Finanziato dall'Unione europea
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Ministero dell'Università e della Ricerca

Italiadomani
PIANO NAZIONALE DI RIPRESA E RESILIENZA

Studio *in vitro* sugli effetti antinfiammatori di nuovi peptidi sintetici leganti i recettori FPR2. Parte I

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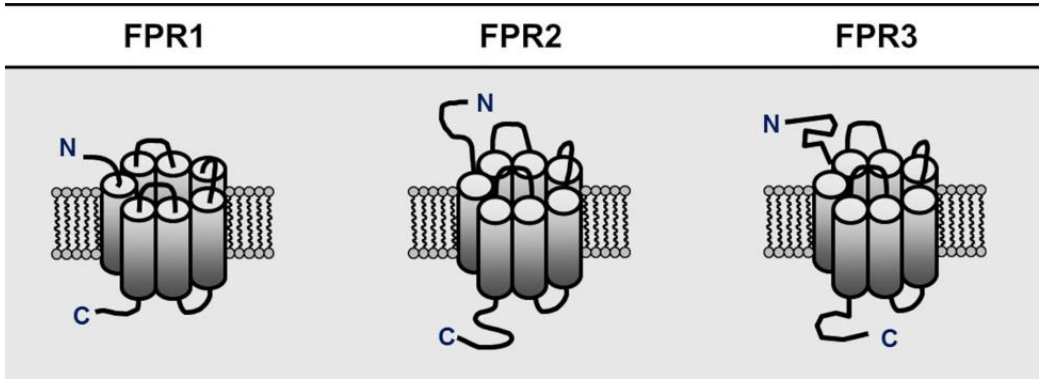
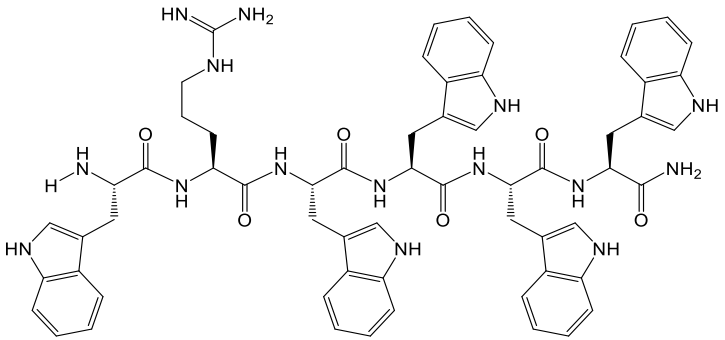
Spoke 8, WP 3, TASK 3.2

Receptors FPR

- G protein-coupled receptor
- 3 isoforms: FPR1, FPR2, FPR3
- Are involved in chemotaxis ⟶ infection and inflammation
- Inflammatory bowel disease, including ulcerative colitis and Crohn's disease
- Mediating inflammatory response

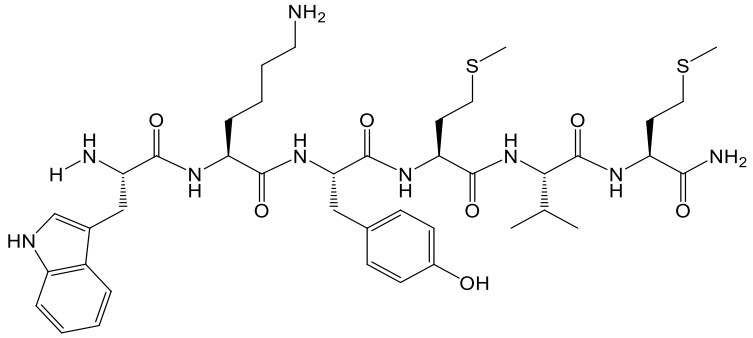


Antagonist: WRWWWW (AMGS3)



Selective FPR2 inhibitor

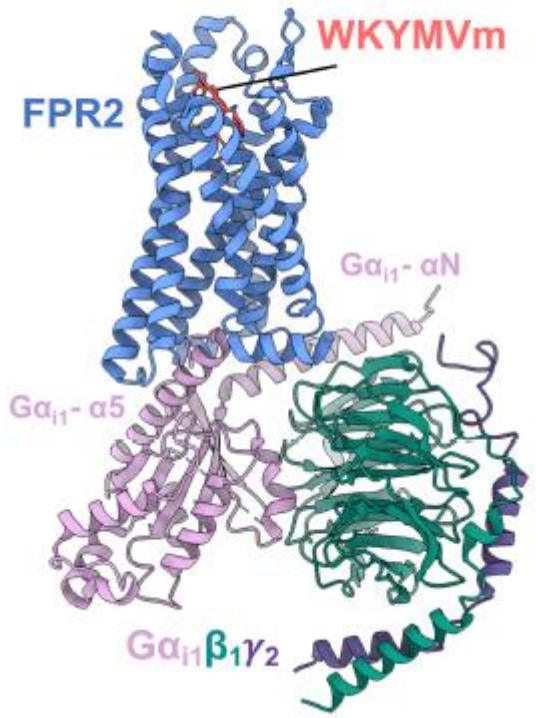
Agonist: WKYMVm (AMGS10)

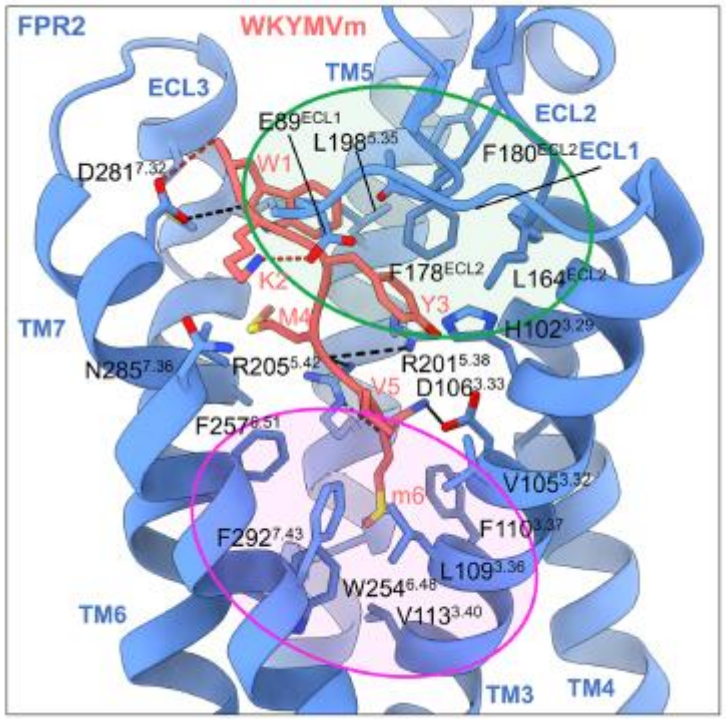


- ✓ **Binds FPR1 and FPR2**
- ✓ **Stimulates phagocyte activity**
- ✓ **Promotes proliferation**
- ✓ **Inhibits intestinal permeability**

[Mormile, Rossi, Prevete , Granata, Pucino, De Paulis "The N-Formyl Peptide Receptors and Rheumatoid Arthritis: A Dangerous Liaison or Confusing Relationship?"]

Receptor FPR2

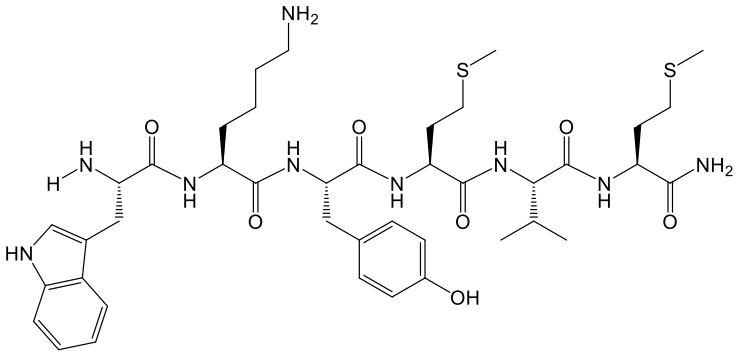




Two hydrophobic clusters:

- **At the top** → **W1 and Y3**
- **At the base** → **V5 and D-Met6**

Agonist AMGS10



W K Y M V m

- Hydrophobic interactions
- Polar interactions
- Hydrogen bonds
- Salt bridges
- Van der Waals

[Cheng Xue Qin, Lucy V. Norling, Elizabeth A. Vecchio, Eoin P. Brennan, Lauren T. May, Denise Wootten, Catherine Godson, Mauro Perretti, Rebecca H. Ritchie "Formylpeptide receptor 2: Nomenclature, structure, signalling and translational perspectives: IUPHAR review 35"]

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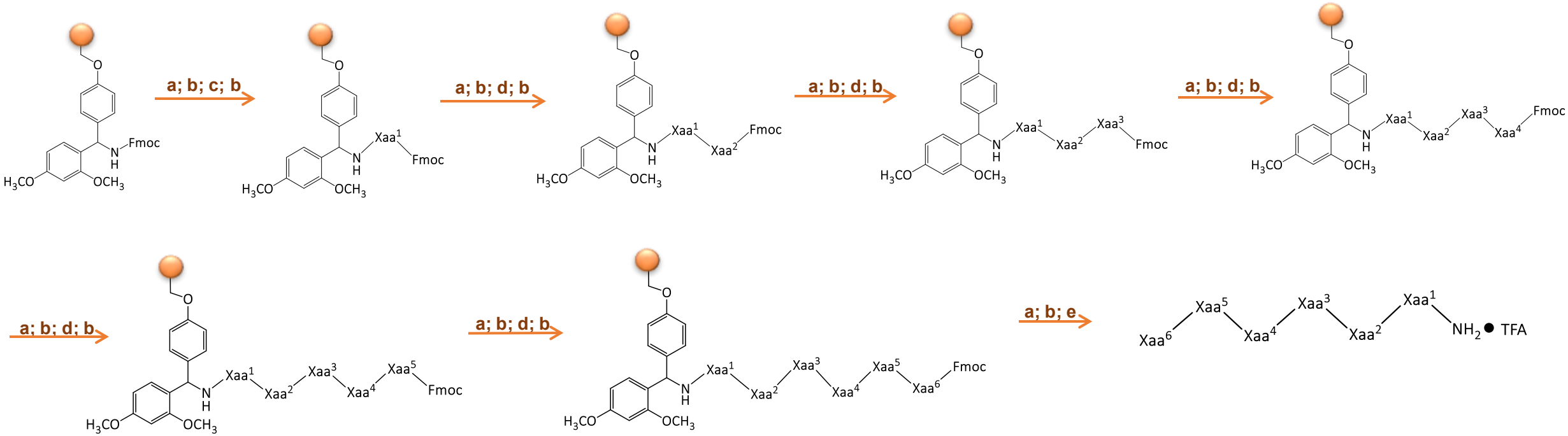
Via Cà Le Suore, Urbino
17 Dicembre 2024

SPPS

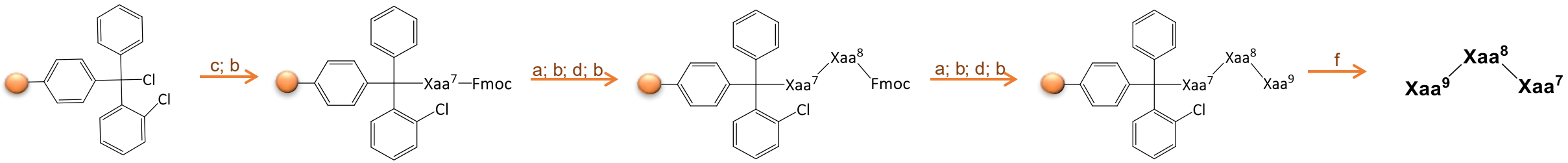


Reagents and conditions:

a) Piperidine 20% DMF;
b) Wash: DMF x3, MeOH x 3, DCM x3;
c) Xaa1 (3 eq.), HOBt (3 eq.), DIPEA (6 eq.), TBTU (3 eq.), DMF (6mL), overnight r.t.;
d) Coupling aminoacid (3 eq.), HOBt (3 eq.), DIPEA (6 eq.), TBTU (3 eq.), DMF (6mL), 2h r.t.;
e) TFA:H_2O:TIPS (95:2.5:2.5), 3h r.t.

PRODUCT	Xaa^1	Xaa^2	Xaa^3	Xaa^4	Xaa^5	Xaa^6
AMGS1	D-Met	L-Trp-(Boc)	L-Met	L-Tyr-(tBut)	L-Lys-(Boc)	L-Trp-(Boc)
AMGS2	L-Met	L-Trp-(Boc)	L-Met	L-Tyr-(tBut)	L-Lys-(Boc)	L-Trp-(Boc)
AMGS3	L-Trp-(Boc)	L-Trp-(Boc)	L-Trp-(Boc)	L-Trp-(Boc)	L-Arg-(Pbf)	L-Trp-(Boc)
AMGS4	D-Met	L-Trp-(Boc)	L-Met	L-Tyr-(tBut)	L-Lys-(Boc)	L-Tyr-(tBut)
AMGS5	L-Trp-(Boc)	L-Lys-(Boc)	L-Tyr-(tBut)	L-Met	L-Leu	For-Met
AMGS6	L-Trp-(Boc)	L-Lys-(Boc)	L-Tyr-(tBut)	L-Met	L-Val	For-Met
AMGS9	D-Met	L-Leu	L-Met	L-Tyr-(tBut)	L-Lys-(Boc)	L-Trp-(Boc)
AMGS10	D-Met	L-Val	L-Met	L-Tyr-(tBut)	L-Lys-(Boc)	L-Trp-(Boc)
AMGS11	D-Met	L-Leu	L-Phe	L-Tyr-(tBut)	L-Lys-(Boc)	L-Trp-(Boc)
AMGS12	D-Met	L-Val	L-Phe	L-Tyr-(tBut)	L-Lys-(Boc)	L-Trp-(Boc)
AMGS13	L-Trp-(Boc)	L-Lys-(Boc)	L-Tyr-(tBut)	L-Phe	L-Leu	For-Met
AMGS14	L-Trp-(Boc)	L-Lys-(Boc)	L-Tyr-(tBut)	L-Phe	L-Val	For-Met

SPPS



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d) Coupling aminoacid (3 eq.), HOBt (3 eq.), DIPEA (6 eq.), TBTU (3 eq.), DMF (6mL), 2h r.t.;
f) 1% TFA/DCM

PRODUCT	Xaa^7	Xaa^8	Xaa^9
AMGS7	L-Phe	L-Leu	For-Met
AMGS8	L-Phe	L-Leu	Boc-Met

Analyses and purification

Semipreparative HPLC

Mass spectrometer

H-NMR

- Yields 30%-50%
- Purity ≥95%

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In vivo tests

Compound	Formula	Early phase	Late phase	PT	EDEMA
Veicol	DMSO:SAL 1:3 v/v	137,3	190,3^{ns}	-53,2	84,6
AMGS1	WKYMWm-NH_2	82,2*	155,3^{ns}	-13***	43,1**
AMGS2	WKYMWM-NH_2	89,8^{ns}	161,0^{ns}	-35	62
AMGS3	WRWWWW-NH_2	103,2^{ns}	146,3^{ns}	-46,7	53*
AMGS4	**YKYMWm-NH_2**	**58,7******	**98,5^{ns}**	**-19,6****	**41,4****
AMGS5	For-MLMYKW-NH_2	67,8***	124,5^{ns}	-30,4	68,8
AMGS6	For-MVMYKW-NH_2	68,0***	119,8^{ns}	-46,9	51,6*
AMGS7	For-MLF-OH	76,5**	165,0^{ns}	-37,2	62,1
AMGS8	Boc-MLF-OH	125,2^{ns}	135,5^{ns}	-56,3	71,6
AMGS9	**WKYMLm-NH_2**	**41,5******	**94,2^{ns}**	**-10,5*****	**29,4******
AMGS10	WKYMVm-NH_2	100,2^{ns}	150,7^{ns}	-42,0	64,3
AMGS11	WKYFLm-NH_2	89,5^{ns}	159,2^{ns}	-42,6	58,1
AMGS12	WKYFVm-NH_2	74,2**	90,0^{ns}	-25,4*	39***
AMGS13	For-MLFYKW-NH_2	72,2**	110,2^{ns}	-20,7*	29,9****
AMGS14	**For-MVFYKW-NH_2**	**65,8*****	**96,3^{ns}**	**-11,1*****	**37,4*****

dose 10 μg/20 μL. ANOVA/Dunnett test. * = P<0.05; ** = P<0.01; *** = P<0.001; **** = P<0.0001; ns = not significant. N=7-9.

Oral and poster communications

Poster presentation: “Synthesis and characterization of FPR ligands for the treatment of Intestinal Bowel syndrome” D’Ingiullo S., D’Amario I., Verginelli F., Stefanucci A., Pieretti S. and Mollica A. Convegno interdisciplinare Giornate Italo-Francesi di Chimica **2024**, Torino, 4-5 Aprile **2024**.

Poster presentation: “Synthesis and characterization of FPR ligands for the treatment of Intestinal Bowel syndrome” D’Ingiullo S., D’Amario I., Verginelli F., Stefanucci A., Pieretti S. and Mollica A. ESMEC School of Medicinal Chemistry **2024** , Urbino, 26-30, Giugno **2024**.

Poster presentation: “Synthesis and characterization of FPR ligands for the treatment of Intestinal Bowel syndrome” D’Ingiullo S., D’Amario I., Verginelli F., Stefanucci A., Pieretti S. and Mollica A. Congresso Nazionale della SCI **2024**, Fiera di Milano, 27-30 Agosto **2024**.


Thank you
for your attention