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Semi-Rigid Tripeptide Agonists of Melanocortin Receptors

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Abstract

A series of 30 RCO-**HfR**-NH_2 derivatives show preference for the mouse MC1R *vs*. MC3-5Rs. *trans*-4-HOC_6H_4CH=CHCO-**HfR**-NH_2 (**13**) [EC_{50} (nM): MC1R 83, MC3R 20500, MC4R 18130 and MC5R 935; ratio 1:246:217:11] is 11 times more potent than the lead compound LK-394 Ph$(CH_2)_3$CO-HfR-NH_2 (**2**) and only 11 times less potent than the native tridecapeptide α-MSH at mMC1R. Differences in conformations of **2** and **13** are discussed.

Keywords

Melanocortin receptors; Melanocortin agonists; N-capping. Peptidomimetics; Conformational analysis; Molecular modeling

Melanocortin receptors (MCRs) are found in different tissues, have a plethora of physiological functions[1] and are a target of intensive pharmacological research.[2] Five MCRs (MC1R – MC5R) have been cloned. From the very onset of research in this area, the role of melanocortins in skin pigmentation has been firmly established and shown to be the result of activation of the MC1R. α-, β- and γ-Melanocyte stimulating hormones (MSH), the natural agonists of MC1, MC3, MC4 and MC5Rs, sharing the common melanocortin core sequence His^6-Phe^7-Arg^8-Trp^9 (HFRW), and their synthetic analogs (NDP-MSH, MT-II) with a similar His^6-D-Phe^7-Arg^8-Trp^9 (HfRW) core are very potent but non-selective. Although many peptide and non-peptide ligands of MCRs have been reported, pharmacological differentiation of properties of MC1, MC3, MC4 and MC5Rs is still a major challenge.[1,2]

α-MSH	Ac-Ser-Tyr-Ser-Met-Glu-**His^6-Phe^7-Arg^8-Trp^9**-Gly-Lys-Pro-Val-NH_2
NDP-MSH	Ac-Ser-Tyr-Ser-Nle-Glu-**His-D-Phe-Arg-Trp**-Gly-Lys-Pro-Val-NH_2
LK-184 (**1**)	Ph$(CH_2)_3$CO-**His-D-Phe-Arg-Trp**-NH_2
LK-394 (**2**)	Ph$(CH_2)_3$CO-**His-D-Phe-Arg**-NH_2

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Compounds **3–32** RCO-**His-D-Phe-Arg**-NH_2

Recently, we have described a superpotent hMC1R agonist LK-184 $Ph(CH_2)_3CO$-**HfR**W-NH_2 (**1**) with an EC_{50} of 0.01 nM and *ca*. 500-fold selectivity for hMC1R compared to hMC3 and hMC4Rs.[3a] Truncation of **1** to $Ph(CH_2)_3CO$-**HfR**-NH_2 gave a full hMC1 agonist LK-394 (**2**) with an EC_{50} of 5 nM and weak partial agonism at hMC3/4Rs.[3b] We have also shown that tetrapeptide **1** was more potent than α-MSH in repairing DNA damage resulting from exposure to solar UV radiation, in addition to stimulating pigmentation that confers further photoprotection and prevents mutagenesis.[4a] Such double-headed action can be used as an effective prevention strategy for the most fatal skin cancer - melanoma.[4b] The goal of this work was to explore modifications of the N-terminal end-capping group on the **HfR**-NH_2 core sequence in order to increase potency and/or selectivity at MC1R compared to the lead compound **2** and provide insight into the interaction of short peptides with MCRs.

Our previous studies on the tetrapeptides RCO-**HfR**W-NH_2 suggested that an aromatic π-binding site in the hMC1R conferred selectivity of the N-capped peptides for this receptor over other hMCRs.[3] Thus, all tripeptides in this study contained an aromatic ring connected to the C=O group *via* C_2 (**3–10, 12–16** and **18–25**), C_3 (**26–28**) or C_4 (**29–31**) spacer, a cyclic C_2 mimic (**11** and **17**) or directly (**32**) (Table 1). For mapping the putative π-binding site, cinnamic derivatives **3–9**, **12**, **13**, **15**, **16**, **18**, **20** and **23–25** provided a shorter and more rigid C_2 link compared to the freely rotating saturated C_3 chain in **2**, while the substituents in their phenyl rings provided an extended conjugated π system overlapping the conformational space with the π system of the phenyl (shown below) in lead compound **2**. In addition, pyridine and imidazole derivatives **21** and **22** with saturated C_2 chain served as flexible probes for potential basic or H-bonding sites at hMC1R near the N-terminal binding region. The N-capping groups with π-conjugated diene system (**29** and **30**) and the corresponding saturated C_4 analog **31** were chosen to explore the space beyond $Ph(CH_2)_3$ in **2**.

As seen in Table 1, the selectivity profile for reference compounds **1** and **2** differs from that previously reported for hMCRs.[3] Tetrapeptide **1** has an EC_{50} in the nanomolar range at mMC1R, MC4R and MC5R (MC1/3/4/5Rs ratio 1:16:0.3:0.2), while its tripeptide analog **2** retains mMC1R selectivity (MC1/3/4/5Rs ratio 1:46:16:12) albeit with 590-fold loss in potency compared to **1**. As we expected on the basis of our data for **1** and **2**[3b] and literature data on the melanocortin core sequence[6], removal of Trp^9 from HfRW-NH_2 resulted in very low potency at mMC3R-MC5R. This desirable effect is most pronounced at mMC3R, where 2/3 of the synthesized tripeptides show no activity. Apparently, the binding site of mMC3R is larger than that of mMC1R, MC4R and MC5Rs and the Ph-C-C-group is too short to interact with the receptor. The elongation of the end-capping group (see the alignment above) due to aromatic substituents (**4**, **7**, **8**, **11–13** and **26**) or spacer extensions (**29–31** *vs*. **18–19**) elicits a weak mMC3R response. This SAR is not straightforward and polar groups (**5**, **6**, **9**, **10**, **14–19, 26** and **28**) cancel the response.

In accordance with our initial reasoning that restrictions in spacer flexibility on the tripeptide core lacking tertiary structure might produce a conformation active at MC1R, we explored an

unsaturated series consisting of **3–9**, **12**, **13**, **15–18**, **20** and **23–26**. The direct unsaturated analog **26** of the parent tripeptide **2** was equipotent to **2** at mMC1R but showed some loss of selectivity (MC1/3/4/5Rs ratio 1:24:5:3). At the same time, the simple cinnamic derivative **3** was more promising - its potency at mMC1R was close to that of **2** and it showed much better selectivity (MC1/4/5Rs 1:44:25 and no activity at mMC3R). Substitution on the cinnamoyl aromatic ring revealed considerable tolerance of the mMC1R for structural changes. *meta*-Substitution (**7–9**) was favorable with a 2- to 3-fold increase in potency, while the *para*-OH derivative **13** was the most potent (11x) and selective (MC1/3/4/5Rs 1:246:217:11). The *p*-OH group was essential for activity since methylation (**15**) or substitution by the isosteric and isoelectronic Cl (**12**) resulted in a substantial drop in potency (Table 1).

In an attempt to pinpoint the location of the OH binding site in mMC1R, we synthesized free rotating analogs (**14** and **10**) of semi-rigid **13** and **9,** as well as their rigid cyclic analogs (**17** and **11**). All of them were less potent than **13**, *i.e*. the cinnamic scaffold provided an optimal positioning of the polar H-bonding group in the aromatic binding region of mMC1R.

To determine factors contributing to high potency of **13**, we did a conformational search on **2, 13**, **11** and **17** using the Monte Carlo Multiple Minimum (MCMM) mixed torsional/low-frequency sampling and the OPLS_2005 force field in water as implemented in Macromodel[7]. The results show that although the number of conformers for **2** (108 within 3 kcal/mol, 293 within 5 kcal/mol) and **13** (55 within 3 kcal/mol, 221 within 5 kcal/mol) does not vary drastically, their 3D distribution is totally different (Figure 1).

While **2** has no preferred conformation (Figure 1a), all 221 conformers of **13** are very well aligned (Figure 1b). Even though the *p*-OH does not contribute to any intramolecular bonds, the semi-rigid cinnamic tail influences the rest of otherwise flexible tripeptide core placing the polar basic Arg and His on the same side of the molecule opposite to the non-polar aromatic D-Phe and cinnamoyl. Together with the existence of **13** in multiple but similar conformations that can morph into the biologically active conformation upon binding, such “pre-alignment” is beneficial for initial recognition of the ligand by the mMC1R that contains known compact acidic (Glu-94, Asp-117 and 121) and aromatic hydrophobic (Phe-175, 179, 196, and 257) binding pockets.[8] The results of the conformational search for the cyclic analog **17** (not shown) are similar to **13,** which demonstrates that its lower potency relative to **13** is related to misplacement of HO in the active site due to an increase in girth or rigidity of the end-capping group. It is interesting to note that the drop in potency of **17** is observed on all mMCRs subtypes with the total loss of activity at mMC3 and mMC4Rs.

Thus, the use of semi-rigid N-capping groups with the C_2 spacer allows for a “soft” control of the peptide conformation without restricting dihedral angles of the backbone or side chains favorable for interaction with an active site. Application of this principle to a very flexible tripeptide **2** resulted in the compound **13** that was more selective than the tridecapeptide α-MSH and only 11 times less potent at mMC1R. Many of the N-capped HfR-NH_2 derivatives show low potency or are inactive at mMC3 and/or MC4Rs (Table 1) and this could be relevant for future design of low molecular weight synthetic drugs acting only on targeted MCRs subtypes.[10]

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References and Notes

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11. Compounds 1–32 (purity >95%) were obtained, analyzed and assayed as previously described.3,5 The EC_{50} values for compounds 1–32 (all of them are full agonists) in Table 1 are an average of at least three separate experiments with the associated standard error of the mean.

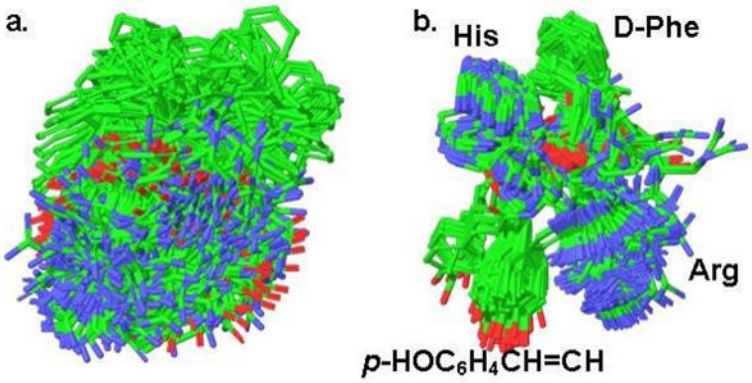
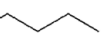
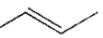
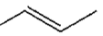


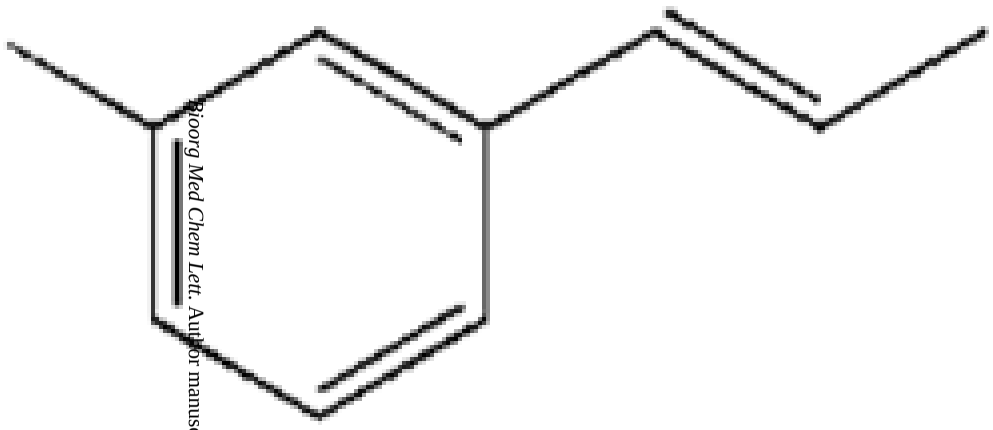
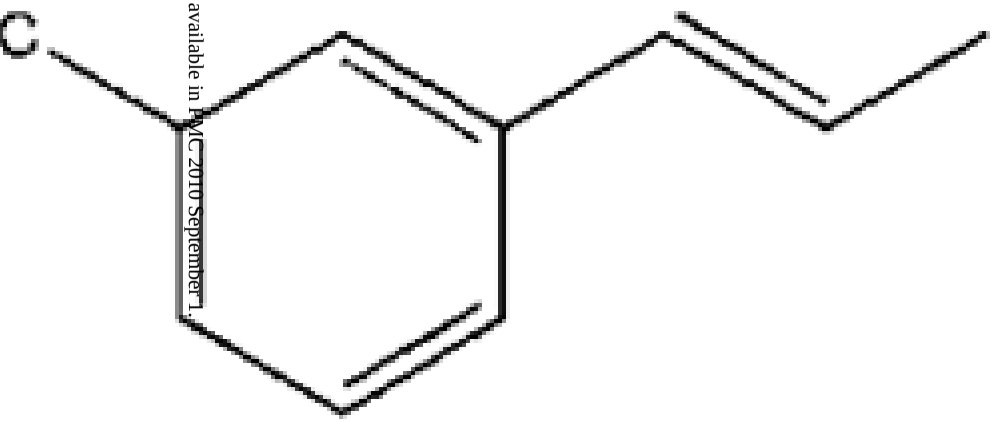
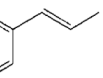
Figure 1.
Conformations within 5 kcal/mol: a. $Ph(CH_2)_3CO$-**His-D-Phe-Arg**-NH_2 (**2**), b. *trans*-4-$HOC_6H_4CH=CHCO$-**His-D-Phe-Arg**-NH_2 (**13**).

Table 1

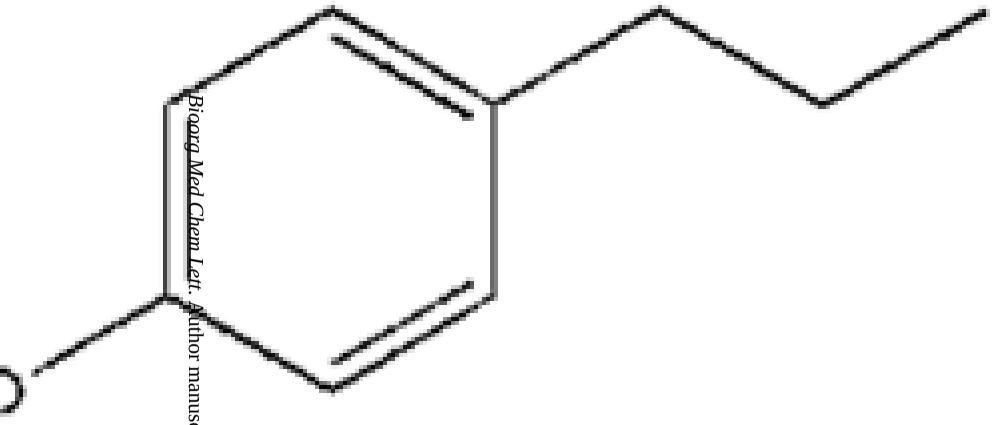
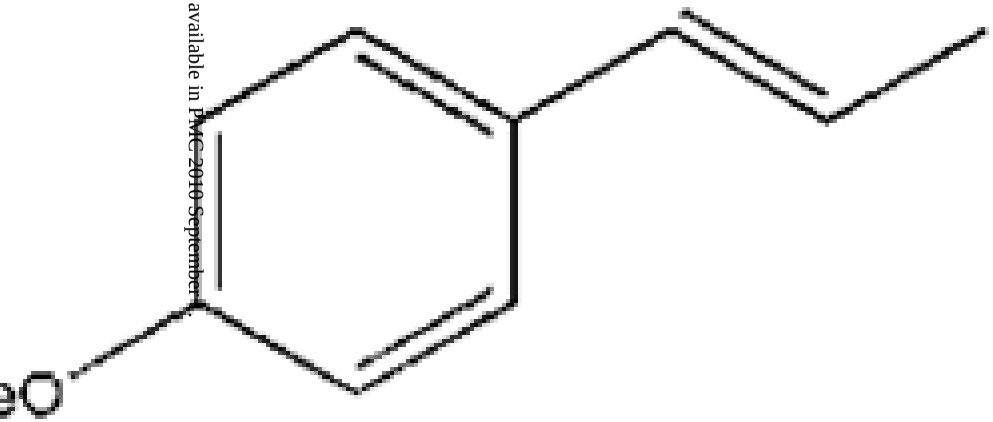
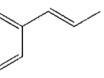
gonist activity of N-capped tripeptides on mouse MC1, MC3, MC4, and MC5 receptors.

g-NH_2	EC_{50}(nM) ± EM			
	mMC1R	**mMC3R**	**mMC4R**	**mMC5R**
O-His-D-Phe-Arg-Trp-NH_2 (**LK-184**)	1.60±0.29	26.0±4.0	0.54±0.19	0.33±0.14
	940±260	43700±1370	15140±8800	11800±5950
	1200±390	*a*	52950±6580	30220±8200
	1160±570	28050±22600	14700±5800	6860±1200

	EC_{50}(nM) ± EM				
	mMC1R	**mMC3R**	**mMC4R**	**mMC5R**
	1690±465	*a*	13700±4700	12850±1710
	3660±1170	*a*	60400±19500	18400±7600

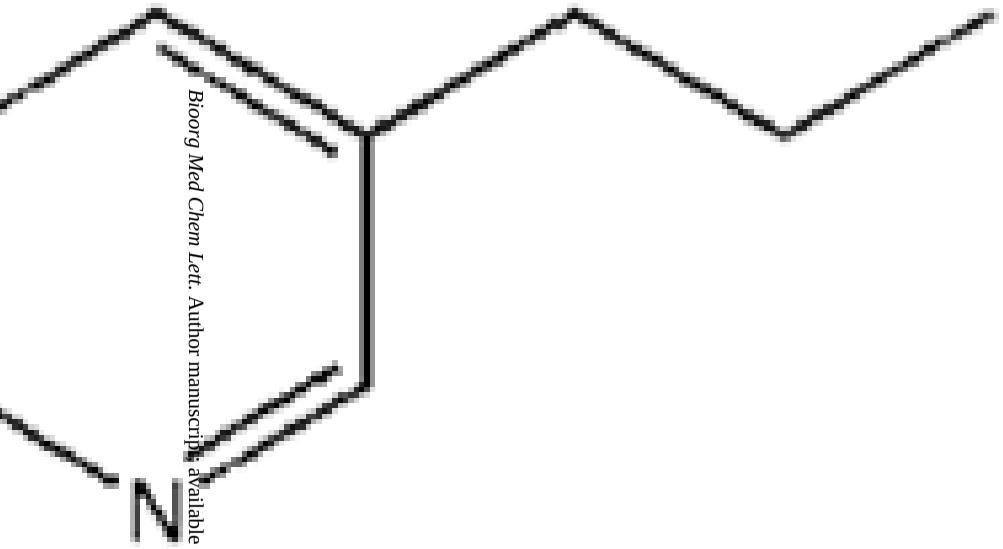
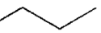
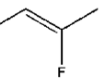
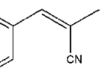
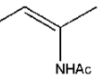
g-NH_2	EC_{50}(nM) ± EM			
	mMC1R	**mMC3R**	**mMC4R**	**mMC5R**
	660±190	33800±13300	4010±240	6390±1100
	310±70	30300±17000	3700±500	6780±490
	274±68	*a*	12740±4120	2770±330

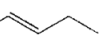
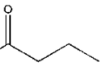
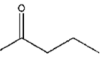
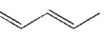
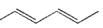
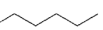
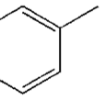
	EC_{50}(nM) ± EM			
	mMC1R	**mMC3R**	**mMC4R**	**mMC5R**
	1120±216	*a*	*a*	9600±3940
	1175±330	7380±1240	17200±2700	2280±615
	1080±300	58900±56000	5200±1700	6900±1400
	83.3±13.7	20500±12600	18130±3258	935±310

g-NH_2	EC_{50}(nM) ± EM			
	mMC1R	**mMC3R**	**mMC4R**	**mMC5R**
	1160±88	*a*	*a*	10540±3200
	420±61	*a*	11500±4920	3410±360
	780±230	*a*	*a*	6640±1830

	EC_{50}(nM) ± EM				
	mMC1R	**mMC3R**	**mMC4R**	**mMC5R**
	620±26	*a*	*a*	4760±1300
g-NH_2	567±253	*a*	23600±1780	6187±1400

	EC_{50}(nM) ± EM				
	mMC1R	**mMC3R**	**mMC4R**	**mMC5R**
	7280±4400	*a*	25500±3400	23000±4700
	1250±130	*a*	23500±9900	17600±3090

g-NH_2	EC_{50}(nM) ± EM			
	mMC1R	**mMC3R**	**mMC4R**	**mMC5R**
	1670±437	*a*	*a*	18050±4510
	3200±670	*a*	38700±6370	70300±11350
	2790±650	*a*	19200±4100	19300±1340
	2060±330	*a*	42800±21600	8500±1720
	8200±2150	*a*	20300±6400	*a*

g-NH_2	EC_{50}(nM) ± EM			
	mMC1R	**mMC3R**	**mMC4R**	**mMC5R**
	990±180	24100±4100	5020±1980	3080±210
	1745±685	*a*	22750±5870	16580±2307
	1230±320	*a*	12400±2240	10500±620
	500±195	16800±4530	4710±1370	6830±1060
	780±430	19500±2900	10050±1270	6230±790
	530±210	25900±15700	*a*	*a*
	3030±1040	*a*	31100±21350	23600±1750
	7.2±3.73	10.2±2.53	19.3±3.59	6.72±2.01
	0.014±0.002	0.09±0.02	0.14±0.03	0.17±0.08

hat no agonist response was observed at up to 100mM concentrations.

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