Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 1035–1038

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Reductive Amination Products Containing Naphthalene and Indole Moieties Bind to Melanocortin Receptors

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Received 22 June 2001; revised 17 December 2001; accepted 31 January 2002

Abstract—Presumed pharmacophoric groups of melanocortin peptides (naphthalene, amino or guanidine, and indole moieties) were combined in mimetics molecules looking for their favorable location for activity at melanocortin (MC) receptors. Twenty-two compounds were prepared and tested. The best of these displayed micromolar affinities for the MC receptors.

Five subtypes of melanocortin receptors, $MC_{1-5}R$, are known.[1–3] The MC_1R regulates skin pigmentation and the immune system. The MC_2R (ACTH receptor) controls steroid production. The MC_3R might be involved in regulation of central sexual behavior, the MC_4R controls feeding behavior and the MC_5R has a role for regulating exocrine gland secretion.[1–3] The melanocortic peptides are the natural ligands for the MCRs and consist of the melanotropins α-MSH, β-MSH and γ-MSH, and the adrenocorticotropin ACTH. These peptides are not very suited for therapeutic applications and there exists a need for non-peptide ligands. Some peptoids,[4] isoquinolines[5] and β-turn related heterocycles[6] were reported to show moderate activity on MCRs. We recently found some MCR active substances whose preparation included reductive amination.[7] Here we report a new series synthesized in a related way.

The diamines **3a**,**b** were synthesized as shown in Scheme 1 by reduction of Schiff bases with $NaCNBH_3$ in trimethylorthoformate in the presence of AcOH (4%). Compound **3b** was prepared starting from *N*-Boc-3-formyl-indole **1b**.[8] The HCl treatment at the end removed Boc groups. Guanidines **4a**,**b** were prepared from **3a**,**b** introducing the guanidine function by using guanylpyrazole (equimolar quantity) in DMF.[9] The products were isolated by reversed phase HPLC using acetonitrile–water–0.1% TFA as eluent and freeze drying.

Ethylenediamine derivative **6a** was prepared on solid phase from carboxylated Wang polymer attached with ethylenediamine **5a**. Piperazine derivatives **6b** and **6c** were similarly synthesized using piperazine-attached polymer. After reductive amination, cleavage from the polymer was achieved using a trifluoroacetic acid based cocktail. Guanidation of both compounds proceeded as described above, giving **7a** and **7b**.

The secondary amine **10a** was prepared from *N*-Boc-3-formyl-indole **1b** and 1-aminomethylnaphthalene **8a** by reductive amination, and deblocking the indole moiety of the intermediate **9a** with trifluoroacetic acid. Similarly, another secondary amine **9b** was obtained from 2-naphthaldehyde **1a** and tryptamine **8b**. Compound **9c** of this type contained two indole moieties and was synthesized from unprotected 3-indolylaldehyde **1c** and tryptamine **8b**. The Schiff base was formed in suspension by stirring for 24 h at room temperature. Compound **9d** was similarly synthesized from 2-naphthaldehyde **1a** and 1-aminomethylnapthalene **8a**.

The tertiary amide **11a** was obtained from Boc protected secondary amine **9a**. The coupling reaction proceeded by a low yield. The product was Boc deprotected and isolated by preparative HPLC and freeze dryed. Compounds **11b** and **11d** were prepared in a similar way as **11a**. Derivative **11c** was obtained from Fmoc-D-Arg(Pbf)–OH. At the end the Fmoc group was removed with piperidine followed by removal of Pbf with trifluoroacetic acid and scavengers. Compound **11e** was obtained from Fmoc-γ-aminocaproic acid. Synthesis of the tertiary amide **11f** proceeded similarly by addition of

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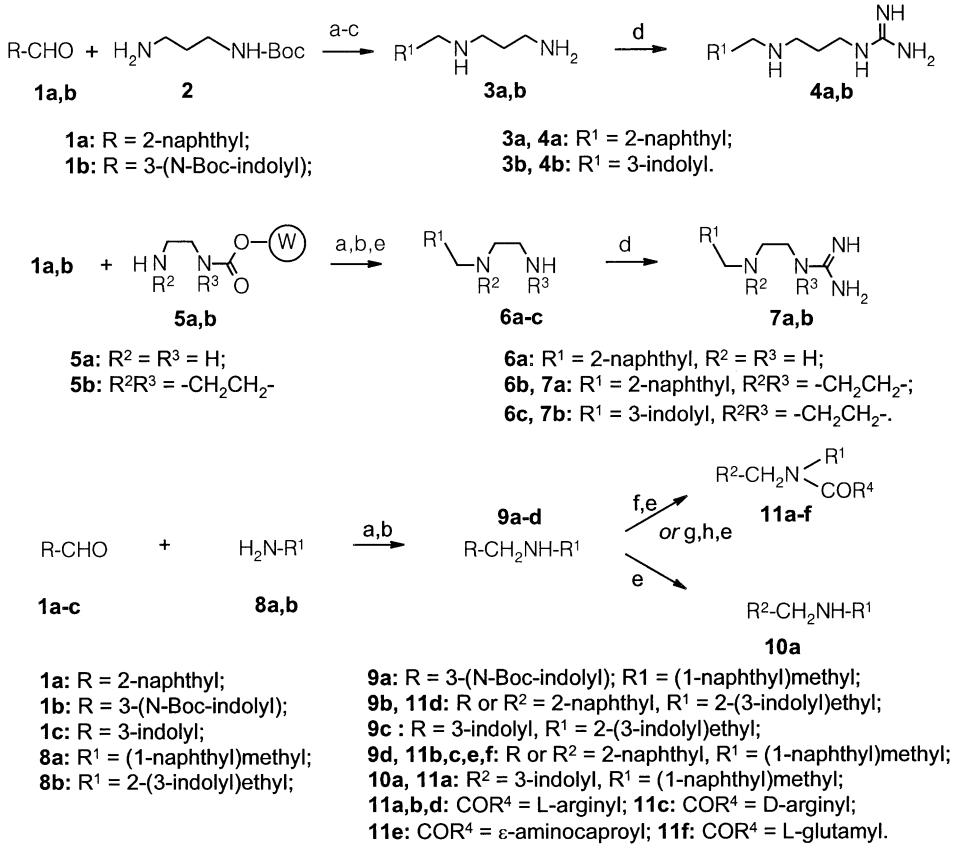
PII: S0960-894X(02)00088-4



Scheme 1. Reagents and conditions: (a) trimethylorthoformate, 1 h; (b) $NaCNBH_3$, AcOH, 10 min; (c) HCl/dioxane; (d) 1H-pyrazole 1-carboxamidine hydrochloride, DIEA, DMF, 20 h; (e) TFA–1,2-ethanedithiol–triisopropylsilane–water (925:25:25:25), 2 h; (f) Boc-Arg-OH·HCl, TFFH, DIEA, DMF, 20 h; (g) Fmoc-amino acid, TFFH, DIEA, DMF, 20 h; (h) 20% piperidine/DMF, 30 min.

Fmoc-Glu(O*t*Bu)–OH and cleavage with piperidine followed by trifluoroacetic acid.

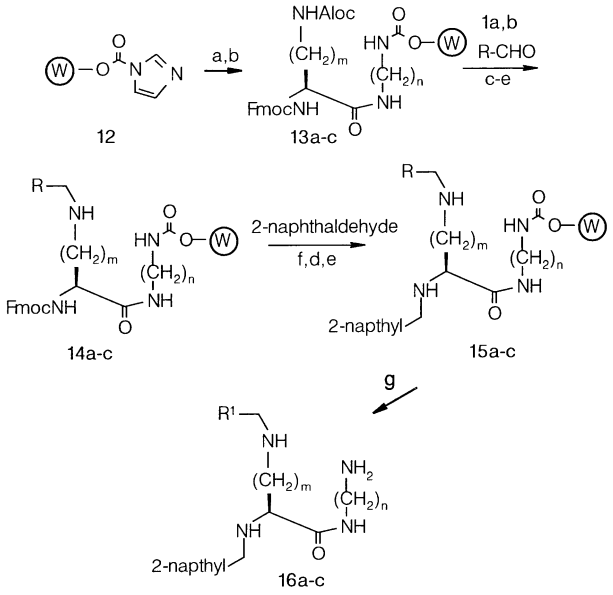
The double reductive amination product **16a** was prepared by loading imidazole carboxylate Wang resin with tetramethylenediamine. The polymeric monoacylated diamine formed was further reacted with Fmoc-Lys(Aloc)–OH (Scheme 2). The polymer **13a** obtained was subjected to palladium derivative deprotection (Scheme 2). The polymeric Lys derivative obtained containing a free α-amino group was further reacted with Boc-indolyl-3-aldehyde. The Schiff base formed was reduced with $NaCNBH_3$ giving secondary amine **14a**. The Fmoc group was then removed deblocking the α-aminofunction of the polymeric lysine derivative. The amino group was introduced into the reaction with 2-naphthaldehyde and the product reduced. The polymer **15a** formed was cleaved by trifluoroacetic acid in the presence of scavengers. The resulting raw product was purified by HPLC yielding pure **16a** (LC/MS and NMR).

Compounds **16b** and **16c** were synthesized in a similar way starting from ethylenediamine or trimethylenediamine attached to carboxylated Wang polymer. Fmoc-L-Dap(Aloc)–OH was used for **16b** and **16c**; for **16b** 2-naphthaldehyde was introduced twice. Yields generally ranged over 25–90%.

Table 1. Binding activities (K_i μM) of compounds for human recombinant MCRs.[a]

Compd	MC_1R	MC_3R	MC_4R	MC_5R
3a	>1000	57	94	43
3b	28	145	104	150
4a	19	31	56	36
4b	12	45	107	47
6a	nb	nb	8.8	83
6b	>1000	>1000	>1000	141
6c	>1000	>1000	>1000	256
7a	>1000	>1000	68	74
7b	>1000	>1000	>1000	>1000
9b	16	59	118	14
9c	32	86	161	18
9d	>1000	>1000	>1000	100
10a	26	214	161	38
11a	11	52	70	12
11b	0.69	9	5.4	2.5
11c	1.2	18	2.1	2.7
11d	1.5	19	15	10
11e	1.5	33	2	4.1
11f	46	294	89	45
16a	9.8	167	24	173
16b	27	58	44	28
16c	0.7	56	27	36

[a]Receptor binding was assessed using radioligand binding on human MCR subtypes[10] (mean values from at least two measurements, deviation between them did not exceed 30%). nb, no binding up to 0.5 mM.



1a, 14a,c 15a,c: R = 3-(N-Boc-indolyl);
1b, 14b, 15b: R = 2-naphthyl;
16a,c: R^1 = 3-indolyl; 16b: R^1 = 2-naphthyl;
13a-16a: m = 4, n = 4; 13b-16b: m = 1, n = 2;
13c-16c: m = 1, n = 3

Scheme 2. Reagents and conditions: (a) $NH_2(CH_2)_nNH_2$, DMF, 2 h, (b) Fmoc-amino acid, HATU, DIEA, DMF, 1 h; (c) tetrakis(triphenylphosphine)-palladium(0) in $CHCl_3$ + 5% AcOH + 2.5% NMM, 2 h; (d) aldehyde, trimethylorthoformate, 1 h; (e) $NaCNBH_3$, trimethylorthoformate, AcOH, 10 min; (f) 20% piperidine/DMF, 30 min; (g) TFA–1,2-ethanedithiol–triisopropylsilane–water (925:25:25:25), 2 h.

Reductive amination followed by acylation allowed us to prepare a series of melanocortin mimetics. They all contain naphthalene/indole and amino/guanidino groups. The secondary and tertiary amines exert relatively low affinity ($K_i > 15$ μM) for the MCRs (Table 1). The secondary amines containing indole and naphthalene functions (**10a**) are better binders than the ones containing two naphthalenes (**9d**), or one naphthalene and one additional primary amine function (**3a**, **6a**). Piperazine derivatives (**6b**,**c**, **7a**,**b**) show very low activity. Obviously, these small and rigid molecules do not fit well to the MCR binding sites. Guanidation of the secondary amine function (**7a**,**b**) did not improve the situation. On the other hand, guanidation of the less rigid **3a**,**b** increased the binding affinity (c.f., **4a**,**b**, Table 1).

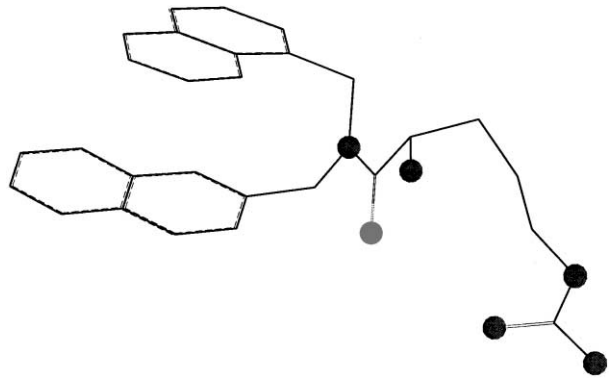


Figure 1. Low energy conformation of **11b**.[11] Hydrogen atoms are omitted for clarity. Nitrogen and oxygen atoms are shown as circles.

Acylation of secondary amines with amino acids contributed much to the activity. This can, for example, be seen by comparing the data for **9d** with the data for **11b** (Table 1). Quite unexpectedly **11b**, containing a naphthalene-naphthalene combination, showed better binding compared to the **11a**,**d** containing naphthalene and indole moieties. It was unexpected also that **11c**, containing a D-arginine residue showed approximately the same activity as **11b**, the latter that was derived from L-arginine. Compound **11e** that has an ε-aminocaproyl group shows also comparable activity to the above mentioned arginine derivatives. However, introduction of the amphoteric L-glutamic acid residue (**11f**) led to a considerable reduction in activity.

As a result of simulated annealing molecular dynamics calculations[11] we found a low energy conformation of **11b** (Fig. 1). Important features of it are the parallel interacting naphthalene ring systems, with the guanidine group being placed at a far distance. The conformation seems useful for explaining structure–affinity relationship of analogues of **11b**. Obviously, both naphthalene groups and the guanidine function, but not the arginine residue α-amino group, are interacting with the MCRs. Such a situation would explain why neither different positions of the α-amino group (i.e., the change of configuration of the asymmetric carbon atom in **11b** and **11c**) nor the removal of this function (**11e**) affects the binding affinity significantly. On the other hand, the importance of a distantly located basic function is illustrated by the lower affinity of **11f**, which contains a carboxylic group instead of a guanidino (**11b**, **11c**) or an amino group (**11e**).

On comparing the data for substances **16a**–**c**, it is seen that **16c** shows considerably higher affinity on the MC_1R than on the other HMCRs. Obviously the geometry of **16c** fits better to the MC_1R than **16a**,**b**.

In conclusion, we have here shown that a wide array of substances exhibit MC receptor binding affinity. The structure–activity relationships obtained will be useful for further developments of MCR subtype selective compounds.

Acknowledgements

This work was supported by a grant from Melacure Therapeutics. Biological parts of studies were supported by the Swedish Medical Research Council (04X-05957).

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11. 3-D structures were modeled using Sybyl 6.5 molecular simulation package (Tripos Inc., 1699 South Hanley Rd., St. Louis, MI 63144, USA). A series of conformers was generated for each compound by randomly assigning angles to rotatable bonds. Each conformer was subjected to simulated annealing, followed by energy minimization using Gasteiger–Hückel charges and Tripos force field.