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Structure–activity relationships of novel piperazines as antagonists for the melanocortin-4 receptor

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Abstract—During the investigation of antagonists for the MC4 receptor, we found that **10ab** having a naphthyl group showed almost the same binding affinity for the MC4 receptor as that of the lead compound **1** with a benzoyl group. We also developed a new type of compounds, namely, bis-piperazines, and found that the bis-piperazines **10** exhibited a high affinity for the MC4 receptor. In particular, (−)-**10bg** exhibited the highest affinity for the MC4 receptor with an IC_{50} value of 8.13 nM. In this paper, we present the design, synthesis, and structure–activity relationships of the novel bis-piperazines as MC4 receptor antagonists.

1. Introduction

The melanocortin peptides, the natural ligands for the melanocortin receptors, consist of the melanocyte-stimulating hormones (α-MSH, β-MSH, and γ-MSH) and the adrenocorticotropic hormone (ACTH), all of which are derived from proopiomelanocortin (POMC).[1] In addition, two endogenous antagonistic peptides, agouti and agouti-related protein (AGRP), have also been indentified.[2,3] All the melanocortin peptides possess a core His-Phe-Arg-Trp (HFRW) terapeptide sequence, which has been shown to be essential for activation of the melanocortin receptors.[4,5]

To date, five subtypes of melanocortin receptors (MC1–MC5 receptors) that belong to seven-transmembrane G-protein-coupled receptor family have been identified.[6] The MC1 receptor, bound mainly by α-MSH, is prominently expressed in the skin and melanoma cells and plays a major role in regulating skin pigmentation. The MC2 receptor, bound by only ACTH, is prominently expressed in the adrenal cortex and is involved in steroidogenesis. The MC3 receptor, bound by both α- and γ-MSH with the same affinity, is widely expressed in the central nervous system as well as placenta, and plays a role in fat metabolism and energy homeostasis together with the MC4 receptor. The MC4 receptor, which exhibits higher affinities for α-MSH and β-MSH than for γ-MSH, is primarily expressed in the brain. The MC5 receptor, bound by α-MSH, is expressed in various peripheral tissues and plays a role in exocrine gland function.

Numerous studies have suggested that the MC4 receptor is involved in the regulation of feeding and metabolism. MC4 receptor deficient-mice are severely obese and hyperphagic. In rodents, while injection of MC4 receptor agonists into the brain inhibits food intake, intracerebroventricular administration of MC4 receptor antagonists stimulates food intake.[7–13] In addition, it has also been reported that the MC4 receptor is involved in the regulation of sexual functions,[14] protection against tumor-induced decrease of body weight,[15] and in the regulation of emotional states such as anxiety and depression.[16–22] These studies indicate that the MC4 receptor could be a promising target for the development of drugs for the above-mentioned conditions, and numerous ligands of the MC4 receptor, both peptidic and nonpeptidic, have been developed to date.

Several peptidic MC4 receptor agonists and antagonists have been reported.[23] Since peptidic compounds tend to have properties unsuitable for an oral administration, such as their low absorption, nonpeptidic ligands of the MC4 receptor have been sought. Recently, nonpeptidic agonists[24] and antagonists[15,25–28] have been

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reported (Chart 1). Compound **1**, having a 1,2-bispiperazinylethane core, inhibited the binding of AGRP to the MC4 receptor (IC_{50} = 52 nM) and also that of NDP-MSH to the MC4 receptor (IC_{50} = 217 nM).[25] Compound **2**, having a succinamide core, exhibited a high affinity for the MC4 receptor (IC_{50} = 1.4 nM) while having no effect of activating the MC4 receptor in a functional assay.[26] Compound **3** (ML00253764) exhibited a moderate affinity (K_i = 160 nM) for the MC4 receptor with antagonist activity (K_i = 103 nM), and following subcutaneous administration in mice protected the animals against tumor-induced weight loss.[15] Compound **4** was a potent MC4 receptor antagonist (K_i = 3.2 nM) with a 240-fold selectivity for this over the MC3 receptor. Intracerebroventricular administration of this compound potently stimulated food intake in satiated mice.[27] Thus, both MC4 receptor agonists and antagonists are attractive targets for the development of CNS drugs to treat obesity, erectile dysfunction, cachexia, anxiety or depression. We report the synthesis and structure–activity relationships of novel bis-piperazine compounds identified as MC4 receptor antagonists.

2. Chemistry

The syntheses of bis-piperazine compounds **10** are shown in Schemes 1 and 2. Compounds **10aa**–**10cj** were prepared by four types of synthetic routes (methods A–D) from haloacetophenone **12**, which was the common starting material for the preparation.

Compounds **10ak**–**10an** were synthesized from compounds **14** via N-protected piperazines **15**. Reaction of **12** with *N*-(Ar_2-$(CH_2)_n$)-piperazine **5** in the presence of $^iPr_2NEt$, followed by reduction of the carbonyl group with $NaBH_4$, yielded alcohols **14**. Chlorination of the hydroxyl group of **14** with thionyl chloride, followed by coupling with *N*-Boc-piperazine **7**, yielded N-protected piperazines **15**. After removal of the Boc group of **15** under acidic conditions (yielded **10ak**), reaction with sodium hydride and alkyl halide yielded compounds **10al**–**10an** (method A, Scheme 1).

Alternatively, compounds **10ao**–**10bd** and **10bk**–**10bm** could be also obtained from alcohols **14** by another pathway. Methanesulfonylation of the hydroxyl group of **14** with MsCl in the presence of Et_3N, followed by coupling with N-R_1-piperazine **6** in the presence of Et_3N, yielded **10ao**–**10bc** and **10bk**–**10bm**. Compound **10bd** was obtained by hydrogenation of **10ba** with PtO_2 as the catalyst (method B, Scheme 1).

Furthermore, compounds **10be** were synthesized via amines **20**. Reaction of **12** with N-CO_2Et-piperazine **8**, followed by reduction of the carbonyl group with $NaBH_4$, yielded alcohols **18**. Chlorination of the hydroxyl group of **18** with thionyl chloride, followed by coupling with N-R_1-piperazine **6** in the presence of $^iPr_2NEt$, yielded N-protected piperazines **19**. Removal of the ethoxycarbonyl group using KOH yielded deprotected amines **20**. Compounds **20** were converted to **10be** by treatment with Ar_2-$(CH_2)_4$-Br (**9b**) in the presence of Et_3N (method C, Scheme 2). Compounds **10aa**–**10aj**, **10bf**, **10bg**, and **10bn**–**10cj** were also obtained by reduction of amides **21**, which had been prepared from **20** by condensation with Ar_2-$(CH_2)_{n-1}CO_2H$ (**9a**), with $LiAlH_4$. Compounds **10bi** and **10bj** were obtained by alkylation of phenol **10bh**, which had been prepared by hydrolysis of **10bg** with HBr (method D, Scheme 2).

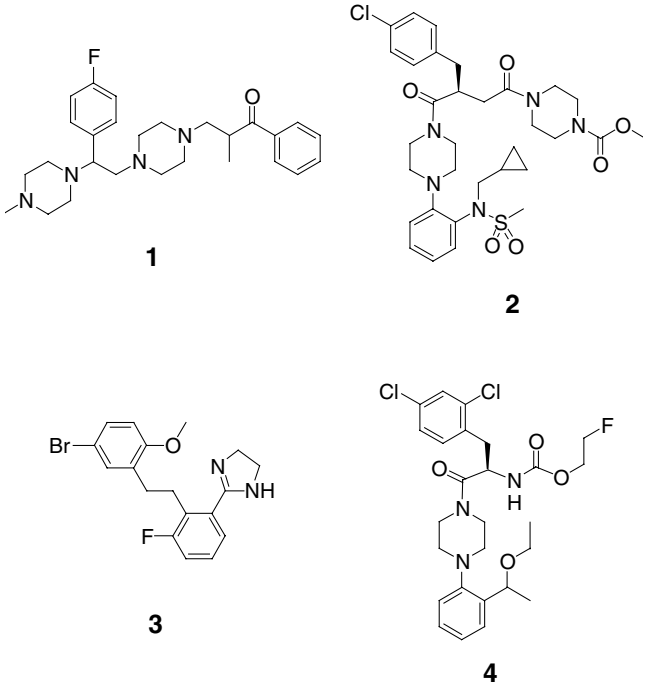
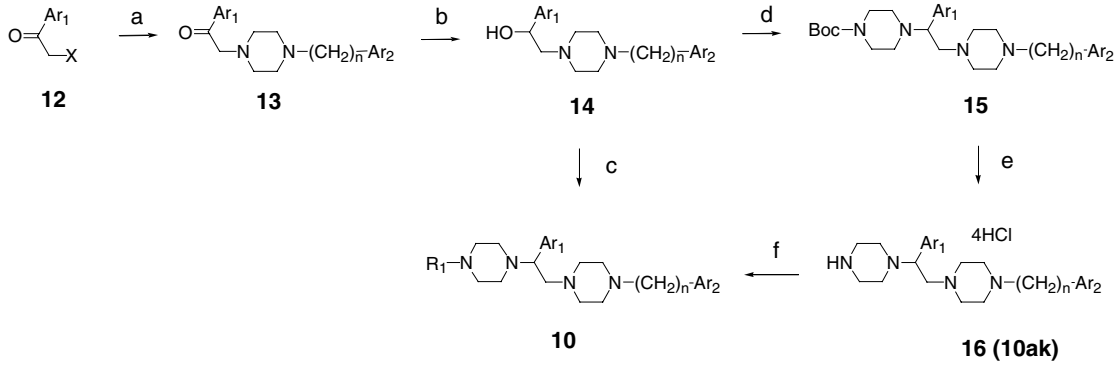


Chart 1.

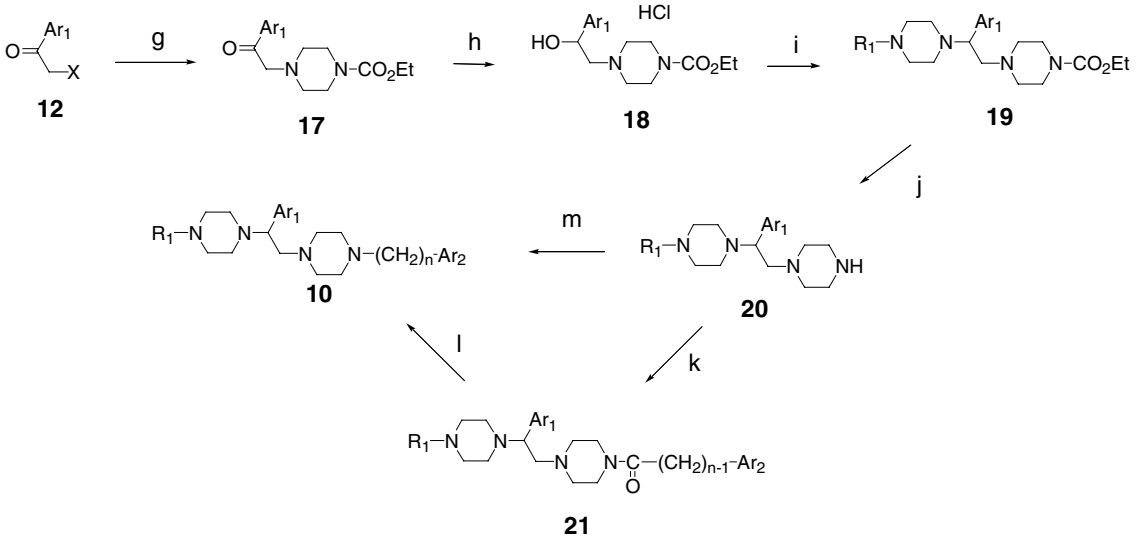
3. Results and discussion

The affinities of all the bis-piperazines for the MC4 receptor were evaluated based on their binding affinity to membranes of COS-1 expressing the human MC4 receptor and calculated from the inhibition curve of [^{125}I]Nle^4-D-Phe^7-α-MSH binding,[19] and the IC_{50} values are shown in Table 1. The affinities of compounds (±)-**10bo**, (±)-**10bs**–(±)-**10bx**, (±)-**10ca**, and (±)-**10cb** were tested as follows; [^{3}H]paroxetine binding to rat cortical membranes (for SET), [^{3}H]nisoxetine binding to rat cortical membranes (for NET), [^{3}H]prazosin binding to rat cortical membranes (for α1),[^{3}H]raclopride binding to rat striatal membranes (for D2), [^{3}H]pyrilamine binding to rat whole brain membranes (for H1), [^{3}H]DAMGO binding to rat brain membranes (for μ), and [^{3}H]DPDPE binding to rat brain membranes (for δ).

We identified compound **1** in a high-throughput screening against our internal compounds library. Since compound **1** exhibited a moderate affinity for the MC4 receptor (IC_{50} = 399 nM), we attempted to explore its analogues to identify compounds with higher affinity for the MC4 receptor.



Scheme 1. Synthesis of bis-piperazine compounds **10** (methods A and B). Reagents and conditions (X = Cl or Br): (a) N-(Ar_2-$(CH_2)_n$)-piperazine (**5**), $^{i}Pr_2NEt$, $CHCl_3$, reflux; (b) $NaBH_4$, EtOH, 50 °C; (c) $MeSO_2Cl$, Et_3N, CH_2Cl_2, in ice-bath and then N-R_1-piperazine (**6**), Et_3N, rt; (d) $SOCl_2$, benzene, 50 °C and then *N*-Boc-piperazine (**7**), $CHCl_3$, 70 °C; (e) 4 M HCl/AcOEt, MeOH, rt; (f) NaH, alkyl halide, DMF, rt; method A: a–f; method B: a–c.



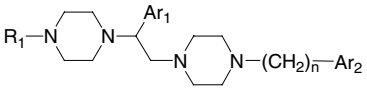
Scheme 2. Synthesis of bis-piperazine compounds **10** (methods C and D). Reagents and conditions (X = Cl or Br): (g) *N*-EtO_2C-piperazine (**8**), $CHCl_3$, reflux; (h) $NaBH_4$, EtOH, 50 °C and then 4 M HCl/EtOAc; (i) $SOCl_2$, benzene, 50 °C and then N-R_1-piperazine (**6**), $^{i}Pr_2NEt$, benzene, 60 °C; (j) KOH, EtOH, reflux; (k) Ar_2-$(CH_2)_{n-1}$-CO_2H (**9a**), EDC, HOBt, DMF, rt; (l) $LiAlH_4$, THF, reflux; (m) Ar_2-$(CH_2)_4$-Br (**9b**) NEt_3, DMF, 60 °C; method C: g–j, and m; method D: g–l.

Bis-piperazine derivatives, MC4 receptor antagonists with a benzoyl group, have been reported by Arasasingham et al.[25] They also showed that the carbonyl group was essential for the binding of these bis-piperazine derivatives to the receptor. We focused on the conformational rigidity of the carbonyl group, and we adapted a naphthyl group for the conformational rigidity as an alternative to the benzoyl group. We prepared a compound with a naphthyl part and evaluated the affinity of the compound for the MC4 receptor. As expected, **10ab** showed almost the same affinity for the MC4 receptor (IC_{50} = 567 nM) as **1**. Then we attempted to explore the naphthyl analogues to identify compounds with higher affinity for the MC4 receptor. To study SAR of bis-piperazines for the MC4 receptor, syntheses of these compounds were accomplished according to Scheme 1 and 2.

Initially, bis-piperazines with various lengths (*n*) of the linking moiety between the naphthyl group and piperazine ring were tested for their affinity for the MC4 receptor (**10aa**–**10af**, Table 1). The results indicated that tetramethylene was the most favored chain length for binding to the receptor (**10ad**, IC_{50} = 118 nM). A similar tendency was observed for the bis-piperazine compounds having a 2-naphthyl group (**10ag**–**10aj**). The 2-naphthyl analogues showed a lower affinity for the MC4 receptor than the 1-naphthyl analogues.

Next, we examined bis-piperazines having various types of Ar_2. The methoxy group on the naphthalene ring at 2-position conferred a slight increase in the affinity for the MC4 receptor (**10bc**: IC_{50} = 57.3 nM) as compared to that of **10ad** with no substitutions in the naphthalene ring, whereas the methoxy group at 4-position conferred a slight decrease in the affinity (**10bf**: IC_{50} = 228 nM). These results suggest that a substitution at 2-position probably increased the binding affinity for the MC4 receptor.

Table 1. Binding affinity for the MC4 receptor of bis-piperazine compounds **10**



Compound	Method	Ar_1	Ar_2	R_1	*n*	IC_{50} (nM)
(±)-**10aa**	D	4-F–Ph	1-Nap	Me	1	594
(±)-**10ab**	D	4-F–Ph	1-Nap	Me	2	567
(±)-**10ac**	D	4-F–Ph	1-Nap	Me	3	208
(±)-**10ad**	D	4-F–Ph	1-Nap	Me	4	118
(±)-**10ae**	D	4-F–Ph	1-Nap	Me	5	218
(±)-**10af**	D	4-F–Ph	1-Nap	Me	6	341
(±)-**10ag**	D	4-F–Ph	2-Nap	Me	1	1250
(±)-**10ah**	D	4-F–Ph	2-Nap	Me	2	1870
(±)-**10ai**	D	4-F–Ph	2-Nap	Me	3	1190
(±)-**10aj**	D	4-F–Ph	2-Nap	Me	4	336
(±)-**10ak**	A	4-F–Ph	1-Nap	H	4	303
(±)-**10al**	A	4-F–Ph	1-Nap	Et	4	105
(±)-**10am**	A	4-F–Ph	1-Nap	*n*-Pr	4	177
(±)-**10an**	A	4-F–Ph	1-Nap	*iso*-Pr	4	72.3
(±)-**10ao**	B	4-F–Ph	1-Nap	cyclo-Pr	4	147
(±)-**10ap**	B	4-F–Ph	1-Nap	cyclo-Hex	4	241
(±)-**10aq**	B	4-F–Ph	1-Nap	Ph	4	>10,000
(±)-**10ar**	B	Ph	1-Nap	Me	4	745
(±)-**10as**	B	3-F–Ph	1-Nap	Me	4	653
(±)-**10at**	B	4-Cl–Ph	1-Nap	Me	4	132
(±)-**10au**	B	2-Br–Ph	1-Nap	Me	4	2300
(±)-**10av**	B	3-Br–Ph	1-Nap	Me	4	565
(±)-**10aw**	B	4-Br–Ph	1-Nap	Me	4	159
(±)-**10ax**	B	4-Me–Ph	1-Nap	Me	4	296
(±)-**10ay**	B	4-MeO–Ph	1-Nap	Me	4	871
(±)-**10az**	B	4-CF_3–Ph	1-Nap	Me	4	226
(±)-**10ba**	B	4-NO_2–Ph	1-Nap	Me	4	263
(±)-**10bb**	B	4-Ph–Ph	1-Nap	Me	4	756
(±)-**10bc**	B	4-F–Ph	2-MeO–1-Nap	Me	4	57.3
(±)-**10bd**	B	4-NH_2–Ph	1-Nap	Me	4	3590
(±)-**10be**	C	4-F–Ph	2-Br–1-Nap	*iso*-Pr	4	36.8
(±)-**10bf**	D	4-F–Ph	4-MeO–1-Nap	Me	4	228
(±)-**10bg**	D	4-F–Ph	2-MeO–1-Nap	*iso*-Pr	4	12.7
(+)-**10bg**	D	4-F–Ph	2-MeO–1-Nap	*iso*-Pr	4	22.0
(−)-**10bg**	D	4-F–Ph	2-MeO–1-Nap	*iso*-Pr	4	8.13
(±)-**10bh**	D	4-F–Ph	2-HO–1-Nap	*iso*-Pr	4	131
(±)-**10bi**	D	4-F–Ph	2-EtO–1-Nap	*iso*-Pr	4	26.0
(±)-**10bj**	D	4-F–Ph	2-*iso*-PrO–1-Nap	*iso*-Pr	4	22.0
(±)-**10bk**	B	4-F–Ph	2-MeO–1-Nap	Et_2CH	4	82.8
(±)-**10bl**	B	4-F–Ph	2-MeO–1-Nap	cyclo-Pen	4	47.2
(±)-**10bm**	B	4-F–Ph	2-MeO–1-Nap	*t*-Bu	4	18.0
(±)-**10bn**	D	4-F–Ph	2-Ph–Ph	*iso*-Pr	2	186
(±)-**10bo**	D	4-F–Ph	2-Ph–Ph	*iso*-Pr	3	11.2
(±)-**10bp**	D	4-F–Ph	2-Ph–Ph	*iso*-Pr	4	65.1
(±)-**10bq**	D	4-F–Ph	3-Ph–Ph	*iso*-Pr	3	156
(±)-**10br**	D	4-F–Ph	4-Ph–Ph	*iso*-Pr	3	243
(±)-**10bs**	D	4-F–Ph	2-Ph–3-F–Ph	*iso*-Pr	3	23.0
(±)-**10bt**	D	4-F–Ph	2-Ph–4-F–Ph	*iso*-Pr	3	33.0
(±)-**10bu**	D	4-F–Ph	2-Ph–5-F–Ph	*iso*-Pr	3	27.1
(±)-**10bv**	D	4-F–Ph	2-Ph–6-F–Ph	*iso*-Pr	3	11.7
(±)-**10bw**	D	4-F–Ph	2-Ph–6-Cl–Ph	*iso*-Pr	3	27.1
(±)-**10bx**	D	4-F–Ph	2-Ph–6-Me–Ph	*iso*-Pr	3	24.3
(±)-**10by**	D	4-F–Ph	2-Ph–6-MeO–Ph	*iso*-Pr	3	15.2
(±)-**10bz**	D	4-F–Ph	2-(2-F–Ph)–Ph	*iso*-Pr	3	36.8
(±)-**10ca**	D	4-F–Ph	2-(3-F–Ph)–Ph	*iso*-Pr	3	23.6
(±)-**10cb**	D	4-F–Ph	2-(4-F–Ph)–Ph	*iso*-Pr	3	10.5
(±)-**10cc**	D	4-F–Ph	2-(4-Cl–Ph)–Ph	*iso*-Pr	3	53.2
(±)-**10cd**	D	4-F–Ph	2-(4-Me–Ph)–Ph	*iso*-Pr	3	41.8
(±)-**10ce**	D	4-F–Ph	2-(4-MeO–Ph)–Ph	*iso*-Pr	3	42.6
(±)-**10cf**	D	4-F–Ph	2-(4-*t*-Bu–Ph)–Ph	*iso*-Pr	3	100
(±)-**10cg**	D	4-F–Ph	2-(4-Ph–Ph)–Ph	*iso*-Pr	3	228
(±)-**10ch**	D	4-F–Ph	2-(4-CF_3–Ph)–Ph	*iso*-Pr	3	62.8
(±)-**10ci**	D	4-F–Ph	2-(4-CF_3O–Ph)–Ph	*iso*-Pr	3	85.9
(±)-**10cj**	D	4-F–Ph	2-(4-Me_2N–Ph)–Ph	*iso*-Pr	3	92.5

We then attempted chemical modifications of Ar_1 to study the effects of substituents on the benzene ring in the binding affinity for the MC4 receptor. Substitution of a chlorine (**10at**) or a bromine (**10aw**) at 4-position of Ar_1 resulted in a slight decrease of the affinity for the MC4 receptor (**10at**: IC_{50} = 132 nM, **10aw**: IC_{50} = 159 nM) as compared to that of **10ad** with a fluorine atom. A halogen group at 4-position of the benzene ring showed a favorable effect in the binding affinity to the MC4 receptor, however, a halogen group at 2- and 3-positions proved to have an unfavorable effect (**10as** vs **10ad**, **10av** vs **10aw**, and **10au** vs **10aw**). Methyl, methoxy, trifluoromethyl, nitro, and phenyl groups at 4-position of Ar_1 decreased a binding affinity for the MC4 receptor as compared to that of **10ad** substituted with a fluorine atom. Although methoxy group at 2-position of the naphthyl group improved a binding affinity, a methoxy group at 4-position of Ar_1 resulted in a much lower affinity than that of the corresponding **10ad**. Compound **10bd** having an amino group showed almost no affinity, whereas nonsubstituted compound **10ar** showed a 6-fold lower affinity for the receptor than **10ad** substituted with a fluorine atom. These results indicate that nonpolar substituents like halogen and alkyl groups at 4-position increase the affinity for the MC4 receptor, while polar substituents like methoxy and amino groups at 4-position decrease the affinity. Thus the most suitable group for Ar_1 to increase the binding affinity for the MC4 receptor was the 4-fluorophenyl group.

At the next step, we examined the effects of replacement of the methyl group at R_1 of **10ad** with other alkyl groups and a phenyl group. Substitution of an *iso*-propyl group resulted in a slightly increased binding affinity (**10an**: IC_{50} = 72.3 nM) as compared to that of methyl-substituted compound **10ad**, whereas that of an ethyl group resulted in almost the same affinity (**10al**: IC_{50} = 105 nM). Compounds **10am**, **10ao**, and **10ap** with *n*-propyl, cyclopropyl, and cyclohexyl groups, respectively, showed between 1.2- and 2.0-fold lower affinity than the corresponding **10ad**, and **10aq** (R_1 = Ph) exhibited no affinity. Removal of the methyl group resulted in **10ak** with a slightly decreased binding affinity (IC_{50} = 303 nM). These results indicate that the *iso*-propyl group at R_1 was the most suitable for optimal binding affinity for the MC4 receptor.

From these structure–activity relationship studies, we designed a compound having the most favorable group at each of R_1, Ar_1, and Ar_2, as follows; *iso*-propyl, 4-fluorophenyl, and 2-methoxy-1-naphthyl, respectively. According to our expectation, **10 bg** having favorable combination of each part showed a higher affinity (IC_{50} = 12.7 nM) for the MC4 receptor than compounds synthesized before.

Next, we studied the substitution effects of the methoxy group on the naphthyl group of **10 bg** with hydroxy, ethoxy, and *iso*-propoxy groups, and a bromine atom (**10bh**, **10bi**, **10bj**, and **10be**, respectively). No compounds having a higher affinity for the MC4 receptor than **10bg** were found, however, compounds **10bi**, **10bj**, and **10be** with ethoxy and *iso*-propoxy groups and a bromine atom, respectively, showed a higher affinity for the receptor than the compound **10an** with no substituent at this position. These results suggest that a substitution at 2-position probably plays an important role in favoring conformation between the naphthalene ring and alkylene part for binding to the MC4 receptor, which may contribute to the increasing affinity.

Then, we studied the substitution effects of the methyl group at R_1 of **10bg** with Et_2CH (**10bk**), cyclopentyl (**10bl**), and *tert*-butyl groups (**10bm**). None of the compounds showed a higher affinity for the MC4 receptor than **10bg**. These results indicate that bulky alkyl and cycloalkyl groups at R_1 decreased the affinity for the MC4 receptor, as described above. Since **10bg** was a racemate, optical resolution was performed to determine the affinity of each enantiomer for the MC4 receptor. The result showed that the (−)-enantiomer exhibited a higher affinity than the (+)-enantiomer (IC_{50}; (−)-**10bg**:8.13 nM, (+)-**10bg**:22.0 nM).

As the next structural conversion, we expected that the naphthyl group could be replaced by a biphenyl (2-Ph–Ph) group (Chart 2). We prepared **10bp** having a 2-Ph–Ph group and evaluated the affinity of this compound for the MC4 receptor. Fortunately, **10bp** showed a high affinity (IC_{50} = 65.1 nM) for the MC4 receptor. This finding therefore led us to explore biphenyl analogues with higher affinity for the receptor.

We investigated the effects of chemical modification of the length (*n*) of the linking moiety between the 2-Ph–Ph group and piperazine ring (**10bn**, **10bo**, and **10bp**). The results indicated that compound **10bo** (IC_{50} = 11.2 nM) having a trimethylene exhibited the highest affinity for the MC4 receptor among these compounds. Interestingly, for the naphthyl compounds, tetramethylene was the most favored chain length in terms of binding affinity for the MC4 receptor, whereas for the 2-Ph–Ph compounds, a trimethylene was the most favored chain length. 3- and 4-Ph–Ph compounds

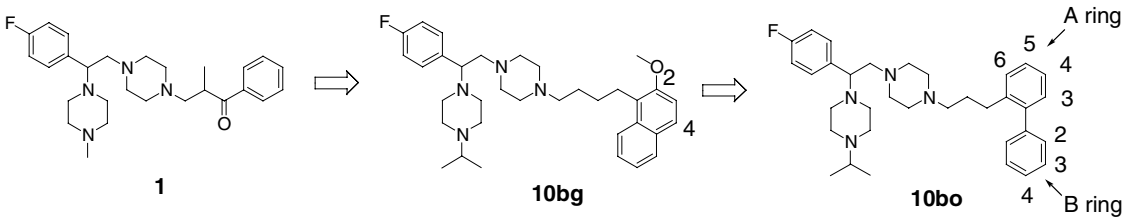


Chart 2.

Table 2. In vitro receptor profiles of bis-piperazine compounds **10**

Compound	IC_{50} (nM)							
	MC4	SET	NET	α1	H1	D2	Opiate μ	Opiate δ
(±)-**10bo**	11.2	3130	671	>1000	>1000	645	>10,000	>10,000
(±)-**10bs**	23.0	3400	2830	>1000	>1000	>1000	>10,000	>10,000
(±)-**10bt**	33.0	1370	1850	>1000	>1000	>1000	>10,000	>10,000
(±)-**10bu**	27.1	1170	369	>1000	>1000	721	>10,000	>10,000
(±)-**10bv**	11.7	1460	206	>1000	>1000	>1000	>10,000	>10,000
(±)-**10bw**	27.1	1290	1770	>1000	>1000	>1000	>10,000	>10,000
(±)-**10bx**	24.3	4960	2400	>1000	>1000	>1000	>10,000	>10,000
(±)-**10ca**	23.6	2110	226	>1000	>1000	>1000	>10,000	>10,000
(±)-**10cb**	10.5	1340	290	>1000	>1000	721	>10,000	>10,000

(**10bq** and **10br**, respectively) showed a much lower affinity than the corresponding 2-Ph–Ph compound **10bo**. These results suggest that the distance between the terminal benzene ring (B-ring, Chart 2) and piperazine ring is significant in relation to the binding affinity for the MC4 receptor, and also that the terminal benzene ring plays an important role.

We then investigated the effects of chemical modification of the substituents on the benzene ring in the biphenyl compounds. Compounds **10bs**–**10bv** substituted with a fluorine atom at 3-, 4-, 5- or 6-position of the core benzene ring (A-ring, Chart 2) exhibited a similar or slightly lower affinity (IC_{50} = 11.7–33.0 nM) for the MC4 receptor as compared to the nonsubstituted compound **10bo**. Introduction of a chlorine atom (**10bw**), a methyl (**10bx**) or a methoxy group (**10by**) at 6-position of the core benzene ring resulted in a slight decrease of the binding affinity (IC_{50} = 15.2–27.1 nM) as compared to that of the corresponding fluorine-substituted compound **10bv**. In the case of naphthyl compounds, introduction of a substituent at the vicinal position (2-position, Chart 2) resulted in an increase in the binding affinity for the MC4 receptor as compared to that of the compound with no substituent at this position. In contrast, for biphenyl compounds, introduction of a substituent at the vicinal position (6-position, Chart 2) did not increase the binding affinity for the receptor. These results suggest that the 2-Ph–Ph structure probably exhibited the optimal conformation between the terminal benzene ring and alkylene part without a substituent at the vicinal position. Compound **10cb**, substituted with a fluorine atom at 4-position of terminal benzene ring (B-ring), exhibited a similar affinity (IC_{50} = 10.5 nM) to that of nonsubstituted compound **10bo**, while compounds **10bz** or **10ca** with a substituent at 2- or 3-position exhibited a slightly lower affinity (IC_{50} = 36.8 and 23.6 nM, respectively) for the MC4 receptor. Compounds **10cc**–**10cj** substituted with chlorine, methyl, methoxy, *tert*-butyl, phenyl, trifluoromethyl, trifluoromethoxy, and dimethylamino groups at 4-position of the terminal benzene ring showed between 3.7- and 20-fold lower affinity than nonsubstituted compound **10bo**. Among these compounds, compounds which have bulky substituents, like phenyl, at 4-position tended to show a lower affinity for the MC4 receptor. These findings suggest that the sterically acceptable space on the benzene ring for binding to the MC4 receptor is limited.

It was reported that (−)-**10bg** (MCL0129) exhibited a high affinity for the MC4 receptor (IC_{50} = 8.13 nM), and exhibited moderate to negligible affinities for other stress- and anxiety/depression-related receptors and transporters.[19] The studies have shown that (−)-**10bg** acts as an antagonist at the MC4 receptor, and also that this compound exhibited anti-depressant like and anxiolytic-like activities in various rodent models.[19] To investigate the in vitro receptor-binding profiles of the compounds described above, several compounds with a high affinity for the MC4 receptor were evaluated for their affinities for the serotonin transporter (SET), norepinephrine transporter (NET), α1-adrenoceptor (α1), dopamine D2 receptor (D2), histamine H1 receptor (H1), and opiate μ and δ receptor (Table 2). Compound (−)-**10bg** did not show an affinity for the NET (>1000 nM),[19] while some biphenyl compounds exhibited a moderate affinity for this receptor (**10bv** and **10ca**, IC_{50} = 206 and 226 nM, respectively). We serendipitously obtained **10ad** (MCL0042), which exhibited a relatively high affinity for the MC4 receptor (IC_{50} = 118 nM), showed a high affinity for the serotonin transporter (IC_{50} = 42.3 nM).[20] Compound **10ad**, which displayed the unique activity of both MC4 receptor antagonism and serotonin transport inhibition, also exhibited antidepressant like and anxiolytic-like effects.[20] None of other tested compounds showed a significant affinity for the receptors or transporters examined.

4. Conclusion

We have reported a new series of MC4 receptor antagonists, namely, bis-piperazines. The lead compound **10ab** was discovered by replacing of a benzoyl group of compound **1** with a naphthyl group. Structural optimization based on the SAR study led to identification of the compound with a high affinity for the MC4 receptor, namely, (−)-**10bg**, which showed a 50-fold higher affinity for the receptor than the lead compound. We also identified some biphenyl compounds which showed a high affinity for the MC4 receptor, and in particular, compound **10bo** exhibited the highest affinity for the MC4 receptor. The various types of bis-piperazine compounds that have been demonstrated to show a high affinity for the MC4 receptor can be excellent tools for exploring the biological and physiological functions of the MC4 receptor, and SAR studies can

serve as a tool to explore new types of MC4 receptor antagonists.

5. Experimental

Melting points were determined on a Yanaco MP-500D melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained using a Varian Gemini 2000 (200 MHz) or Varian Unity Inova 300 (300 MHz). Chemical shifts are reported in parts per million relative to tetramethylsilane as an internal standard. Mass spectra (MS) were obtained on Micromass Platform LC (IonSpray). Elemental analyses were performed by a PerkinElmer 2400 or a Yanaco MT-6. Silica gel C-200 (100–200 mesh, Wako Pure Chemical) and Chromatorex NH (100–200 mesh, Fuji Silysia Chemical Ltd) were used for column chromatography, using the solvent systems (volume ratios) indicated below.

5.1. General methods for the synthesis of 10ak–10an (method A)

5.1.1. 1-(4-Fluorophenyl)-2-{4-[4-(1-naphthyl)butyl]piperazin-1-yl} ethanone (13). A mixture of 2-chloro-1-(4-fluorophenyl)ethanone **12** (486 mg, 2.87 mmol), 1-[4-(1-naphthyl)butyl]piperazine 2 hydrochloride **5** (980 mg, 2.87 mmol), and ^{i}Pr$_2$NEt (2.24 g, 17.2 mmol) in $CHCl_3$ (10 mL) was heated at reflux for 3 h. The mixture was partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$. The separated organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was chromatographed on Silica gel C-200 ($CHCl_3$/MeOH 40:1) to obtain 1-(4-fluorophenyl)-2-{4-[4-(1-naphthyl)butyl]piperazin-1-yl}ethanone **13** (1.25 g, quantitative yield) as an oily product: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.53–1.82 (4H, m), 2.42 (2H, t, J = 8.5 Hz), 2.50–2.82 (8H, m), 3.12 (2H, t, J = 7.5 Hz), 3.79 (2H, s), 7.14 (2H, t, J = 8.9 Hz), 7.20–7.56 (4H, m), 7.71 (1H, d, J = 7.6 Hz), 7.80–7.92 (1H, m), 7.99–8.12 (3H, m); MS (ESI, Pos) m/z 405 $(M+H)^+$.

5.1.2. (±)-1-(4-Fluorophenyl)-2-{4-[4-(1-naphthyl)butyl]piperazin-1-yl}ethanol (14). 1-(4-Fluorophenyl)-2-{4-[4-(1-naphthyl)butyl]piperazin-1-yl}ethanone **13** (1.25 g, 2.87 mmol) was dissolved in EtOH (4 mL), and to the solution were added $NaBH_4$ (130 mg, 3.44 mmol) and a mixture of H_2O (1 mL) and 1 drop of 10% aqueous NaOH. After stirring at 50 °C for 1 h, the reaction mixture was partitioned between $CHCl_3$ and H_2O. The separated organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo to obtain (±)-1-(4-fluorophenyl)-2-{4-[4-(1-naphthyl)butyl]piperazin-1-yl}ethanol **14** (1.25 g, quantitative yield) as a powder: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.60–1.82 (4H, m), 2.26–2.62 (10H, m), 2.66–2.82 (2H, m), 3.08 (2H, t, J = 7.5 Hz), 3.90–4.15 (1H, br s), 4.70 (1H, dd, J = 4.4, 5.3 Hz), 7.02 (2H, t, J = 8.8 Hz), 7.20–7.55 (6H, m), 7.73 (1H, d J = 7.7 Hz), 7.79–7.92 (1H, m), 7.98–8.10 (1H, m); MS (ESI, Pos) m/z 407 $(M+H)^+$.

5.1.3. (±)-*tert*-Butyl 4-(1-(4-fluorophenyl)-2-{4-[4-(1-naphthyl)butyl]piperazin-1-yl}ethyl)piperazine-1-carboxylate (15). A mixture of (±)-1-(4-fluorophenyl)-2-{4-[4-(1-naphthyl)butyl]piperazin-1-yl}ethanol **14** (1.25 g, 2.87 mmol) and $SOCl_2$ (0.450 mL, 6.19 mmol) in benzene (8 mL) was stirred at 50 °C for 3 h. The mixture was partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$. The separated organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo. A mixture of the residue and *tert*-butyl piperazine-1-carboxylate **7** (1.12 g, 6.03 mmol) in benzene (10 mL) was stirred at 70 °C for 3 h. The mixture was partitioned between EtOAc and saturated aqueous $NaHCO_3$. The separated organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was chromatographed on Silica gel C-200 ($CHCl_3$/MeOH 20:1) to obtain (±)-*tert*-butyl 4-(1-(4-fluorophenyl)-2-{4-[4-(1-naphthyl)butyl]piperazin-1-yl}ethyl)piperazine-1-carboxylate **15** (619 mg, 38%) as an oily product: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.42 (9H, s), 1.60–1.82 (4H, m), 2.20–2.70 (15H, m), 2.85 (1H, dd, J = 6.2, 13.0 Hz), 3.07 (2H, t, J = 7.5 Hz), 3.37 (4H, t, J = 5.1 Hz), 3.60 (1H, t, J = 6.7 Hz), 7.00 (2H, t, J = 8.8 Hz), 7.14–7.56 (6H, m), 7.70 (1H, d J = 7.5 Hz), 7.79–7.90 (1H, m), 8.01–8.10 (1H, m); MS (ESI, Pos) m/z 575 $(M+H)^+$.

5.1.4. (±)-1-[2-(4-Fluorophenyl)-2-piperazin-1-ylethyl]-4-[4-(1-naphthyl)butyl]piperazine 4 hydrochloride (16, (±)-10ak). To a solution of (±)-*tert*-butyl 4-(1-(4-fluorophenyl)-2-{4-[4-(1-naphthyl)butyl]piperazin-1-yl}ethyl)piperazine-1-carboxylate **15** (619 mg, 1.08 mmol) in a mixture of AcOEt (3 mL) and MeOH (3 mL) was added 4 M HCl in AcOEt (4 mL) and the mixture was stirred at room temperature for 6 h. The resulting precipitate was collected by filtration and washed with AcOEt to obtain (±)-1-[2-(4-fluorophenyl)-2-piperazin-1-ylethyl]-4-[4-(1-naphthyl)butyl]piperazine 4 hydrochloride (**16**) ((±)-**10ak**, 425 mg, 64%) as a crystal: mp 180–182 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.60–2.03 (4H, m), 2.38–2.75 (2H, m), 2.79–2.98 (1H, m), 3.01–4.62 (21H, m), 7.21–7.62 (8H, m), 7.69–7.83 (1H, m), 7.83–7.97 (1H, m), 8.02–8.17 (1H, m), 8.88–9.13 (3H, m) 11.6–11.8 (1H, m); MS (ESI, Pos) m/z 475 $(M+H)^+$; Anal. ($C_{30}H_{39}FN_4$·4HCl·2.5H_2O) C, H, N.

5.1.5. (±)-1-[2-(4-Fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]-4-[4-(1-naphthyl)butyl]piperazine 3 maleate ((±)-10an). (±)-1-[2-(4-Fluorophenyl)-2-piperazin-1-ylethyl]-4-[4-(1-naphthyl)butyl]piperazine 4 hydrochloride **16** (200 mg, 0.308 mmol) was dissolved in DMF (0.7 mL) and the solution was cooled in ice-bath. To the cooled solution was added 60% NaH in oil (74.0 mg, 1.85 mmol) and the suspension was stirred at room temperature for 10 min. After addition of 2-iodopropane (203 mg, 1.19 mmol) to the mixture, the resulting mixture was stirred at room temperature overnight. The mixture was partitioned between AcOEt and water. The separated organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was chromatographed on Chromatorex NH (hexane/EtOAc 1:1) to obtain (±)-1-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]-4-[4-(1-naphthyl)butyl]piperazine (130 mg, 82%) as an oily product: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.00 (6H, d, J = 6.4 Hz), 1.52–1.88 (4H, m), 2.28–2.64 (19H, m), 2.64 (1H, dd, J = 7.0, 13.0 Hz), 2.86 (1H, dd, J = 5.7, 13.0 Hz), 3.07 (2H, dd, J = 7.0, 8.1 Hz), 3.54 (1H, t, J = 6.4 Hz), 6.93–7.02 (2H, m), 7.07 (2H, d,

J = 7.9 Hz), 7.16–7.53 (5H, m), 7.82–7.87 (1H, m), 8.02–8.07 (1H, m); MS (ESI, Pos) m/z 517 $(M+H)^+$. The above free amine was dissolved in EtOH (1.5 mL) and a solution of maleic acid (88 mg, 0.76 mmol) in EtOH (1 mL) was added. After stirred for 2 h, precipitated crystal was collected by filtration to obtain (±)-1-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]-4-[4-(1-naphthyl)butyl]piperazine 3 maleate (±)-**10an** (182 mg, 68%) as a crystal: mp 127–129 °C (EtOH); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.19 (6H, d, J = 6.6 Hz), 1.60–1.78 (4H, m), 2.58–3.53 (23H, m), 3.90–4.02 (1H, m), 6.13 (6H, s), 7.23 (2H, t, J = 7.5 Hz), 7.32–7.61 (6H, m), 7.80 (1H, d, J = 7.5 Hz), 7.91–7.96 (1H, m), 8.06–8.10 (1H, m); MS (ESI, Pos) m/z 517 $(M+H)^+$; Anal. ($C_{33}H_{45}FN_4{\cdot}3C_4H_4O_4{\cdot}0.5H_2O$) C, H, N.

Compounds (±)-**10al** and (±)-**10am** were prepared by using methods of Sections 5.1.1–5.1.5.

5.1.6. (±)-1-Ethyl-4-(1-(4-fluorophenyl)-2-{4-[4-(1-naphthyl)butyl]piperazin-1-yl}ethyl)piperazine 3 maleate ((±)-10al). Compound (±)-**10al** was obtained as a crystal. Mp 120–122 °C (EtOH); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.18 (3H, t, J = 7.3 Hz), 1.58–1.80 (4H, m), 2.60–3.70 (24H, m), 3.88–4.01 (1H, m), 6.11 (6H, s), 7.22 (2H, d, J = 8.8 Hz), 7.25–7.61 (6H, m), 7.80 (1H, d, J = 7.7 Hz), 7.88– 7.98 (1H, m), 8.01– 8.07 (1H, m); MS (ESI, Pos) m/z 503 $(M+H)^+$; Anal. ($C_{32}H_{43}FN_4{\cdot}3C_4H_4O_4{\cdot}1.0H_2O$) C, H, N.

5.1.7. (±)-1-(1-(4-Fluorophenyl)-2-{4-[4-(1-naphthyl)butyl]piperazin-1-yl}ethyl)-4-propylpiperazine 3 maleate ((±)-10am). Compound (±)-**10am** was obtained as a crystal. Mp 150–152 °C (EtOH); ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.88 (3H, t, J = 7.5 Hz), 1.45–1.82 (6H, m), 2.55–3.50 (24H, m), 3.83–4.01 (1H, m), 6.11 (6H, s), 7.22 (2H, d, J = 8.9 Hz), 7.27–7.62 (6H, m), 7.80 (1H, d, J = 7.5 Hz), 7.91–7.99 (1H, m), 8.02–8.09 (1H, m); MS (ESI, Pos) m/z 517 $(M+H)^+$; Anal. ($C_{33}H_{45}FN_4{\cdot}3C_4H_4O_4{\cdot}0.7H_2O$) C, H, N.

5.2. General methods for the synthesis of 10ao–10bd and 10bk–10bm (method B)

5.2.1. (±)-1-(1-(4-Fluorophenyl)-2-{4-[4-(2-methoxy-1-naphthyl)butyl]piperazin-1-yl}ethyl)-4-methylpiperazine 3 maleate ((±)-10bc). (±)-1-(4-Fluorophenyl)-2-{4-[4-(2-methoxy-1-naphthyl)butyl]piperazin-1-yl}ethanol **14** (2.00 g, 4.60 mmol) was dissolved in CH_2Cl_2 (20 mL), and to the cooled solution in ice-bath were added Et_3N (1.28 mL, 9.20 mmol) and MsCl (0.710 mL, 9.20 mmol). After stirring at the same temperature for 30 min, to the mixture were added Et_3N (0.640 mL, 4.60 mmol) and 1-methylpiperazine **6** (1.02 mL, 9.20 mmol), and the mixture was stirred at room temperature for 3 h. The mixture was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$. The separated organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was chromatographed on Chromatorex NH (hexane/EtOAc 4:1) to obtain (±)-1-(1-(4-fluorophenyl)-2-{4-[4-(2-methoxy-1-naphthyl)-butyl]piperazin-1-yl}ethyl)-4-methylpiperazine (2.30 g, 96%) as an oily product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.49–1.66 (4H, m), 2.24 (3H, s), 2.24–2.60 (18H, m), 2.62 (1H, dd, J = 7.0, 12.9 Hz), 2.85 (1H, dd, J = 5.9, 12.9 Hz), 2.99–3.13 (2H, m), 3.55 (1H, dd, J = 5.9, 7.0 Hz), 3.90 (3H, s), 6.94–7.02 (2H, m), 7.18–7.36 (4H, m), 7.41–7.50 (1H, m), 7.69–7.80 (2H, m), 7.95 (1H, d, J = 8.4 Hz); MS (ESI, Pos) m/z 519 $(M+H)^+$. The above free amine (370 mg, 0.713 mmol) was dissolved in EtOH (3.7 mL), and to the solution was added a solution of maleic acid (248 mg, 2.14 mmol) in EtOH (2.5 mL). After stirred at room temperature for 1 h, the resulting precipitate was collected by filtration to obtain (±)-1-(1-(4-fluorophenyl)-2-{4-[4-(2-methoxy-1-naphthyl)butyl]piperazin-1-yl}ethyl)-4-methylpiperazine 3 maleate (±)-**10bc** (480 mg, 78%) as a crystal: mp 173–175 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.48–1.76 (4H, m), 2.73 (3H, s), 2.63–3.51 (22H, m), 3.91 (3H, s), 3.87–3.95 (1H, m), 6.11 (6H, s), 7.21 (2H, t, J = 8.9 Hz), 7.28–7.58 (4H, m), 7.85–7.89 (3H, m), 7.95 (1H, d, J = 8.6 Hz); MS (ESI, Pos) m/z 519 $(M+H)^+$; Anal. ($C_{32}H_{43}FN_4O{\cdot}3C_4H_4O_4{\cdot}0.5H_2O$) C, H, N.

5.2.2. (±)-1-Cyclopropyl-4-(1-(4-fluorophenyl)-2-{4-[4-(1-naphthyl)butyl]piperazin-1-yl}ethyl)piperazine 3 maleate ((±)-10ao). Compound (±)-**10ao** was obtained as a crystal. Mp 161–163 °C (EtOH); ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.55–0.72 (4H, m), 1.52–1.79 (4H, m), 2.50–3.55 (23H, m), 4.03–4.21 (1H, m), 6.13 (6H, s), 7.24 (2H, t, J = 8.8 Hz), 7.32–7.61 (6H, m), 7.79 (1H, d, J = 7.3 Hz), 7.88–7.99 (1H, m), 8.02–8.16 (1H, m); MS (ESI, Pos) m/z 515 $(M+H)^+$; Anal. ($C_{33}H_{43}FN_4{\cdot}3C_4H_4O_4{\cdot}0.1H_2O$) C, H, N.

5.2.3. (±)-1-Cyclohexyl-4-(1-(4-fluorophenyl)-2-{4-[4-(1-naphthyl)butyl]piperazin-1-yl}ethyl)piperazine 3 maleate ((±)-10ap). Compound (±)-**10ap** was obtained as a crystal. Mp 171–173 °C (EtOH); ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.96–1.41 (4H, m), 1.50–1.82 (6H, m), 1.84–2.10 (4H, m), 2.55–3.50 (19H, m), 3.90–4.02 (1H, m), 6.11 (6H, s), 7.22 (2H, d, J = 8.9 Hz), 7.28–7.61 (6H, m), 7.80 (1H, d, J = 8.1 Hz), 7.89–7.99 (1H, m), 8.03–8.13 (1H, m); MS (ESI, Pos) m/z 557 $(M+H)^+$; Anal. ($C_{36}H_{49}FN_4{\cdot}3C_4H_4O_4{\cdot}0.5H_2O$) C, H, N.

5.2.4. (±)-1-(1-(4-Fluorophenyl)-2-{4-[4-(1-naphthyl)butyl]-piperazin-1-yl}ethyl)-4-phenylpiperazine 2 maleate ((±)-10aq). Compound (±)-**10aq** was obtained as a crystal. Mp 172–174 °C (EtOH/Et_2O); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.55–1.80 (4H, m), 2.55–3.60 (22H, m), 4.08–4.30 (1H, m), 6.11 (4H, s), 6.88 (1H, t, J = 7.1 Hz), 6.90 (2H, d, J = 7.9 Hz), 7.12–7.36 (3H, m), 7.38–7.63 (7H, m), 7.80 (1H, d, J = 7.7 Hz), 7.88–7.99 (1H, m), 8.02–8.16 (1H, m); MS (ESI, Pos) m/z 551 $(M+H)^+$; Anal. ($C_{36}H_{43}FN_4{\cdot}2C_4H_4O_4{\cdot}0.5H_2O$) C, H, N.

5.2.5. (±)-1-Methyl-4-(2-{4-[4-(1-naphthyl)butyl]piperazin-1-yl}-1-phenylethyl)piperazine 3 maleate ((±)-10ar). Compound (±)-**10ar** was obtained as a crystal. Mp 167–169 °C (EtOH); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.58–1.80 (4H, m), 2.60–3.80 (22H, m), 2.74 (3H, s), 3.91–3.99 (1H, m), 6.10 (6H, s), 7.22–7.60 (9H, m), 7.78 (1H, d, J = 7.7 Hz), 7.88–7.98 (1H, m), 8.02–8.11 (1H, m); MS (ESI, Pos) m/z 471 $(M+H)^+$; Anal. ($C_{31}H_{42}N_4{\cdot}3C_4H_4O_4$) C, H, N.

5.2.6. (±)-1-[2-(3-Fluorophenyl)-2-(4-methylpiperazin-1-yl)ethyl]-4-[4-(1-naphthyl)butyl]piperazine 3 maleate ((±)-10as). Compound (±)-**10as** was obtained as a crystal. Mp 173–175 °C (EtOH); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.60–1.78 (4H, m), 2.65–3.47 (22H, m), 2.73 (3H, s), 3.92–3.99 (1H, m), 6.11 (6H, s), 7.04–7.22 (3H, m), 7.35–7.61 (5H, m), 7.79 (1H, d, J = 8.6 Hz), 7.88–7.99 (1H, m), 8.01–8.13 (1H, m); MS (ESI, Pos) m/z 489 $(M+H)^+$; Anal. ($C_{31}H_{41}FN_4{\cdot}3C_4H_4O_4$) C, H, N.

5.2.7. (±)-1-[2-(4-Chlorophenyl)-2-(4-methylpiperazin-1-yl)ethyl]-4-[4-(1-naphthyl)butyl]piperazine 3 maleate ((±)-10at). Compound (±)-**10at** was obtained as a crystal. Mp 175–177 °C (EtOH); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.60–1.79 (4H, m), 2.62–3.44 (22H, m), 2.74 (3H, s), 3.91–3.97 (1H, m), 6.10 (6H, s), 7.28–7.59 (8H, m), 7.79 (1H, d, J = 7.3 Hz), 7.87–7.98 (1H, m), 8.02–8.11 (1H, m); MS (ESI, Pos) m/z 505 $(M+H)^+$; Anal. ($C_{31}H_{41}ClN_4{\cdot}3C_4H_4O_4{\cdot}1.0H_2O$) C, H, N.

5.2.8. (±)-1-[2-(2-Bromophenyl)-2-(4-methylpiperazin-1-yl)ethyl]-4-[4-(1-naphthyl)butyl]piperazine 3 maleate ((±)-10au). Compound (±)-**10au** was obtained as an amorphous. ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.58–1.78 (4H, m), 2.40–3.65 (22H, m), 2.77 (3H, s), 4.05–4.24 (1H, m), 6.05 (6H, s), 7.19–7.30 (1H, m), 7.36–7.58 (6H, m), 7.63 (1H, d, J = 8.0 Hz), 7.78 (1H, d, J = 7.5 Hz), 7.88–7.98 (1H, m), 8.02–8.12 (1H, m); MS (ESI, Pos) m/z 549 $(M+H)^+$, 551 $(M^+{+}2{+}H)^+$; Anal. ($C_{31}H_{41}BrN_4{\cdot}3C_4H_4O_4{\cdot}1.0H_2O$) C, H, N.

5.2.9. (±)-1-[2-(3-Bromophenyl)-2-(4-methylpiperazin-1-yl)ethyl]-4-[4-(1-naphthyl)butyl]piperazine 3 maleate ((±)-10av). Compound (±)-**10av** was obtained as a crystal. Mp 119–121 °C (EtOH); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.59–1.79 (4H, m), 2.60–3.60 (22H, m), 2.74 (3H, s), 3.93–4.00 (1H, m), 6.12 (6H, s), 7.23–7.61 (8H, m), 7.78 (1H, d, J = 7.7 Hz), 7.85–7.98 (1H, m), 8.02–8.14 (1H, m); MS (ESI, Pos) m/z 549 $(M+H)^+$, 551 $(M^+{+}2{+}H)$; Anal. ($C_{31}H_{41}BrN_4{\cdot}3C_4H_4O_4{\cdot}1.0H_2O$) C, H, N.

5.2.10. (±)-1-[2-(4-Bromophenyl)-2-(4-methylpiperazin-1-yl)ethyl]-4-[4-(1-naphthyl)butyl]piperazine 3 maleate ((±)-10aw). Compound (±)-**10aw** was obtained as a crystal. Mp 168–170 °C (EtOH); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.59–1.80 (4H, m), 2.55–3.70 (22H, m), 2.73 (3H, s), 3.91–3.98 (1H, m), 6.12 (6H, s), 7.25 (2H, d, J = 8.6 Hz), 7.36–7.63 (6H, m), 7.78 (1H, d, J = 7.7 Hz), 7.89–7.99 (1H, m), 8.01–8.12 (1H, m); MS (ESI, Pos) m/z 549 $(M+H)^+$, 551 $(M^+{+}2{+}H)^+$; Anal. ($C_{31}H_{41}BrN_4{\cdot}3C_4H_4O_4{\cdot}1.0H_2O$) C, H, N.

5.2.11. (±)-1-Methyl-4-(1-(4-methylphenyl)-2-{4-[4-(1-naphthyl)butyl]piperazin-1-yl}ethyl)piperazine 3 maleate ((±)-10ax). Compound (±)-**10ax** was obtained as a crystal. Mp 186–188 °C (EtOH); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.58–1.78 (4H, m), 2.30 (3H, s), 2.60–3.50 (22H, m), 2.75 (3H, s), 3.89–3.93 (1H, m), 6.10 (6H, s), 7.10–7.23 (4H, m), 7.36–7.60 (4H, m), 7.78 (1H, d, J = 7.9 Hz), 7.89–7.99 (1H, m), 8.03–8.11 (1H, m); MS (ESI, Pos) m/z 485 $(M+H)^+$; Anal. ($C_{32}H_{44}N_4{\cdot}3C_4H_4O_4$) C, H, N.

5.2.12. (±)-1-[2-(4-Methoxyphenyl)-2-(4-methylpiperazin-1-yl)ethyl]-4-[4-(1-naphthyl)butyl]piperazine 3 maleate ((±)-10ay). Compound (±)-**10ay** was obtained as a crystal. Mp 138–140 °C (EtOH); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.59–1.78 (4H, m), 2.60–3.50 (22H, m), 2.72 (3H, s), 3.75 (3H, s), 3.80–3.95 (1H, m), 6.11 (6H, s), 6.93 (2H, d, J = 8.8 Hz), 7.20 (2H, d, J = 8.6 Hz), 7.36–7.61 (4H, m), 7.79 (2H, d, J = 7.7 Hz), 7.91–7.98 (1H, m), 8.03–8.11 (1H, m); MS (ESI, Pos) m/z 501 $(M+H)^+$; Anal. ($C_{32}H_{44}N_4O{\cdot}3C_4H_4O_4{\cdot}1.0H_2O$) C, H, N.

5.2.13. (±)-1-Methyl-4-{2-{4-[4-(1-naphthyl)butyl]piperazin-1-yl}-1-[4-(trifluoromethyl)phenyl]ethyl}piperazine 3 maleate ((±)-10az). Compound (±)-**10az** was obtained as a crystal. Mp 128–130 °C (EtOH); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.60–1.80 (4H, m), 2.74 (3H, s), 2.60–3.55 (22H, m), 3.96–4.06 (1H, m), 6.12 (6H, s), 7.33–7.59 (6H, m), 7.70–7.83 (3H, m), 7.88–7.96 (1H, m), 8.02–8.11 (1H, m); MS (ESI, Pos) m/z 539 $(M+H)^+$; Anal. ($C_{32}H_{41}F_3N_4{\cdot}3C_4H_4O_4{\cdot}1.0H_2O$) C, H, N.

5.2.14. (±)-1-Methyl-4-[2-{4-[4-(1-naphthyl)butyl]piperazin-1-yl}-1-(4-nitrophenyl)ethyl]piperazine 3 maleate ((±)-10ba). Compound (±)-**10ba** was obtained as a crystal. Mp 176–178 °C (EtOH); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.62–1.76 (4H, m), 2.77 (3H, s), 2.60–3.65 (22H, m), 3.97–4.07 (1H, m), 6.10 (6H, s), 7.34–7.63 (6H, m), 7.78 (1H, d, J = 7.7 Hz), 7.89–7.96 (1H, m), 8.03–8.11 (1H, m), 8.24 (2H, d, J = 8.8 Hz); MS (ESI, Pos) m/z 516 $(M+H)^+$; Anal. ($C_{31}H_{41}N_5O_2{\cdot}3C_4H_4O_4$) C, H, N.

5.2.15. (±)-4-(1-(4-Methylpiperazin-1-yl)-2-{4-[4-(1-naphthyl)butyl]piperazin-1-yl}ethyl)aniline 3 maleate ((±)-10bd). A suspension of (±)-1-methyl-4-[2-{4-[4-(1-naphthyl)butyl]piperazin-1-yl}-1-(4-nitrophenyl)ethyl]piperazine (free of (±)-**10ba**) (331 mg, 0.540 mmol) and PtO_2 (10 mg) in EtOH (20 mL) was stirred under a hydrogen atmosphere for 2 h. The mixture was filtered through Celite and the filtrate was concentrated in vacuo. The residue was dissolved in EtOH (2 mL), and to the solution was added a solution of maleic acid (187 mg, 1.51 mmol) in EtOH (1 mL). After stirred for at room temperature 1 h, the resulting precipitate was collected by filtration to obtain (±)-4-(1-(4-methylpiperazin-1-yl)-2-{4-[4-(1-naphthyl)butyl]piperazin-1-yl}ethyl)aniline 3 maleate (±)-**10bd** (350 mg, 78% yield) as a crystal: mp 152–154 °C (EtOH); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.50–1.80 (4H, m), 2.30–3.20 (27H, m), 3.56–3.63 (1H, m), 6.01 (6H, s), 6.35–6.60 (2H, m), 6.63 (1H, s), 6.90–7.05 (1H, m), 7.25–7.60 (4H, m), 7.65–7.81 (1H, m), 7.83–7.98 (1H, m), 8.00–8.15 (1H, m); MS (ESI, Pos) m/z 486 $(M+H)^+$; Anal. ($C_{31}H_{43}N_5{\cdot}3C_4H_4O_4{\cdot}0.5H_2O$) C, H, N.

5.2.16. (±)-1-[2-Biphenyl-4-yl-2-(4-methylpiperazin-1-yl)ethyl]-4-[4-(1-naphthyl)butyl]piperazine 3 maleate ((±)-10bb). Compound (±)-**10bb** was obtained as a crystal. Mp 123–125 °C (EtOH); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.58–1.81 (4H, m), 2.60–3.70 (22H, m), 2.73 (3H, s), 3.90–4.03 (1H, m), 6.11 (6H, s), 7.30–7.60 (8H, m), 7.61–7.77 (5H, m), 7.78 (1H, d, J = 8.4 Hz), 7.88–7.99 (1H, m), 8.01–8.13 (1H, m); MS (ESI, Pos) m/z 547 $(M+H)^+$; Anal. ($C_{37}H_{46}N_4{\cdot}3C_4H_4O_4{\cdot}1.1H_2O$) C, H, N.

5.2.17. (±)-1-(1-Ethylpropyl)-4-(1-(4-fluorophenyl)-2-{4-[4-(2-methoxy-1-naphthyl)butyl]piperazin-1-yl}ethyl)piperazine 3 maleate ((±)-10bk). Compound (±)-**10bk** was obtained as a crystal. Mp 141–143 °C (EtOH); ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.90 (6H, t, J = 7.3 Hz), 1.40–1.80 (8H, m), 2.80–3.40 (23H, m), 3.92 (3H, s), 3.95–4.05 (1H, m), 6.12 (6H, s), 7.22 (2H, d, J = 8.8 Hz), 7.29–7.54 (5H, m), 7.80–7.88 (2H, m), 7.96 (1H, d, J = 8.6 Hz); MS (ESI, Pos) m/z 575 $(M+H)^+$; Anal. ($C_{36}H_{51}FN_4O{\cdot}3C_4H_4O_4{\cdot}0.5H_2O$) C, H, N.

5.2.18. (±)-1-Cyclopentyl-4-(1-(4-fluorophenyl)-2-{4-[4-(2-methoxy-1-naphthyl)butyl]piperazin-1-yl}ethyl)piperazine 3 maleate ((±)-10bl). Compound (±)-**10bl** was obtained as a crystal. Mp 163–164 °C (EtOH); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.40–1.80 (10H, m), 1.90–2.10 (2H, m), 2.60–3.60 (23H, m), 3.92 (3H, s), 3.95–4.01 (1H, m), 6.22 (6H, s), 7.22 (2H, t, J = 8.9 Hz), 7.30–7.54 (5H, m), 7.80–7.88 (2H, m), 7.96 (1H, d, J = 8.4 Hz); MS (ESI, Pos) m/z 573 $(M+H)^+$; Anal. ($C_{36}H_{49}FN_4O{\cdot}3C_4H_4O_4{\cdot}0.5H_2O$) C, H, N.

5.2.19. (±)-1-*tert*-Butyl-4-(1-(4-fluorophenyl)-2-{4-[4-(2-methoxy-1-naphthyl)butyl]piperazin-1-yl}ethyl)piperazine 3 maleate ((±)-10bm). Compound (±)-**10bm** was obtained as a crystal. Mp 116–118 °C (EtOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.27 (9H, s), 1.49–1.62 (2H, m), 1.63–1.79 (2H, m), 2.80–3.30 (19H, m), 3.33–3.49 (3H, m), 3.92 (3H, s), 3.98–4.06 (1H, m), 6.16 (6H, s), 7.24 (2H, t, J = 9.0 Hz), 7.31–7.56 (5H, m), 7.83 (2H, m), 7.96 (1H, d, J = 8.9 Hz); MS (ESI, Pos) m/z 547 $(M+H)^+$; Anal. ($C_{35}H_{49}FN_4O{\cdot}3C_4H_4O_4{\cdot}1.5H_2O$) C, H, N.

5.3. General methods for the synthesis of 10be (method C)

5.3.1. (±)-Ethyl 4-[2-(4-fluorophenyl)-2-hydroxyethyl]piperazine-1-carboxylate hydrochloride (18). A solution of 2-chloro-1-(4-fluorophenyl)ethanone **12** (8.63 g, 50.0 mmol), ethyl piperazine-1-carboxylate **8** (16.0 g, 101 mmol) in $CHCl_3$ (60 mL) was heated at reflux for 3 h. The mixture was concentrated in vacuo, and the residue was partitioned between Et_2O and 25% aqueous NH_3. The separated organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo to obtain crude (±)-ethyl 4-[2-(4-fluorophenyl)-2-oxoethyl]piperazine-1-carboxylate **17**. The crude **17** was dissolved in EtOH (80 mL), and to the solution were added $NaBH_4$ (2.00 g, 52.6 mmol) and a mixture of H_2O (10 mL) and one drop of 5% aqueous KOH. After stirring the mixture at 50 °C for 1 h, the resulting mixture was concentrated in vacuo. The residue was partitioned between Et_2O and 25% aqueous NH_3. The separated organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo. To the residue was added 4 M HCl in AcOEt (30 mL), the mixture was concentrated in vacuo. The resulting solid was collected by filtration with washed with Et_2O to obtain crude (±)-ethyl 4-[2-(4-fluorophenyl)-2-hydroxyethyl]piperazine-1-carboxylate hydrochloride **18** (18.0 g, quantitative yield): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.20 (3H, t, J = 7.0 Hz), 3.00–3.68 (8H, m), 3.90–4.11 (5H, m), 5.17–5.27 (1H, m), 7.22 (2H, t, J = 8.9 Hz), 7.42–7.51 (2H, m), 9.45–9.60 (1H, m), 10.70–10.85 (1H, m); MS (ESI, Pos) m/z 297 $(M+H)^+$.

5.3.2. (±)-Ethyl 4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine-1-carboxylate (19). A mixture of (±)-ethyl 4-[2-(4-fluorophenyl)-2-hydroxyethyl]piperazine-1-carboxylate hydrochloride **18** (10.0 g, 30.1 mmol) and $SOCl_2$ (3.30 mL, 45.2 mmol) in benzene (30 mL) was stirred at 50 °C for 10 min and concentrated in vacuo. The residue was partitioned between AcOEt and 5% aqueous NH_3. The separated organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to obtain crude (±)-ethyl 4-[2-chloro-2-(4-fluorophenyl)ethyl]piperazine-1-carboxylate. A mixture of the above crude product, 1-isopropylpiperazine 2 hydrochloride (12.1 g, 60.2 mmol), and ^{i}Pr$_2$NEt (21.3 mL, 120 mmol) in benzene (50 mL) was stirred at 60 °C for 6 h. The mixture was partitioned between Et_2O and saturated aqueous $NaHCO_3$. The separated organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was chromatographed on Chromatorex NH (hexane/EtOAc 4:1) to obtain (±)-ethyl 4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine-1-carboxylate **19** (9.60 g, 78%) as an oily product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.01 (6H, d, J = 6.5 Hz), 1.22 (3H, t, J = 7.0 Hz), 2.25–2.89 (14H, m), 3.29–3.43 (5H, m), 3.50–3.58 (1H, m), 4.02–4.13 (2H, m), 6.89–7.01 (2H, m), 7.12–7.21 (2H, m); MS (ESI, Pos) m/z 407 $(M+H)^+$.

5.3.3. (±)-1-[1-(4-Fluorophenyl)-2-piperazin-1-ylethyl]-4-isopropylpiperazine (20). A mixture of (±)-ethyl 4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine-1-carboxylate **19** (2.00 g, 4.92 mmol) and KOH (2.00 g) in EtOH (4 mL) was stirred at reflux for 1 h. The mixture was partitioned between EtOAc and water, and separated organic phase was washed with brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo to obtain crude (±)-1-[1-(4-fluorophenyl)-2-piperazin-1-ylethyl]-4-isopropylpiperazine **20** (1.39 g, 85%) as an oily product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.01 (6H, d, J = 6.5 Hz), 1.22 (3H, t, J = 7.0 Hz), 2.25–2.89 (14H, m), 3.29–3.43 (5H, m), 3.50–3.58 (1H, m), 4.02–4.13 (2H, m), 6.89–7.01 (2H, m), 7.12–7.21 (2H, m); MS (ESI, Pos) m/z 335 $(M+H)^+$. This product was used in the next step without further purification.

5.3.4. (±)-1-[4-(2-Bromo-1-naphthyl)butyl]-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine 3 maleate ((±)-10be). A mixture of a crude (±)-1-[1-(4-fluorophenyl)-2-piperazin-1-ylethyl]-4-isopropylpiperazine **20** (0.39 g, 1.17 mmol), 2-bromo-1-(4-bromobutyl)naphthalene **9b** (0.56 g, 1.64 mmol), and Et_3N (0.48 mL, 3.47 mmol) in DMF (5 mL) was heated at 60 °C for 5 h. The mixture was partitioned between EtOAc and saturated aqueous $NaHCO_3$, and separated organic phase was washed with brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was chromatographed on Chromatorex NH (hexane/EtOAc 9:1) to obtain (±)-1-[4-(2-bromo-1-naphthyl)butyl]-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine (0.14 g, 20%) as an oily product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.02 (6H, d, J = 6.5 Hz), 1.60–1.73 (4H, m), 2.30–2.61 (20H, m), 2.62 (1H, dd, J = 7.0, 13.0 Hz), 2.85 (1H, dd, J = 5.7,

13.0 Hz), 3.18–3.29 (1H, m), 3.53 (1H, t, J = 6.4 Hz), 6.99 (2H, t, J = 8.5 Hz), 7.18–7.23 (2H, m), 7.43–7.62 (4H, m), 7.80 (1H, d, J = 8.0 Hz), 8.06 (2H, d, J = 8.9 Hz); MS (ESI, Pos) m/z 595 $(M+H)^+$, 597 $(M+2+H)^+$, 617 $(M+Na)^+$, 619 $(M+2+Na)^+$. The above free base (0.14 g, 0.24 mmol) was dissolved in EtOH (2.0 mL), and to the solution was added a solution of maleic acid (82 mg, 0.71 mmol) in EtOH (1.0 mL). After stirred at room temperature for 1 h, the resulting precipitate was collected by filtration to obtain (±)-1-[4-(2-bromo-1-naphthyl)butyl]-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine 3 maleate (±)-**10be** (0.16 g, 72%) as a crystal: mp 147–150 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.20 (6H, d, J = 6.6 Hz), 1.55–1.70 (2H, m), 1.75–1.85 (2H, m), 2.90–3.50 (23H, m), 3.98–4.06 (1H, m), 6.16 (6H, s), 7.23 (2H, d, J = 8.8 Hz), 7.28–7.42 (2H, m), 7.55–7.80 (4H, m), 7.94– 8.02 (1H, m), 8.08–8.20 (1H, m); MS (ESI, Pos) m/z 595 $(M+H)^+$, 597 $(M+2+H)^+$; Anal. ($C_{33}H_{46}N_4OBrF\cdot3C_4H_4O_4\cdot2.5H_2O$) C, H, N.

5.4. General methods for the synthesis of 10aa–10aj, 10bf–10bj, and 10bn–10cj (method D)

5.4.1. (±)-1-[2-(4-Fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]-4-[4-(2-methoxy-1-naphthyl)butanoyl]piperazine (21). To a mixture of a crude (±)-1-[1-(4-fluorophenyl)-2-piperazin-1-ylethyl]-4-isopropylpiperazine **20** (0.74 g, 2.2 mmol), 4-(2-methoxy-1-naphthyl)butanoic acid (0.59 g, 2.4 mmol), and HOBt·H_2O (0.51 g, 3.3 mmol) in DMF (10 mL) was added EDC·HCl (0.63 g, 3.3 mmol) and the mixture was stirred at room temperature overnight. The mixture was partitioned between EtOAc and saturated aqueous $NaHCO_3$, and separated organic phase was washed with brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was chromatographed on Silica gel C-200 ($CHCl_3$/MeOH 10:1) to obtain (±)-1-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]-4-[4-(2-methoxy-1-naphthyl)butanoyl]piperazine **21** (0.61 g, 49%) as an oily product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.02 (6H, d, J = 6.5 Hz), 1.65–1.99 (6H, m), 2.22–2.59 (11H, m), 2.62 (1H, dd, J = 7.0, 13.0 Hz), 2.82 (1H, dd, J = 5.7, 13.0 Hz), 3.12 (2H, t, J = 8.0 Hz), 3.21–3.27 (2H, m), 3.41–3.58 (3H, m), 3.92 (3H, s), 6.89–7.01 (2H, m), 7.10–7.21 (2H, m), 7.24–7.35 (2H, m), 7.40–7.49 (1H, m), 7.70–7.79 (2H, m), 8.01 (1H, d, J = 9.0 Hz); MS (ESI, Pos) m/z 361 $(M+H)^+$.

5.4.2. (±)-1-[2-(4-Fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]-4-[4-(2-methoxy-1-naphthyl)butyl]piperazine 3 maleate ((±)-10bg). A mixture of (±)-1-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]-4-[4- (2-methoxy-1-naphthyl)butanoyl]piperazine **21** (0.53 g, 0.95 mmol) and $LiAlH_4$ (72 mg, 1.9 mmol) in THF (10 mL) was stirred at reflux for 1 h. To the mixture were added dropwise H_2O (2.0 mL) and 1 M NaOH aqueous (8.0 mL) at room temperature, and the mixture was extracted with Et_2O. The organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was chromatographed on Chromatorex NH (hexane/EtOAc 1:2) to obtain (±)-1-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]-4-[4-(2-methoxy-1-naphthyl)butyl]piperazine (0.47 g, 90%) as an oily product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.01 (6H, d, J = 6.5 Hz), 1.56–1.83 (5H, m), 2.25–3.70 (17H, m), 2.62 (1H, dd, J = 7.0, 13.0 Hz), 2.83 (1H, dd, J = 5.7, 13.0 Hz), 3.01–3.13 (2H, m), 3.55 (2H, t, J = 8.0 Hz), 3.94 (3H, s), 6.98 (2H, t, J = 8.0 Hz), 7.18–7.37 (4H, m), 7.42 (1H, t, J = 8.0 Hz), 7.69–7.80 (2H, m), 7.93 (1H, d, J = 8.0 Hz); MS (ESI, Pos) m/z 547 $(M+H)^+$. The above free base (0.45 g, 0.82 mmol) was dissolved in EtOH (3 mL), and to the solution was added a solution of maleic acid (0.29 g, 2.5 mmol) in EtOH (3 mL). After stirred at room temperature for 1 h, the resulting precipitate was collected by filtration to obtain (±)-1-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]-4-[4-(2-methoxy-1-naphthyl)butyl]piperazine 3 maleate (±)-**10bg** (0.49 g, 67%) as a crystal: mp 154–156 °C (EtOH); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.20 (6H, d, J = 6.6 Hz), 1.50–1.80 (4H, m), 2.80–3.50 (23H, m), 3.91 (3H, s), 3.90–4.02 (1H, m), 6.10 (6H, s), 7.22 (2H, t, J = 8.9 Hz), 7.30–7.53 (5H, m), 7.79–7.98 (3H, m); MS (ESI, Pos) m/z 547 $(M+H)^+$; Anal. ($C_{34}H_{47}FN_4O\cdot3C_4H_4O_4\cdot0.5H_2O$) C, H, N.

Each enantiomer was obtained from free base of (±)-**10bg** by using HPLC for optical resolution [Chiralpak AD (Daicel Chemical Industries, Ltd), 2.0 × 25 cm, mobile phase: hexane/2-propanol/diethylamine = 98:2:0.1, flow rate: 5.0 mL/min]. Compound **10bg** detected at a shorter retention time (0.21 g, 0.38 mmol) was dissolved in EtOH (4 mL), treated with 4 M HCl in AcOEt (1 mL) at room temperature, and the mixture was concentrated in vacuo. The residue was crystallized in EtOAc, and the precipitate was collected by filtration to obtain (−)-1-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]-4-[4-(2-methoxy-1-naphthyl)butyl]piperazine 4 hydrochloride (−)-**10bg** (0.15 g, 60%) as a crystal: $[\alpha]_D^{26}$ −13.1 (c 0.24, MeOH), mp 183–185 °C (EtOAc); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.23 (6H, d, J = 6.6 Hz), 1.48–1.70 (2H, m), 1.76–2.00 (2H, m), 2.10–2.28 (1H, m), 2.53–2.77 (1H, m), 2.90–3.81 (23H, m), 3.94 (3H, s), 3.98–4.62 (8H, m), 7.20–7.55 (7H, m), 7.77–8.02 (3H, m); MS (ESI, Pos) m/z 547 $(M+H)^+$; Anal. ($C_{34}H_{47}FN_4O\cdot4HCl\cdot1.0H_2O$) C, H, N. Compound **10bg** detected at a longer retention time gave (+)-**10bg** as a crystal. $[\alpha]_D^{26}$ +12.1 (c 0.20, MeOH), mp 183–185 °C (EtOAc); m/z 547 $(M+H)^+$; Anal. ($C_{34}H_{47}FN_4O\cdot4HCl\cdot1.0H_2O$) C, H, N. ^{1}H NMR was corresponding to that of (−)-**10bg**.

Compounds **10aa**–**10aj**, **10bf**, **10bh**–**10bj**, and **10bn**–**10cj** were prepared by using methods of Sections 5.4.1 and 5.4.2.

5.4.3. (±)-1-[2-(4-Fluorophenyl)-2-(4-methylpiperazin-1-yl)ethyl]-4-(1-naphthylmethyl)piperazine 3.5 maleate ((±)-10aa). Compound (±)-**10aa** was obtained as a crystal. Mp 173–175 °C (Et_2O); ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.60–3.63 (20H, m), 2.71 (3H, s), 3.98–4.31 (1H, m), 6.17 (6H, s), 7.21–7.38 (4H, m), 7.43–7.62 (4H, m), 7.89–8.01 (2H, m), 8.28–8.37 (1H, m); MS (ESI, Pos) m/z 447 $(M+H)^+$; Anal. ($C_{28}H_{35}FN_4\cdot3.5C_4H_4O_4$) C, H, N.

5.4.4. (±)-1-[2-(4-Fluorophenyl)-2-(4-methylpiperazin-1-yl)-ethyl]-4-[2-(1-naphthyl)ethyl]piperazine 3 maleate ((±)-10ab). Compound (±)-**10ab** was obtained as a crystal. Mp 174–176 °C (EtOH); ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.60–3.58 (25H, m), 3.69–3.79 (1H, m), 6.30 (6H, s), 6.99–7.12 (2H, m), 7.15–7.29 (2H, m), 7.31–7.62 (4H, m), 7.78–7.92 (2H, m), 7.93–8.02 (1H, m); MS (ESI, Pos) *m*/*z* 461 $(M+H)^+$; Anal. ($C_{29}H_{37}FN_4{\cdot}3C_4H_4O_4{\cdot}0.7H_2O$) C, H, N.

5.4.5. 1-[2-(4-Fluorophenyl)-2-(4-methylpiperazin-1-yl)ethyl]-4-[3-(1-naphthyl)propyl]piperazine 3 maleate ((±)-10ac). Compound (±)-**10ac** was obtained as a crystal. Mp 175–177 °C; NMR (200 MHz, DMSO-d_6) δ 1.95–2.05 (2H, m), 2.61–3.58 (22H, m), 2.73 (3H, s), 3.85–3.95 (1H, m), 6.11 (6H, s), 7.18–7.63 (8H, m), 7.81 (1H, d, J = 7.9 Hz), 7.90–7.99 (1H, m), 8.03–8.12 (1H, m); MS (ESI) *m*/*z* 475 $(M+H)^+$; Anal. ($C_{30}H_{39}FN_4{\cdot}3C_4H_4O_4$) C, H, N.

5.4.6. (±)-1-[2-(4-Fluorophenyl)-2-(4-methylpiperazin-1-yl)ethyl]-4-[4-(1-naphthyl)butyl]piperazine 3 maleate ((±)-10ad). Compound (±)-**10ad** was obtained as a crystal. Mp 174–176 °C (EtOH); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.58–1.80 (4H, m), 2.80–3.55 (22H, m), 2.71 (3H, s), 3.89–4.02 (1H, m), 6.12 (6H, s), 7.18–7.58 (8H, m), 7.79 (1H, d, J = 7.9 Hz), 7.88–7.97 (1H, m), 8.02–8.11 (1H, m); MS (ESI, Pos) *m*/*z* 489 $(M+H)^+$; Anal. ($C_{31}H_{41}FN_4{\cdot}3C_4H_4O_4$) C, H, N.

5.4.7. (±)-1-[2-(4-Fluorophenyl)-2-(4-methylpiperazin-1-yl)ethyl]-4-[5-(1-naphthyl)pentyl]piperazine 3 maleate ((±)-10ae). Compound (±)-**10ae** was obtained as a crystal. Mp 170–172 °C (EtOH); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.30–1.49 (2H, m), 1.50–1.79 (4H, m), 2.74 (3H, s), 2.80–3.43 (22H, m), 3.93–3.97 (1H, m), 6.11 (6H, s), 7.19–7.58 (8H, m), 7.79 (1H, d, J = 8.0 Hz), 7.86–7.97 (1H, m), 8.01–8.07 (1H, m); MS (ESI, Pos) *m*/*z* 503 $(M+H)^+$; Anal. ($C_{32}H_{43}FN_4{\cdot}3C_4H_4O_4$) C, H, N.

5.4.8. (±)-1-[2-(4-Fluorophenyl)-2-(4-methylpiperazin-1-yl)ethyl]-4-[6-(1-naphthyl)hexyl]piperazine 3 maleate ((±)-10af). Compound (±)-**10af** was obtained as a crystal. Mp 182–184 °C (EtOH); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.21–1.79 (8H, m), 2.60–3.75 (22H, m), 2.76 (3H, s), 3.92–3.98 (1H, m), 6.11 (6H, s), 7.18–7.60 (8H, m), 7.78 (1H, d, J = 8.0 Hz), 7.86–7.97 (1H, m), 8.01–8.07 (1H, m); MS (ESI, Pos) *m*/*z* 517 $(M+H)^+$; Anal. ($C_{33}H_{45}FN_4{\cdot}3C_4H_4O_4$) C, H, N.

5.4.9. (±)-1-[2-(4-Fluorophenyl)-2-(4-methylpiperazin-1-yl)ethyl]-4-(2-naphthylmethyl)piperazine 3 maleate ((±)-10ag). Compound (±)-**10ag** was obtained as a crystal. Mp 189–191 °C (EtOH/Et_2O); ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.60–3.82 (20H, m), 2.72 (3H, s), 3.88–4.12 (1H, m), 6.17 (6H, s), 7.20–7.39 (4H, m), 7.50–7.63 (3H, m), 7.86–8.02 (4H, m); MS (ESI, Pos) *m*/*z* 447 $(M+H)^+$; Anal. ($C_{28}H_{35}FN_4{\cdot}3C_4H_4O_4{\cdot}0.3H_2O$) C, H, N.

5.4.10. (±)-1-[2-(4-Fluorophenyl)-2-(4-methylpiperazin-1-yl)ethyl]-4-[2-(2-naphthyl)ethyl]piperazine 3 maleate ((±)-10ah). Compound (±)-**10ah** was obtained as a crystal. Mp 187–189 °C (EtOH); ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.62–3.60 (25H, m), 3.68–3.80 (1H, m), 6.31 (6H, s), 7.06 (2H, t, J = 8.6 Hz), 7.11–7.28 (2H, m), 7.30–7.38 (1H, m), 7.43–7.53 (2H, m), 7.70 (1H, s), 7.79–7.88 (3H, m); MS (ESI, Pos) *m*/*z* 461 $(M+H)^+$; Anal. ($C_{29}H_{37}FN_4{\cdot}3C_4H_4O_4{\cdot}0.7H_2O$) C, H, N.

5.4.11. (±)-1-[2-(4-Fluorophenyl)-2-(4-methylpiperazin-1-yl)ethyl]-4-[3-(2-naphthyl)propyl]piperazine 3 maleate ((±)-10ai). Compound (±)-**10ai** was obtained as a crystal. Mp 180–182 °C (EtOH); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.93–2.08 (2H, m), 2.65–3.62 (22H, m), 2.71 (3H, s), 3.89–4.02 (1H, m), 6.12 (6H, s), 7.08–7.53 (8H, m), 7.71 (1H, s), 7.80–7.92 (2H, m); MS (ESI, Pos) *m*/*z* 475 $(M+H)^+$; Anal. ($C_{30}H_{39}FN_4{\cdot}3C_4H_4O_4$) C, H, N.

5.4.12. (±)-1-[2-(4-Fluorophenyl)-2-(4-methylpiperazin-1-yl)ethyl]-4-[4-(2-naphthyl)butyl]piperazine 3 maleate ((±)-10aj). Compound (±)-**10aj** was obtained as a crystal. Mp 185–187 °C (EtOH); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.50–1.78 (4H, m), 2.30–3.80 (22H, m), 2.70 (3H, s), 3.91–3.98 (1H, m), 6.11 (6H, s), 7.18–7.57 (8H, m), 7.73 (1H, s), 7.78–7.92 (2H, m); MS (ESI, Pos) *m*/*z* 489 $(M+H)^+$; Anal. ($C_{31}H_{41}FN_4{\cdot}3C_4H_4O_4{\cdot}0.1H_2O$) C, H, N.

5.4.13. (±)-1-(1-(4-Fluorophenyl)-2-{4-[4-(4-methoxy-1-naphthyl)butyl]piperazin-1-yl}ethyl)-4-methylpiperazine 3 maleate ((±)-10bf). Compound (±)-**10bf** was obtained as a crystal. Mp 178–181 °C (EtOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.58–1.73 (4H, m), 2.73 (3H, s), 2.79–3.31 (22 H, m), 3.88–3.94 (1H, m), 3.94 (3H, s), 6.11 (6H, s), 6.88 (1H, d, J = 7.9 Hz), 7.18–7.36 (5H, m), 7.48–7.60 (2H, m), 8.00 (1H, d, J = 7.6 Hz), 8.17– 8.21 (1H, m); MS (ESI, Pos) *m*/*z* 519 $(M+H)^+$; Anal. ($C_{32}H_{43}FN_4O{\cdot}3C_4H_4O_4$) C, H, N.

5.4.14. (±)-1-(2-Biphenyl-2-ylethyl)-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine 3.5 hydrochloride ((±)-10bn). Compound (±)-**10bn** was obtained as a crystal. Mp 233–237 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.22 (6H, d, J = 6.7 Hz), 2.40–2.70 (1H, m), 2.93–3.05 (2H, m), 3.10–3.80 (19H, m), 3.99–4.18 (1H, m), 4.38–4.56 (1H, m), 7.21–7.78 (13H, m); MS (ESI, Pos) *m*/*z* 515 $(M+H)^+$; Anal. ($C_{33}H_{43}FN_4{\cdot}3.3HCl$) C, H, N.

5.4.15. (±)-1-(3-Biphenyl-2-ylpropyl)-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine 4 hydrochloride ((±)-10bo). Compound (±)-**10bo** was obtained as a crystal. Mp 190–193 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.22 (6H, d, J = 6.7 Hz), 1.82–2.01 (2H, m), 2.10–2.22 (1H, m), 2.50–2.70 (1H, m), 2.92–3.03 (2H, m), 3.08–3.77 (14H, m), 3.98–4.56 (6H, m), 7.19 (1H, d, J = 7.3 Hz), 7.21–7.48 (12H, m); MS (ESI, Pos) *m*/*z* 529 $(M+H)^+$; Anal. ($C_{34}H_{45}FN_4{\cdot}4HCl{\cdot}1.5H_2O$) C, H, N.

5.4.16. (±)-1-(4-Biphenyl-2-ylbutyl)-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine 4 hydrochloride ((±)-10bp). Compound (±)-**10bp** was obtained as a crystal. Mp 193–196 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.22 (6H, d, J = 6.5 Hz), 1.42–1.71 (4H, m), 2.04–2.12 (1H, m), 2.52–2.67 (1H, m),

2.93–3.03 (2H, m), 3.06–3.76 (14H, m), 4.02–4.21 (1H, m), 4.40–4.81 (5H, m), 7.17 (1H, d, J = 7.2 Hz), 7.20–7.49 (12H, m); MS (ESI, Pos) m/z 543 $(M+H)^+$; Anal. ($C_{35}H_{47}FN_4 \cdot 4HCl \cdot 2.1H_2O$) C, H, N.

5.4.17. (±)-1-(3-Biphenyl-3-ylpropyl)-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine 4 hydrochloride ((±)-10bq). Compound (±)-**10bq** was obtained as a crystal. Mp 173–176 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.22 (6H, d, J = 6.5 Hz), 2.03–2.13 (3H, m), 2.51–2.77 (1H, m), 3.09–3.93 (16H, m), 4.07–4.80 (6H, m), 7.21–7.59 (11H, m), 7.63–7.69 (2H, m); HRMS 529.3707 (M+1).

5.4.18. (±)-1-(3-Biphenyl-4-ylpropyl)-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine 4 hydrochloride ((±)-10br). Compound (±)-**10br** was obtained as a crystal. Mp 182–185 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.22 (6H, d, J = 6.5 Hz), 2.01–2.21 (2H, m), 2.50–2.78 (2H, m), 3.05–3.43 (8H, m), 3.45–3.92 (8H, m), 3.95–4.40 (5H, m), 4.51–4.60 (1H, m), 7.22–7.49 (9H, m), 7.58–7.65 (4H, m); MS (ESI, Pos) m/z 529 $(M+H)^+$; Anal. ($C_{34}H_{45}FN_4 \cdot 4HCl \cdot 1.0H_2O$) C, H, N.

5.4.19. (±)-1-[3-(6-Fluorobiphenyl-2-yl)propyl]-4-[2-(4-fluorophenyl)-2-4-isopropylpiperazin-1-yl)ethyl]piperazine 4 hydrochloride ((±)-10bs). Compound (±)-**10bs** was obtained as a crystal. Mp 196–199 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.22 (6H, d, J = 6.4 Hz), 1.79–2.01 (2H, m), 2.04–2.31 (2H, m), 2.50–2.77 (2H, m), 3.15–3.93 (18H, m), 3.98–4.22 (1H, m), 4.41–4.60 (1H, m), 7.09–7.58 (12H, m); MS (ESI, Pos) m/z 547 $(M+H)^+$; Anal. ($C_{34}H_{44}F_2N_4 \cdot 4HCl \cdot 0.4H_2O$) C, H, N.

5.4.20. (±)-1-[3-(5-Fluorobiphenyl-2-yl)propyl]-4-[2-(4-fluorophenyl)-2-4-isopropylpiperazin-1-yl)ethyl]piperazine 3.8 hydrochloride ((±)-10bt). Compound (±)-**10bt** was obtained as a crystal. Mp 205–208 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.22 (6H, d, J = 6.7 Hz), 1.81–1.99 (2H, m), 2.03–2.20 (1H, m), 2.53–2.72 (1H, m), 2.95–3.03 (2H, m), 3.08–3.77 (18H, m), 4.01–4.20 (1H, m), 4.44–4.56 (1H, m), 7.02 (1H, dd, J = 2.8, 6.8 Hz), 7.19–7.49 (11H, m); MS (ESI, Pos) m/z 547 $(M+H)^+$; Anal. ($C_{34}H_{44}F_2N_4 \cdot 3.8HCl$) C, H, N.

5.4.21. (±)-1-[3-(4-Fluorobiphenyl-2-yl)propyl]-4-[2-(4-fluorophenyl)-2-4-isopropylpiperazin-1-yl)ethyl]piperazine 4 hydrochloride ((±)-10bu). Compound (±)-**10bu** was obtained as a crystal. Mp 197–199 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.22 (6H, d, J = 6.5 Hz), 1.88–2.02 (2H, m), 2.07–2.22 (1H, m), 2.53–2.70 (1H, m), 2.96–3.04 (2H, m), 3.09–3.78 (18H, m), 4.03–4.21 (1H, m), 4.43–4.58 (1H, m), 7.12 (1H, dt, J = 2.6, 5.9 Hz), 7.19–7.48 (11H, m); MS (ESI, Pos) m/z 547 $(M+H)^+$; Anal. ($C_{34}H_{44}F_2N_4 \cdot 4HCl$) C, H, N.

5.4.22. (±)-1-[3-(3-Fluorobiphenyl-2-yl)propyl]-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine 3.9 hydrochloride ((±)-10bv). Compound (±)-**10bv** was obtained as a crystal. Mp 178–181 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.22 (6H, d, J = 6.5 Hz), 1.81–2.00 (2H, m), 2.06–2.20 (1H, m), 2.51–2.68 (1H, m), 2.94–3.03 (2H, m), 3.08–3.76 (18H, m), 4.01–4.20 (1H, m), 4.42–4.58 (1H, m), 7.06 (1H, d, J = 6.7 Hz), 7.19–7.55 (11H, m); MS (ESI, Pos) m/z 547 $(M+H)^+$; Anal. ($C_{34}H_{44}F_2N_4 \cdot 3.9HCl$) C, H, N.

5.4.23. (±)-1-[3-(3-Chlorobiphenyl-2-yl)propyl]-4-[2-(4-fluorophenyl)-2-4-isopropylpiperazin-1-yl)ethyl]piperazine 4 hydrochloride ((±)-10bw). Compound (±)-**10bw** was obtained as a crystal. Mp 185–187 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.23 (6H, d, J = 6.4 Hz), 1.81–2.01 (2H, m), 2.14–2.23 (1H, m), 2.58–2.78 (1H, m), 2.93–3.02 (2H, m), 3.08–3.75 (14H, m), 4.06–4.21 (1H, m), 4.22–4.79 (5H, m), 7.17 (1H, dd, J = 1.2, 6.4 Hz), 7.23–7.51 (11H, m); MS (ESI, Pos) m/z 563 $(M+H)^+$; Anal. ($C_{34}H_{44}ClFN_4 \cdot 4HCl \cdot 3.5H_2O$) C, H, N.

5.4.24. (±)-1-[2-(4-Fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]-4-[3-(3-methylbiphenyl-2-yl)propyl]piperazine 4 hydrochloride ((±)-10bx). Compound (±)-**10bx** was obtained as a crystal. Mp 178–180 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.23 (6H, d, J = 6.5 Hz), 1.77–1.92 (2H, m), 2.14–2.23 (1H, m), 2.40 (3H, s), 2.61–2.78 (1H, m), 2.92–3.00 (2H, m), 3.07–3.74 (14H, m), 4.07–4.21 (1H, m), 4.40–4.90 (5H, m), 6.98 (1H, dd, J = 1.6, 6.6 Hz), 7.17–7.50 (11H, m); MS (ESI, Pos) m/z 543 $(M+H)^+$; Anal. ($C_{35}H_{47}FN_4 \cdot 4HCl \cdot 4H_2O$) C, H, N.

5.4.25. (±)-1-[2-(4-Fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]-4-[3-(3-methoxybiphenyl-2-yl)propyl]piperazine 4 hydrochloride ((±)-10by). Compound (±)-**10by** was obtained as a crystal. Mp 175–177 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.22 (6H, d, J = 6.4 Hz), 1.81–1.98 (2H, m), 2.07–2.22 (1H, m), 2.60–2.77 (1H, m), 2.87–3.01 (2H, m), 3.09–3.75 (18H, m), 2.92 (3H, s), 4.05–4.21 (1H, m), 4.43–4.58 (1H, m), 6.79 (1H, d, J = 7.7 Hz), 7.04 (1H, d, J = 8.4 Hz), 7.19–7.50 (10H, m); MS (ESI, Pos) m/z 559 $(M+H)^+$; Anal. ($C_{35}H_{47}FN_4O \cdot 4HCl \cdot 2.0H_2O$) C, H, N.

5.4.26. (±)-1-[3-(2′-Fluorobiphenyl-2-yl)propyl]-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine 4 hydrochloride ((±)-10bz). Compound (±)-**10bz** was obtained as a crystal. Mp 171–173 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.22 (6H, d, J = 6.5 Hz), 1.83–1.99 (2H, m), 2.08–2.21 (1H, m), 2.58–2.71 (1H, m), 2.89–3.03 (2H, m), 3.07–3.78 (18H, m), 4.04–4.21 (1H, m), 4.42–4.56 (1H, m), 7.15–7.50 (12H, m); MS (ESI, Pos) m/z 547 $(M+H)^+$; Anal. ($C_{34}H_{44}F_2N_4 \cdot 4HCl \cdot 0.5H_2O$) C, H, N.

5.4.27. (±)-1-[3-(3′-Fluorobiphenyl-2-yl)propyl]-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine 4 hydrochloride ((±)-10ca). Compound (±)-**10ca** was obtained as a crystal. Mp 171–173 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.22 (6H, d, J = 6.6 Hz), 1.83–2.01 (2H, m), 2.07–2.23 (1H, m), 2.56–2.70 (1H, m), 2.93–3.05 (2H, m), 3.09–3.77 (18H, m), 4.06–4.21 (1H, m), 4.44–4.58 (1H, m), 7.16–7.52 (12H, m); MS (ESI, Pos) m/z 547 $(M+H)^+$; Anal. ($C_{34}H_{44}F_2N_4 \cdot 4HCl \cdot 0.5H_2O$) C, H, N.

5.4.28. (±)-1-[3-(4′-Fluorobiphenyl-2-yl)propyl]-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine 4 hydrochloride ((±)-10cb). Compound (±)-**10cb** was obtained as a crystal. Mp 197–199 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.21 (6H, d, J = 6.6 Hz), 1.82–2.01 (2H, m), 2.06–2.25 (1H, m), 2.51–2.79 (3H, m), 2.95–3.05 (2H, m), 3.08–3.78 (16H, m), 4.08–4.21 (1H, m), 4.49–4.61 (1H, m), 7.16–7.52 (12H, m); MS (ESI, Pos) m/z 547 $(M+H)^+$; Anal. ($C_{34}H_{44}F_2N_4$·4HCl·0.5H_2O) C, H, N.

5.4.29. (±)-1-[3-(4′-Chlorobiphenyl-2-yl)propyl]-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine 4 hydrochloride ((±)-10cc). Compound (±)-**10cc** was obtained as a crystal. Mp 175–177 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.21 (6H, d, J = 6.6 Hz), 1.82–2.01 (2H, m), 2.06–2.25 (1H, m), 2.51–2.79 (3H, m), 2.95–3.05 (2H, m), 3.08–3.78 (16H, m), 4.08–4.21 (1H, m), 4.49–4.61 (1H, m), 7.16–7.52 (12H, m); MS (ESI, Pos) m/z 563 $(M+H)^+$; Anal. ($C_{34}H_{44}ClFN_4$·4HCl·2.5H_2O) C, H, N.

5.4.30. (±)-1-[2-(4-Fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]-4-[3-(4′-methylbiphenyl-2-yl)propyl]piperazine 4 hydrochloride ((±)-10cd). Compound (±)-**10cd** was obtained as a crystal. Mp 172–174 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.22 (6H, d, J = 6.6 Hz), 1.84–2.01 (2H, m), 2.11–2.30 (1H, m), 2.38 (3H, s), 2.53–2.78 (3H, m), 2.93–3.03 (2H, m), 3.07–3.74 (16H, m), 4.06–4.22 (1H, m), 4.40–4.62 (1H, m), 7.09–7.42 (12H, m); MS (ESI, Pos) m/z 543 $(M+H)^+$; Anal. ($C_{35}H_{47}FN_4$·4HCl·1.6H_2O) C, H, N.

5.4.31. (±)-1-[2-(4-Fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]-4-[3-(4′-methoxybiphenyl-2-yl)propyl]piperazine 4 hydrochloride ((±)-10ce). Compound (±)-**10ce** was obtained as a crystal. Mp 168–170 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.21 (6H, d, J = 6.6 Hz), 1.88–2.01 (2H, m), 2.05–2.30 (1H, m), 2.57–2.78 (3H, m), 2.95–3.04 (2H, m), 3.08–3.77 (16H, m), 3.91 (3H, s), 4.05–4.23 (1H, m), 4.42–4.57 (1H, m), 7.00 (2H, d, J = 7.5 Hz), 7.14–7.43 (10H, m); MS (ESI, Pos) m/z 559 $(M+H)^+$; Anal. ($C_{35}H_{47}FN_4O$·4HCl·2H_2O) C, H, N.

5.4.32. (±)-1-[3-(4′-*tert*-Butylbiphenyl-2-yl)propyl]-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine 4 hydrochloride ((±)-10cf). Compound (±)-**10cf** was obtained as a crystal. Mp 177–179 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.22 (6H, d, J = 6.7 Hz), 1.33 (9H, s), 1.89–2.03 (2H, m), 2.08–2.22 (1H, m), 2.58–2.68 (3H, m), 2.97–3.04 (2H, m), 3.09–3.79 (16H, m), 4.07–4.21 (1H, m), 4.47–4.57 (1H, m), 7.19 (2H, dd, J = 1.6, 5.9 Hz), 7.21–7.49 (10H, m); MS (ESI, Pos) m/z 585 $(M+H)^+$; Anal. ($C_{38}H_{53}FN_4$·4HCl·2.5H_2O) C, H, N.

5.4.33. (±)-1-[2-(4-Fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]-4-[3-(1,1′:4′,1″-terphenyl-2-yl)propyl]piperazine 3.8 hydrochloride ((±)-10cg). Compound (±)-**10cg** was obtained as a crystal. Mp 192–195 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.21 (6H, d, J = 6.7 Hz), 1.90–2.18 (3H, m), 2.59–2.73 (3H, m), 2.97–3.12 (2H, m), 3.18–4.21 (17H, m), 4.37–4.52 (1H, m), 7.21–7.56 (13H, m), 7.72–7.81 (4H, m); MS (ESI, Pos) m/z 605 $(M+H)^+$; Anal. ($C_{40}H_{49}FN_4$·3.8HCl·0.5H_2O) C, H, N.

5.4.34. (±)-1-[2-(4-Fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]-4-{3-[4′-(trifluoromethyl)biphenyl-2-yl]propyl}-piperazine 4 hydrochloride ((±)-10ch). Compound (±)-**10ch** was obtained as a crystal. Mp 183–186 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.21 (6H, d, J = 6.6 Hz), 1.91–2.06 (2H, m), 2.13–2.29 (1H, m), 2.53–2.68 (3H, m), 2.97–3.09 (2H, m), 3.18–3.81 (16H, m), 4.09–4.25 (1H, m), 4.49–4.62 (1H, m), 7.20–7.49 (8H, m), 7.69 (2H, d, J = 7.5 Hz), 7.81 (2H, d, J = 7.5 Hz); MS (ESI, Pos) m/z 597 $(M+H)^+$; Anal. ($C_{35}H_{44}F_4N_4$·4HCl·3H_2O) C, H, N.

5.4.35. (±)-1-[2-(4-Fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]-4-{3-[4′-(trifluoromethoxy)biphenyl-2-yl]propyl}-piperazine 4 hydrochloride ((±)-10ci). Compound (±)-**10ci** was obtained as a crystal. Mp 172–174 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.22 (6H, d, J = 6.6 Hz), 1.91–2.03 (2H, m), 2.15–2.29 (1H, m), 2.53–2.78 (3H, m), 2.97–3.11 (2H, m), 3.18–3.78 (16H, m), 4.09–4.22 (1H, m), 4.48–4.62 (1H, m), 7.19–7.52 (12H, m); MS (ESI, Pos) m/z 613 $(M+H)^+$; Anal. ($C_{35}H_{44}F_4N_4O$·4HCl·2H_2O) C, H, N.

5.4.36. (±)-2′-(3-{4-[2-(4-Fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazin-1-yl}propyl)-*N*,*N*-dimethylbiphenyl-4-amine 5 hydrochloride ((±)-10cj). Compound (±)-**10cj** was obtained as a crystal. Mp 194–197 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.22 (6H, d, J = 6.6 Hz), 1.90–2.01 (2H, m), 2.11–2.22 (1H, m), 2.59–2.68 (3H, m), 2.98–3.03 (2H, m), 3.09 (6H, s), 3.19–3.74 (16H, m), 4.08–4.21 (1H, m), 4.50–4.59 (1H, m), 7.18 (2H, d, J = 7.3 Hz), 7.19–7.52 (10H, m); MS (ESI, Pos) m/z 572 $(M+H)^+$; Anal. ($C_{36}H_{50}FN_5$·5HCl·2H_2O) C, H, N.

5.4.37. (±)-1-(4-{4-[2-(4-Fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazin-1-yl}butyl)-2-naphthol 3 maleate ((±)-10bh). A mixture of (±)-1-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]-4-[4-(2-methoxy-1-naphthyl)butyl]piperazine (±)-**10bg** (61 mg, 0.11 mmol) in 48% HBr aqueous (10 mL) was stirred at 105 °C for 3 h and concentrated in vacuo. The residue was partitioned between 1 M NaOH aqueous and Et_2O. The separated organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to obtain crude (±)-1-(4-{4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazin-1-yl}butyl)-2-naphthol (50 mg, 85%) as an oily product. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (6H, d, J = 6.5 Hz), 1.50–1.62 (2H, m), 1.65–1.79 (2H, m), 2.30–2.76 (21H, m), 2.88–3.02 (3H, m), 3.57 (1H, t, J = 6.2 Hz), 6.99 (2H, t, J = 8.8 Hz), 7.14–7.29 (4H, m), 7.42 (1H, t, J = 7.4 Hz), 7.60 (1H, d, J = 7.8 Hz), 7.75 (1H, d, J = 7.7 Hz), 7.85 (1H, d, J = 7.9 Hz). The above free base (50 mg, 0.094 mmol) was dissolved in EtOH (2 mL), and to the solution was added a solution of maleic acid (38 mg, 0.31 mmol) in EtOH (1 mL). After stirred at room temperature for 1 h, the resulting precipitate was collected by filtration to obtain (±)-1-(4-{4-[2-(4-fluoro-

phenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazin-1-yl}butyl)-2-naphthol 3 maleate (±)-**10bh** (61 mg, 74%) as an amorphous: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22 (6H, d, J = 6.5 Hz), 1.58–1.82 (4H, m), 2.50–3.31 (21H, m), 3.53–3.62 (1H, m), 6.25 (6H, s), 3.57 (1H, t, J = 6.2 Hz), 6.98 (2H, t, J = 8.9 Hz), 7.10–7.30 (4H, m), 7.42 (1H, t, J = 7.6 Hz), 7.59 (1H, d, J = 7.9 Hz), 7.74 (1H, d, J = 7.7 Hz), 7.85 (1H, d, J = 7.9 Hz); HRMS 533.3656 (M+1).

5.4.38. (±)-1-[4-(2-Ethoxy-1-naphthyl)butyl]-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine 3.5 maleate ((±)-10bi). A mixture of (±)-1-(4-{4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazin-1-yl}butyl)-2-naphthol (free base of (±)-**10bh**) (0.10 g, 0.19 mmol), iodoethane (15 μL, 0.19 mmol), and K_2CO_3 (26 mg, 0.19 mmol) in DMF (5 mL) was stirred at 80 °C for 6 h. The mixture was partitioned between EtOAc and H_2O, and separated organic phase was washed with saturated aqueous $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was chromatographed on Chromatorex NH (hexane/EtOAc 2:1) to obtain (±)-1-[4-(2-ethoxy-1-naphthyl)butyl]-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine (20 mg, 19%) as an oily product. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (6H, d, J = 6.5 Hz), 1.42 (3H, d, J = 7.0 Hz), 1.50–1.72 (4H, m), 2.25–2.71 (21H, m), 2.88–2.92 (1H, m), 3.00–3.12 (2H, m), 3.58 (1H, t, J = 6.4 Hz), 3.58 (1H, q, J = 7.0 Hz), 6.97 (2H, t, J = 8.0 Hz), 7.16–7.31 (4H, m), 7.42 (1H, t, J = 7.0 Hz), 7.67 (1H, d, J = 8.0 Hz), 7.78 (1H, d, J = 7.5 Hz), 7.95 (1H, d, J = 8.0 Hz). The above free base (20 mg, 0.036 mmol) was dissolved in EtOH (1 mL), and to the solution was added a solution of maleic acid (13 mg, 0.11 mmol) in EtOH (1 mL). After stirred at room temperature for 1 h, the resulting precipitate was collected by filtration to obtain (±)-1-[4-(2-ethoxy-1-naphthyl)butyl]-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)-ethyl]piperazine 3 maleate (±)-**10bi** (10 mg, 31%) as an amorphous: ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.20 (6H, d, J = 6.6 Hz), 1.37 (3H, t, J = 6.9 Hz), 1.45–1.79 (4H, m), 2.20–3.60 (23H, m), 3.88–4.03 (1H, m), 4.18 (2H, q, J = 6.8 Hz), 6.12 (6H, s), 7.15–7.22 (2H, t, J = 8.8 Hz), 7.55 (5H, m), 7.82 (2H, t, J = 9.0 Hz), 7.94 (1H, d, J = 8.4 Hz); MS (ESI, Pos.) m/z 561 $(M+H)^+$; Anal. ($C_{35}H_{49}FN_4O{\cdot}3.5C_4H_4O_4{\cdot}1.0H_2O$) C, H, N.

5.4.39. 1-(1-(4-Fluorophenyl)-2-{4-[4-(2-isopropoxy-1-naphthyl)butyl]piperazin-1-yl}ethyl)-4-isopropylpiperazine 3 maleate ((±)-10bj). Compound (±)-**10bj** was obtained by using the method of Section 5.4.38 as a crystal. Mp 167–169 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.20 (6H, d, J = 6.6 Hz), 1.30 (6H, d, J = 6.0 Hz), 1.50–1.80 (4H, m), 2.70–3.50 (23H, m), 3.94–4.02 (1H, m), 4.54 (1H, m), 6.10 (6H, s), 7.22 (2H, t, J = 8.8 Hz), 7.30–7.53 (5H, m), 7.78–7.97 (3H, m); MS (ESI, Pos.) m/z 575 $(M+H)^+$; Anal. ($C_{36}H_{51}FN_4O{\cdot}3C_4H_4O_4$) C, H, N.

5.5. Binding test

5.5.1. Material. [^{125}I][Nle^4,D-Phe^7]α-Melanocyte-stimulating hormone ([Nle^4,D-Phe^7]α-MSH) (specific radioactivity: 81.4 TBq/mmol) was purchased from Amersham International (Buckinghamshire, England). COS-1 cells were purchased from American Type Culture Collection (Rocksville, MD, USA). [Nle^4,D-Phe^7]α-MSH was purchased from Peninsula Laboratories (Belmont, CA, USA). All other chemicals used in this study were obtained commercially, and all were of the highest purity available.

5.5.2. [^{125}I][Nle^4,D-Phe^7]α-MSH binding to recombinant MC4 receptor. COS-1 cells expressing the MC4 receptor, prepared according to the method reported previously,[19] were washed with phosphate buffered saline, scraped, and pelleted by centrifugation. Cell pellets were homogenized with 50 mM Tris–HCl buffer (pH 7.4) containing 2 mM EDTA, 10 mM $CaCl_2$, and 100 μM phenylmethylsulfonyl fluoride, and centrifuged at 48,000g for 20 min at 4 °C. The pellet was washed twice with the buffer, and the final pellet was suspended in an assay buffer (50 mM Tris–HCl buffer (pH 7.4) containing 2 mM EDTA, 10 mM $CaCl_2$, 100 μM phenylmethylsulfonyl fluoride, and 0.1% bovine serum albumin (BSA)), and served as crude membrane preparation for binding studies. Binding assays of [^{125}I][Nle^4,D-Phe^7]α-MSH were performed according to Chaki et al.[19] Membranes were incubated with [^{125}I][Nle^4,D-Phe^7]α-MSH (0.2 nM) for 120 min at 25 °C, and the reaction was terminated by rapid filtration over a GF/C filter presoaked with 0.5% BSA, after which the filters were washed three times with the buffer. Radioactivity was quantified in a γ-counter. Nonspecific binding was determined in the presence of 1 μM [Nle^4,D-Phe^7]α-MSH. Specific binding was determined by subtracting nonspecific from total binding. In the competition assay, concentration of the test compound that caused 50% inhibition of the specific binding (IC_{50} value) was determined from each concentration–response curve.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2006.12.039.

References and notes

1. Irani, B. G.; Holder, J. R.; Todorovic, A.; Wilczynski, A. M.; Joseph, C. G.; Wilson, K. R.; Haskell-Luevano, C. *Curr. Pharm. Des.* **2004**, *10*, 3443.
2. Lu, D.; Willard, D.; Patel, I. R.; Kadwell, S.; Overton, L.; Kost, T.; Luther, M.; Chen, W.; Yowchik, R. P.; Wilkinson, W. O.; Cone, R. D. *Nature* **1994**, *371*, 799.
3. Ollmann, M. M.; Wilson, B. D.; Yang, Y.-K.; Kerns, J. A.; Chen, Y.; Gantz, I.; Barsh, G. S. *Science* **1997**, *278*, 135.
4. Holder, J. R.; Haskell-Luevano, C. *Med. Res. Rev.* **2004**, *24*, 325.
5. Hruby, V. J.; Wilkes, B. C.; Hadley, M. E.; Al-Obeidi, F.; Sawyer, T. K.; Staples, D. J.; DeVaux, A.; Dym, O.; Castrucci, A. M.; Hintz, M. F.; Riehm, J. P.; Rao, K. R. *J. Med. Chem.* **1987**, *30*, 2126.

6. (a) Wikberg, J. E. S. *Eur. J. Pharmacol.* **1999**, *375*, 295; (b) Chaki, S.; Nakazato, A. *Drugs future* **2004**, *29*(10), 1065; (c) Chaki, S.; Okuyama, S. *Peptides* **2005**, *26*, 1952.
7. Fan, W.; Boston, B. A.; Kesterson, R. A.; Hruby, V. J.; Cone, R. D. *Nature* **1997**, *385*, 165.
8. Forbes, S.; Bui, S.; Robinson, B. R.; Hochgeschwendr, U.; Brenna, M. B. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 4233.
9. Huszar, D.; Lynch, C. A.; Fairchild-Huntress, V.; Dunmore, J. H.; Fang, Q.; Berkemeier, L. R.; Gu, W.; Kesterson, R. A.; Boston, B. A.; Cone, R. D.; Smith, F. J.; Campfield, L. A.; Burn, P.; Lee, F. *Cell* **1997**, *88*, 131.
10. Kask, A.; Rago, L.; Wikberg, J. E. S.; Schiotch, H. B. *Eur. J. Pharmacol.* **1998**, *360*, 15.
11. Murphy, B.; Nunes, C. N.; Ronan, J. J.; Harper, C. M.; Beall, M. J.; Hanaway, M.; Fairhurst, A. M.; Van der Ploeg, L. H. T.; MacIntyre, D. E.; Mellin, T. N. *Neuropeptides* **1998**, *32*, 491.
12. Giraudo, S. Q.; Billington, C. J.; Levine, A. S. *Brain Res.* **1998**, *809*, 302.
13. Kask, A.; Rago, L.; Mutulis, F.; Pahkla, R.; Wikberg, J. E. S.; Schiotch, H. B. *Biochem. Biophys. Res. Commun.* **1998**, *245*, 90.
14. van der Ploeg, L. H. T.; Martin, W. J.; Howard, A. D.; Nargund, R. P.; Austin, C. P.; Guan, X.; Drisko, J.; Cashen, D.; Sebhat, I.; Patchett, A. A.; Figueroa, D. J.; DiLella, A. G.; Connolly, B. M.; Weinberg, D. H.; Tan, C. T.; Palyha, O. C.; Pong, S.; MacNeil, T.; Rosenblum, C.; Vongs, A.; Tang, R.; Yu, H.; Sailer, A. W.; Fong, T. M.; Huang, C.; Tota, M.; Chang, R. S.; Stearns, R.; Tamvakopoulos, C.; Christ, G.; Drazen, D. L.; Spar, B. D.; Nelson, R. J.; MacIntyre, D. E. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 11381.
15. Vos, T. J.; Caracoti, A.; Che, J. L.; Dai, M.; Farrer, C. A.; Forsyth, N. E.; Drabic, S. V.; Horlick, R. A.; Lamppu, D.; Yowe, D. L.; Balani, S.; Li, P.; Zeng, H.; Joseph, I. B. J. K.; Rodriguez, L. E.; Maguire, M. P.; Patane, M. A.; Claiborne, C. F. *J. Med. Chem.* **2004**, *47*, 1602.
16. Adan, R. A.; Szklarczyk, A. W.; Oosterom, J.; Brakkee, J. H.; Nijenhuis, W. A.; Schaaper, W. M.; Meloen, R. H.; Gispen, W. H. *Eur. J. Pharmacol.* **1999**, *378*, 249.
17. Gonzalez, M. I.; Vaziri, S.; Wilson, C. A. *Peptides* **1996**, *17*, 171.
18. Vergoni, A. V.; Bertolini, A.; Wikberg, J. E.; Schioth, H. B. *Eur. J. Pharmacol.* **1999**, *369*, 11.
19. Chaki, S.; Hirota, S.; Funakoshi, T.; Suzuki, Y.; Suetake, S.; Okubo, T.; Ishii, T.; Nakazato, A.; Okuyama, S. *J. Pharmcol. Exp. Ther.* **2003**, *304*, 818.
20. Chaki, S.; Oshida, Y.; Ogawa, S.; Funakoshi, T.; Shimazaki, T.; Okubo, T.; Nakazato, A.; Okuyama, S. *Pharmacol. Biochem. Behav.* **2005**, *82*, 621.
21. Chaki, S.; Ogawa, S.; Toda, Y.; Funakoshi, T.; Okuyama, S. *Eur. J. Pharmacol.* **2003**, *474*, 95.
22. Shimazaki, T.; Chaki, S. *Pharmacol. Biochem. Behav.* **2005**, *80*, 395.
23. (a) Todorovic, A.; Holder, J. R.; Bauzo, R. M.; Scott, J. W.; Kavanagh, A.; Abdel-Malek, Z.; Haskell-Luevano, C. *J. Med. Chem.* **2005**, *48*, 3328; (b) Wilczynski, A.; Wilson, K. R.; Scott, J. W.; Edison, A. S.; Haskell-Luevano, C. *J. Med. Chem.* **2005**, *48*, 3060; (c) Joseph, C. G.; Wang, X. S.; Scott, J. W.; Bauzo, R. M.; Xiang, Z.; Richards, N. G.; Haskell-Luevano, C. *J. Med. Chem.* **2004**, *47*, 6702; (d) Wilczynski, A.; Wang, X. S.; Joseph, C. G.; Xiang, Z.; Bauzo, R. M.; Scott, J. W.; Sorensen, N. B.; Shaw, A. M.; Millard, W. J.; Richards, N. G.; Haskell-Luevano, C. *J. Med. Chem.* **2004**, *47*, 2194; (e) Han, G.; Haskell-Luevano, C.; Kendall, L.; Bonner, G.; Hadley, M. E.; Cone, R. D.; Hruby, V. J. *J. Med. Chem.* **2004**, *47*, 1514; (f) Balse-Srinivasan, P.; Grieco, P.; Cai, M.; Trivedi, D.; Hruby, V. J. *J. Med. Chem.* **2003**, *46*, 4965; (g) Grieco, P.; Lavecchia, A.; Cai, M.; Trivedi, D.; Weinberg, D.; MacNeil, T.; Van der Ploeg, L. H.; Hruby, V. J. *J. Med. Chem.* **2002**, *45*, 5287; (h) Holder, J. R.; Bauzo, R. M.; Xiang, Z.; Haskell-Luevano, C. *J. Med. Chem.* **2002**, *45*, 3073; (i) Holder, J. R.; Bauzo, R. M.; Xiang, Z.; Haskell-Luevano, C. *J. Med. Chem.* **2002**, *45*, 2801; (j) Kavarana, M. J.; Trivedi, D.; Cai, M.; Ying, J.; Hammer, M.; Cabello, C.; Grieco, P.; Han, G.; Hruby, V. J. *J. Med. Chem.* **2002**, *45*, 2644; (k) Bednarek, M. A.; MacNeil, T.; Kalyani, R. N.; Tang, R.; Van der Ploeg, L. H.; Weinberg, D. H. *J. Med. Chem.* **2001**, *45*, 3665; (l) Fotsch, C.; Smith, D. M.; Adams, J. A.; Cheetham, J.; Croghan, M.; Doherty, E. M.; Hale, C.; Jarosinski, M. A.; Kelly, M. G.; Norman, M. H.; Tomayo, N. A.; Xi, N.; Baumgartner, J. W. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2337; (m) Haskell-Luevano, C.; Hendrata, S.; North, C.; Sawyer, T. K.; Hadley, M. E.; Hruby, V. J.; Dickinson, C.; Gantz, I. *J. Med. Chem.* **1997**, *40*, 2133; (n) Cheung, A. W.-H.; Gore, L. Q. V.; Chu, X.-J.; Bartkovitz, D.; Kurylko, G.; Swistok, J.; Danho, W.; Chen, L.; Yagaloff, K. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5504; (o) Yan, L. Z.; Flora, D.; Edwards, P.; Smiley, D. L.; Emmerson, P. J.; Hsiung, H. M.; Gadski, R.; Hertel, J.-A.; Heiman, M. L.; Husain, S.; O'Brien, T. P.; Kahl, S. D.; Zhang, L.; DiMarchi, R. D.; Mayer, J. P. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4611; (p) Odagami, T.; Tsuda, Y.; Kogami, Y.; Kouji, H.; Okada, Y. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3723.
24. (a) Sebhat, I. K.; Martin, W. J.; Ye, Z.; Barakat, K.; Mosley, R. T.; Johnston, D. B. R.; Bakshi, R.; Palucki, B.; Weinberg, D. H.; MacNeil, T.; Kalyani, R. N.; Tang, R.; Stearns, R. A.; Miller, R. R.; Tamvakopoulos, C.; Strack, A. M.; McGowan, E.; Cashen, D. E.; Drisko, J. E.; Hom, G. J.; Howard, A. D.; MacIntyre, D. E.; van der Ploeg, L. H. T.; Patchett, A. A.; Nargund, R. P. *J. Med. Chem.* **2002**, *45*, 4589; (b) Palucki, B. L.; Park, M. K.; Nargund, R. P.; Ye, Z.; Sebhat, I. K.; Pollard, P. G.; Kalyani, R. N.; Tang, R.; MacNeil, T.; Weinberg, D. H.; Vongs, A.; Rosenblum, C. I.; Doss, G. A.; Miller, R. R.; Stearns, R. A.; Peng, Q.; Tamvakopoulos, C.; McGowan, E.; Martin, W. J.; Metzger, J. M.; Shepherd, C. A.; Strack, A. M.; MacIntyre, D. E.; van der Ploeg, L. H. T.; Patchett, A. A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 171; (c) Ye, Z.; Guo, L.; Barakat, K. J.; Pollard, P. G.; Palucki, B. L.; Sebhat, I. K.; Bakshi, R. K.; Tang, R.; Kalyani, R. N.; Vongs, A.; Chen, A. S.; Chen, H. Y.; Rosenblum, C. I.; MacNeil, T.; Weinberg, D. H.; Peng, Q.; Tamvakopoulos, C.; Miller, R. R.; Stearns, R. A.; Cashen, D. E.; Martin, W. J.; Metzger, J. M.; Strack, A. M.; MacIntyre, D. E.; van der Ploeg, L. H. T.; Patchett, A. A.; Wyvratt, M. J.; Nargund, R. P. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3501; (d) Dyck, B.; Parker, J.; Phillips, T.; Carter, L.; Murphy, B.; Summers, R.; Hermann, J.; Baker, T.; Cismowski, M.; Saunders, J.; Goodfellow, Val. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3793; (e) Pontillo, J.; Tran, J. A.; Arellano, M.; Fleck, B. A.; Huntley, R.; Marinkovic, D.; Lanier, M.; Nelson, J.; Parker, J.; Saunders, J.; Tucci, F. C.; Jiang, W.; Chen, C. W.; White, N. S.; Foster, A. C.; Chen, C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4417; (f) Chen, C.; Pontillo, J.; Fleck, B. A.; Gao, Y.; Wen, J.; Tran, J. A.; Tucci, F. C.; Marinkovic, D.; Foster, A. C.; Saunders, J. *J. Med. Chem.* **2004**, *47*, 6821; (g) Richardson, T. I.; Ornstein, P. L.; Briner, K.; Fisher, M. J.; Backer, R. T.; Biggers, C. K.; Clay, M. P.; Emmerson, P. J.; Hertel, L. W.; Hsiung, H. M.; Husain, S.; Kahl, S. D.; Lee, J. A.; Lindstrom, T. D.; Martinelli, M. J.; Mayer, J. P.; Mullaney, J. T.; O'Brien, T. P.; Pawlak, J. M.; Revell, K. D.; Shah, J.; Zgombick, J. M.; Herr, R. J.; Melekhov, A.; Sampson, P. B.; King, C.-H. R. *J. Med. Chem.* **2004**, *47*, 744; (h) Fisher, M. J.;

Backer, R. T.; Husain, S.; Hsiung, H. M.; Mullaney, J. T.; O'Brien, T. P.; Ornstein, P. L.; Rothhaar, R. R.; Zgombick, J. M.; Briner, K. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4459; (i) Pan, K.; Scott, M. K.; Lee, D. H. S.; Fitzpatrick, L. J.; Crooke, J. J.; Rivero, R. A.; Rosenthal, D. I.; Vaidya, A. H.; Zhao, B.; Reitz, A. B. *Bioorg. Med. Chem.* **2003**, *11*, 185; (j) Fotsch, C.; Han, N.; Arasasingham, P.; Bo, Y.; Carmouche, M.; Chen, N.; Davis, J.; Goldberg, M. H.; Hale, C.; Hsieh, F.-Y.; Kelly, M. G.; Liu, Q.; Norman, M. H.; Smith, D. M.; Stec, M.; Tamayo, N.; Xi, N.; Xu, S.; Bannon, A. W.; Baumgartner, J. W. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1623.

25. Arasasingham, P. N.; Fotsch, C.; Ouyang, X.; Norman, M. H.; Kelly, M. G.; Stark, K. L.; Karbon, B.; Hale, C.; Baumgartner, J. W.; Zambrano, M.; Cheettham, J.; Tomayo, N. *J. Med. Chem.* **2003**, *46*, 9.
26. Xi, N.; Hale, C.; Kelly, M. G.; Norman, M. H.; Stec, M.; Xu, S.; Baumgartner, J. W.; Fotsch, C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 377.
27. Pontillo, J.; Tran, J. A.; Markison, S.; Joppa, M.; Fleck, B. A.; Marinkovic, D.; Arellano, M.; Tucci, F. C.; Lanier, M.; Nelson, J.; Saunders, J.; Hoare, S. R. J.; Foster, A. C.; Chen, C. *Bioorg. Med. Chem. Lett.* **2005**, *14*, 2541.
28. (a) Pontillo, J.; Marinkovic, D.; Tran, J. A.; Arellano, M.; Fleck, B. A.; Wen, J.; Tucci, F. C.; Nelson, J.; Saunders, J.; Foster, A. C.; Chen, C. *Bioorg. Med. Chem. Lett.* **2005**, *14*, 4615; (b) Tucci, F. C.; White, N. S.; Markison, S.; Joppa, M.; Tran, J. A.; Fleck, B. A.; Madan, A.; Dyck, B. P.; Parker, J.; Pontillo, J.; Arellano, L. M.; Marinkovic, D.; Jiang, W.; Chen, C. W.; Gogas, K. R.; Goodfellow, V. S.; Saunders, J.; Foster, A. C.; Chen, C. *Bioorg. Med. Chem. Lett.* **2005**, *14*, 4389; (c) Pontillo, J.; Tran, J. A.; Fleck, B. A.; Marinkovic, D.; Arellano, M.; Tucci, F. C.; Lanier, M.; Nelson, J.; Parker, J.; Saunders, J.; Murphy, B.; Foster, A. C.; Chen, C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5605; (d) Tran, J. A.; Pontillo, J.; Arellano, M.; White, N. S.; Fleck, B. A.; Marinkovic, D.; Tucci, F. C.; Lanier, M.; Nelson, J.; Saunders, J.; Foster, A. C.; Chen, C. *Bioorg. Med. Chem. Lett.* **2005**, *14*, 833; (e) Marsilje, T. H.; Roses, J. B.; Calderwood, E. F.; Stroud, S. G.; Forsyth, N. E.; Blackburn, C.; Yowe, D. L.; Miao, W.; Drabic, S. V.; Bohane, M. D.; Daniels, J. S.; Li, P.; Wu, L.; Patane, M. A.; Claiborne, C. F. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3721.