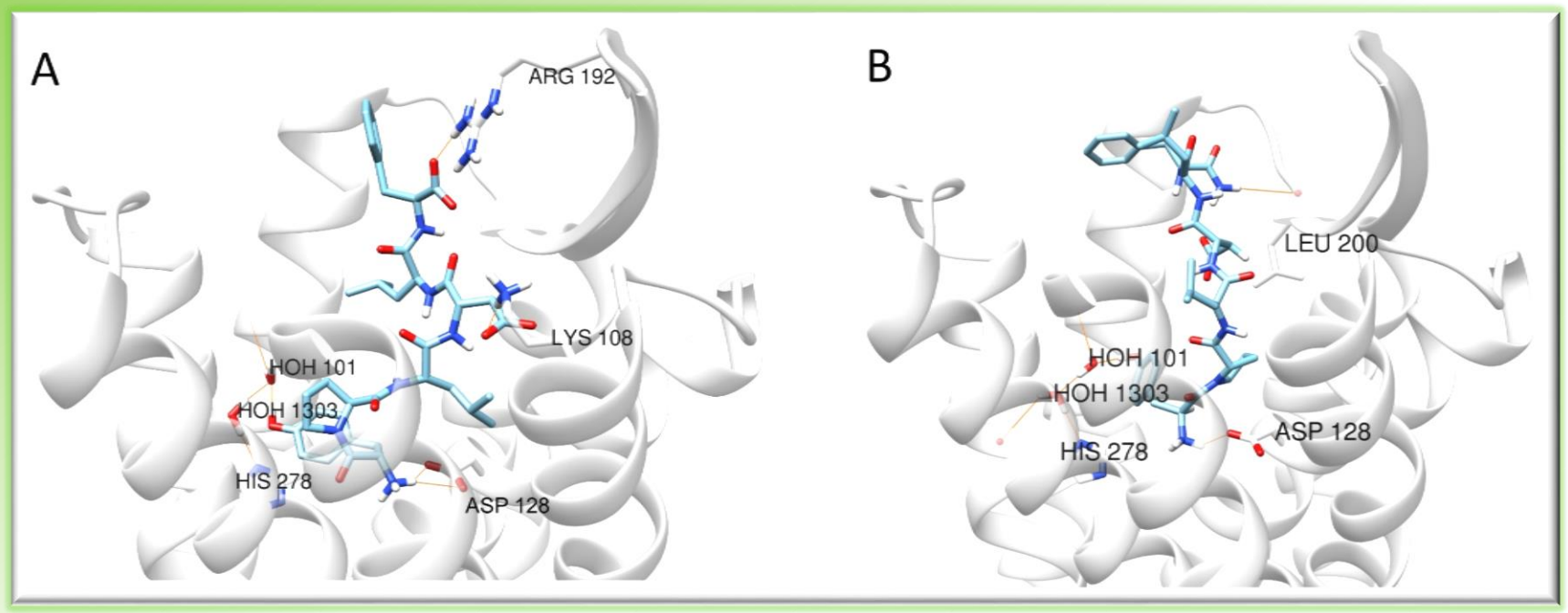

Figure 3. Best ranked docking poses of **Rubiscolin-6** (A) **and Rubiscolin-6 *C*-amide** (B) docked at the DOR (4RWD).

DOR interactions					
Compounds	Asp128	His278	Trp284	Leu200	Arg192
TIPP-NH_2	H bond	H bond through water network	**π-π**	**H bond**	**Cat-π**
Rubiscolin-6	Ionic + H bond	H bond through water network	--	--	H bond
Rubiscolin-6 *C*-amide	Ionic + H bond	H bond through water network	--	H bond	--

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CONCLUSION

The tested peptides exhibit low antioxidant ability.

All our peptides exhibit moderate tyrosinase inhibitory effect.

Calcium mobilization assay data reveal that all compounds are able to stimulate the DOR and MOR at 100 μM concentration.

Rubiscolin-6 *C*-amide centrally administered demonstrates a strong antinociceptive effect higher than the parent compound, and it is effective after subcutaneous administration.

All peptides were absorbed intact through CaCo2 monolayer however, rubiscolin-6 *C*-amide shows the most interesting *in vivo* biological profile, which prompt us to further investigate its effect after oral administration.

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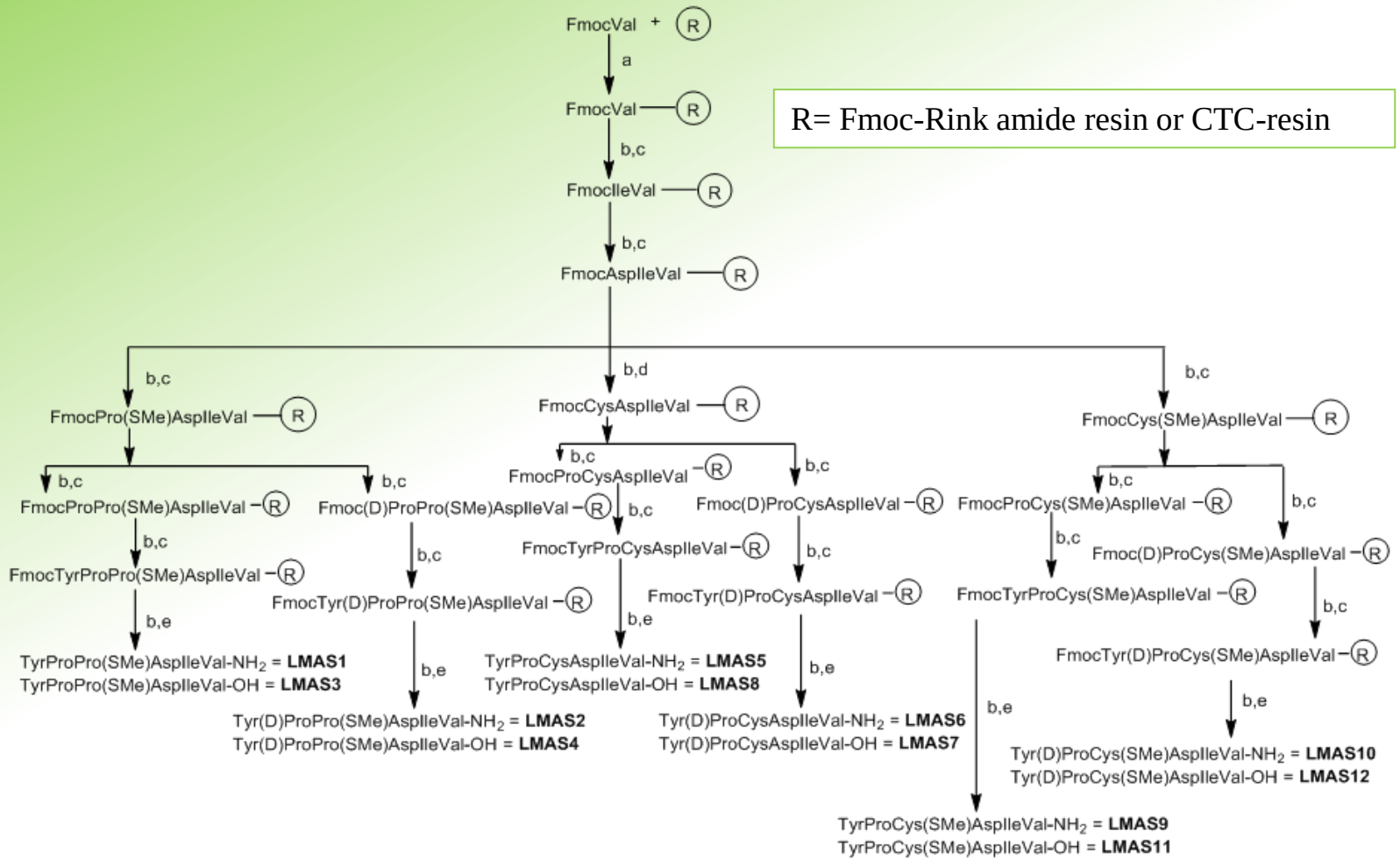
FUTURE PERSPECTIVES



R= Fmoc-Rink amide resin or CTC-resin

Reagents and conditions: (a) Fmoc-Rink-Amide resin: Piperidine 20% in DMF (15min x2); FmocValOH, HOBt, DIPEA, TBTU, DMF. Resina CTC: FmocValOH, HOBt, DIPEA, TBTU, DMF; then DCM/MeOH/DIPEA 17:2:1; (b) Piperidine 20% in DMF (15min x2); (c) FmocXaaOH, HOBt, DIPEA, TBTU, DMF (Xaa = Ile, Asp, Pro/Met, Cys(SMe), Pro, (D)Pro, Tyr; (d) FmocCys(Trt)OH, DMF, TBTU, Collidine; (e) TFA/H_2O/TES or TFA/H_2O/TIPS 95:2.5:2.5.

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