Discovery of Stable and Potent Chemerin Analogs Selective for CMKLR1

Yan Wang[a], Yvonne Angell[a], Yun Wu[b], Yanfen Teng[b], Meng Li[b], Krishna Kodukula[c] and Robert Drakas[c]

[a.] Department of Peptide Chemistry, ChemPartner SSF; [b.] ChemPartner Shanghai;

[c.] ShangPharma Innovation, 280 Utah Avenue, Suite 250, SSF, CA 94080

Introduction

Chemerin is an immunomodulating factor secreted in many tissues including the spleen, lymph nodes, adipose tissue and epithelia. It was identified as the natural ligand of the Chemerin receptor (ChemR23 / CMKLR1), a chemokine-like G protein-coupled receptor (GPCR) and induces chemotaxis in natural killer cells, macrophages, and immature dendritic cells.[1-2] Chemerin also binds to GPR1 and CCRL2 with high affinity, but the signaling is inefficient. It is secreted as an inactive precursor, pro-chemerin, and is activated by proteolytic removal of amino acids from the c-terminus by proteases such as elastase, cathepsin G, or kallikrein 7. The nonapeptide, chemerin-9, derived from chemerin C-terminus retains significant bioactivity of full-size protein, with regard to agonism toward the receptor.[3] Chemerin is involved in a variety of functions including cell differentiation. However, its mechanism and detailed signaling pathway remain unclear. Also, chemerin and chemerin-9 are rapidly degraded and inactivated in plasma, which has limited their use in *in vivo* experiments and as potential therapies. There is a need to develop stable and selective molecular tools to accelerate pharmacological analysis of Chemerin–CMKLR1 interactions, better define signaling pathways, and screen *in vivo* to validate CMKLR1 as a potential therapeutic target.

Materials and Methods

Peptide synthesis:

All the peptides and non-commerical available building blocks were synthesized at ChemPartner Shanghai. Peptides were synthesized using CTC or MBHA resin. Couplings were performed with 3 equiv. of amino acid derivative / coupling reagent / base or additive (5 equiv.). Fmoc-deprotection was carried out using 20 % piperidine in DMF. Peptide was cleaved from resin using TFA cocktail. Peptides were purified by reverse-phase HPLC (RP-HPLC). Purity was ≥ 95% as estimated by RP-HPLC and mass spectrometric analysis.

***In vitro* assay of activity:**

Tango™ CMKLR1-bla U2OS cells was purchased from Thermo Fisher Scientific. On day 1, 40 µL cells (2.5 x 10^4 cells / mL) that stably over-express hCMKLR1 were seeded into a 384-well cell culture plate then incubated overnight at 37 °C / 5% CO_2. On day 2, cells were then stimulated with indicated compound in 0.5% DMSO at ten different concentrations for 4 h at 37 °C / 5% CO_2 prior to loading with the LiveBLAzer™ FRET-B/G substrate. Tango™ beta-lactamase activity was determined by fluorescence measurement from each well by an Enspire microplate detector (λex = 409 nm, λem = 460/530 nm). The data was analyzed by XLfit and EC_{50} values were calculated using Y=Bottom + (Top-Bottom)/(1+(EC_{50}/X)^Hill Slope).

ADME studies:

All ADME studies were carried out at ChemPartner Shanghai, DMPK group.

Mouse C57BL/6 blood or plasma were used for stability test.

CYP inhibition: Human liver microsomes were purchased from Corning. Phenacetin, diclofenac, S-mephenytoin, bufuralol, midazolam and testosterone are used as the probe substrate for CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 respectively.

hERG safety assay: A CHO cell line stably transfected with hERG cDNA and expressing hERG channels were used for the study. HEKA EPC 10 USB patch clamp amplifier (from HEKA Elektronik, Germany) was used in the whole cell recording.

GPCR panel screening:

GPCR panel screening was performed by DiscoverX. Full GPCR profiling was performed at a single peptide concentration of 10 µM. 168 GPCRs were screened against two selected peptides.

Results and Discussion

Structure and relationship study (SAR)

Shimamura et al reported the identification of metabolically stable-mouse chemerin analog (analog 17, Fig. 1) based on the modification of chemerin-9[4]. To use this analog as reference peptide, we did SAR study and screened more than 230 peptides using Tango β-arrestin assay. We first did single position replacement in the sequence using various strategies, such as unnatural amino acids replacement, N-methylation scan, peptoid incorporation, peptide cyclization, N/C-terminus modification, backbone modification, and lipidation, etc.

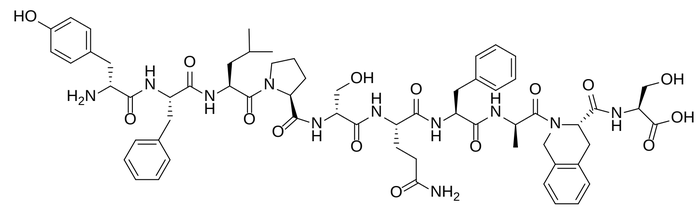

Figure 1. Peptide structure of Chemerin-9 analog, analog 17

yF[148]L[149]P[150]s[151]Q[152]F[153]a[154]Tic[155]S[156]

Single modification which lead to more potent ligand was combined to multi-replacements of the sequence. More than 10 peptides with EC_{50} at ~1 nM range were discovered (Fig. 2). YW-111 and YW-125 were chosen for further pharmacological characterization.

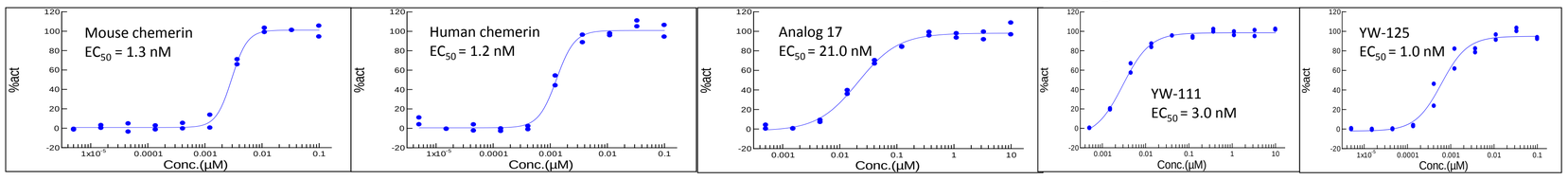

Figure 2. EC_{50} curve of human chemerin, mouse chemerin, analog 17 , YW-111 and YW-125

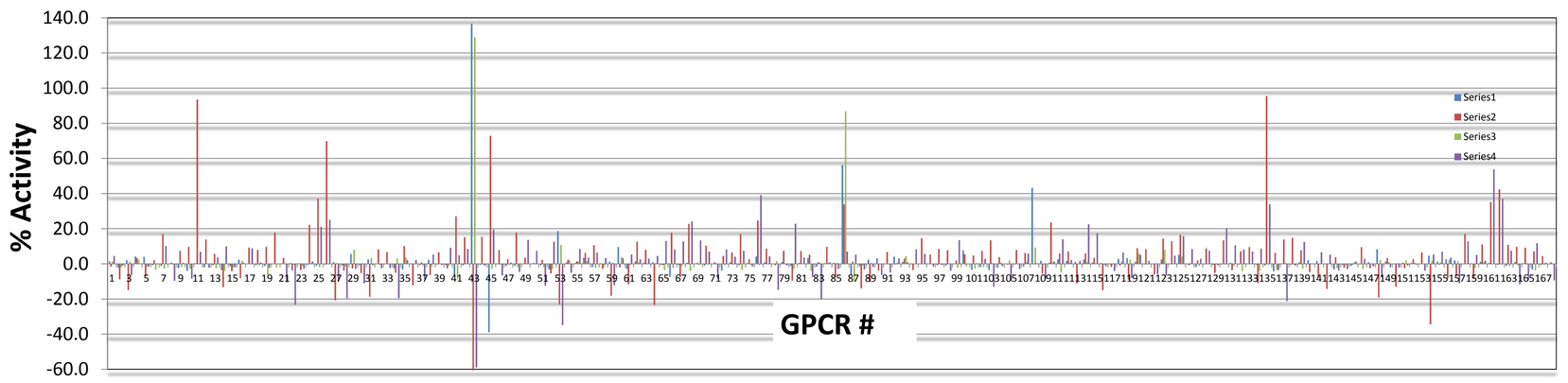

Figure 3. GPCR panel primary screening results. Series 1: YW-111 was screened as agonist; Series 2: YW-111 was screened as antagonist; Series 3: YW-125 was screened as agonist; Series 4: YW-125 was screened as antagonist.

Pharmacological characterization

YW-111 contains modification at two positions which improved potency dramatically, and YW-125 is a potent peptide that combines multi-replacement of the sequence. They are ~7 to 21 fold more potent than the reference compound (Fig. 1 and 2). These peptides were selected for multiple ADME studies and GPCR panel screening.

In general both of them have good solubility in DMSO (data not shown) and PBS. YW-111 has better solubility than YW-125 in PBS at pH 7. Two of them have excellent stability in mouse plasma, human plasma, and in mouse whole blood. They are also shown to have good human liver microsome and hepatocyte stability. The peptides had low renal clearance (data not shown).

The two peptides were screened as agonist and antagonist against 168 GPCRs at 10 µM concentration (Fig. 3). To use 40% as activity cutoff, both of them only hit a few other GPCRs at such high concentration. They all indicated good binding to CMKLR1 and binding to GPR1 as expected. We are following up on testing IC_{50} of the peptides to against other GPCRs.

Table 1 ADME studies of YW-111 and YW-125

	Solubility (PBS, pH=7.4) (mM)	Stability in mouse whole blood, $T_{1/2}$ (h)	Human liver microsome stability		Hepatocyte Stability $T_{1/2}$ (h)		CYP (1A2/2C9/2C19 /2D6/3A4) inhibition IC_{50} (µM)	hERG IC_{50} (µM)	Log D	PAMPA (Log Pe)	Human plasma protein binding (%)
			$T_{1/2}$ (h)	Clint (mL/min/kg)	Mouse	Human					
YW-111	3.76	>96	8.07	3.59	>96	11.9	> 10	>30	0.76	-10.27	90.6
YW-125	0.90	>96	3.64	7.96	5.4	10.26	> 10	>30	0.52	-9.9	94.4

Conclusion

After an SAR study and modification on multiple positions based on the original sequence, we were able to identify multiple sequences which are at least 7 fold potent than analog 17. To use YW-111 and YW-125 as examples, we report here our discovery of potent and stable lead peptides which have excellent selectivity towards CMKLR1 during GPCR panel screening. They are currently under further investigation in potential therapeutic indications. These analogs have emerged as useful tools for further interrogation of chemerin's biological role and as potential lead structures for continued development.

References

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ShangPharma and ChemPartner West Coast Main Contacts

SHANGPHARMA INNOVATION

Robert A. Drakas, PhD
Executive Director
ShangPharma Innovation
m: 215-720-6650 (US EDT)
w: 215-343-3134
rdrakas@spiivc.com
www.spiivc.com

Sarah E Lively, PhD
Executive Director, Site Head
ChemPartner San Francisco
US mobile: 650-830-1761
sarah@chempartner.com
South San Francisco, CA94080
www.chempartner.com

Yvonne M. Angell, PhD
Director of Peptide Chemistry
ChemPartner San Francisco
US mobile: 408-768-2218
yangell@chempartner.com
South San Francisco, CA94080
www.chempartner.com

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