Synthesis of Diphenylmethyl Analogues and Their Affinity for the Melanocortin-4 Receptor and the Serotonin Transporter

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While examining antagonists of the melanocortin-4 receptor (MC4 receptor), we found that compound 12b, containing a diphenylmethyl moiety, had a relatively high affinity for the MC4 receptor. When diphenylmethyl analogues were further examined, compounds 12c and 18 were also found to exhibit a high affinity for the MC4 receptor (IC_{50}=46.7 nM and 33.2 nM, respectively). Furthermore, compound 12c was also found to show a high affinity for the serotonin transporter (IC_{50}=10.7 nM). Here, we describe the synthesis and biological evaluation of various diphenylmethyl analogues in relation to their actions on the MC4 receptor and the serotonin transporter.

Key words melanocortin-4 receptor; serotonin transporter; depression; anxiety

Adrenocorticotropic hormone (ACTH) and melanocyte-stimulating hormones (α-MSH, β-MSH and γ-MSH) are derived by enzymatic processing from proopiomelanocortin (POMC) and are collectively referred to as the melanocortins. Five melanocortin receptors (MC1—MC5 receptors) belonging to the seven-transmembrane G-protein-coupled receptor family have been reported to date.[1—3] 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 equal affinity, is widely expressed in the central nervous system as well as the 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 behavior, energy homeostasis, sexual functions and protection against tumor-induced reductions in body weight.[4—13] In addition, this receptor has been the focus of interest for its possible relationship to stress and the regulation of emotional behavior[14—16] in relation to conditions such as depression and anxiety.[17—20] The findings to date indicate that the MC4 receptor may be a promising target for the development of drugs for the above-mentioned conditions, and numerous agonists[25—29] and antagonists[22—24,30—40] of the MC4 receptor have been developed. We previously reported that the MC4 receptor antagonist **3** (MCL0129; Fig. 1) exhibited antidepressant, anxiolytic, and anti-stress effects in a variety of animal models.[17] These findings suggest that MC4 receptor blockade may be a useful approach for treating subjects with depressive and anxiety disorders. This hypothesis is supported by the demonstration in various rodent models of depression and anxiety that other MC4 receptor antagonists also exert antidepressant and anxiolytic effects.[19]

Serotonin transporter blockade has been recognized as being effective for the treatment of depression, and selective serotonin reuptake inhibitors (SSRIs) have since become first-line drugs for the treatment of not only depression, but also anxiety disorders.[21] Although most patients are successfully treated with SSRIs, the existence of non-responders and of patients who respond slowly to the drug remains a drawback of SSRIs therapy. Therefore, much effort is being expended on the development of new antidepressants with mono-aminergic mechanisms of action. Previously, we reported that **2** (MCL0042; Fig. 1) exhibited relatively high affinities for both the MC4 receptor and the serotonin transporter. This compound, with its unique activity for both the MC4 receptor and the serotonin transporter, was also shown to exhibit antidepressant and anxiolytic-like effects.[18] Thus, drugs exhibiting a combination of MC4 antagonism and serotonin transporter inhibition might represent a new class of antidepressants.

In this paper, we report the synthesis and biological evaluation of various diphenylmethyl analogues in relation to their actions on the MC4 receptor. Moreover, selected compounds with a high affinity for the MC4 receptor were also examined to determine their affinity for the serotonin transporter.

Chemistry Charts 1—3 show the synthesis of compounds **12a**—**p** and **18**—**20**. Compounds **12a**—**p** were prepared using two synthetic routes (Methods A and B) from

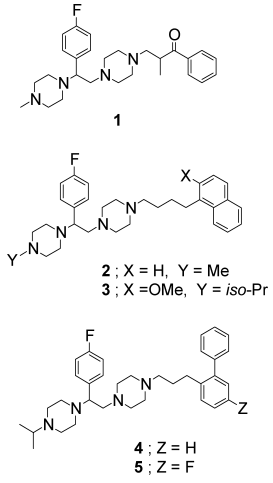

Fig. 1. Bis-Piperazine Compounds as MC4 Receptor Antagonists

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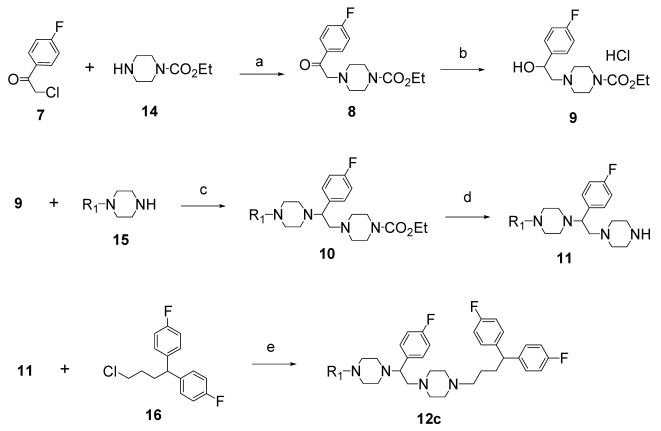
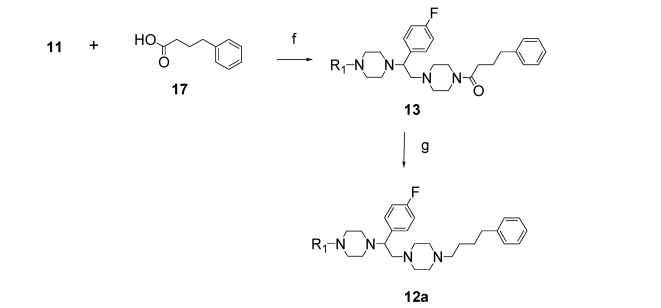


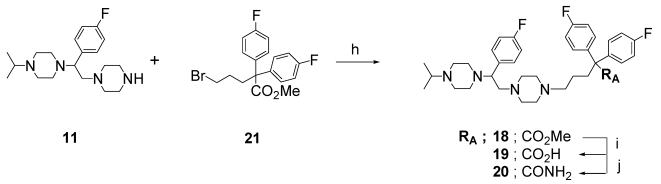
Reagents and conditions: (a) $CHCl_3$, reflux; (b) $NaBH_4$, EtOH, 50 °C and then 4 M HCl/EtOAc; (c) **9**, $SOCl_2$, benzene, 50 °C and then N-R_1-piperazine (**15**), $^iPr_2NEt$, benzene, 60 °C (78% from **7**); (d) KOH, EtOH, reflux (85%); (e) $^iPr_2NEt$, DMF, 60 °C (58%)

Chart 1. Synthesis of Compound **12c**; General Synthetic Method for **12b**, **12c**, **12e** and **12h**—**o** (Method A)



Reagents and conditions: (f) EDC, $CHCl_3$, rt; (g) $LiAlH_4$, THF, reflux (73% from **11**)

Chart 2. Synthesis of Compound **12a**; General Synthetic Method for **12a**, **12f**, **12g** and **12p** (Method B)



Reagents and conditions: (h) $^iPr_2NEt$, DMF, 70 °C (89%); (i) HCl, reflux (72%); (j) $SOCl_2$, benzene, 80 °C and then aq. NH_3, rt (quant.)

Chart 3. Synthesis of Compounds **18**—**20** (Method C)

fluoroacetophenone **7** *via* compound **11**[23] (Charts 1, 2). The reaction of **7** with N-CO_2Et-piperazine **14** followed by the reduction of the carbonyl group with $NaBH_4$ yielded **9**. Chlorination of the hydroxyl group of **9** with thionyl chloride followed by coupling with N-R_1-piperazine **15** in the presence of $^iPr_2NEt$ yielded **10**. Removal of the ethoxycarbonyl group of **10** using KOH yielded **11**. Compounds **11** were converted to **12b**, **12c**, **12e** and **12h**—**o** by treatment with alkyl halides (**16** for **12c**) in the presence of $^iPr_2NEt$ (Method A; Chart 1). Compound **12d** was obtained by the hydrogenation of **12j** using Pd–C as a catalyst. Furthermore, compound **12a** was prepared by the reduction of **13**, which had been prepared from **11** by condensation with carboxylic acid **17**, using $LiAlH_4$. Compounds **12f**, **12g** and **12p** were also prepared from **11** by the same synthetic method as that for **12a** (Method B; Chart 2).

Compounds **18**—**20** were also synthesized from compound **11** (Chart 3). The reaction of **11** with alkyl halide **21** in the presence of $^iPr_2NEt$ (yielding **18**) and followed by hydrolysis yielded **19**. Compound **20** was prepared from **19** by treatment with thionyl chloride, followed by treatment with aqueous NH_3 (Method C; Chart 3).

Results and Discussion

The affinities of all the compounds were evaluated by assessing their binding to the membranes of COS-1 cells expressing the human MC4 receptor and estimating the resulting affinities using the inhibition curve for [^{125}I]Nle^4-D-Phe^7-α-MSH binding[17]; the respective IC_{50} values are shown in Tables 1 and 2. In addition, selected compounds were tested for their affinity to the serotonin transporter (SERT) by assessing [^{3}H]Paroxetine binding to the rat cortical membrane (Table 3).

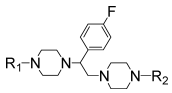
We previously reported that compound **1** (Fig. 1), a bis-piperazine compound with a carbonyl group that was identified in a high-throughput screening against an internal compounds library, exhibited moderate affinity for the MC4 receptor (IC_{50}=399 nM).[23] Bis-piperazine analogues with a carbonyl group (including **1**), antagonists of the MC4 receptor, have also been reported by Arasasingham *et al.*[22] They also reported that bis-piperazine compound **6** (Table 1), without the carbonyl group, exhibited almost no affinity for the MC4 receptor. These findings indicate that the carbonyl group is crucial for the binding of bis-piperazine compounds to the MC4 receptor. However, compound **1** has a beta amino ketone moiety in its structure, possibly resulting in the beta-elimination of the amine part. Therefore, we attempted to design non-carbonyl compounds with a higher affinity for the MC4 receptor.

At first, to increase the binding affinity of non-carbonyl compound **6** for the MC4 receptor, we investigated a non-carbonyl compound with a longer length of an alkylene linker moiety between the piperazine ring and the benzene ring, designed based on the results of previously reported information regarding the structure–activity relationships (SARs) on the bis-piperazine compounds.[23] As expected, the resultant non-carbonyl compound **12a**, with a four-carbon linker, exhibited a higher affinity for the MC4 receptor (**12a**: IC_{50}=1050 nM) than compound **6** with a three-carbon linker.

We next focused attention on π-electron of carbonyl group, and anticipated that a phenyl group having π-electron could be used as an alternative to the carbonyl group. We prepared compound **12b**, in which a phenyl group was introduced into the benzyl position of compound **12a**, and evaluated the affinity of the resultant compound for the MC4 receptor. Fortunately, compound **12b** showed a much higher affinity for the MC4 receptor (IC_{50}=153 nM) than the lead compound **12a** (IC_{50}=1050 nM) and also had a higher affinity than compound **1** (IC_{50}=399 nM). Therefore, we started to examine diphenylmethyl analogues with a higher affinity for the MC4 receptor.

Next, to increase the binding affinity for the MC4 receptor, we investigated an *iso*-propyl group at R_1, because the *iso*-propyl group was previously reported to be an optimized group in bis-piperazine analogues.[23] Moreover, we explored the effects of introducing fluorine atoms at the benzene ring on the binding affinity for the receptor. The resultant **12c**,

Table 1. Binding Affinity for the MC4 Receptor



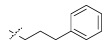
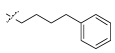
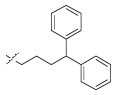
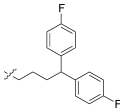
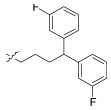
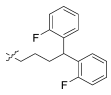
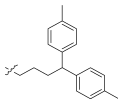
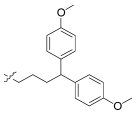
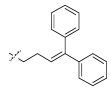
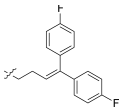
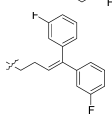
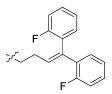
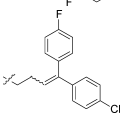
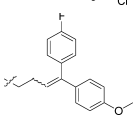
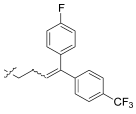
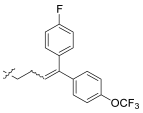
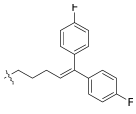
Compound	R_1	R_2	IC_{50} (nM)
6	Me		3829
12a	Me		1050
12b	Me		153
12c	isoPr		46.7
12d	isoPr		43.8
12e	isoPr		90.8
12f	isoPr		97.7
12g	isoPr		224
12h	Me		92.0
12i	isoPr		64.5
12j	isoPr		45.1
12k	isoPr		68.2
12l	isoPr		83.6
12m	isoPr		148
12n	isoPr		365
12o	isoPr		87.5
12p	isoPr		52.2

Table 2. Binding Affinity for the MC4 Receptor

Compound	IC_{50} (nM)
18	33.2
19	2960
20	618

Table 3. Binding Affinities for the MC4 Receptor and SERT

Compound	IC_{50} (nM)	
	MC4	SERT
2	118	42.3
3	8.13	383
4	11.2	3130
5	33.0	1370
12c	46.7	10.7
12i	64.5	444

with the *iso*-propyl group and fluorine atoms at the benzene ring, showed a high affinity (IC_{50}=46.7 nM) for the MC4 receptor, and the *iso*-propyl group was employed in subsequent research efforts to explore diphenylmethyl analogues further.

We then explored the effects of benzene ring substituents in **12c** on the binding affinity for the MC4 receptor. Substitution with a methyl (**12f**) or methoxy (**12g**) group at position 4 of the benzene ring resulted in a decrease in affinity for the MC4 receptor (**12f**: IC_{50}=97.7 nM and **12g**: IC_{50}=224 nM), compared to that of a fluorine-substituted compound, **12c** (IC_{50}=46.7 nM). Compound **12d** substituted with a fluorine atom at position 3 of the benzene ring exhibited almost the same affinity for the MC4 receptor (IC_{50}=43.8 nM) as that of **12c** with fluorine substitution at position 4, whereas substitution with a fluorine atom at position 2 resulted in a 2-fold decrease in the binding affinity (**12e**: IC_{50}=90.8 nM). These results suggest that substitution at positions 3 or 4 of the benzene ring might be more favorable for obtaining an increased binding affinity for the MC4 receptor than substitution at position 2.

Next, we examined diphenylmethyl analogues with other alkylene linkers between the piperazine ring and the diphenylmethyl moiety. At first, we focused on the conformational rigidity of the carbonyl group of compound **1** and introduced a carbon–carbon double bond to **12b**. The resultant **12h**, containing a carbon–carbon double bond, was tested for its affinity for the MC4 receptor. The result revealed that **12h** exhibited a slightly higher affinity for the MC4 receptor (IC_{50}=92.0 nM) than **12b**, without a carbon–carbon double bond. This finding encouraged us to optimize the binding affinity of this series of compounds containing a carbon–carbon double bond. Compounds **12i**, **12j** and **12k**, substituted with a fluorine atom at positions 4, 3 or 2 of the benzene ring, respectively, exhibited similar affinities (IC_{50}=64.5, 45.1 and 68.2 nM, respectively) to those of compounds without a carbon–carbon double bond (**12i** *vs.* **12c**, **12j** *vs.* **12d**, **12k** *vs.* **12e**). We next prepared compounds **12l**—**o** substituted with a chlorine atom (**12l**), methoxy (**12m**), trifluoromethyl (**12n**) or trifluoromethoxy (**12o**) group at position 4 of the benzene ring and tested the binding affinities of these compounds for the MC4 receptor. The results showed that

none of these compounds exhibited a higher affinity for the receptor than **12i** (**12l**: IC_{50}=83.6 nM, **12m**: IC_{50}=148 nM, **12n**: IC_{50}=365 nM, **12o**: IC_{50}=87.5 nM). These results suggest that a steric bulk at this position may not be well tolerated. We then investigated diphenylmethyl analogues with longer alkylene linkers between the piperazine ring and the diphenylmethyl moiety. The results showed that **12p**, with a four-carbon linker, exhibited a similar affinity for the MC4 receptor (IC_{50}=52.2 nM) to **12i**, with a three-carbon linker.

Since the introduction of the carbon–carbon double bond had no significant decrease of the binding affinity for MC4 receptor, we attempted to explore the effects of introducing R_A (Chart 3) on the binding affinity for the receptor (Table 2). The introduction of a methoxycarbonyl group at R_A resulted in a slight increase in affinity for the MC4 receptor (**18**: IC_{50}=33.2 nM), compared with that of a non-substituted compound, **12c**. Thus, a methoxycarbonyl group at R_A had a favorable effect on the binding affinity for the MC4 receptor: however, a carboxyl or carbamoyl group at R_A proved to be unfavorable (**19**: IC_{50}=2960 nM and **20**: IC_{50}=618 nM).

Selected compounds with a high affinity for the MC4 receptor were also examined for their affinity to SERT (Table 3). Although **3**, **4** and **5** (Fig. 1), antagonists of the MC4 receptor previously reported by us,[23] and **12i** had a higher affinity for the MC4 receptor than for SERT, **12c** exhibited a high affinity (IC_{50}=10.7 nM) for SERT. Interestingly, the only difference between **12c** and **12i** was the presence of a carbon–carbon double bond, yet **12c** exhibited a high affinity for SERT (IC_{50}=10.7 nM) while **12i** had a low affinity for SERT (IC_{50}=444 nM). These results suggest that, in the case of these analogues, the carbon–carbon double bond might significantly impact the affinity for SERT. Furthermore, the finding that the affinities of **12c** for both the MC4 receptor and SERT were greater than those of **2**, which exhibited antidepressant and anxiolytic-like effects,[18] suggests that **12c** may represent a promising tool for investigating the pathogenesis of stress-related disorders, such as depression and anxiety.

Conclusion

We have reported the synthesis and evaluation of a new series of MC4 receptor ligands, diphenylmethyl analogues. Removal of a carbonyl and methyl group from compound **1** provided our lead compound **12a**. Structural optimization based on the SAR study around the piperazine side chain moiety led to the identification of diphenylmethyl analogues with high affinities for the MC4 receptor, namely, **12c** and **18**, which showed an over 20-fold higher affinity for the receptor than our lead compound **12a**. Among these compounds, the introduction of a carbon–carbon double bond, such as in **12i**, resulted in a higher selectivity for the MC4 receptor over SERT. In contrast, compound **12c** without a carbon–carbon double bond exhibited high affinities for both the MC4 receptor and SERT. Considering that SSRIs are widely prescribed for the treatment of both depressive and anxiety disorders in clinical settings, this new class of compounds, such as **12c**, displaying dual high affinities for both the MC4 receptor and SERT might also be useful for the treatment of these disorders.

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 (TMS) as an internal standard. IR spectra were recorded on a Perkin–Elmer Spectrum One FT-IR spectrometer. Mass spectra (MS) were obtained on Micromass Platform LC (ES). High resolution spectra were recorded on a Micromass Q-TOF2 instrument. Elemental analyses were performed by a Perkin–Elmer 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.

General Methods for the Synthesis of (±)-12b—e and (±)-12h—o (Method A). (±)-1-[4,4-Bis(4-fluorophenyl)butyl]-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine 3 Maleate ((±)-12c) A mixture of (±)-1-[1-(4-fluorophenyl)-2-piperazin-1-ylethyl]-4-isopropylpiperazine (±)-**11**[23] (0.20 g, 0.6 mmol), 1,1′-(4-chlorobutane-1,1-diyl)bis(4-fluorobenzene) **16** (0.42 g, 1.5 mmol) and ^{i}Pr$_2$NEt (0.26 ml, 1.5 mmol) in DMF (5 ml) was heated at 60 °C for 6 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-[4,4-bis(4-fluorophenyl)butyl]-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine (0.20 g, 58%) as an oily product. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.01 (6H, d, J=6.5 Hz), 1.39—1.44 (2H, m), 1.90—2.02 (2H, m), 2.18—2.67 (20H, m), 2.82 (1H, dd, J=6.2, 12.8 Hz), 3.51 (1H, t, J=6.8 Hz), 3.83 (1H, t, J=7.6 Hz), 6.88—7.01 (6H, m), 7.09—7.21 (6H, m). MS (ESI, Pos) m/z: 579 $(M+H)^+$. The above free base (0.18 g, 0.31 mmol) was dissolved in EtOH (2 ml), and to the solution was added a solution of maleic acid (0.11 g, 0.95 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-bis(4-fluorophenyl)butyl]-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine 3 maleate (±)-**12c** (0.20 g, 70%) as a crystal. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.20 (6H, d, J=6.5 Hz), 1.41—1.52 (2H, m), 1.88—2.12 (2H, m), 2.55—3.65 (22H, m), 3.95—4.03 (1H, m), 6.12 (6H, s), 7.11—7.32 (12H, m). MS (ESI, Pos) m/z: 579 $(M+H)^+$. *Anal*. Calcd for $C_{35}H_{45}F_3N_4\cdot 3C_4H_4O_4\cdot 1.0H_2O$: C, 59.74; H, 6.29; N, 5.93. Found: C, 59.80; H, 6.11; N, 5.66. mp 148—150 °C.

(±)-**12b**, **12e** and (±)-**12h**—**o** were prepared by using method of (±)-**12c**.

(±)-1-(4,4-Diphenylbutyl)-4-[2-(4-fluorophenyl)-2-(4-methylpiperazin-1-yl)ethyl]piperazine 3 Maleate ((±)-12b) (±)-**12b** was obtained as a crystal. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.41—1.55 (2H, m), 1.98—2.08 (2H, m), 2.72 (3H, s), 2.74—3.46 (20H, m), 3.85—3.96 (2H, m), 6.10 (6H, s), 7.13—7.33 (14H, m). MS (ESI, Pos) m/z: 515 $(M+H)^+$. *Anal*. Calcd for $C_{33}H_{43}FN_4\cdot 3C_4H_4O_4$: C, 62.63; H, 6.42; N, 6.49. Found: C, 62.36; H, 6.42; N, 6.53. mp 181—182 °C (crystallized from EtOH).

(±)-1-[4,4-Bis(2-fluorophenyl)butyl]-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine 4 Hydrochloride ((±)-12e) (±)-**12e** was obtained as a crystal. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.20 (6H, d, J=6.5 Hz), 1.61—1.76 (2H, m), 2.02—2.21 (2H, m), 2.51—2.70 (1H, m), 3.09—3.75 (21H, m), 4.03—4.21 (1H, m), 4.42—4.57 (2H, m), 7.07—7.43 (12H, m), 9.80 (1H, br s), 11.3—11.5 (1H, m). MS (ESI, Pos) m/z: 579 $(M+H)^+$. *Anal*. Calcd for $C_{35}H_{45}F_3N_4\cdot 4HCl\cdot 1.2H_2O$: C, 56.33; H, 6.94; N, 7.51. Found: C, 56.08; H, 7.08; N, 7.50. mp 170—173 °C (crystallized from a mixture of EtOAc and MeOH).

(±)-1-(4,4-Diphenylbut-3-en-1-yl)-4-[2-(4-fluorophenyl)-2-(4-methylpiperazin-1-yl)ethyl]piperazine 3 Maleate ((±)-12h) (±)-**12h** was obtained as a crystal. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.33—2.41 (2H, m), 2.72 (3H, s), 2.62—3.59 (20H, m), 3.91—3.99 (1H, m), 6.05 (1H, t, J=7.2 Hz), 6.10 (6H, s), 7.14—7.47 (14H, m). MS (ESI, Pos) m/z: 513 $(M+H)^+$. *Anal*. Calcd for $C_{33}H_{41}FN_4\cdot 3C_4H_4O_4$: C, 62.78; H, 6.21; N, 6.51. Found: C, 62.50; H, 6.15; N, 6.57. mp 182—184 °C (crystallized from EtOH).

(±)-1-[4,4-Bis(4-fluorophenyl)but-3-en-1-yl]-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine 3 Maleate ((±)-12i) (±)-**12i** was obtained as a crystal. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.20 (6H, d, J=6.5 Hz), 2.18—3.82 (23H, m), 3.96—4.08 (1H, m), 6.04 (1H, t, J=7.2 Hz), 6.12 (6H, s), 7.09—7.32 (12H, m). MS (ESI, Pos) m/z: 577 $(M+H)^+$. *Anal*. Calcd for $C_{35}H_{43}F_3N_4\cdot 3C_4H_4O_4\cdot 1.5H_2O$: C, 59.30; H, 6.14; N, 5.89. Found: C, 59.31; H, 5.90; N, 5.62. mp 161—164 °C (crystallized from EtOH).

(±)-1-[4,4-Bis(3-fluorophenyl)but-3-en-1-yl]-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine 4 Hydrochloride ((±)-12j) (±)-**12j** was obtained as a crystal. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.20 (6H, d, J=6.5 Hz), 2.17—2.30 (2H, m), 2.62—2.77 (2H, m), 3.10—3.78 (18H, m), 4.09—4.22 (1H, m), 4.49—4.61 (1H, m), 6.32 (1H, t, J=7.1 Hz), 6.70—6.94 (2H, m), 6.99—7.07 (5H, m), 7.20—7.54 (7H, m), 10.2 (1H, br s), 11.9 (1H, br s). MS (ESI, Pos) m/z: 577 $(M+H)^+$. *Anal*. Calcd for $C_{35}H_{43}F_3N_4\cdot 4HCl\cdot 0.5H_2O$: C, 57.46; H, 6.61; N, 7.66. Found: C, 57.51; H, 6.72; N, 7.62. mp 198—202 °C (crystallized from a mixture of EtOAc and MeOH).

(±)-1-[4,4-Bis(2-fluorophenyl)but-3-en-1-yl]-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine 3.8 Hydrochloride ((±)-12k) (±)-**12k** was obtained as a crystal. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.20 (6H, d, J=6.5 Hz), 2.08—2.26 (1H, m), 2.51—2.59 (1H, m), 3.12—3.77 (22H, m), 4.01—4.20 (1H, m), 4.42—4.58 (1H, m), 6.12 (1H, t, J=7.2 Hz), 7.05—7.41 (12H, m), 9.90 (1H, br s), 11.7 (0.8H, br s). MS (ESI, Pos) m/z: 577 $(M+H)^+$. *Anal*. Calcd for $C_{35}H_{43}F_3N_4\cdot 3.8HCl\cdot 0.3H_2O$: C, 58.33; H, 6.63; N, 7.77. Found: C, 58.56; H, 6.89; N, 7.71. mp 200—203 °C (crystallized from a mixture of EtOAc and MeOH).

(±)-1-[4-(4-Chlorophenyl)-4-(4-fluorophenyl)but-3-en-1-yl]-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine 3.8 Hydrochloride ((±)-12l) (a Mixture of *E* and *Z*) (±)-**12l** was obtained as a crystal. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.21 (6H, d, J=6.5 Hz), 2.12—2.31 (1H, m), 2.45—2.59 (1H, m), 2.60—2.77 (1H, m), 3.18—3.41 (9H, m), 3.43—3.77 (10H, m), 4.12 (1H, t, J=7.2 Hz), 4.38—4.95 (3H, m), 6.09—6.22 (1H, m), 7.09—7.53 (12H, m), 10.2 (1H, br s), 11.8 (0.8H, br s). MS (ESI, Pos) m/z: 593 $(M+H)^+$. *Anal*. Calcd for $C_{35}H_{43}F_2ClN_4\cdot 3.8HCl\cdot 0.5H_2O$: C, 56.90; H, 6.62; N, 7.58. Found: C, 56.71; H, 6.76; N, 7.49. mp 187—190 °C (crystallized from a mixture of EtOAc and MeOH).

(±)-1-(1-(4-Fluorophenyl)-2-{4-[4-(4-fluorophenyl)-4-(4-methoxyphenyl)but-3-en-1-yl]piperazin-1-yl}ethyl)-4-isopropylpiperazine 3.8 Hydrochloride ((±)-12m) (a Mixture of *E* and *Z*) (±)-**12m** was obtained as a crystal. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.21 (6H, d, J=6.5 Hz), 2.09—2.22 (1H, m), 3.19—3.77 (24H, m), 4.06—4.20 (1H, m), 4.30—4.60 (3H, m), 6.09—6.22 (1H, m), 7.09—7.49 (12H, m), 9.80 (1H, br s), 11.5—11.7 (0.8H, m). MS (ESI, Pos) m/z: 589 $(M+H)^+$. *Anal*. Calcd for $C_{36}H_{46}F_2N_4O\cdot 3.8HCl\cdot 0.9H_2O$: C, 58.15; H, 6.99; N, 7.54. Found: C, 58.34; H, 7.28; N, 7.53. mp 172—174 °C (crystallized from a mixture of EtOAc and MeOH).

(±)-1-[1-(4-Fluorophenyl)-2-(4-{4-(4-fluorophenyl)-4-[4-(trifluoromethyl)phenyl]but-3-en-1-yl}piperazin-1-yl)ethyl]-4-isopropylpiperazine 3.8 Hydrochloride ((±)-12n) (a Mixture of *E* and *Z*) (±)-**12n** was obtained as a crystal. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.21 (6H, d, J=6.5 Hz), 2.05—2.22 (1H, m), 3.18—3.80 (21H, m), 4.00—4.55 (4H, m), 6.18—6.32 (1H, m), 7.10—7.49 (10H, m), 7.64—7.83 (2H, m), 9.80 (1H, br s), 11.6—11.7 (0.8H, m). MS (ESI, Pos) m/z: 527 $(M+H)^+$. *Anal*. Calcd for $C_{36}H_{43}F_5N_4\cdot 3.8HCl\cdot 2.0H_2O$: C, 53.96; H, 6.39; N, 6.99. Found: C, 54.23; H, 6.66; N, 7.15. mp 173—175 °C (crystallized from a mixture of EtOAc and MeOH).

(±)-1-[1-(4-Fluorophenyl)-2-(4-4-(4-fluorophenyl)-4-[4-(trifluoromethoxy)phenyl]but-3-en-1-yl}piperazin-1-yl)ethyl]-4-isopropylpiperazine 4 Hydrochloride ((±)-12o) (a Mixture of *E* and *Z*) (±)-**12o** was obtained as a crystal. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.21 (6H, d, J=6.5 Hz), 2.09—2.21 (1H, m), 3.18—4.23 (25H, m), 6.11—6.22 (1H, m), 7.10—7.44 (12H, m), 9.85 (1H, br s), 11.6—11.8 (1H, m). MS (ESI, Pos) m/z: 643 $(M+H)^+$. *Anal*. Calcd for $C_{36}H_{43}FN_4O\cdot 4HCl\cdot 0.1H_2O$: C, 54.71; H, 6.02; N, 7.09. Found: C, 54.47; H, 5.97; N, 6.92. mp 212—215 °C (crystallized from a mixture of EtOAc and MeOH).

(±)-1-[4,4-Bis(3-fluorophenyl)butyl]-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine 4 Hydrochloride ((±)-12d) A mixture of (±)-1-[4,4-bis(3-fluorophenyl)but-3-en-1-yl]-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine 4 hydrochloride (±)-**12j** (0.25 g, 0.34 mmol), 5% Pd–C (25 mg) in MeOH (2.5 ml) was stirred at room temperature under H_2 atmosphere for 12 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-[4,4-bis(3-fluorophenyl)butyl]-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine (53 mg, 27%) as an oily product. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.01 (6H, d, J=6.5 Hz), 1.31—1.44 (2H, m), 1.92—2.04 (2H, m), 2.10—2.66 (20H, m), 2.82 (1H, dd, J=5.8, 12.7 Hz), 3.53 (1H, t, J=7.0 Hz), 3.84 (1H, t, J=8.0 Hz), 6.80—7.02 (8H, m), 7.10—7.25 (4H, m). MS (ESI, Pos) m/z: 579 $(M+H)^+$. The above free base (53 mg, 0.092 mmol) was dissolved in EtOH (1 ml), treated with 4 M HCl in AcOEt (0.10 ml) at room temperature, and the mixture was concentrated *in vacuo*. The residue was crystallized in a mixture of AcOEt and MeOH, and the precipitate was collected by filtration to obtain (±)-1-[4,4-bis(3-fluorophenyl)butyl]-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]-piperazine 4 hydrochloride (±)-**12d** (45 mg, 66%) as a crystal. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.20 (6H, d, J=6.5 Hz), 1.56—1.69 (2H, m), 2.03—2.22 (2H, m), 2.52—2.69 (1H, m), 3.10—3.79 (22H, m), 3.99—4.21 (1H, m), 4.45—4.57 (1H, m), 6.98—7.05 (2H, m), 7.17—7.41 (10H, m), 9.90 (1H, br s), 11.5 (1H, br s). MS (ESI, Pos) m/z: 579 $(M+H)^+$. *Anal*. Calcd for $C_{35}H_{45}F_3N_4\cdot 4HCl\cdot 0.5H_2O$: C, 57.30; H, 6.87; N, 7.64. Found: C, 57.34; H, 7.08; N, 7.60. mp 196—198 °C.

General Methods for the Synthesis of 12a, 12f, 12g and 12p (Method B). (±)-1-[2-(4-Fluorophenyl)-2-(4-methylpiperazin-1-yl)ethyl]-4-(4-phenylbutyl)piperazine 3 Maleate ((±)-12a) To a mixture of (±)-1-[1-(4-fluorophenyl)-2-piperazin-1-ylethyl]-4-methylpiperazine (±)-**11** (0.385 g, 1.26 mmol) and 4-phenylbutanoic acid **17** (0.217 g, 1.32 mmol) in $CHCl_3$ (5 ml) was added EDC·HCl (0.253 g, 1.32 mmol) and the mixture was stirred at room temperature for 15 h. The mixture was partitioned between EtOAc and saturated aqueous $NaHCO_3$, and separated organic phase was washed with brine. The organic phase was concentrated *in vacuo* to obtain crude (±)-1-[2-(4-fluorophenyl)-2-(4-methylpiperazin-1-yl)ethyl]-4-(4-phenylbutanoyl)piperazine (±)-**13** (0.555 g, quantitative) as an oily product. A mixture of the above crude (±)-**13** (0.555 g, 1.26 mmol) and $LiAlH_4$ (48 mg, 1.3 mmol) in THF (5 ml) was refluxed for 1 h. To the mixture were added Et_2O (5 ml) and 25% aqueous NH_3 (1 ml) at room temperature. After stirred for 1 h, the mixture was filtered with Celite and the filtrate was concentrated *in vacuo*. The residue was chromatographed on Silica gel C-200 ($CHCl_3$/MeOH 4 : 1) to obtain (±)-1-[2-(4-fluorophenyl)-2-(4-methylpiperazin-1-yl)ethyl]-4-(4-phenylbutyl)piperazine (0.40 g, 73%) as an oily product. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.60—1.72 (4H, m), 2.22—2.69 (24H, m), 2.84 (1H, dd, J=6.2, 12.8 Hz), 3.56 (1H, d, J±6.5 Hz), 6.91—7.05 (2H, m) 7.10—7.35 (7H, m). MS (ESI, Pos) m/z: 439 $(M+H)^+$. The above free base (0.31 g, 0.71 mmol) was dissolved in EtOH (2 ml), and to the solution was added a solution of maleic acid (0.25 g, 2.1 mmol) in EtOH (1 ml). After stirred at room temperature for 1 h, the resulting precipitate was collected by filtration to obtain (±)-1-[2-(4-fluorophenyl)-2-(4-methylpiperazin-1-yl)ethyl]-4-(4-phenylbutyl)piperazine 3 maleate (±)-**12a** (0.45 g, 90%) as a crystal. ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 1.50—1.70 (4H, m), 2.55—3.98 (26H, m), 6.10 (6H, s), 7.16—7.40 (9H, m). MS (ESI, Pos) m/z: 439 $(M+H)^+$. *Anal*. Calcd for $C_{27}H_{39}FN_4\cdot 3C_4H_4O_4$: C, 59.53; H, 6.53; N, 7.12. Found: C, 59.41; H, 6.55; N, 7.06. mp 185—187 °C.

12f, **12g** and **12p** were prepared by using method of (±)-**12a**.

(±)-1-[4,4-Bis(4-methylphenyl)butyl]-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine 4 Hydrochloride ((±)-12f) (±)-**12f** was obtained as a crystal. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.22 (6H, d, J=6.5 Hz), 1.52—1.69 (2H, m), 1.97—2.10 (2H, m), 2.13 (6H, s), 2.55—2.72 (1H, m), 3.10—3.73 (19H, m), 3.83 (1H, t, J=7.6 Hz), 4.09—4.22 (1H, m), 4.35—4.60 (3H, m), 7.07 (4H, d, J=8.1 Hz), 7.17 (4H, d, J=8.2 Hz), 7.25 (2H, t, J=8.6 Hz), 7.34—7.43 (2H, m), 10.1 (1H, br s), 11.6 (1H, br s). MS (ESI, Pos) m/z: 571 $(M+H)^+$. *Anal*. Calcd for $C_{37}H_{51}FN_4\cdot 4HCl\cdot 1.2H_2O$: C, 60.19; H, 7.84; N, 7.59. Found: C, 60.18; H, 7.91; N, 7.54. mp 174—176 °C (crystallized from a mixture of EtOAc and MeOH).

(±)-1-[4,4-Bis(4-methoxyphenyl)butyl]-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine 4 Hydrochloride ((±)-12g) (±)-**12g** was obtained as a crystal. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.22 (6H, d, J=6.5 Hz), 1.53—1.70 (2H, m), 1.96—2.09 (2H, m), 2.11—2.28 (1H, m), 2.57—2.73 (1H, m), 3.09—3.42 (9H, m), 3.44—3.78 (9H, m), 3.72 (6H, s), 3.82 (1H, t, J=7.8 Hz), 4.17 (1H, t, J=12.4 Hz), 4.49—4.59 (1H, m), 5.15—5.55 (2H, m), 6.82 (4H, d, J=8.7 Hz), 7.20 (4H, d, J=8.7 Hz), 7.25 (2H, t, J=8.7 Hz), 7.35—7.44 (2H, m), 10.2 (1H, br s), 11.1 (1H, br s). MS (ESI, Pos) m/z: 603 $(M+H)^+$. *Anal*. Calcd for $C_{37}H_{51}FN_4O_2\cdot 4HCl\cdot 1.5H_2O$: C, 57.29; H, 7.54; N, 7.22. Found: C, 57.20; H, 7.57; N, 7.18. mp 169—172 °C (crystallized from a mixture of EtOAc and MeOH).

(±)-1-[5,5-Bis(4-fluorophenyl)pent-4-en-1-yl]-4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazine 4 Hydrochloride ((±)-12p) (±)-**12p** was obtained as a crystal. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.20 (6H, d, J=6.5 Hz), 1.82—1.98 (2H, m), 2.03—2.22 (3H, m), 2.58—2.76 (1H, m), 3.02—3.16 (2H, m), 3.18—3.43 (8H, m), 3.45—3.79 (8H, m), 4.14 (1H, t, J=8.7 Hz), 4.30—4.90 (3H, m), 6.12 (1H, t, J=7.2 Hz), 7.06—7.43 (12H, m), 10.0 (1H, br s), 11.8 (1H, br s). MS (ESI, Pos) m/z: 591 $(M+H)^+$. *Anal*. Calcd for $C_{36}H_{45}F_3N_4\cdot 4HCl\cdot 0.5H_2O$: C, 55.96; H, 6.91; N, 7.25. Found: C, 55.91; H, 6.95; N, 7.25. mp 203—207 °C (crystallized from a mixture of EtOAc and MeOH).

Synthesis of 18—20 (Method C). (±)-Methyl 2,2-bis(4-fluorophenyl)-5-{4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazin-1-yl}pentanoate 3.5 Maleate ((±)-18) A mixture of (±)-1-[1-(4-fluorophenyl)-2-piperazin-1-ylethyl]-4-isopropylpiperazine (±)-**11** (1.0 g, 3.0 mmol), methyl 5-bromo-2,2-bis(4-fluorophenyl)pentanoate **21** (1.2 g, 3.1 mmol) and $^{i}Pr_2NEt$ (0.55 ml, 3.1 mmol) in DMF (10 ml) was heated at 70 °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 4 : 1) to obtain (±)-methyl 2,2-bis(4-fluorophenyl)-5-{4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazin-1-yl}pentanoate (1.7 g, 89%) as an oily product. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.00 (6H, d, J=6.5 Hz), 1.11—1.29 (2H, m), 1.58—1.70 (2H, m), 2.15—2.64 (20H, m), 2.82 (1H, dd, J=6.0, 12.8 Hz), 3.53 (1H, t, J=6.8 Hz), 3.74 (3H, s), 6.97 (2H, t, J=8.8 Hz), 7.12—7.23 (10H, m). MS (ESI, Pos) m/z: 637 $(M+H)^+$. The above free base (1.2 g, 1.9 mmol) was dissolved in EtOH (10 ml), and to the solution was added a solution of maleic acid (0.66 g, 5.7 mmol) in EtOH (5 ml). After stirred at room temperature for 1 h, the resulting precipitate was collected by filtration to obtain (±)-methyl 2,2-bis(4-fluorophenyl)-5-{4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazin-1-yl}pentanoate 3.5 maleate (±)-**18** (0.46 g, 18%) as a crystal. IR (KBr) cm^{-1}: 1729, 1623, 1605, 1576, 1511, 1480, 1386, 1356, 1228, 1165. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.20 (6H, d, J=6.5 Hz), 1.22—1.36 (2H, m), 1.91—2.10 (1H, m), 2.22—2.37 (2H, m), 2.65—3.15 (16H, m), 3.22—3.45 (4H, m), 3.63 (3H, s), 3.96—4.03 (1H, m), 6.13 (7H, s), 7.17—7.29 (9H, m), 7.30—7.39 (3H, m). MS (ESI, Pos) m/z: 637 $(M+H)^+$. *Anal*. Calcd for $C_{37}H_{47}F_3O_2N_4 \cdot 3.5C_4H_4O_4 \cdot 0.5H_2O$: C, 58.22; H, 5.94; N, 5.33. Found: C, 57.92; H, 6.02; N, 5.13. mp 104—106 °C.

(±)-2,2-Bis(4-fluorophenyl)-5-{4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazin-1-yl}pentanoic Acid 4 Hydrochloride ((±)-19) A solution of (±)-methyl 2,2-bis(4-fluorophenyl)-5-{4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazin-1-yl}pentanoate (±)-**18** (0.50 g, 0.79 mmol) in conc. HCl (20 ml) was refluxed for 12 h. After the mixture was concentrated *in vacuo*, the residue was stirred in a mixture of Et_2O and EtOH. The resulting precipitate was collected by filtration to obtain (±)-2,2-bis(4-fluorophenyl)-5-{4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazin-1-yl}pentanoic acid 4 hydrochloride (±)-**19** (0.44 g, 72%) as an amorphous. IR (KBr) cm^{-1}: 1718, 1606, 1510, 1469, 1448, 1230, 1165. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.22 (6H, d, J=6.5 Hz), 1.41—1.56 (2H, m), 2.15—2.32 (1H, m), 2.34—2.42 (2H, m), 2.61—2.78 (1H, m), 3.08—3.79 (21H, m), 4.09—4.23 (1H, m), 4.50—4.72 (1H, m), 7.17 (4H, t, J=8.8 Hz), 7.22—7.32 (5H, m), 7.35—7.43 (3H, m), 10.3 (1H, br s), 11.7 (1H, br s). MS (ESI, Pos) m/z: 623 $(M+H)^+$. *Anal*. Calcd for $C_{36}H_{45}F_3N_4O_2 \cdot 4HCl \cdot 1.5H_2O$: C, 54.35; H, 6.59; N, 7.04. Found: C, 54.14; H, 6.70; N, 6.90.

(±)-2,2-Bis(4-fluorophenyl)-5-{4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazin-1-yl}pentanamide 4 Hydrochloride ((±)-20) A mixture of (±)-2,2-bis(4-fluorophenyl)-5-{4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazin-1-yl}pentanoic acid 4 hydrochloride (±)-**19** (0.20 g, 0.26 mmol) and one drop of DMF in $SOCl_2$ (5 ml) was stirred at 80 °C for 2 h, and concentrated *in vacuo* to obtain crude product as an amorphous. A mixture of the above crude product in a mixture of 25% aqueous NH_3 (15 ml) and THF (15 ml) was stirred at room temperature for 1 h. The mixture was partitioned between EtOAc and brine. The separated organic phase was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was chromatographed on Chromatorex NH (hexane/EtOAc 1 : 1) to obtained (±)-2,2-bis(4-fluorophenyl)-5-{4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazin-1-yl}pentanamide (80 mg, 50%) as an oily product. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.01 (6H, d, J=6.5 Hz), 1.62 (4H, br s), 2.20—2.68 (20H, m), 2.81 (1H, dd, J=5.8, 12.8 Hz), 3.52 (1H, t, J=7.5 Hz), 5.38 (1H, br s), 5.99 (1H, br s), 6.93—7.04 (5H, m), 7.12—7.21 (2H, m), 7.23—7.31 (5H, m). MS (ESI, Pos) m/z: 622 $(M+H)^+$. The above free base (80 mg, 0.13 mmol) was dissolved in EtOH (1 ml), treated with 4 M HCl in 1,4-dioxane (0.20 ml) at room temperature, and the mixture was concentrated *in vacuo* to give (±)-2,2-bis(4-fluorophenyl)-5-{4-[2-(4-fluorophenyl)-2-(4-isopropylpiperazin-1-yl)ethyl]piperazin-1-yl}pentanoic acid 4 hydrochloride (±)-**20** (90 mg, quantitative) as an amorphous. IR (KBr) cm^{-1}: 1668, 1605, 1510, 1469, 1454, 1229, 1165. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.21 (6H, d, J=6.5 Hz), 1.31—1.56 (2H, m), 2.12—2.42 (3H, m), 2.60—2.79 (1H, m), 3.00—4.23 (23H, m), 4.41—4.58 (1H, m), 7.01—7.20 (4H, m), 7.22—7.43 (8H, m), 10.2 (1H, br s), 11.5 (1H, br s). MS (ESI, Pos) m/z: 622 $(M+H)^+$. HR-MS m/z: 622.3745 (Calcd for $C_{36}H_{46}F_3N_5O$: 622.3733).

Binding Test. 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, U.S.A.). [Nle^4,D-Phe^7]α-MSH was purchased from Peninsula Laboratories (Belmont, CA, U.S.A.). All other chemicals used in this study were obtained commercially, and all were of the highest purity available.

[^{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 (Chaki *et al.* 2003),[17] 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 phenylmethylsulfonylfluoride, and centrifuged at 48000×g 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 phenylmethylsulfonylfluoride 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.*[17] 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.

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