Discovery of Tyrosine-Based Potent and Selective Melanocortin-1 Receptor Small-Molecule Agonists with Anti-inflammatory Properties

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Abstract: The melanocortin-1 receptor (MC-1R) is a G-protein-coupled receptor involved in inflammation and skin pigmentation. Compound **2** is the first highly potent and selective MC-1R small-molecule agonist reported. Compound **2** showed efficacy in an acute model of inflammation, which has demonstrated the role of MC-1R in modulation of inflammation.

Introduction. There are five receptor subtypes known in the melanocortin family. Activation of these G-protein-coupled receptors (GPCR) stimulates the production of cAMP in various tissues.[1] The melanocortin-1 receptor (MC-1R) is expressed in melanocytes, monocytes, mast cells, and neutrophils and is involved in blocking inflammation and augmenting pigmentation.[2] The melanocortin 2 receptor (MC-2R) is expressed in adipocytes and adrenal cells and is involved in steroidogenesis.[3] MC-3R is present in the brain, hypothalamus, heart, gut, and placenta and has been associated with energy homeostasis and inflammation.[4] MC-4R is expressed in the brain and has been associated with feeding behavior, energy homeostasis, and erectile function.[5] MC-5R is found in a wide range of tissues and is involved in exocrine gland function.[6] With the plethora of melanocortin receptors, there is a significant need for receptor-selective agents to further define and delineate the roles of the individual receptors.

The endogenous agonists for these receptors are all derived from the pro-opiomelanocortin (POMC) gene transcript by post-translational cleavage.[7] Differential processing of the gene products generates α-melanocyte stimulating hormone (α-MSH), β-MSH, γ-MSH, δ-MSH, and adrenocorticotropic hormone (ACTH). All of these melanocortin peptide agonists contain the His-Phe-Arg-Trp sequence that is believed to be essential for stimulation of four out of five melanocortin receptors.[8] There also exists two endogenous antagonists for the melanocortin receptors, agouti and the agouti-related protein (AGRP).[9] Several synthetic peptides or peptide analogues have been used to characterize the function of these receptors. NDP-αMSH (also called melanotan-I or MT-I) is a high-affinity, nonselective agonist of MC-1R, MC-3R, MC-4R, and MC-5R.[10] Melanotan-II (MT-II),[11] a cyclic peptide currently in phase II clinical trial for the treatment of erectile dysfunction, is an agonist with a similar nonselective profile.[12] SHU-9119, another cyclic peptide, has full agonist activity at MC-1R and MC-5R and a weak partial agonist/antagonist activity at the MC-3R and MC-4R receptors.[13] HP-228 is a peptide analogue with affinity for all four receptors but a preference for MC-1R.[14] HP-228 was in clinical trials for the treatment of pain and inflammation associated with surgery.[15] There is a clear opportunity for the discovery of receptor-selective melanocortin receptor agents.

Several small-molecule ligands for the melanocortin receptors have been disclosed.[15] Most of the activity in small-molecule drug discovery has been directed at MC-4R agonists as a treatment for obesity or sexual dysfunction. Scientists at Merck have disclosed a series of potent and selective MC-4R agonists exemplified by structure **1**.[16] Trega and Melacure also have patented small-molecule ligands for melanocortins.[17] Recently, selective small-molecule MC-1R agonists with micromolar affinity have been identified from β-turn libraries.[18]

Although MC-4R has received most of the medicinal chemistry attention to date, we were interested in MC-1R as a target for the development of anti-inflammatory agents. MC-1R is expressed on the surfaces of monocytes, and activation by α-MSH has been shown to increase expression of the anti-inflammatory cytokine IL-10 and stabilization of I-κB. These effects, in turn, negatively modulate the activation of NF-κB, resulting in the suppression of cytokine production and i-NOS activity.[19,20] In addition, α-MSH has been demonstrated to be a potent anti-inflammatory agent in vivo in both acute and chronic inflammatory disease models (e.g., inflammatory bowel disease, rheumatoid arthritis, and renal ischemia/reperfusion injury).[21] Administration of α-MSH in these disease models resulted in substantial tissue protection from inflammation-mediated damage, a significant decrease in leukocyte infiltration, and a dramatic reduction (near baseline level) of elevated levels of cytokines (e.g., TNF-α), chemokines (e.g., MCP-1, IL-8), and inflammatory mediators (e.g., i-NOS and ICAM-1). Although there are strong indications that these effects were mediated by MC-1R, the lack of potent and selective MC-1R agonists has prevented an unambiguous identification of MC-1R as the melanocortin receptor mediating this anti-inflammatory response. Recently, highly selective and potent peptide MC-1R agonists have been reported,[22] but to the best of our knowledge, their in vivo evaluation has not been published.

Starting from the known[16] MC-4R agonist **1** (Figure 1) and through extensive use of parallel synthesis techniques, we have identified compound **2** as a low-nanomolar, selective MC-1R full agonist. Compound **2** was evaluated in a mouse model of lipopolysaccharide-induced (LPS-induced) TNF-α production, where it dose-dependently inhibited cytokine production.

Chemistry. The chemistry to prepare compound **2** and its analogues is depicted in Scheme 1. Typical EDC–HOAt coupling was used to assemble the three fragments: the piperidine moiety (**4**), *O*-methyltyrosine (**3**), and the histidine derivative (**6**). For the production of libraries, SCX cartridge purification was used to

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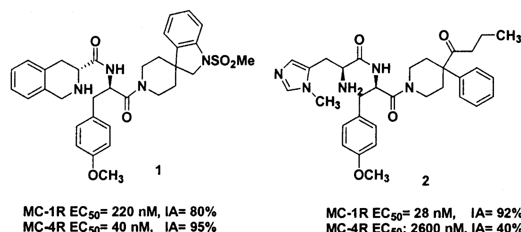
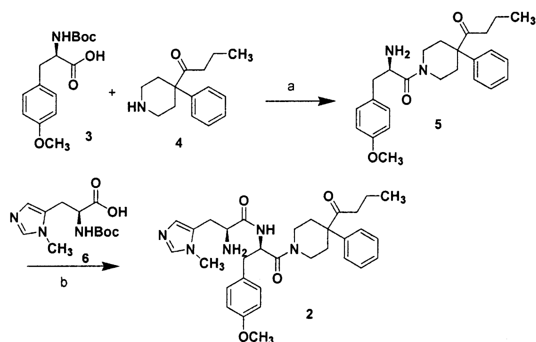


Figure 1. Known MC-4R agonist and novel selective MC-1R agonist (IA = intrinsic activity).

Scheme 1. Synthesis of Compound **2**



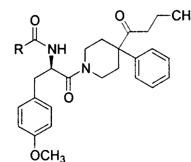
[a] (a) (i) EDC, HOAt, DCM–DMF (1:1), overnight, SCX extraction, (ii) TFA/DCM, 1 h, SCX extraction; (b) (i) EDC, HOAt, DCM–DMF (1:1), overnight, SCX extraction, (ii) TFA/DCM, 1 h.

remove most of the byproducts, followed by reverse-phase HPLC purification.

Biology. Functional activity at human MC-1R was measured by a SPA-based cAMP assay in HBL melanoma cells. HBL cells express only MC-1R among the melanocortin receptors.[23] Functional activity at MC-3R, MC-4R, and MC-5R was tested in the SPA-based cAMP assay using CHO cells overexpressing the individual melanocortin receptors. Functional activity at MC-2R was measured in a mouse Y1-adenocarcinoma cell line, which expresses MC-2R endogenously. Results were normalized to the level of c-AMP obtained after stimulation with either 100 nM NDP-αMSH (for MC-1,2,3, and 4R) or 100 nM ACTH (for MC-2R). Binding affinities were measured with a SPA-based radioligand binding assay in intact cells using [^{125}I]-NDP-α-MSH.

In Vitro SAR. An initial library designed around the known MC-4R agonist **1** was executed. This effort identified the combination of the piperidine moiety **4** linked with tyrosine and histidine (compound **10**, Table 1) as a potent and full agonist at MC-1R. A subsequent small library of close analogues was then used to probe the structure–activity relationship of the basic moiety in this series. Table 1 summarizes the functional and binding activities of selected compounds from this effort. The α-amino group of the histidine in **10** could be removed (compound **8**) or replaced by a hydroxy group (compound **9**) without a loss in potency or intrinsic activity. However, when the amino group was present, the *S* configuration (**10**) was much more potent than the corresponding *R* isomer (compound **11**). N-methylation of the imidazole ring of histidine provided two isomers with very different activity. Isomer **2** retained most of the activity of the parent compound, whereas isomer **12** was only a partial agonist and 50-fold less active than **10**. Finally, acylation of the α-amino group of the histidine of compound **2** resulted in an improvement in functional activity by about 2 orders of magnitude (compounds **6** and **7**).

Table 1. In Vitro Activity of *O*-Methyltyrosine MC-1R Agonists ($n = 4$ Unless Otherwise Stated)



Cpd	R	MC-1R EC50 (nM)[a]	MC-1R IA (%)[b]	MC-1R IC50 (nM)[c]
2	NH2, N-CH3	28 ± 12	92 ± 3	120 ± 15[d]
6	NHCOPh	0.19 ± 0.02[d]	98 ± 2[d]	3.1 ± 0.2
7	NHCOCH3	0.35 ± 0.07[d]	96 ± 4[d]	5.1 ± 2
8		2.5 ± 1.3	97 ± 1	18 ± 3
9	OH	3.8 ± 1.3	90 ± 1	5.5 ± 0.6[d]
10	NH2	1.5 ± 0.7	93 ± 4	32 ± 4[d]
11	NH2	400 ± 60	67 ± 10	1300 ± 400[d]
12	H3C–N, NH2	510 ± 95[d]	34 ± 6[d]	1500 ± 50[d]

[a] EC50 ± SEM (nM). [b] IA = intrinsic activity ± SEM (%). [c] IC50 ± SEM (nM). [d] $n = 2$.

Table 2 summarizes the selectivity data for some of these compounds. All compounds had no agonist activity at MC-2R when tested at concentrations up to 40 μM. Compounds **6**–**10** were found to activate the MC-3R and MC-4R receptors, albeit with EC_{50} values that were 2 orders of magnitude less potent than for MC-1R. They also had mixed activities at MC-5R with **6** and **7** being full agonists and **8**–**10** being only partial agonists. Compound **2** was found to be the most selective among the MC-1R agonists. It did not activate MC-3R and was a very weak partial agonist at MC-4R and MC-5R with potencies of 3 and 4 μM. Compound **2** was selected for in vivo evaluation based on its selectivity profile and on results from a preliminary pharmacokinetic screen in mice (data not shown).

PK Profile of Compound 2. Before the compound was subjected to in vivo efficacy studies, the pharma-

Table 2. In Vitro Selectivity for All Four Melanocortin Receptors ($n = 2$ Unless Otherwise Stated)[a]

compd	MC-1R		MC-3R		MC-4R		MC-5R	
	EC_{50} (nM)[b]	IA (%)[c]	EC_{50} (nM)[b]	IA (%)[c]	EC_{50} (nM)[b]	IA (%)[c]	EC_{50} (nM)[b]	IA (%)[c]
2	28 ± 12[d]	92 ± 3[d]	NA	2.1 ± 0.2[e]	2600 ± 200	39 ± 3	4400 ± 1300	15 ± 3
6	0.19 ± 0.02	98 ± 2	250 ± 170	100 ± 6	2.9 ± 0.04	95 ± 0.01	2100 ± 400	65 ± 12
7	0.35 ± 0.07	96 ± 4	2200 ± 1000	90 ± 0.01	23 ± 12	96 ± 0.8	3900 ± 900	90 ± 15
8	2.5 ± 1.3[d]	97 ± 1[d]	7000 ± 1700	85 ± 14	840 ± 150	92 ± 3	9400 ± 200	32 ± 0.2
9	3.8 ± 1.3[d]	90 ±1.3[d]	3900 ± 3000	85 ± 14	77 ± 25	95 ± 0.4	2700 ± 100	20 ± 2
10	1.5 ± 0.7[d]	93 ± 4[d]	1700 ± 1000	98 ± 0.01	630 ± 340	103 ± 3	10000 ± 500	40 ± 4

[a] All of these compounds had no activity at MC-2R when tested at concentrations up to 40 μM. [b] EC_{50} ± SEM (nM). [c] IA = intrinsic activity ± SEM (%). [d] $n = 4$. [e] Tested up to 40 μM.

Table 3. Pharmacokinetic (PK) Profile of Compound **2** Dosed Intravenously in Balb/c Mice ($n = 3$)[a]

dose, μmol/kg	C_{max}, nM	AUC,[b] nM h	$T_{1/2}$, h	MRT, h	Cl, mL min^{-1} kg^{-1}	V_{ss}, L kg^{-1}
6.8	2200	1000	1.5	1.2	110	8.2

[a] Coefficient of variation of the concentration determinations is less than 26%. [b] Area under the curve, 0−6 h.

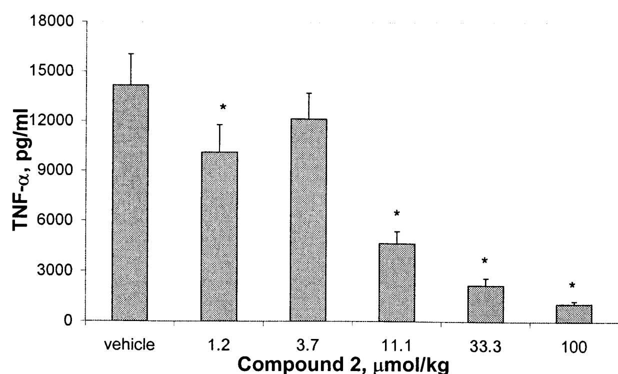


Figure 2. Compound **2** was administered subcutaneously to Balb/C mice ($n = 5$). One hour later, the mice were injected with LPS iv, and TNF-α levels were measured after 1 h by ELISA. $p < 0.0001$.

cokinetic characteristics of compound **2** were evaluated in Balb/c mice. The compound was dosed intravenously in 50:50 PEG400−water. Results are summarized in Table 3. Compound **2** had a half-life of 1.5 h, suggesting that the compound was suitable for further in vivo studies.

In Vivo Evaluation of Compound 2. The ability of an MC-1R selective agonist to elicit an anti-inflammatory effect in vivo was evaluated using a murine LPS (lipopolysaccharide) induced cytokine accumulation model.[24] Balb/c mice were injected subcutaneously with compound **2** at various doses. LPS was injected intravenously 1 h later. After another hour, mice were bled and TNF-α levels were measured by an enzyme linked immunosorbent assay (ELISA). Figure 2 shows the dose-dependent decrease of TNF-α production in response to compound **2**. Compound **2** at 11 μmol/kg reduced TNF-α levels by 65% and at 33 μmol/kg by 82% (there was no observable toxicity at these doses). By comparison, 1 μmol/kg of NDP-αMSH co-injected intravenously with LPS in the same model elicited a 75% reduction in TNF-α production (data not shown).

Conclusion. In summary, the first small-molecule potent MC-1R selective agonists have been discovered. Compound **2** showed efficacy in an acute model of inflammation, which has clearly demonstrated a role of MC-1R in modulation of inflammation. Further structure−activity relationships are being investigated for this class of compounds and will be reported elsewhere. Evaluation of compound **2** in other animal models of acute and chronic inflammation is currently underway.

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Supporting Information Available: Experimental procedure for the preparation of **10**, high-temperature ^{1}H NMR for compound **8**, and ^{1}H NMR, HPLC, and HRMS data for compounds **2**, **6**−**10**, and assay protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

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