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β-Alanine Dipeptides as MC4R Agonists

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Abstract—β-Alanine derivative **2** ($IC_{50}=16$ nM) and related compounds were found to be potent MC4R agonists.

The melanocortin (MC) receptors belong to the G-protein coupled receptor superfamily and are involved in a number of physiological functions including feeding behavior, sexual behavior, immune regulation and skin pigmentation.[1] There are five MC receptors (MC1R–MC5R) which, except for MC2R, all recognize the melanocyte-stimulating hormones (α-, β-, and γ-MSH) and their activation leads to a downstream elevation of cAMP.[1h,2]

Based on experiments in various rodent feeding models, the MC4R of the hypothalamus and brain stem may play an important role in food intake and energy homeostasis. For example, it has been shown that icv administration (<10 μg/animal) of the agonist peptide α-MSH resulted in long lasting (6–9 h) inhibition of spontaneous feeding in rats.[3] The non-selective MC4R agonist MTII has also been shown to reduce food consumption in rats.[4] Furthermore, MC4R knockout mice were shown to develop morbid obesity,[5] and this phenotype was insensitive to administration of the cyclic α-MSH analogue MTII.[6]

Researchers from Merck recently reported a number of MC4R agonists exemplified by structure **1** (Fig. 1).[7] These lipophilic, tetrahydroisoquinoline (TIC)-based dipeptides share a common *para*-substituted D-phenylalanine unit and a 4,4-disubstituted piperidine moiety. Using **1** as a guide, we initiated a program to identify related bis-amide MC4R agonists with improved physicochemical properties (M_r, lipophilicity, solubility). Herein we report our efforts at the identification and optimization of novel N-terminal analogues of **1**, which have enhanced physicochemical properties.

We first examined simple tetrahydroisoquinoline replacements with lower molecular weights, lower lipophilicities and enhanced solubilities compared to **1**.[8] The simple β-alanine derivative **2**[9,13] was found to retain potent binding affinity for MC4R ($IC_{50}=16$ nM)[10] compared to **1** ($IC_{50}=7$ nM, Table 1). The calculated lipophilicity of **2** [cLog D (pH 7.4) = 2.94] was reduced by at least three log units compared to **1** [cLog D (pH 7.4) = 5.92].[8] Furthermore, β-alanine **2** was found to be a full agonist in a functional cAMP assay[11] ($EC_{50}=170$ nM and Intrinsic Activity (IA) = 98%). These results prompted us to expand the SAR around this lead. The structure/activity around substitution of the basic nitrogen atom of the β-alanine is summarized in Table 1. The *N*-methyl and *N*,*N*-dimethyl derivatives (**3a** and **3b**, respectively) were equipotent with the primary amine **2**. Additionally, there appeared to be some steric tolerance at this position since the mono-benzylated analogue **3c** was also equipotent with the primary amine **2**. However, additional large substitution of the nitrogen atom led to a significant diminution of binding affinity as demonstrated by the *N*,*N*-dibenzyl analogue **3d**.

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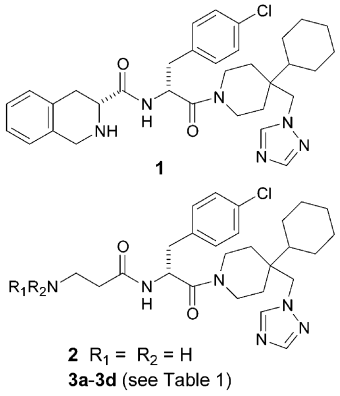


Figure 1.

Table 1. MC4R binding of **1** and β-alanine derivatives **2** and **3**

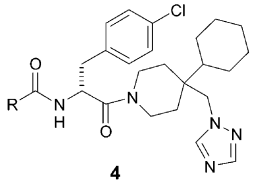
Compd	R_1	R_2	MC4R (IC_{50}, nM)[a]
1	—	—	9±3
2	H	H	16±8
3a	Me	H	10±8
3b	Me	Me	10±8
3c	H	Bn	14±4
3d	Bn	Bn	110[b]

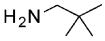
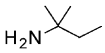
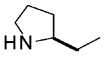
[a]Values are means of three or more experiments unless otherwise noted.
[b]Values are means of two experiments.

Substitution on the sp^3 carbon atoms of the β-alanine pharmacophore was also investigated (Table 2). The *gem*-dimethyl analogues **4a** and **4b** and the cyclic analogues **4c**–**4f** were similarly potent to β-alanine **2**. Also, the configuration of the chiral center in the cyclic diastereomers **4d** and **4e** had no influence on MC4R binding affinity. Thus, this region of the agonists was reasonably promiscuous as a variety of basic amine moieties led to potent MC4R agonists (Table 2).

In order to examine the effect of basicity of **2** on MC4R affinity, we prepared a series of related amide derivatives **5**, and the results are summarized in Table 3. Based on the hypothesis that the minimal core sequence of His-Phe-Arg-Trp was necessary for MC4R recognition,[12] we anticipated that the corresponding amides **5** would show reduced potency at MC4Rs compared with their amine counterparts. Surprisingly, acetamide **5a** was found to be equipotent with the primary amine **2**. The methoxymethyl ether **5b** was also equipotent with **2** and **5a**, but the bulkier benzamide **5c** had reduced MC4R affinity compared to the simple acetamide **5a**. The more lipophilic trifluoroacetamide **5d** also lost significant binding affinity. Interestingly, re-introduction of a basic nitrogen in the β-alanine amide side chain afforded compounds (**5e**–**5h**) with potent MC4R affinities comparable to that of the original β-alanine **2** (Table 3). The fact that smaller and hydrophilic substituents (i.e., **4a** – **4f**, **5a**, **5g** and **5h**) were tolerated at this position, but that larger hydrophobic substituents were not (i.e., **3d**, **5c**, **5d**), coupled with the lack of stereogenic differentiation at this position (i.e., **4d** and **4e**,

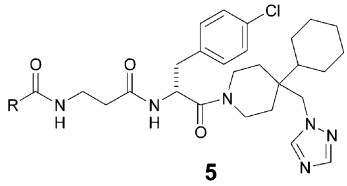
Table 2. MC4R binding of substituted and cyclic β-alanine derivatives **4**

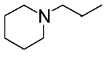


Compd	R	MC4R (IC_{50}, nM)[a]
4a		30[b]
4b		18±10
4c		16[b]
4d		9[b]
4e		12±4
4f		22[b]

[a]Values are means of three or more experiments unless otherwise noted.
[b]Values are means of two experiments.

Table 3. MC4R binding of β-alanine amide derivatives **5**



Compd	R	MC4R (IC_{50}, nM)[a]
5a	Me	12[b]
5b	$MeOCH_2$	21±19
5c	Ph	90[b]
5d	CF_3	180[b]
5e	$Me_2NCH_2CH_2$	11±6
5f		12[b]
5g		25[b]
5h		22±14

[a]Values are means of three or more experiments unless otherwise noted.
[b]Values are means of two experiments.

Table 4. Functional activity of selected compounds

Compd	MC4R (IC_{50}, nM)[a]	MC4R (EC_{50}, nM)[a]	IA (%)[b]
1	7	3.6	99±3
2	16	170	92±5
3a	10	70	98±10
3b	10	40	98±7

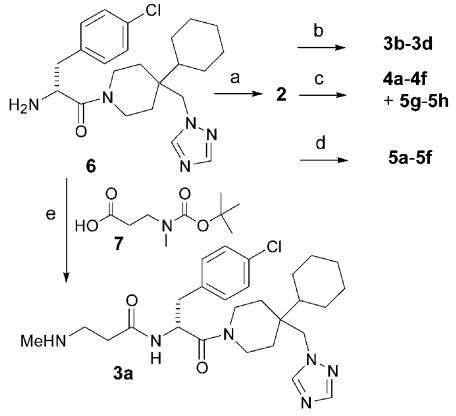
[a]Values are means of three or more experiments.
[b]Values are means of four or more experiments.

5g and **5h**) could suggest that this portion of the molecule was solvent-exposed in the receptor. It would then follow that potency was driven by that portion of the molecule's hydrophilicity more than anything else. Our present SAR studies are being directed to examine this hypothesis.

The functional properties of β-alanines **2**, **3a** and **3b** are provided in Table 4. All compounds were found to be full agonists with EC_{50} values ranging from 40 to 170 nM[11] and with intrinsic activities of >90%. Intrinsic activity was expressed as % cAMP stimulation compared to that of 100 nM NDP-α-MSH, which causes maximal stimulation in this system. For each compound the assays were performed twice or three times in duplicate. NDP-α-MSH and the natural ligand, α-MSH, stimulated cAMP to similar levels, with an EC_{50} of 0.3 and 5.5 nM, respectively. NDP-α-MSH was used as a reference compound in these studies due to its higher affinity and greater stability. Intrinsic activity of >90% was general for all compounds reported in this manuscript (data not shown).

Synthesis

Scheme 1 describes the synthesis of the β-alanines **2**–**5**. Amine **6**[7] was coupled with *N*-Boc-protected β-alanine, and the resulting carbamate was treated with trifluoroacetic acid to give β-alanine **2**.[13] This compound served as the common intermediate for the synthesis of compounds **3**–**5** (Scheme 1). *N*-Methyl β-alanine **3a** was prepared using an EDCI-induced coupling of amine **6** and *N*-Boc-*N*-methyl-β-alanine **7**, followed by treatment with trifluoroacetic acid. *N*,*N*-dimethyl-β-alanine **3b** was prepared via a reductive amination of β-alanine **2** using excess formaldehyde. Reductive amination of **2** with stoichiometric and excess benzaldehye afforded **3c** and **3d**, respectively. Standard amide coupling of **2** with the appropriate activated acids, followed by deprotection (where needed), afforded compounds **4a**–**f** and compounds **5a**–**h**.



Scheme 1. Synthesis of the β-alanine derivatives: (a) (1) *N*-Boc-β-ALA, EDCI, HOBt, CH_2Cl_2; (2) TFA, CH_2Cl_2; (b) RCHO, NaBH$(OAc)_3$, DMF; (c) (1) *N*-Boc-β-aminoacid, EDCl, HOBt, CH_2Cl_2; (2) TFA, CH_2Cl_2; (d) RCOOH, EDCl, HOBt, CH_2Cl_2; (e) (1) **7**, EDCl, HOBt, CH_2Cl_2; (2) TFA, CH_2Cl_2.

Conclusion

Replacement of the tetrahydroisoquinoline moiety in **1** by a simple β-alanine moiety provided compounds with potent affinity for the MC4 receptor and enhanced physicochemical properties. Optimization of this series provided several compounds with low nanomolar binding affinity for MC4R and full agonist activity.[14] Our SAR study described herein indicated that a wide range of substitution are tolerated on the N-terminus of these β-alanine dipeptides. Small hydrophilic substituents seemed to be tolerated the best, possibly suggesting that this portion of the agonist was solvent-exposed. Our future efforts will be directed at further testing and evaluating this hypothesis.

Acknowledgements

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8. **1**: $M_r = 589$, clogD (pH 7.4) = 5.92, polar surface area (PSA) = 85.1 Å^2. **2**: $M_r = 501$, clogD (pH 7.4) = 2.94, PSA = 97.6 Å^2. Windows XP ACD Software version 5.0 was used to perform these calculations.
9. Related glycine and 4-aminobutyryl amide derivatives were also studied. This work will be reported in due course.
10. Binding activity of compounds was measured using membranes of Hi5 cells expressing the human MC4R receptors. Homogenates were incubated with 100 pM [^{125}I]-NDP-α-MSH and increasing concentrations of unlabeled test compound (at final DMSO concentration of 1%) for 90 min at 37 °C in buffer consisting of 25 mM HEPES (pH 7.4), 140 mM NaCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, and 0.1% BSA. Assays were stopped by addition of ice cold wash buffer (20 mM HEPES, 5 mM $MgCl_2$) and filtrated over GF/B glass fiber filters that were previously soaked in 1% PEI. Non-specific binding was defined in the presence of 1 μM NDP-α-MSH. Filters were measured by liquid scintillation counting, and the data was analyzed by non-linear regression using the XLfit function of EXCEL.
11. Stimulation of adenylate cyclase in HEK293 cells expressing the human MC4R receptor was determined by measuring cAMP accumulation using the RPA559 SPA assay kit from Amersham. Recombinant HEK293 cells were cultured in MEM with 400 μg/mL G418, 100 mM sodium pyruvate and 10% heat inactivated FBS. Cells were seeded on 96-well plates at 5×10^4 cells/well and incubated overnight. The culture medium was aspirated and changed to the assay medium containing 0.1 mM IBMX, and increasing concentrations of the test compound at final DMSO concentration of 1%. The cells were incubated for 10 min at 37 °C, then the medium was aspirated and replaced with the lysis medium, and cAMP concentration was measured according to the manufacturer's direction. Intrinsic activity was expressed as percent of maximal activity achieved by NDP-α-MSH. Data was analyzed by non-linear regression using the PRIZM program.
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13. Synthesis of **2**: To a solution of α-amino amide **6** (1.1 g, 2.56 mmol) and *N*-Boc-β-alanine (531 mg, 2.81 mmol) in dichloromethane (12 mL) was added 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI) (736 mg, 3.8 mmol) and 1-hydroxybenzotriazole hydrate (HOBt) (518 mg, 3.8 mmol) at room temperature. The mixture was stirred at room temperature overnight and a saturated solution of ammonium chloride (15 mL) was added. The separated aqueous layer was extracted with dichloromethane (3×25 mL) and the combined organic layers were dried ($MgSO_4$), filtered, and evaporated to afford the *tert*-butylcarbamate which was used in the next step without further purification. To a solution of this carbamate (1.0 g, 1.7 mmol) in dichloromethane (10 mL) was added a 20% (v/v) solution of trifluoroacetic acid in dichloromethane (1.6 mL) at room temperature. The mixture was stirred at room temperature for 8 h and then evaporated under reduced pressure. The residue was purified using preparative (Column: YMC-PACK S5-C18 30×100 mm) HPLC (acetonitrile–0.1% TFA/water: 7 min gradient from 10% AcCN to 90% AcCN at 220 nm. Flow rate: 30 mL/min) and, after evaporation, the residue was lyophilized to afford 0.9 g (47% yield) of the trifluoroacetate salt. HPLC/MS (Column: Premisphere C18 4.6×30 mm; Flow rate: 4 mL/min, Solvent system: 0–100% B in 2 min. Solvent A: 10% CH_3CN– 90%H_2O – 5 mM NH_4OAc; Solvent B: 90% CH_3CN– 10%H_2O – 5 mM NH_4OAc; UV: 220 nm; Micromass ZMD 2000, ESI): retention time 1.50 min, MS pos. *m*/*z* 501 $(M+H)^+$; IR (ν_{max}, KBr) 3600–2880, 1695, 1620 cm^{-1}; ^{1}H NMR (400 MHz, MeOH-d_4) δ ppm (two rotamers; 1:2 ratio) 8.43 (1H, s, minor rotamer), 8.42 (1H, s, major rotamer), 7.96 (1H, s, minor rotamer), 7.92 (1H, s, major rotamer), 7.26 (2H, d, $J = 8.3$ Hz, major rotamer), 7.23 (2H, d, $J = 8.4$ Hz, minor rotamer), 7.18 (2H, d, $J = 8.3$ Hz, major rotamer), 7.15 (2H, d, $J = 8.6$ Hz, minor rotamer), 4.98 (1H, t, $J = 7.8$ Hz), 4.21 (2H, s, major rotamer), 4.18 (2H, s, minor rotamer), 3.60 (1H, m), 3.31 (3H, m), 3.08 (2H, m), 2.87 (2H, m), 2.54 (2H, t, $J = 6.5$ Hz), 1.95–0.82 (15H, m). Anal. calcd for $C_{26}H_{37}ClN_6O_2\cdot3HCl\cdot H_2O$: C, 49.69; H, 6.74; N, 13.37. Found: C, 49.96; H, 6.75; N, 12.88.
14. Selectivity towards the MC1 receptor[15] of selected compounds was also measured. The IC_{50} values (nM) for MC1R of selected compounds are provided along with the MC1R/MC4R ratio given in parentheses: **2**: 690 (43), **3a**: 500 (55), **3b**: 110 (12) and **5a**: 4400 (367).
15. For a recent disclosure of related compounds with high affinity on MC1 receptor, see: Herpin, T. F.; Yu, G.; Carlson, K. E.; Morton, G. C.; Wu, X.; Kang, L.; Tuerdi, H.; Khanna, H.; Tokarski, J. S.; Lawrence, R. M.; Macor, J. E. *J. Med. Chem.* **2003**, *46*, 1123.