Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 2375–2385
Bioorganic & Medicinal Chemistry

Novel piperazines: Potent melanocortin-4 receptor antagonists with anxiolytic-like activity

Dai Nozawa,* Taketoshi Okubo, Takaaki Ishii, Kazuaki Takamori, Shigeyuki Chaki, Shigeru Okuyama and Atsuro Nakazato

Medicinal Research Laboratories, Taisho Pharmaceutical Co., Ltd, 1-403 Yoshino-cho, Kita-ku, Saitama, Saitama 331-9530, Japan

Received 22 December 2006; revised 12 January 2007; accepted 13 January 2007
Available online 18 January 2007

Abstract—In the present study, we found that a novel piperazine compound, **11a**, showed a moderate affinity (IC_{50} = 333 nM) for the MC4 receptor. We developed the new type of piperazine compounds and found that mono-piperazine **11b** exhibited a high-affinity (IC_{50} = 40.3 nM) for the MC4 receptor. We also found that a series of biphenyl analogues exhibited a high-affinity for the receptor, and in particular, compound **11j** exhibited the highest affinity for the MC4 receptor with an IC_{50} value of 14.5 nM. Furthermore, some of these compounds, when administered orally, significantly reversed the stress-induced anxiety-like behavior in rats. In this paper, we report the synthesis, structure-activity relationships, and oral activity of the novel mono-piperazines as MC4 receptor antagonists.

1. Introduction

Since five subtypes of melanocortin receptors (MC1–MC5 receptors) belonging to a family of seven-trans-membrane G-protein-coupled receptors were first cloned and characterized, numerous studies have been conducted to investigate the distribution and functions of these receptors.[1–3] The MC1 receptor is prominently expressed in the skin and melanoma cells, and plays a major role in regulating skin pigmentation. The MC2 receptor is prominently expressed in the adrenal cortex and is involved in steroidogenesis. The MC3 receptor 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, which is primarily in the brain. The MC5 receptor is expressed in various peripheral tissues and plays a role in exocrine gland function.[1–3]

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).[4] Two endogenous antagonistic proteins, namely, agouti and agouti-related protein (AGRP), have also been identified.[5,6] All of 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.[7,8]

Among the MC1–MC5 receptors, the MC4 receptor plays an important role in the regulation of feeding behavior and energy homeostasis.[9–15] It has also been reported to be involved in the regulation of sexual functions,[16,17] and to protect against tumor-induced decrease of body weight.[18,19] In addition, this receptor has been a focus of interest in terms of its relationship to stress and regulation of emotional behavior such as depression and anxiety.[20–26] These findings untill date 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. Although several peptidic MC4 receptor agonists and antagonists designed based on the core His-Phe-Arg-Trp terapeptide sequence have been reported,[27] nonpeptidic ligands of the MC4 receptor have been sought since peptidic ligands tend to have properties unsuitable for oral administration, such as poor absorption from the gastrointestinal tract.

Keywords: Melanocortin-4 receptor antagonist; Piperazine; Anxiety; Depression.
* Corresponding author. Tel.: +81 48 663 1111; fax: +81 48 652 7254; e-mail: dai.nozawa@po.rd.taisho.co.jp

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.01.019

Numerous studies focused on the development of non-peptidic agonists[28] and antagonists[29–36] for the MC4 receptor have been reported (Fig. 1). Compound **1**, having a 1,2-bispiperazinylethane core, inhibited the binding of AGRP (IC_{50} = 52 nM), and also Nle^4-D-Phe^7-α-MSH (NDP-MSH) (IC_{50} = 217 nM), to the MC4 receptor.[29] Compound **2**, having a succinamide core, exhibited a high-affinity for the MC4 receptor (IC_{50} = 1.4 nM) although it showed no effect of activating the receptor in a functional assay.[30] Compound **3** (ML00253764) exhibited a moderate affinity (K_i = 160 nM) and antagonist activity (IC_{50} = 103 nM) for the receptor; subcutaneous administration of this compound in mice protected the animals against tumor-induced weight loss,[18] suggesting its potential clinical usefulness for the treatment of cachexia.[18,19] Compound **4**, having an imidazole ring instead of the amidine moiety of compound **3**, antagonized the MC4 receptor with a functional IC_{50} value of 51 nM.[32] Compound **5**, designed by using information derived from the SAR of the MC4 agonists, and mutagenesis results of the MC4 receptor structure and peptidic ligands, exhibited a high binding affinity (K_i = 1.8 nM) for the MC4 receptor.[33] Compound **6** also exhibited a high-affinity for the MC4 receptor (K_i = 3.2 nM), with a 240-fold selectivity for this receptor over the MC3 receptor; intracerebroventricular administration of this compound potently stimulated food intake in satiated mice.[31] Compound **7**, having a guanidine part, exhibited a high-affinity for the MC4 receptor (K_i = 0.58 nM).[34] Furthermore, 3-arylpropionylpiperazine, compound **8**, exhibited a good binding affinity (K_i = 13 nM) for the MC4 receptor and also good oral bioavailability (F = 26.1%).[35]

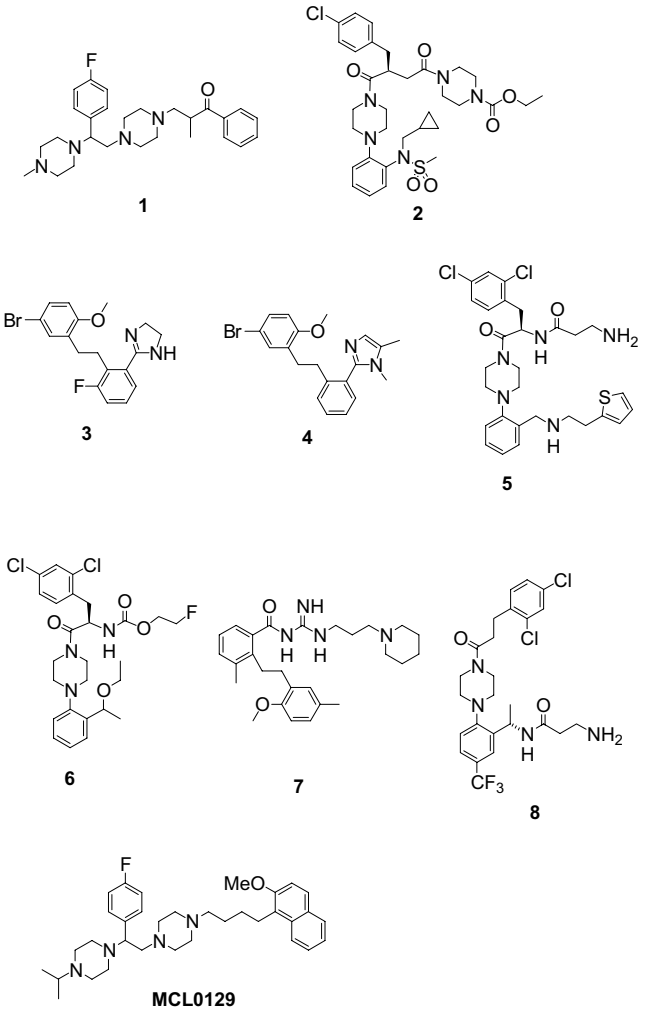


Figure 1. Small molecule antagonists of the MC4 receptor.

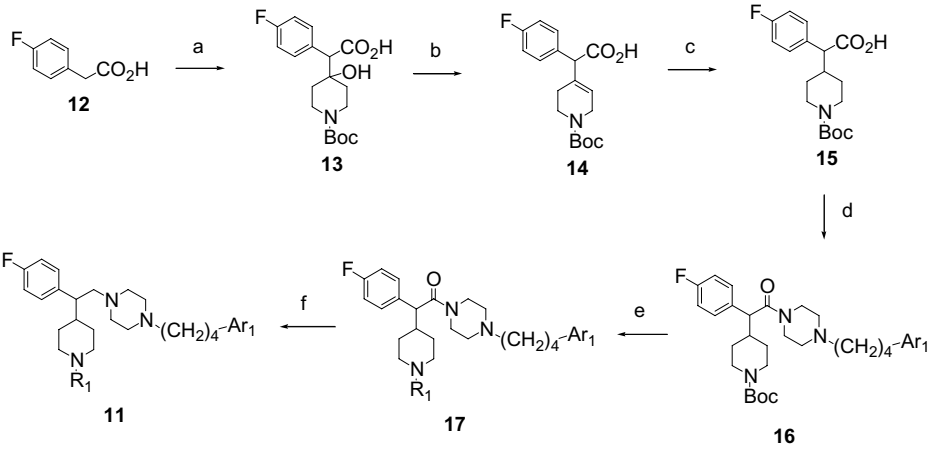
We previously demonstrated that the bis-piperazine compound, MCL0129 (Fig. 1), exhibited a high-affinity (IC_{50} = 8.13 nM) for the MC4 receptor, and acts as an antagonist at the receptor.[23] Studies have also shown that this compound exhibits antidepressant and anxiolytic-like activities in various rodent models.[23] These findings suggest that blockade of the MC4 receptor may be a useful approach for treating subjects with depressive disorders and anxiety. This hypothesis is supported by the results of studies showing that other MC4 receptor antagonists also exhibit antidepressant and anxiolytic effects in various rodent models of depression and anxiety.[24,25] In this paper, we report a new class of MC4 receptor antagonists, namely, the mono-piperazine compounds, with reduced basicity relative to the bis-piperazines. The synthesis, structure–activity relationships, and anxiolytic-like activities of the novel mono-piperazine compounds are described in this study.

2. Chemistry

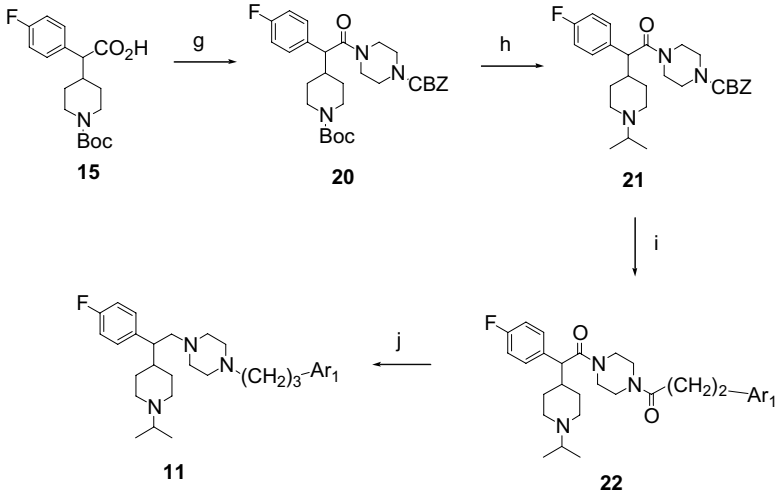
Compounds **11a–j** were prepared via two synthetic routes (Methods A and B) from 4-fluorophenylacetic acid **12**, which was the common starting material for both (Schemes 1 and 2). Compounds **11a** and **11b** were synthesized from compound **15** (Scheme 1). Reaction of **12** with LDA and *N*-Boc-piperizin-4-one **9** yielded **13**. Dehydration of **13** with H_2SO_4 in $CHCl_3$, followed by protection with a Boc group, yielded **14**. After hydrogenation of **14** with $Pd(OH)_2$ as the catalyst, condensation with *N*-(Ar_1–$(CH_2)_4$)-piperazine **10** in the presence of HOBt and EDC yielded **16**. After removal of the Boc group of **16** under acidic conditions, treatment with alkyl iodide in the presence of K_2CO_3 yielded **17**. Compounds **11a** and **11b** were obtained by reduction of **17** with $LiAlH_4$ (Method A).

Compounds **11c–j** were synthesized from compound **15** by another synthetic pathway (Scheme 2). Compound **20** was obtained by coupling **15** with *N*-CBZ piperazine **18** in the presence of HOBt and EDC. After removal of the Boc group of **20** under acidic conditions, reaction with *iso*-propyl iodide in the presence of K_2CO_3 yielded **21**. After removal of the CBZ group of **21** with $Pd(OH)_2$ as the catalyst, condensation with Ar_1–$(CH_2)_2$– CO_2H **19** in the presence of HOBt and EDC yielded **22**. Compounds **11c–j** were obtained by reduction of **22** with $LiAlH_4$ (Method B).

The syntheses of **27** and **30** are shown in Scheme 3. Condensation of **13** with *N*-(1-Nap-$(CH_2)_4$)-piperazine **23** in the presence of HOBt and EDC yielded **25**. After



Scheme 1. Synthesis of mono-piperazine compound **11** (Method A). Reagents and conditions: (a) *N*-Boc-piperizin-4-one (**9**), LDA, THF, HMPA, ice-cooling to rt; (b) H_2SO_4, $CHCl_3$, reflux and then Boc_2O, 4 M aq NaOH, 1,4-dioxane, rt; (c) H_2, $Pd(OH)_2$, MeOH, rt; (d) *N*-(Ar_1–$(CH_2)_4$)-piperazine-2HCl (**10**), EDC–HCl, HOBt–H_2O, Et_3N, DMF; (e) 4 M HCl/1,4-dioxane, MeOH, rt and then R_1-I, K_2CO_3, DMF, rt; (f) $LiAlH_4$, THF, 50 °C.



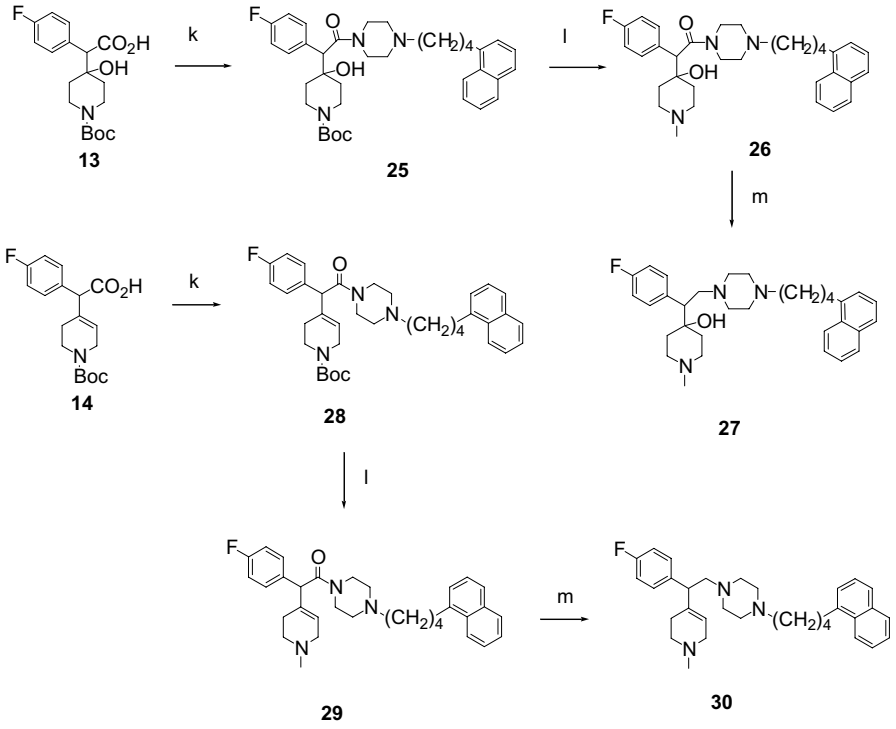
Scheme 2. Synthesis of mono-piperazine compounds **11** (Method B). Reagents and conditions: (g) *N*-CBZ-piperazine (**18**), EDC–HCl, HOBt–H_2O, Et_3N, DMF; (h) 4 M HCl/1,4-dioxane, MeOH, rt; and then *iso*-propyl iodide, K_2CO_3, DMF, rt; (i) H_2, $Pd(OH)_2$, MeOH, rt, and then Ar_1–$(CH_2)_2$–CO_2H (**19**), EDC–HCl, HOBt–H_2O, Et_3N, DMF; (j) $LiAlH_4$, THF, 50 °C.

removal of the Boc group of **25** under acidic conditions, treatment with formaldehyde and $NaBH(OAc)_3$ in the presence of AcOH yielded **26**. Compound **27** was obtained by reduction of **26** with $LiAlH_4$. Compound **30** was also prepared from **14** by the same synthetic method as that for **27** (Method C).

3. Results and discussion

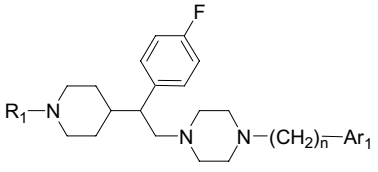
The affinities of all the mono-piperazines for the MC4 receptor were evaluated by assessing their binding affinity to the membranes of COS-1 cells expressing the human MC4 receptor, calculated from the inhibition curve of [^{125}I]Nle^4-D-Phe^7-α-MSH binding,[23] and the IC_{50} values are shown in Table 1. In addition, the affinities of compounds (±)-**11b**–**d**, **11g**, **11i**, and **11j** were tested as follows: [^{3}H]paroxetine binding to the rat cortical membrane (for SET), [^{3}H]nisoxetine binding to the rat cortical membrane (for NET), [^{3}H]prazopsin binding to the rat cortical membrane (for α1), [^{3}H]raclopride binding to the rat striatal membrane (for D2), [^{3}H]pyrilamine binding to the rat whole brain membrane (for H1), [^{3}H]DAMGO binding to the rat brain membrane (for μ), and [^{3}H]DPDPE binding to the rat brain membrane (for δ) (Table 2).

Since the bis-piperazine compound, MCL0042, reported previously by us,[24] had very strong basicity, we attempted to explore MC4 antagonists with varied physicochemical properties (Fig. 2). During our investigation of other structural types of compounds with less basic amines, we found that mono-piperazine compound, **11a**, in which one of the piperazine nitrogen atoms of the bis-piperazine compound MCL0042 is replaced with a carbon, showed no significant decrease of binding affinity (IC_{50} = 333 nM) as compared to MCL0042 (IC_{50} = 118 nM). These results suggest that at least



Scheme 3. Synthesis of mono-piperazine compounds **27** and **30** (Method C). Reagents and conditions: (k) *N*-(Nap-$(CH_2)_4$)-piperazine-2HCl (**23**), EDC–HCl, HOBt–H_2O, Et_3N, DMF; (l) 4 M HCl/1,4-dioxane, MeOH, rt and then HCHO, AcOH, $NaBH(OAc)_3$, $CHCl_3$, rt; (m) $LiAlH_4$, THF, 50 °C.

Table 1. Binding affinity for the MC4 receptor of compounds **11**



Compound[a]	Method	Ar_1	R_1	*n*	IC_{50} (nM)
(±)-**11a**	A	1-Nap	Me	4	333
(±)-**11b**	A	2-MeO-1-Nap	*i*-Pr	4	40.3
(±)-**11c**	B	2-Ph–Ph	*i*-Pr	3	19.7
(±)-**11d**	B	2-Ph-3-F-Ph	*i*-Pr	3	19.8
(±)-**11e**	B	2-Ph-4-F-Ph	*i*-Pr	3	52.7
(±)-**11f**	B	2-Ph-5-F-Ph	*i*-Pr	3	52.2
(±)-**11g**	B	2-Ph-6-F-Ph	*i*-Pr	3	18.3
(±)-**11h**	B	2-(2-F-Ph)-Ph	*i*-Pr	3	98.6
(±)-**11i**	B	2-(3-F-Ph)-Ph	*i*-Pr	3	23.4
(±)-**11j**	B	2-(4-F-Ph)-Ph	*i*-Pr	3	14.5

[a] All compounds are of 3 hydrochloride form.

Table 2. In vitro receptor profiles of compounds **11**

Compound	IC_{50} (nM)							
	MC4	SET	NET	α1	H1	D2	Opiate μ	Opiate δ
(±)-**11b**	40.3	877	2830	>1000	>1000	>1000	>10,000	>10,000
(±)-**11c**	19.7	953	1180	>1000	>1000	423	>10,000	>10,000
(±)-**11d**	19.8	3130	3940	>1000	>1000	482	>10,000	>10,000
(±)-**11g**	18.3	723	491	>1000	>1000	>1000	>10,000	>10,000
(±)-**11i**	23.4	2140	543	>1000	>1000	805	>10,000	>10,000
(±)-**11j**	14.5	3940	729	>1000	>1000	820	>10,000	>10,000

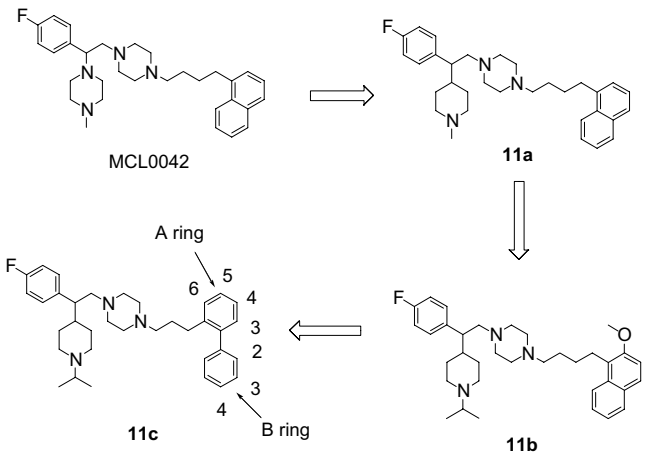


Figure 2. Lead discovery and optimization.

one nitrogen atom in the piperazine ring does not play an important role in binding to the MC4 receptor. Thus, we began to explore the mono-piperazine analogues with reduced basicity relative to the bis-piperazines to identify compounds with higher affinity for the MC4 receptor.

Initially, the binding affinity for the MC4 receptor was optimized by replacement of a methyl group at R_1 (Table 1). We prepared compound **11b** with an *iso*-propyl group at R_1, designed based on the results of our previous investigations,[23] and evaluated the affinity of the compound for the receptor. As expected, compound **11b** showed a much higher affinity (IC_{50} = 40.3 nM) for the MC4 receptor as compared to compound **11a** (IC_{50} = 333 nM).

As the next structural conversion to increase the affinity, we considered that the naphthyl group could be replaced with a biphenyl (2-Ph–Ph) group. We prepared **11c** with the biphenyl (2-Ph–Ph) group and evaluated the affinity of this compound for the MC4 receptor. Fortunately, **11c** showed a higher affinity (IC_{50} = 19.7 nM) for the MC4 receptor than compound **11b** (IC_{50} = 40.3 nM), therefore, we started to explore biphenyl analogues further.

We investigated the effects of substituents on the core benzene ring (A ring) in the biphenyl compounds (Fig. 2). Compounds **11d** and **11g** substituted with a fluorine atom at 3- or 6-position of the core benzene ring exhibited almost the same affinity (IC_{50} = 19.8 and 18.3 nM, respectively) for the MC4 receptor as the nonsubstituted compound **11c** (IC_{50} = 19.7 nM), while **11e** and **11f** substituted with a fluorine atom at 4- or 5-position of the core benzene ring showed a decreased affinity (IC_{50} = 52.7 and 52.2 nM, respectively) as compared to **11c**. These findings suggest that the sterically acceptable space at 4- or 5-position on the core benzene ring for binding to the MC4 receptor was limited.

To optimize the binding affinity of this series of compounds, we next investigated the effects of substitution on the terminal benzene ring (B ring) in the biphenyl compounds (Fig. 2). Introduction of a fluorine atom at 4-position of the terminal benzene ring resulted in a slightly higher affinity (**11j**; IC_{50} = 14.5 nM) as compared with that of the nonsubstituted compound **11c**, while **11i** with a substitution at 3-position exhibited a slightly lower affinity (IC_{50} = 23.4 nM). In contrast, compound **11h** with a substitution at 2-position of the terminal benzene ring showed a much lower affinity (IC_{50} = 98.6 nM) as compared with that of the nonsubstituted compound **11c**, indicating that a substituent at 2-position might have a negligible effect on the conformation for binding to the MC4 receptor.

Finally, we focused our attention on compounds having different frameworks related structurally to the original mono-piperazine **11a**. We prepared compounds **27** and **30** as shown in Scheme 1, and evaluated the binding affinity of the compounds for the MC4 receptor. These compounds did not show any significant decrease of the affinity (**27**, IC_{50} = 487 nM and **30**, 563 nM) for the receptor as compared to that of **11a** (IC_{50} = 333 nM), supporting our hypothesis that the presence of one nitrogen atom in the piperazine may have little impact on the binding affinity for the MC4 receptor. These results indicate that these structural changes also do not cause any important change in the conformation of the molecule.

3.1. Selectivity for other stress- and anxiety/depression-related receptors and transporters

To investigate the in vitro receptor-binding profiles of the mono-piperazine 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 receptors (Table 2). The results indicate that none of the compounds showed a significant affinity for the receptors or transporters examined, revealing the high selectivity of these mono-piperazine compounds for the MC4 receptor.

3.2. Effect on stress-induced anxiety-like behavior in rats

We examined the ability of selected compounds from this series to reverse anxiety-like behavior in the elevated plus-maze task in rats. Exposure of rats to swim stress for 2 min markedly reduced the time spent on the open arms of the maze in the elevated plus-maze task, suggestive of anxiety-like behavior (Fig. 3). The stress-induced anxiety-like behavior in the elevated plus-maze task has been used as a useful model to evaluate the anxiolytic and antistress activity of such compounds.[23] Compounds **11c** and **11d**, when administered orally at 1 mg/kg, significantly reversed the stress-induced reduction in the time spent in the open arms of the maze (Fig. 3), while bis-piperazine compound, MCL0129, reversed at 10 mg/kg.[23] These results indicate that these mono-piperazine compounds exhibited anxiolytic effects in the rats.

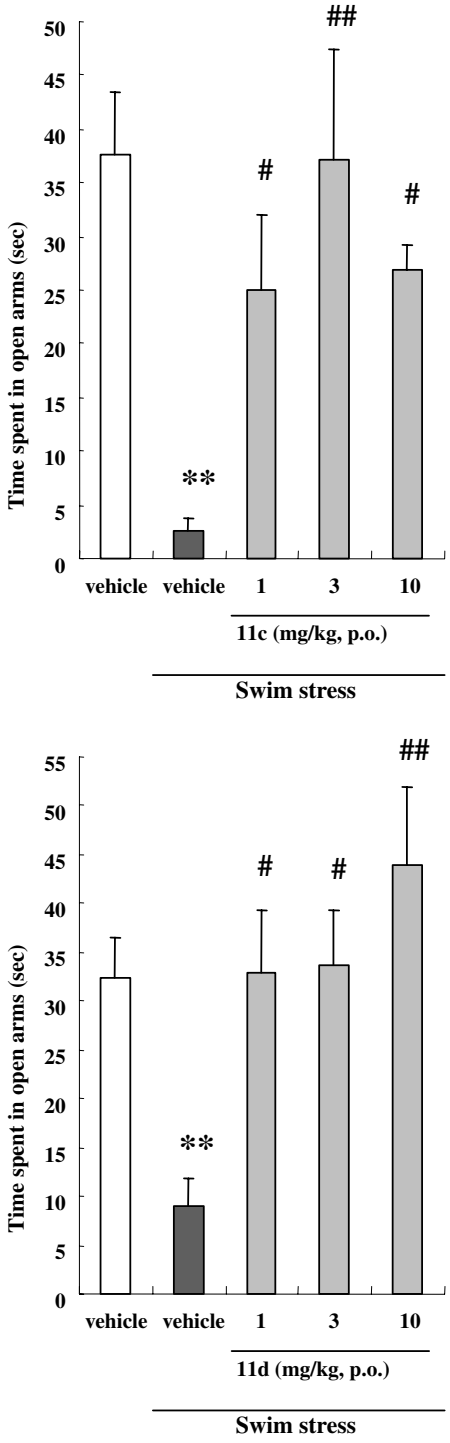


Figure 3. Effects of **11c** and **11d** on swim stress-induced reduction of time spent in open arms in the elevated plus-maze task in the rats. Data represent means ± SE ($n = 10$ or 15). $^{**}P < 0.01$ versus nonstress group (Dunnett's test). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus swim stress group (Dunnett's test).

4. Conclusion

We have reported on the synthesis, SAR, and biological activity of a new series of MC4 receptor antagonists, namely, the mono-piperazines, with reduced basicity relative to the bis-piperazines. Compound **11a**, obtained by substituting one of the piperazine nitrogen atoms of MCL0042 with a carbon, showed a moderate affinity for the MC4 receptor. Then, structural conversion led to the development of compound **11b**, which showed optimal binding affinity ($IC_{50} = 40.3$ nM) for the MC4 receptor. We also found another class of compounds, the biphenyl analogues, some of which showed a high-affinity for the MC4 receptor. Among these compounds, **11j** exhibited the highest affinity for the MC4 receptor ($IC_{50} = 14.5$ nM), with an over 20-fold higher affinity for this receptor than compound **11a**. In addition, compounds **11c** and **11d**, having a high affinity for the MC4 receptor, significantly reversed anxiety-like behavior caused by swim stress when administered po, indicating that these mono-piperazine compounds exhibited anxiolytic effects in the rats. These various mono-piperazine compounds that have been demonstrated to show a high affinity for the MC4 receptor can be useful tools for investigating the involvement of the MC4 receptor in the development of stress-related disorders, such as anxiety, and for elucidating the actions mediated by this receptor.

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 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 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.

5.1. General methods for the synthesis of 11a and 11b (Method A)

5.1.1. (±)-[1-(*tert*-Butoxycarbonyl)-4-hydroxypiperidin-4-yl](4-fluorophenyl)acetic acid ((±)-13). A solution of (4-fluorophenyl)acetic acid **12** (25.2 g, 163 mmol) in THF (100 mL) was added dropwise to a solution of LDA prepared by treatment of ^{i}Pr$_2$NH (48.3 mL, 343 mmol) in THF (200 mL) with 2.50 M solution of *n*-BuLi in hexane (137 mL, 343 mmol) with ice-cooling. To the mixture was added HMPA (28.4 mL) and the mixture was stirred at room temperature for 30 min. The mixture was cooled in ice-bath, and a solution of *tert*-butyl 4-oxopiperidine-1-carboxylate **9** (32.5 g, 163 mmol) in THF (100 mL) was added dropwise to the cooled mixture. After stirring at room temperature for 3 h, the mixture was acidified by treatment with 5% $KHSO_4$ aq and extracted with AcOEt. The extract was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was stirred in Et_2O and the resulting precipitate was collected by filtration to obtain (±)-[1-(*tert*-butoxycarbonyl)-4-hydroxypiperi-

din-4-yl](4-fluorophenyl)acetic acid (±)-**13** (30.0 g, 50%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18–1.30 (2H, m), 1.42 (9H, s), 1.59–1.81 (2H, m), 2.91–3.22 (2H, m), 3.27–3.43 (1H, m), 3.70–3.99 (3H, m), 6.98–7.07 (2H, m), 7.29–7.42 (2H, m); MS (ESI, Neg) *m*/*z* 352 $(M-H)^-$.

5.1.2. (±)-[1-(*tert*-Butoxycarbonyl)-1,2,3,6-tetrahydropyridin-4-yl](4-fluorophenyl)acetic acid ((±)-14). To a solution of (±)-[1-(*tert*-butoxycarbonyl)-4-hydroxypiperidin-4-yl](4-fluorophenyl)acetic acid (±)-**13** (20.0 g, 56.7 mmol) in $CHCl_3$ (40 mL) was added dropwise concentrated H_2SO_4 (40 mL). The mixture was stirred at reflux for 3 h and cooled in ice-bath. To the cooled mixture were added 4 M NaOH aq (250 mL), 1,4-dioxane (200 mL), and $(Boc)_2O$ (14.8 g, 68.0 mmol). The mixture was stirred at room temperature for 30 min. The mixture was acidified by treatment with 5% $KHSO_4$ aq and extracted with $CHCl_3$. The extract was washed with brine, dried over Na_2SO_4, filtered, concentrated in vacuo, and chromatographed on Silica gel C-200 ($CHCl_3$/MeOH, 10:1) to obtain (±)-[1-(*tert*-butoxycarbonyl)-1,2,3,6-tetrahydropyridin-4-yl](4-fluorophenyl)-acetic acid (±)-**14** (18.0 g, 95%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 (9H, s), 2.01–2.10 (2H, m), 3.22–3.40 (2H, m), 3.81–4.02 (2H, m), 4.27–4.32 (1H, m), 5.51–5.62 (1H, m), 6.99–7.08 (2H, m), 7.21–7.29 (2H, m); MS (ESI, Pos) *m*/*z* 358 $(M+Na)^+$.

5.1.3. (±)-[1-(*tert*-Butoxycarbonyl)piperidin-4-yl](4-fluorophenyl)acetic acid ((±)-15). A suspension of (±)-[1-(*tert*-butoxycarbonyl)-1,2,3,6-tetrahydropyridin-4-yl](4-fluorophenyl)acetic acid (±)-**14** (5.00 g, 14.9 mmol) and $Pd(OH)_2$/C (20 wt% Pd on carbon, wet) (0.50 g) in MeOH (50 mL) was stirred under hydrogen atmosphere for 2 days. The mixture was filtered through Celite and the filtrate was concentrated in vacuo to obtain crude [1-(*tert*-butoxycarbonyl)piperidin-4-yl](4-fluorophenyl)-acetic acid (±)-**15** (3.60 g, 72%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.77–1.09 (1H, m), 1.17–1.29 (2H, m), 1.42 (9H, s), 1.79–1.90 (1H, m), 1.98–2.20 (1H, m), 2.47–2,.82 (2H, m), 3.18–3.25 (1H, m), 3.28–3.55 (1H, m), 3.87–4.20 (1H, m), 6.96–7.08 (2H, m), 7.18–7.36 (2H, m); MS (ESI, Neg) *m*/*z* 336 $(M-H)^-$.

5.1.4. (±)-*tert*-Butyl 4-(1-(4-fluorophenyl)-2-{4-[4-(2-methoxy-1-naphthyl)butyl]piperazin-1-yl}-2-oxoethyl)piperidine- 1-carboxylate ((±)-16). To a mixture of crude (±)-[1-(*tert*-butoxycarbonyl)piperidin-4-yl](4-fluorophenyl)-acetic acid (±)-**15** (2.2 g, 6.5 mmol), 1-[4-(2-methoxy-1-naphthyl)butyl]piperazine 2 hydrochloride **10** (2.0 g, 5.4 mmol), HOBt·H_2O (1.5 g, 9.8 mmol), and Et_3N (3.5 mL, 25 mmol) in DMF (20 mL) was added EDC·HCl (1.9 g, 9.8 mmol) and the mixture was stirred at room temperature for 15 h. The mixture was partitioned between EtOAc and satd aq $NaHCO_3$, 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, 3:1) to obtain (±)-*tert*-butyl 4-(1-(4-fluorophenyl)-2-{4-[4-(2-methoxy-1-naphthyl)butyl]-piperazin-1-yl}-2-oxoethyl)piperidine-1-carboxylate (±)-**16** (2.4 g, 73%) as an oily product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86–1.22 (2H, m), 1.42 (9H, s), 1.50–1.70 (6H, m), 1.80–2.02 (1H, m), 2.19–2.41 (6H, m), 2.48–2.82 (2H, m), 3.02–3.09 (2H, m), 3.35–3.72 (5H, m), 3.92 (3H, s), 3.94–4.15 (2H, m), 6.96–7.04 (2H, m), 7.42 (5H, t, J = 7.5 Hz), 7.70–7.90 (2H, m), 7.92 (1H, d, J = 9.5 Hz); MS (ESI, Pos) *m*/*z* 618 $(M+H)^+$.

5.1.5. (±)-1-[(4-Fluorophenyl) (1-isopropylpiperidin-4-yl)acetyl]-4-[4-(2-methoxy-1-naphthyl)butyl]piperazine ((±)-17). To a solution of (±)-*tert*-butyl 4-(1-(4-fluorophenyl)-2-{4-[4-(2-methoxy-1-naphthyl)butyl]piperazin-1-yl}-2-oxoethyl)piperidine-1-carboxylate (±)-**16** (2.1 g, 3.4 mmol) in MeOH (10 mL) was added 4 M HCl in 1,4-dioxane (10 mL), and the mixture was stirred at room temperature for 2 h. The mixture was concentrated in vacuo and the resulting residue was crystallized from Et_2O to obtain (±)-1-[(4-fluorophenyl) (piperidin-4-yl)-acetyl]-4-[4-(2-methoxy-1-naphthyl)butyl]piperazine 2 hydrochloride (1.7 g, 85%). ^{1}H NMR (DMSO-d_6) δ 1.02–1.61 (4H, m), 1.65–1.92 (3H, m), 2.02–2.31 (2H, m), 2.60–3.82 (16H, m), 3.92 (3H, m), 4.10–4.52 (2H, m), 7.11–7.24 (2H, m), 7.30–7.53 (5H, m), 7.79–7.98 (3H, m); MS (ESI, Pos) *m*/*z* 518 $(M + H)^+$. A mixture of (±)-1-[(4-fluorophenyl) (piperidin-4-yl)acetyl]-4-[4-(2-methoxy-1-naphthyl)butyl]piperazine 2 hydrochloride (0.20 g, 0.34 mmol), 2-iodopropane (41 μL, 0.41 mmol), K_2CO_3 (93 mg, 0.68 mmol) in DMF (2 mL) was stirred at room temperature for 15 h. The mixture was partitioned between EtOAc and satd aq $NaHCO_3$, 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 (±)-1-[(4-fluorophenyl) (1-isopropylpiperidin-4-yl)acetyl]-4-[4-(2- methoxy-1-naphthyl)butyl]piperazine (±)-**17** (0.15 g, 78%) as an oily product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (6H, d, J = 6.5 Hz), 1.10–1.30 (2H, m), 1.50–1.70 (5H, m), 1.84–2.42 (8H, m), 2.59–3.10 (6H, m), 3.37–3.70 (6H, m), 3.91 (3H, s), 6.95–7.01 (2H, m), 7.19–7.36 (4H, m), 7.42 (1H, t, J = 7.5 Hz), 7.68–7.79 (2H, m), 7.91 (1H, d, J = 9.4 Hz); MS (ESI, Pos) *m*/*z* 560 $(M+H)^+$.

5.1.6. (±)-1-[2-(4-Fluorophenyl)-2-(1-isopropylpiperidin-4-yl)ethyl]-4-[4-(2-methoxy-1-naphthyl)butyl]piperazine 3 hydrochloride ((±)-11b). A mixture of (±)-1-[(4-fluorophenyl) (1-isopropylpiperidin-4-yl)acetyl]-4-[4-(2-methoxy-1-naphthyl)butyl]piperazine (±)-**17** (0.14 g, 0.25 mmol) and $LiAlH_4$ (10 mg, 0.25 mmol) in THF (5 mL) was stirred at 50 °C for 15 min. To the mixture was added dropwise 25% aq NH_3 (1.0 mL) at room temperature. The resulting solid was excluded by the filtration through Celite. The filtrate was concentrated in vacuo and chromatographed on Chromatorex NH (hexane/EtOAc, 4:1) to obtain (±)-1-[2-(4-fluorophenyl)-2-(1-isopropylpiperidin-4-yl)ethyl]-4-[4-(2-methoxy-1-naphthyl)-butyl]piperazine (0.13 g, 95%) as an oily product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (6H, d, J = 6.5 Hz), 1.03–1.18 (1H, m), 1.30–1.52 (2H, m), 1.55–1.68 (4H, m), 1.73–1.85 (1H, m), 1.90–2.05 (1H, m), 2.20–2.50 (10H, m), 2.50–2.92 (8H, m), 3.01–3.12 (2H, m), 3.91 (3H, s), 6.88–7.09 (4H, m), 7.22–7.32 (2H, m), 7.42 (1H, t, J = 7.5 Hz), 7.69–7.81 (2H, m), 7.97 (1H, d, J = 8.5 Hz); MS (ESI, Pos) *m*/*z* 546 $(M+H)^+$. The above

free base (0.11 g, 0.20 mmol) was dissolved in MeOH (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 from AcOEt, and the precipitate was collected by filtration to obtain (±)-1-[2-(4-fluorophenyl)-2-(1-isopropylpiperidin-4-yl)ethyl]-4-[4-(2-methoxy-1-naphthyl)butyl]piperazine 3 hydrochloride (±)-**11b** (0.12 g, 91%) as a crystal: mp 233–235 °C (EtOAc); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.20 (6H, d, J = 6.6 Hz), 1.20–1.70 (5H, m), 1.70–2.10 (4H, m), 2.60–4.00 (20H, m), 3.92 (3H, s), 7.19–7.52 (7H, m), 7.79–7.88 (2H, m), 7.96 (1H, d, J = 8.6 Hz); MS (ESI, Pos) m/z 546 $(M + H)^+$; Anal.($C_{35}H_{48}FN_3O{\cdot}3HCl{\cdot}2.5H_2O$) C, H, N.

Compound (±)-**11a** was prepared by the same synthetic method as that for (±)-**11b**.

5.1.7. (±)-1-[2-(4-Fluorophenyl)-2-(1-methylpiperidin-4-yl)ethyl]-4-[4-(1-naphthyl)butyl]piperazine 3 hydrochloride ((±)-11a). Compound (±)-**11a** was obtained as a crystal. Mp 190–193 °C (AcOEt); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.22–1.55 (2H, m), 1.62–1.93 (7H, m), 2.64 (3H, s), 2.70–3.75 (19H, m), 7.21 (2H, t, J = 8.9 Hz), 7.33–7.58 (6H, m), 7.78 (1H, d, J = 7.8 Hz), 7.90–7.94 (1H, m), 8.07 (1H, d, J = 7.8 Hz); MS (ESI, Pos) m/z 519 $(M + H)^+$; Anal. ($C_{32}H_{42}FN_3{\cdot}3HCl{\cdot}1.2H_2O$) C, H, N.

5.2. General methods for the synthesis of 11c–j (Method B)

5.2.1. (±)-Benzyl 4-[[1-(*tert*-butoxycarbonyl)piperidin-4-yl](4- fluorophenyl)acetyl]piperazine-1-carboxylate ((±)-20). To a mixture of (±)-[1-(*tert*-butoxycarbonyl)piperidin-4-yl](4-fluorophenyl)acetic acid (±)-**15** (9.2 g, 27 mmol), benzyl piperazine-1-carboxylate **18** (6.6 g, 30 mmol), HOBt·H_2O (6.3 g, 41 mmol), and Et_3N (4.5 mL, 33 mmol) in DMF (20 mL) was added EDC·HCl (6.3 g, 33 mmol) and the mixture was stirred at room temperature for 3 h. The mixture was partitioned between EtOAc and satd aq $NaHCO_3$, 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 (±)-benzyl 4-[[1-(*tert*-butoxycarbonyl)piperidin-4-yl](4-fluorophenyl)acetyl]piperazine-1-carboxylate (±)-**20** (11 g, 75%) as an oily product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.83–1.19 (3H, m), 1.43 (9H, s), 1.81–1.92 (1H, m), 2.19–2.37 (1H, m), 2.49–2.92 (3H, m), 3.18–3.61 (8H, m), 3.70–3.93 (1H, m), 3.93–4.17 (1H, m), 5.10 (2H, m), 6.97–7.03 (2H, m), 7.18–7.40 (7H, m); MS (ESI, Pos) m/z 562 $(M+Na)^+$.

5.2.2. (±)-Benzyl 4-[(4-fluorophenyl) (1-isopropylpiperidin-4-yl)acetyl]piperazine-1- carboxylate ((±)-21). Compound (±)-Benzyl 4-[[1-(*tert*-butoxycarbonyl)piperidin-4-yl](4-fluorophenyl)acetyl]piperazine-1-carboxylate (±)-**20** (10.6 g, 19.6 mmol) was added with 4 M HCl in 1,4-dioxane (50 mL), and the mixture was stirred at room temperature for 1 h. The mixture was concentrated in vacuo and the resulting residue was partitioned between EtOAc and NaOH aq, and separated organic phase was washed with brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo to give crude (±)-benzyl 4-[(4-fluorophenyl) (piperidin-4-yl)acetyl]piperazine-1-carboxylate (9.30 g, quantitative yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.83–1.19 (3H, m), 1.43 (9H, s), 1.81–1.92 (1H, m), 2.19–2.37 (1H, m), 2.49–2.92 (3H, m), 3.18–3.61 (8H, m), 3.70–3.93 (1H, m), 3.93–4.17 (1H, m), 5.10 (2H, m), 6.97–7.03 (2H, m), 7.18–7.40 (7H, m). This product was used in the next step without further purification. A mixture of crude (±)-benzyl 4-[(4-fluorophenyl) (piperidin-4-yl)acetyl]piperazine-1-carboxylate (9.30 g, 19.6 mmol), 2-iodopropane (2.95 mL, 29.5 mmol), and K_2CO_3 (8.14 g, 59.0 mmol) in DMF (100 mL) was stirred at room temperature for 15 h. The mixture was partitioned between EtOAc and saturated water, 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, 1:1) to obtain (±)-benzyl 4-[(4-fluorophenyl) (1-isopropylpiperidin-4-yl)acetyl]piperazine-1-carboxylate (±)-**21** (6.20 g, 66%) as an oily product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.01 (6H, d, J = 6.6 Hz), 1.05–1.19 (2H, m), 1.83–2.22 (3H, m), 2.59–2.98 (5H, m), 3.18–3.61 (8H, m), 3.63–3.83 (1H, m), 5.10 (2H, m), 6.96–7.03 (2H, m), 7.18–7.39 (7H, m); MS (ESI, Pos) m/z 482 $(M+H)^+$.

5.2.3. (±)-1-[3-(4′-Fluorobiphenyl-2-yl)propanoyl]-4-[(4-fluorophenyl) (1-isopropylpiperidin-4-yl)acetyl]piperazine ((±)-22). A suspension of (±)-benzyl 4-[(4-fluorophenyl) (1-isopropylpiperidin-4-yl)acetyl]piperazine-1-carboxylate (±)-**21** (6.1 g, 13 mmol) and $Pd(OH)_2$/C (20 wt% Pd on carbon, wet) (0.61 g) in MeOH (60 mL) was stirred under a hydrogen atmosphere for 15 h. The mixture was filtered through Celite and the filtrate was concentrated in vacuo to obtain crude (±)-1-[(4-fluorophenyl) (1-isopropylpiperidin-4-yl)acetyl]piperazine (4.8 g, quantitative yield). This product was used in the next step without further purification. To a mixture of crude (±)-1-[(4-fluorophenyl) (1-isopropylpiperidin-4-yl)acetyl]-piperazine (0.30 g, 0.86 mmol), 3-(4′-fluorobiphenyl-2-yl)propanoic acid **19** (0.23 g, 0.95 mmol), HOBt·H_2O (0.20 g, 1.3 mmol), and Et_3N (0.14 mL, 1.0 mmol) in DMF (5 mL) was added EDC·HCl (0.20 g, 1.0 mmol) and the mixture was stirred at room temperature for 3 h. The mixture was partitioned between EtOAc and satd aq $NaHCO_3$, 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, 1:2) to obtain (±)-1-[3-(4′-fluorobiphenyl-2-yl)propanoyl]-4-[(4-fluorophenyl) (1-isopropylpiperidin-4-yl)acetyl]piperazine (±)-**22** (0.30 g, 61%) as an oily product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.02 (6H, d, J = 6.5 Hz), 1.04–1.22 (2H, m), 1.84–2.51 (9H, m), 2.59–3.01 (8H, m), 3.03–3.76 (3H, m), 6.95–7.42 (12H, m); MS (ESI, Pos) m/z 574 $(M+H)^+$.

5.2.4. (±)-1-[3-(4′-Fluorobiphenyl-2-yl)propyl]-4-[2-(4-fluorophenyl)-2-(1-isopropylpiperidin-4-yl)ethyl]piperazine 3 hydrochloride ((±)-11j). A mixture of (±)-1-[3-(4′-fluorobiphenyl-2-yl)propanoyl]-4-[(4-fluorophenyl) (1-isopro-

pylpiperidin-4-yl)acetyl]piperazine (±)-**22** (0.29 g, 0.52 mmol) and $LiAlH_4$ (41 mg, 1.0 mmol) in THF (5 mL) was stirred at 50 °C for 30 min. To the mixture was added dropwise 25% aq NH_3 (1.0 mL) at room temperature. The resulting solid was excluded by the filtration through Celite. The filtrate was concentrated in vacuo and chromatographed on Chromatorex NH (hexane/EtOAc, 1:1) to obtain (±)-1-[3-(4′-fluorobiphenyl-2-yl)propyl]-4-[2-(4-fluorophenyl)-2-(1-isopropylpiperidin-4-yl)ethyl]piperazine (0.25 g, 88%) as an oily product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (6H, d, J = 6.5 Hz), 1.03–1.16 (1H, m), 1.31–1.82 (6H, m), 1.91–2.41 (9H, m), 2.50–2.91 (10H, m), 6.89–6.99 (2H, t, J = 7.5 Hz), 7.00–7.27 (10H, m); MS (ESI, Pos) m/z 546 $(M+H)^+$. The above free base (0.22 g, 0.40 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 from a mixture of AcOEt and MeOH, and the precipitate was collected by filtration to obtain (±)-1-[3-(4′-fluorobiphenyl-2-yl)propyl]-4-[2-(4-fluorophenyl)-2-(1-isopropylpiperidin-4-yl)ethyl]piperazine 3 hydrochloride (±)-**11j** (0.23 g, 81%) as a crystal: mp 221–223 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.19 (6H, d, J = 6.5 Hz), 1.23–1.60 (3H, m), 1.80–2.02 (4H, m), 2.50–2.59 (1H, m), 2.70–4.10 (19H, m), 7.15–7.60 (12H, m); MS (ESI, Pos) m/z 546 $(M+H)^+$; Anal. ($C_{35}H_{45}F_2N_3$·3HCl·0.5H_2O) C, H, N.

Compound (±)-**11c**–**i** were prepared by the same synthetic method as that for (±)-**11j**.

5.2.5. (±)-1-(3-Biphenyl-2-ylpropyl)-4-[2-(4-fluorophenyl)-2-(1-isopropylpiperidin-4-yl)ethyl]piperazine 3 hydrochloride ((±)-11c). Compound (±)-**11c** was obtained as a crystal. Mp 211–213 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.18 (6H, d, J = 6.5 Hz), 1.22–1.60 (3H, m), 1.78–2.03 (4H, m), 2.52–2.61 (1H, m), 2.77–4.03 (19H, m), 7.17–7.50 (13H, m); MS (ESI, Pos) m/z 528 $(M+H)^+$; Anal. ($C_{35}H_{46}FN_3$·3HCl·0.5H_2O) C, H, N.

5.2.6. (±)-1-[3-(6-Fluorobiphenyl-2-yl)propyl]-4-[2-(4-fluorophenyl)-2-(1-isopropylpiperidin-4-yl)ethyl]piperazine 3 hydrochloride ((±)-11d). Compound (±)-**11d** was obtained as a crystal. Mp 227–229 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.19 (6H, d, J = 6.5 Hz), 1.23–1.61 (3H, m), 1.77–2.03 (4H, m), 2.41–2.61 (1H, m), 2.70–4.93 (19H, m), 7.17–7.50 (12H, m); MS (ESI, Pos) m/z 546 $(M+H)^+$; Anal. ($C_{35}H_{45}F_2N_3$·3HCl) C, H, N.

5.2.7. (±)-1-[3-(5-Fluorobiphenyl-2-yl)propyl]-4-[2-(4-fluorophenyl)-2-(1-isopropylpiperidin-4-yl)ethyl]piperazine 3 hydrochloride ((±)-11e). Compound (±)-**11e** was obtained as a crystal. Mp 219–221 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.19 (6H, d, J = 6.5 Hz), 1.23–1.60 (3H, m), 1.78–2.03 (4H, m), 2.51–2.59 (1H, m), 2.70–3.65 (18H, m), 4.00–4.18 (1H, m), 6.99 (2H, dd, J = 1.5, 6.0 Hz), 7.12–7.49 (10H, m); MS (ESI, Pos) m/z 546 $(M+H)^+$; Anal. ($C_{35}H_{45}F_2N_3$·3HCl·0.5H_2O) C, H, N.

5.2.8. (±)-1-[3-(4-Fluorobiphenyl-2-yl)propyl]-4-[2-(4-fluorophenyl)-2-(1-isopropylpiperidin-4-yl)ethyl]piperazine 3 hydrochloride ((±)-11f). Compound (±)-**11f** was obtained as a crystal. Mp 229–231 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.19 (6H, d, J = 6.5 Hz), 1.23–1.60 (3H, m), 1.79–2.03 (4H, m), 2.52–2.61 (1H, m), 2.70–3.90 (18H, m), 4.20–4.45 (1H, m), 7.03–7.45 (12H, m); MS (ESI, Pos) m/z 546 $(M+H)^+$; Anal. ($C_{35}H_{45}F_2N_3$·3HCl·0.5H_2O) C, H, N.

5.2.9. (±)-1-[3-(3-Fluorobiphenyl-2-yl)propyl]-4-[2-(4-fluorophenyl)-2-(1-isopropylpiperidin-4-yl)ethyl]piperazine 3 hydrochloride ((±)-11g). Compound (±)-**11g** was obtained as a crystal. Mp 226–228 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.19 (6H, d, J = 6.5 Hz), 1.22–1.60 (3H, m), 1.74–2.03 (4H, m), 2.50–2.59 (1H, m), 2.70–3.70 (18H, m), 4.18–4.37 (1H, m), 7.03 (2H, d, J = 7.5 Hz), 7.13–7.47 (10H, m); MS (ESI, Pos) m/z 546 $(M+H)^+$; Anal. ($C_{35}H_{45}F_2N_3$·3HCl·0.5H_2O) C, H, N.

5.2.10. (±)-1-[3-(2′-Fluorobiphenyl-2-yl)propyl]-4-[2-(4-fluorophenyl)-2-(1-isopropylpiperidin-4-yl)ethyl]piperazine 3 hydrochloride ((±)-11h). Compound (±)-**11h** was obtained as a crystal. Mp 221–223 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.19 (6H, d, J = 6.5 Hz), 1.22–1.59 (3H, m), 1.78–2.03 (4H, m), 2.40–2.49 (1H, m), 2.70–4.00 (19H, m), 7.14–7.49 (12H, m); MS (ESI, Pos) m/z 546 $(M+H)^+$; Anal. ($C_{35}H_{45}F_2N_3$·3HCl·0.7H_2O) C, H, N.

5.2.11. (±)-1-[3-(3′-Fluorobiphenyl-2-yl)propyl]-4-[2-(4-fluorophenyl)-2-(1-isopropylpiperidin-4-yl)ethyl]piperazine 3 hydrochloride ((±)-11i). Compound (±)-**11i** was obtained as a crystal. Mp 218–220 °C (AcOEt–MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.20 (6H, d, J = 6.5 Hz), 1.22–1.61 (3H, m), 1.79–2.03 (4H, m), 2.52–2.61 (1H, m), 2.75–3.70 (19H, m), 7.10–7.52 (12H, m); MS (ESI, Pos) m/z 546 $(M+H)^+$; Anal. ($C_{35}H_{45}F_2N_3$·3HCl·0.5H_2O) C, H, N.

5.3. General methods for the synthesis of (±)-27 and (±)-30 (Method C)

5.3.1. 4-(1-(4-Fluorophenyl)-2-{4-[4-(1-naphthyl)butyl]-piperazin-1-yl}ethyl)- 1-methylpiperidin-4-ol 3 hydrochloride ((±)-27). Compound (±)-**27** was prepared from (±)-**13** by the same synthetic method as that for (±)-**11b**. Compound (±)-**27** was obtained as an amorphous. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.21–1.34 (1H, m), 1.59–1.84 (6H, m), 2.68 (3H, d, J = 4.7 Hz), 2.90–3.85 (21H, m), 7.14–7.25 (2H, m), 7.37–7.57 (6H, m), 7.78 (1H, d, J = 7.6 Hz), 7.91–7.94 (1H, m), 8.07 (1H, d, J = 7.6 Hz); MS (ESI, Pos) m/z 504 $(M+H)^+$; Anal. ($C_{32}H_{42}FN_3O$·3HCl·3.5H_2O) C, H, N.

5.3.2. 1-[2-(4-Fluorophenyl)-2-(1-methyl-1,2,3,6-tetrahydropyridin-4-yl)ethyl]-4-[4-(1-naphthyl)butyl]piperazine 3 hydrochloride ((±)-30). Compound (±)-**30** was prepared from (±)-**14** by the same synthetic method as that for (±)-**11b**. Compound (±)-**30** was obtained as an amorphous. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.63–1.91 (4H, m), 2.68–2.86 (3H, m), 2.94–3.90 (19H, m),

3.91–4.18 (2H, m), 7.16–7.25 (2H, m), 7.33–7.59 (6H, m), 7.79 (1H, d, $J = 7.8$ Hz), 7.91–7.94 (1H, m), 8.08 (1H, d, $J = 7.8$ Hz); HRMS; 486.3287 (M+1).

5.4. Binding test

5.4.1. Materials. [^{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.

5.4.2. [^{125}I][Nle^4,D-Phe^7]α-MSH binding to the recombinant MC4 receptor. COS-1 cells expressing the MC4 receptor, prepared according to the method reported previously,[23] 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 48,000*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.[23] 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.

5.5. In vivo test

5.5.1. Animals. Male Sprague–Dawley rats (220–240 g, Charles River, Yokohama, Japan) were housed three per cage and used to assess stress-induced anxiogenic-like behavior in the elevated plus-maze task Animals were maintained under a 12-h light/dark cycle (light on 7:00 AM) in a temperature- and humidity-controlled holding room. Food and water were available ad libitum. Behavioral studies were carried out during 9:00 AM–4:00 PM. All studies were reviewed by the Taisho Pharmaceutical Co., Ltd. Animal Care Committee and met the Japanese Experimental Animal Research Association standards, as defined in the *Guidelines for Animal Experiments* (1987).

For behavioral studies, these compounds were dissolved in 0.3% Tween 80/saline solution.

5.5.2. Stress-induced anxiogenic-like behavior in rats. The swim stress consists of placing rats in a 40-cm tall, 20 cm wide cylindrical plastic container containing 25 cm of water maintained at 25 ± 1 °C. Duration of the swim stress was 2 min, and the elevated plus-maze test was performed 5 min after the swim stress. The elevated plus-maze test was based on that validated for the rat by Guimaraes et al.[37] The apparatus consisted of a plus-maze elevated 50 cm high from the floor. The maze has four arms such that two opposite open arms, 50 × 10 cm, were crossed at right angles by the same dimensions and enclosed by 40 cm-high walls with an open roof. In addition, a 1-cm high edge made of Plexiglas surrounded the open arms to avoid falls. Luminosity measured at the center of the maze was 40 lux. During the observation, the experimenter always sat in the same place, next to the apparatus. Each rat was placed in the center of the plus-maze facing one enclosed arm. The amount of time spent in open arms of the maze was recorded. Rats were naive to the apparatus. Testing compounds were administered po 30 min prior to swim stress.

5.5.3. Statistical analysis. Data from in vivo experiment were analyzed by one-way ANOVA and significant differences between groups were determined, using Dunnett's test.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.01.019.

References and notes

1. Chaki, S.; Okuyama, S. *Peptides* **2005**, *26*, 1952.
2. Chaki, S.; Nakazato, A. *Drugs of the future* **2004**, *29*, 1065.
3. Wikberg, J. E. S. *Eur. J. Pharmacol.* **1999**, *375*, 295.
4. 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.
5. 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.
6. Ollmann, M. M.; Wilson, B. D.; Yang, Y.-K.; Kerns, J. A.; Chen, Y.; Gantz, I.; Barsh, G. S. *Science* **1997**, *278*, 135.
7. Holder, J. R.; Haskell-Luevano, C. *Med. Res. Rev.* **2004**, *24*, 325.
8. 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.
9. Fan, W.; Boston, B. A.; Kesterson, R. A.; Hruby, V. J.; Cone, R. D. *Nature* **1997**, *385*, 165.
10. Forbes, S.; Bui, S.; Robinson, B. R.; Hochgeschwendr, U.; Brenna, M. B. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 4233.

11. 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.
12. Kask, A.; Rago, L.; Wikberg, J. E. S.; Schiotch, H. B. *Eur. J. Pharmacol.* **1998**, *360*, 15.
13. 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.
14. Giraudo, S. Q.; Billington, C. J.; Levine, A. S. *Brain Res.* **1998**, *809*, 302.
15. Kask, A.; Rago, L.; Mutulis, F.; Pahkla, R.; Wikberg, J. E. S.; Schiotch, H. B. *Biochem. Biophys. Res. Commun.* **1998**, *245*, 90.
16. 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.
17. Chen, W.; Kelly, M. A.; Opitz-Araya, X.; Thomas, R. E.; Low, M. J.; Cone, R. D. *Cell* **1997**, *91*, 789.
18. 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.
19. Marks, D. L.; Ling, N.; Cone, R. D. *Cancer Res.* **2001**, *61*, 1432.
20. 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.
21. Vergoni, A. V.; Bertolini, A.; Wikberg, J. E.; Schioth, H. B. *Eur. J. Pharmacol.* **1999**, *369*, 11.
22. Von Frijtag, J. C.; Croiset, G.; Gispen, W. H.; Adan, R. A.; Wiegant, V. M. *Br. J. Pharmacol.* **1998**, *123*, 1503.
23. 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.
24. Chaki, S.; Oshida, Y.; Ogawa, S.; Funakoshi, T.; Shimazaki, T.; Okubo, T.; Nakazato, A.; Okuyama, S. *Pharmacol. Biochem. Behav.* **2005**, *82*, 621.
25. Chaki, S.; Ogawa, S.; Toda, Y.; Funakoshi, T.; Okuyama, S. *Eur. J. Pharmacol.* **2003**, *474*, 95.
26. Shimazaki, T.; Chaki, S. *Pharmacol. Biochem. Behav.* **2005**, *80*, 395.
27. (a) 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; (b) 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; (c) 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; (d) Odagami, T.; Tsuda, Y.; Kogami, Y.; Kouji, H.; Okada, Y. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3723.
28. (a) Bakshi, R. K.; Hong, Q.; Tang, R.; Kalyani, R. N.; MacNeil, T.; Weinberg, D. H.; Van der Ploeg, L. H. T.; Patchett, A. A.; Nargund, R. P. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1130; (b) Shi, Q.; Ornstein, P. L.; Briner, K.; Richardson, T. I.; Arnold, M. B.; Backer, R. T.; Buckmaster, J. L.; Canada, E. J.; Doecke, C. W.; Hertel, L. W.; Honigschmidt, N.; Hsiung, H. M.; Husain, S.; Kuklish, S. L.; Martinelli, M. J.; Mullaney, J. T.; O'Brien, T. P.; Reinhard, M. R.; Rothhaar, R.; Shah, J.; Wu, Z.; Xie, C.; Zgombick, J. M.; Fisher, M. J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2341; (c) Briner, K.; Collado, I.; Fisher, M. J.; García-Paredes, C.; Husain, S.; Kuklish, S. L.; Mateo, A. I.; O'Brien, T. P.; Ornstein, P. L.; Zgombick, J.; De Frutos, O. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3449; (d) Kuklish, S. L.; Backer, R. T.; Briner, K.; Doecke, C. W.; Husain, S.; Mullaney, J. T.; Ornstein, P. L.; Zgombick, J. M.; O'Brien, T. P.; Fisher, M. J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3843; (e) Tian, X.; Mishra, R. K.; Switzer, A. G.; Hu, X. E.; Kim, N.; Mazur, A. W.; Ebetino, F. H.; Wos, J. A.; Crossdoersen, D.; Pinney, B. B.; Farmer, J. A.; Sheldon, R. J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4668.
29. 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. A. *J. Med. Chem.* **2003**, *46*, 9.
30. 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.
31. 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.
32. 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.
33. 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.
34. Vos, T. J.; Balani, S.; Blackburn, C.; Chau, R. W.; Danca, M. D.; Drabic, S. V.; Farrer, C. A.; Patane, M. A.; Stroud, S. G.; Yowe, D. L.; Claiborne, C. F. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2302.
35. Jiang, W.; Tucci, F. C.; Chen, C. W.; Arellano, M.; Tran, J. A.; White, N. S.; Marinkovic, D.; Pontillo, J.; Fleck, B. A.; Wen, J.; Saunders, J.; Madan, A.; Foster, A. C.; Chen, C. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4674.
36. (a) 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; (b) 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; (c) 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; (d) 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; (e) 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; (f) Tran, J. A.; Pontillo, J.; Fleck, B. A.; Marinkovic, D.; Arellano, M.; Tucci, F. C.; Lanier, M.; Saunders, J.; Jiang, W.; Chen, C. W.; Foster, A. C.; Chen, C. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3693.
37. Guimaraes, F. S.; Carobrez, A. P.; De Aguiar, J. C.; Graeff, F. G. *Psychopharmacology* **1991**, *103*, 91.