Bioorganic & Medicinal Chemistry Letters 19 (2009) 2835–2839

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Identification and characterization of a selective radioligand for melanin-concentrating hormone 1-receptor (MCH1R)

Masahiko Ito [a,*], Toshihiro Sakamoto [b], Takao Suzuki [b], Shinichiro Egashira [a], Minoru Moriya [b], Akane Ishihara [c], Hisashi Iwaasa [a], Hiroko Matsushita [c], Kazuho Nakase [a], Michael A. Wallace [d], Dennis Dean [d], Nagaaki Sato [b], Shigeru Tokita [a], Akio Kanatani [a]

[a] Department of Metabolic Disorder, Tsukuba Research Institute, BANYU Pharmaceutical Co., Ltd, Okubo 3, Tsukuba, Ibaraki 300-2611, Japan
[b] Department of Medicinal Chemistry, Tsukuba Research Institute, BANYU Pharmaceutical Co., Ltd, Okubo 3, Tsukuba, Ibaraki 300-2611, Japan
[c] Department of Pharmacology, Tsukuba Research Institute, BANYU Pharmaceutical Co., Ltd, Okubo 3, Tsukuba, Ibaraki 300-2611, Japan
[d] Merck Research Laboratories, Rahway, NJ 07065, USA

ARTICLE INFO

Article history:
Received 19 February 2009
Revised 18 March 2009
Accepted 21 March 2009
Available online 26 March 2009

Keywords:
Melanin-concentrating hormone 1-receptor
Antagonist
Radioligand
Receptor occupancy

ABSTRACT

We have developed and characterized [^{35}S]**4a** as a potent and selective radioligand for melanin-concentrating hormone 1-receptor (MCH1R). Compound [^{35}S]**4a** showed appreciable specific signals in brain slices prepared from wild-type mice but not from MCH1R deficient mice, confirming the specificity and utility of [^{35}S]**4a** as a selective MCH1R radioligand for ex vivo receptor occupancy assays.

© 2009 Elsevier Ltd. All rights reserved.

Melanin-concentrating hormone (MCH) is a cyclic neuropeptide that is predominantly expressed in the central nervous system (CNS). Although MCH neurons are exclusively found in the hypothalamus, they project widely throughout the CNS, suggesting that MCH plays a variety of functions in CNS. Two G-protein coupled receptors (GPCRs), named MCH1R and MCH2R, are identified as specific receptors for MCH in mammals.[1–5] Higher species (e.g., human, rhesus, and dog) have both MCH1R and 2R while rodents (e.g., rat and mouse) have only MCH1R, indicating that physiological functions of MCH are mediated solely through MCH1R in rodents.[6] In rodents, consistent with the widespread projections of MCH neurons, MCH1Rs are widely expressed in various brain regions.[7] Accumulated evidence has demonstrated that MCH plays a variety of physiological roles and provides therapeutic opportunities for several CNS disorders such as obesity, depression and anxiety.[8–11] Regarding the roles of MCH in obesity, it is reported that continuous central administration of MCH potently stimulated feeding behavior resulting in the development of obesity and insulin resistance in rodents.[12–15] In addition, MCH1R-deficient mice are resistant to diet-induced obesity (DIO).[16,17] Furthermore, several MCH1R antagonists with diverse structures (i.e., T-226296[18,19] and SNAP-7941[20,21] in Fig. 1) displayed anti-obesity efficacy in DIO rats. Collectively, these genetic and pharmacological evidences strongly support the idea that MCH1R might be a promising therapeutic target for the treatment of obesity. Currently, GW856464 (structure undisclosed),[22] AMG-076[23] (Fig. 1) and NGD-4715 (structure undisclosed)[24] have entered clinical trials for the treatment of obesity.

From a drug discovery point of view, a selective radioligand is an essential tool for pharmacodynamic analyses of antagonists (i.e., assessment of relationship between target occupancy levels and pharmacological efficacy). It could facilitate pre-clinical and clinical studies.[25] As radioligands for MCH1R, [^{125}I]-S36057[26,27] (a peptide agonist) (Fig. 2) and [^{3}H]-SNAP-7941 have been identified and used for ex vivo receptor occupancy (RO) studies.[28] However, the selectivity of these radioligands in vivo has not been validated in MCH1R deficient mice (i.e., loss of specific signal in MCH1R deficient mice).

In this study, we report the discovery and characterization of a potent and selective [^{35}S]-radioligand for MCH1R that showed specific signals in an ex vivo RO study using wild-type mice brains but not MCH1R deficient mice brains.

Compounds **1**–**5** were synthesized as outlined in Scheme 1. Commercially available ester **7** was brominated to give **8**. The bromo group of **8** was displaced by 4-(methylsulfonyl)piperazin-2-one or *tert*-butyl 3-oxopiperazine-1-carboxylate in the presence of base followed by hydrolysis of the ester group to afford carboxylic acid

* Corresponding author. Tel.: +81 29 877 2000; fax: +81 29 877 2027.
E-mail address: masahiko_ito2@merck.com (M. Ito).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.03.102

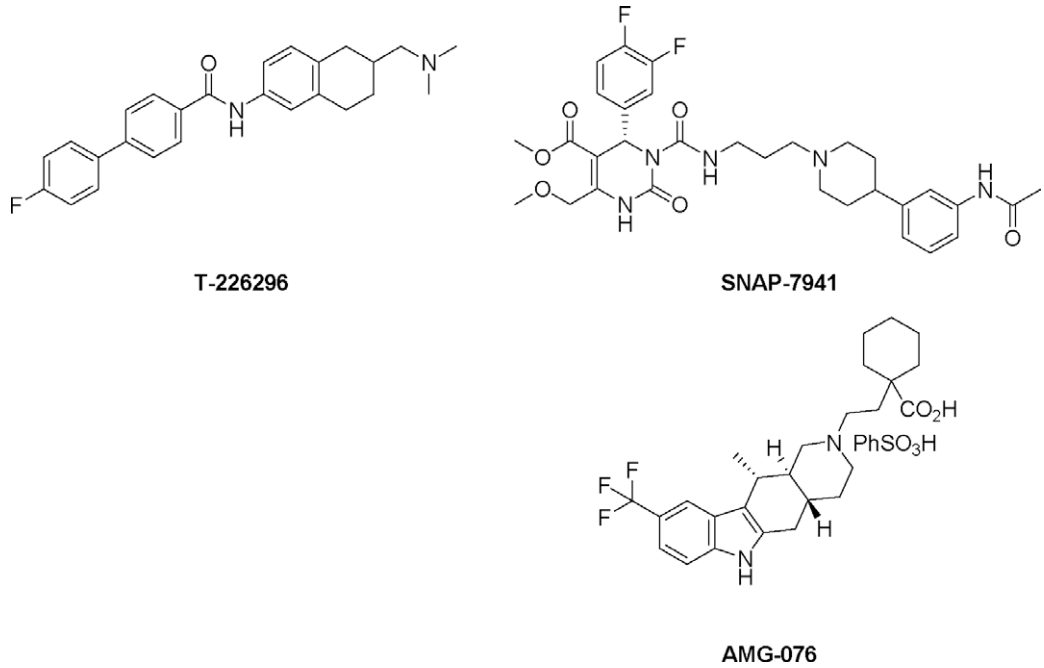


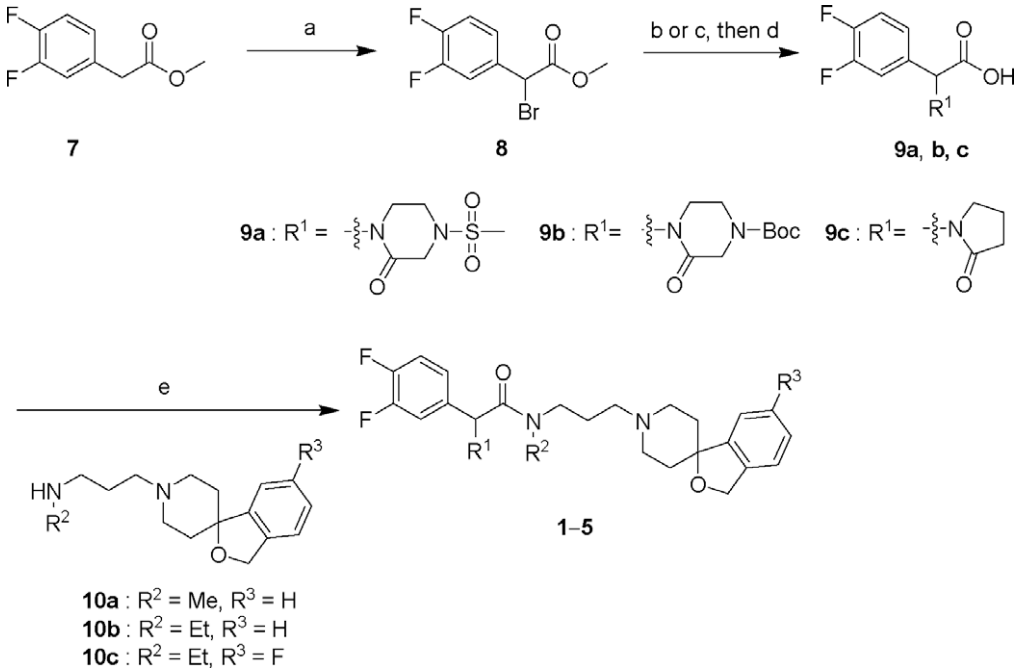
Figure 1. Structures of MCH1R antagonists that were tested in a clinical trial.

(^{125}I)Tyr-ADO^{a}-Arg-Cys-Met-Leu-Gly-Arg-Val-Phe-Arg-Pro-Cys-Trp

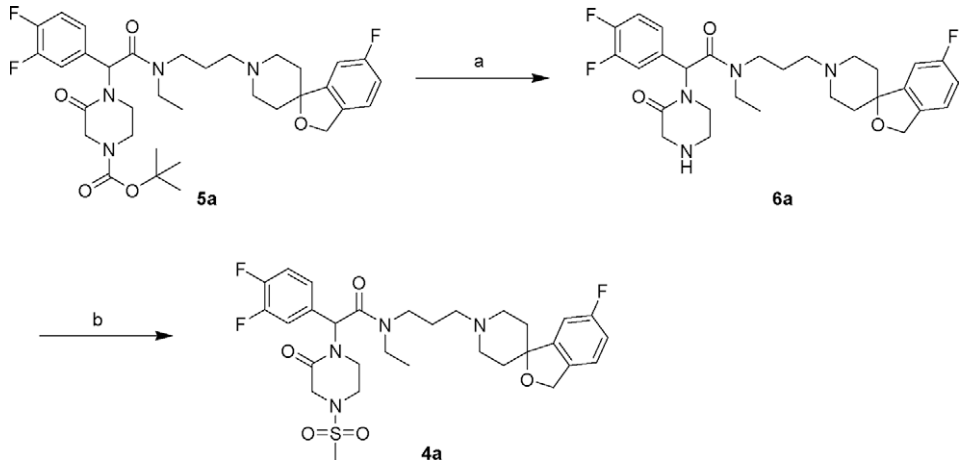
Figure 2. Amino acid sequence of [^{125}I]-S36057. ^{a}8-Amino-3,6-dioxyoctanoyl.

9a, **b**. Preparation of **9c** was reported previously from our laboratory.[29] Carboxylic acids **9a**–**c** were coupled with amines **10a**, **b**, or **c** to afford target compounds **1–5**. Racemates **4** and **5** were resolved by HPLC to give single isomers **4a**, **b** and **5a**, **b**, respectively.[30] Cleavage of the *tert*-butylcarbonyl group of the active isomer **5a** provided **6a**. Mesylation of **6a** gave the active isomer **4a** without racemization (Scheme 2), establishing the synthetic route of [^{35}S]**4a** from the corresponding precursor **5a** using [^{35}S]methanesulfonyl chloride as a radiolabel donor.

We previously reported that compound **1** is a significant human and mouse P-gp substrate; hence, further development of **1** was abandoned.[29] However, the relatively low log*D* value (log$D_{7.4}$ = 2.2) and potent intrinsic activity (IC_{50} = 2.7 nM) of **1** prompted us to utilize compound **1** as a template for the identification of radioligands for rodent RO studies if a sulfur atom is appropriately incorporated into the molecule. The result of the present struc-



Scheme 1. Reagents and conditions: (a) NBS, HBr, CCl_4, reflux, 17 h, 98%; (b) 4-(methylsulfonyl)piperazin-2-one, NaH, DMF–THF, 0 °C, 30 min, 31%; (c) *tert*-butyl 3-oxopiperazine-1-carboxylate, *n*-BuLi, THF, 0 °C, 30 min, 46%; (d) 4 N NaOH aq, MeOH, rt, 2 h, 99%; (e) EDCI·HCl, HOBt·H_2O, Et_3N, $CHCl_3$, DMF, rt, 17 h, 20–68%.

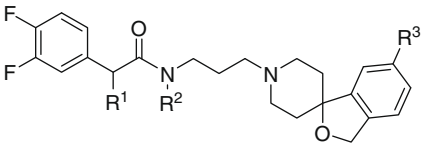


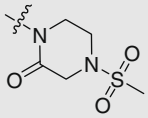
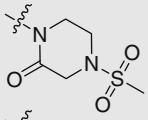
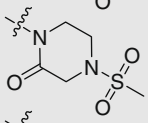
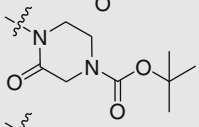
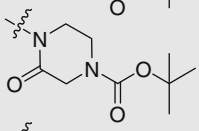
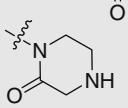
Scheme 2. Reagents and conditions: (a) TFA, rt, 10 min, 85%; (b) MsCl, $NaHCO_3$, THF, rt, 10 min, 99%.

ture–activity relationship study is shown in Table 1. Ethyl derivative **2** (R^2 = Et) showed improved activity (IC_{50} = 1.0 nM), and the substitution of the right hand spiropiperidine portion with a fluorine atom (R^3 = F) as in **3** additionally improved potency (IC_{50} = 0.71 nM). Subsequently, we found that 4-(methanesulfonyl)-piperidin-2-one-1-yl structure was tolerated as in **4** (IC_{50} = 0.71). After the resolution of racemate **4**, the active isomer **4a** was found to have an IC_{50} value of 0.36 nM. Thus, compound **4a** was identified as a markedly potent radioligand candidate that is amenable to ^{35}S labeling. In addition, corresponding *tert*-butylcarbonyl derivatives **5a** and precursor **6a** were shown to retain MCH1R potency. However, compound **4a** was a significant human and mouse P-gp substrate. Therefore, the radioligand might be useful for ex vivo RO study, but it might be difficult to use for in vivo RO assay.

In vitro binding profiles of **4a** are summarized in Table 2. Compound **4a** showed high affinity to human MCH1R (K_i = 0.42 nM) while having negligible affinity to MCH2R. In addition, the K_i value of compound **4a** for mouse MCH1R was comparable to that for human MCH1R, indicating no significant species differences in receptor binding. In the cellular functional assay using CHO cells expressing human MCH1R (i.e., FLIIPR), compound **4a** inhibited MCH-induced $[Ca^{2+}]i$ mobilization, indicating the antagonistic activity of **4a**. Compound **4a** was selective over a panel of 60 unrelated receptors, enzymes and transporters (IC_{50} > 1 μM for all the items tested). The binding profile of [^{35}S]**4a** (specific activity: 1200 mCi/mmol) at human and mouse MCH1Rs was examined and summarized in Figure 3. The CHO cell membranes overexpressing human or mouse MCH1R were mixed with various concentrations of [^{35}S]**4a** in the presence or absence of an excessive amount of cold compound **4a** to determine non-specific or total binding, respectively. After 1 h incubation at room temperature, the membranes were filtrated, and the residual radioactivities on the filters were measured by microplate scintillation counter. Compound [^{35}S]**4a** bound to human MCH1R with a *K*d value of 0.18 ± 0.02 nM and a B_{max} value of 23 ± 1 pmol/mg protein (Fig. 3A and B). The receptor density (i.e., the B_{max} value) determined by [^{35}S]**4a** was comparable to that obtained using [^{125}I]MCH (24 ± 4 pmol/mg protein) (Fig. 3C and D). No specific binding of [^{35}S]**4a** to CHO cell membranes was observed (data not shown), suggesting that [^{35}S]**4a** recognized MCH1R in a specific manner. Next, we investigated the kinetic profile of [^{35}S]**4a** (i.e., association and dissociation rates) for human MCH1R in order to further assess the potential of [^{35}S]**4a** as a radioligand for receptor occupancy assays. Non-specific binding was determined in the presence of an excess amount of compound **4a**. As a result, the spe-

Table 1
Human MCH1R binding of compounds **1**–**5**[a]



Compound	R^1	R^2	R^3	hMCH1R[b] (nM)
1		Me	H	2.7
2		Et	H	1.0
3		Et	F	0.71
4		Et	F	0.71
4a		Et	F	0.36
4b		Et	F	480
5a		Et	F	0.24
5b		Et	F	145
6a		Et	F	0.87

[a] Inhibitory activities of compounds **1**–**5** against [^{125}I]MCH binding to human MCH1R in CHO cells were determined.
[b] The values represent the mean for $n \geqslant 2$.

Table 2
In vitro profile of compound **4a**

	Binding assay[a] K_i (nM)			$[Ca^{2+}]i$ mobilization[b] IC_{50} (nM)
	Human MCH1R	Mouse MCH1R	Human MCH2R	Human MCH1R
Compound **4a**	0.42 ± 0.02	0.50 ± 0.08	>9700 ± 100	3.0 ± 0.3
MCH	0.034 ± 0.009	0.052 ± 0.009	1.6 ± 0.6	

[a] K_i values of the compound **4a** and human MCH for human and mouse MCH1R receptors were determined by ligand binding assay. The values are mean ± SD from three independent tests.
[b] Inhibition activity of compound **4a** on MCH-induced $[Ca^{2+}]i$ increase in CHO cells expressing human MCH1R was determined by FLIPR.

cific binding of [^{35}S]**4a** to MCH1R reached equilibrium within 5 min (Fig. 4). The kinetic parameters, k_{on} and k_{off}, were determined to be 2.3 ± 1.0 nM min^{-1} and 0.54 ± 0.06 min^{-1}, respectively. These data suggest that [^{35}S]**4a** binds rapidly to MCH1R when compared to [^{125}I]MCH that requires 1 h for equilibrium (data not shown).

Finally, we examined the binding of [^{35}S]**4a** to brain coronal slices prepared from wild type and MCH1R-knockout mice (C57BL/6j background). In the RO assay, striatum was used as a target area because MCH1R is abundantly and widely expressed in this region, thus making it feasible to perform the RO assay.[7,25]

Mice coronal brain sections including striatum were incubated with 500 pM of [^{35}S]**4a** for 5 min at room temperature. After washing the treated brain slices, the radioactivities on the striatum area were imaged and measured by BAS5000. As shown in Figure 5, [^{35}S]**4a** gave appreciable signals in the striatum region of wild-type brains while showing negligible signals in the knockout mice brains. These results demonstrate the selectivity of [^{35}S]**4a** not only in in vitro but also in vivo, which strongly suggest that [^{35}S]**4a** is a very useful tool for the RO assay.

In summary, we developed and characterized a potent and selective radioligand [^{35}S]**4a** that rapidly and specifically binds to MCH1R in both in vitro and in vivo systems. The use of [^{35}S]**4a** will

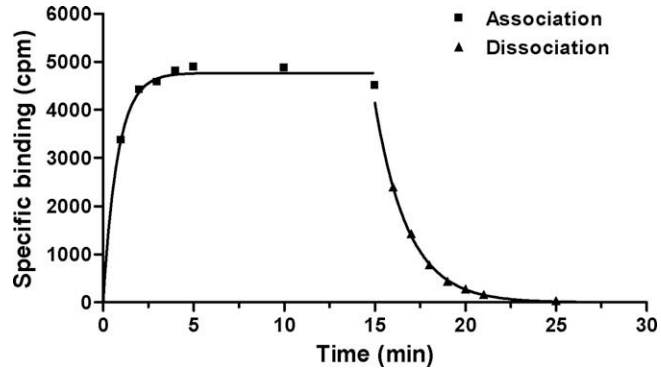


Figure 4. Kinetic profiles of [^{35}S]**4a** for human MCH1R. The association and dissociation kinetic studies were performed with 300 pM [^{35}S]**4a** as described in Figure 2 with slight modification. The association rate (K_{on}) was determined by analyzing time-dependent changes of the specific binding of [^{35}S]**4a** to human MCH1R (from 1 min to 15 min). The non-specific binding was determined in the presence of an excessive amount of cold **4a** (1 μM). Regarding the dissociation rate, the membranes were incubated with [^{35}S]**4a** for 1 h and then mixed with cold **4a** (1 μM) to initiate the dissociation step. The dissociation rate was determined by analyzing time-dependent changes of the specific binding of [^{35}S]**4a** to human MCH1R (from 1 min to 10 min). The binding experiment follows the procedure described in the legend of Figure 3.

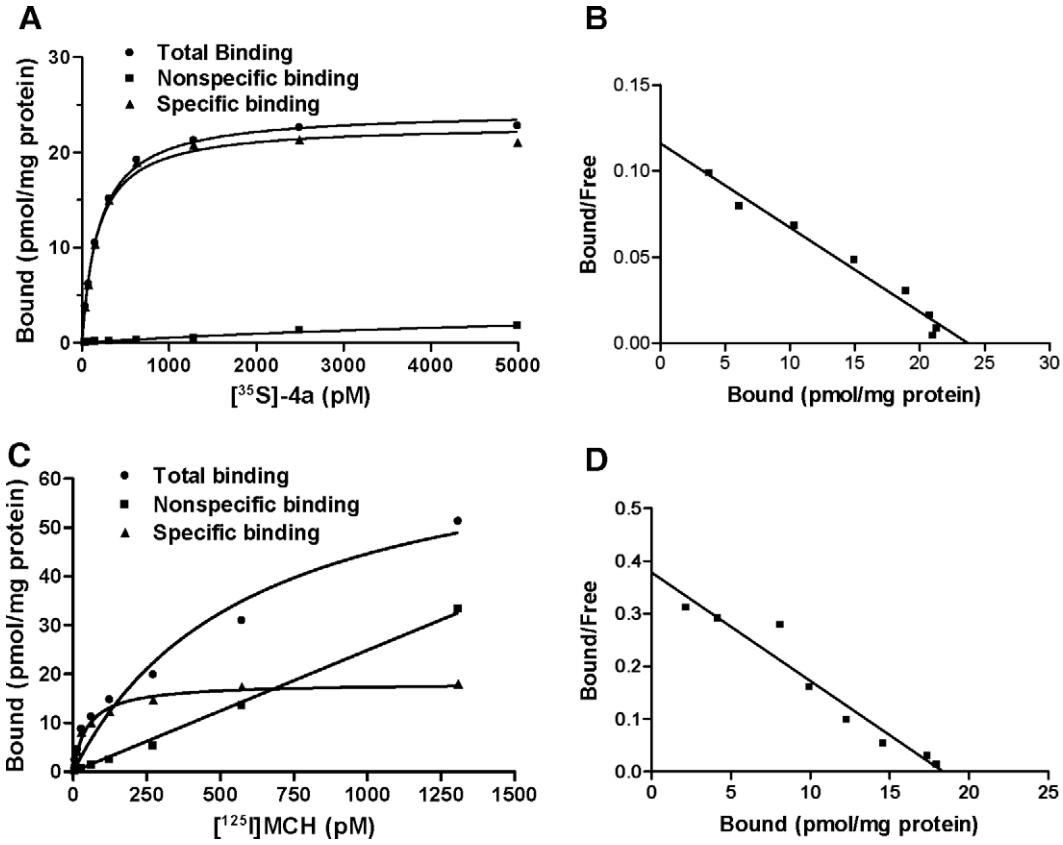


Figure 3. Saturation binding of [^{35}S]**4a** to human and mouse MCH1R CHO cell membranes. Membranes from CHO cells expressing human or mouse MCH1R were incubated with several concentrations of [^{35}S]**4a** in the presence of DMSO (total binding) or 1 μM compound **4a** in assay buffer (pH 7.4, 50 mM Tris–HCl, 10 mM $MgCl_2$, 2 mM EDTA, 50 μg/ml bacitracin, 0.2% bovine serum albumin (BSA)) at room temperature for 1 h. The membranes were filtrated onto GF/C filter and washed with washing buffer (pH 7.4, 50 mM Tris–HCl, 10 mM $MgCl_2$, 2 mM EDTA, 0.02% Tween-20) three times. The residual radioactivities on the dried filter were measured by microplate scintillation counter.

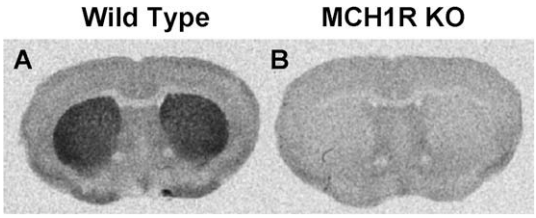


Figure 5. Binding of [^{35}S]**4a** to striatum of wild type and MCH1R deficient mice. Mice brain coronal slices including striatum region were incubated with 500 pM [^{35}S]**4a** for 5 min in assay buffer (pH 7.4, 50 mM Tris–HCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM KCl, 0.5% BSA). The slices were then washed with assay buffer at 4 °C three times for 4 min, then briefly dipped in chilled distilled water and air-dried. The images and residual radioactivities were analyzed by BAS5000 (Fujifilm, Tokyo, Japan).

facilitate the development of MCH1R antagonists through elucidation of mechanism-based efficacy and pharmacodynamic profiles of target compounds.

References and notes

1. Rodriguez, M.; Beauverger, P.; Naime, I.; Rique, H.; Ouvry, C.; Souchaud, S.; Dromaint, S.; Nagel, N.; Suply, T.; Audinot, V.; Boutin, J. A.; Galizzi, J. P. *Mol. Pharmacol.* **2001**, *60*, 632.
2. Saito, Y.; Nothacker, H. P.; Wang, Z. W.; Lin, S. H. S.; Leslie, F.; Civelli, O. *Nature* **1999**, *400*, 265.
3. Chambers, J.; Ames, R. S.; Bergsma, D.; Muir, A.; Fitzgerald, L. R.; Hervieu, G.; Dytko, G. M.; Foley, J. J.; Martin, J.; Liu, W. S.; Park, J.; Ellis, C.; Ganguly, S.; Konchar, S.; Cluderay, J.; Leslie, R.; Wilson, S.; Sarau, H. M. *Nature* **1999**, *400*, 261.
4. Hill, J.; Duckworth, M.; Murdock, P.; Rennie, G.; Sabido-David, C.; Ames, R. S.; Szekeres, P.; Wilson, S.; Bergsma, D. J.; Gloger, I. S.; Levy, D. S.; Chambers, J. K.; Muir, A. I. *J. Biol. Chem.* **2001**, *276*, 20125.
5. Sailer, A. W.; Sano, H.; Zeng, Z. Z.; McDonald, T. P.; Pan, J.; Pong, S. S.; Feighner, S. D.; Tan, C. P.; Fukami, T.; Iwaasa, H.; Hreniuk, D. L.; Morin, N. R.; Sadowski, S. J.; Ito, M.; Ito, M.; Bansal, A.; Ky, B.; Figueroa, D. J.; Jiang, Q. P.; Austin, C. P.; MacNeil, D. J.; Ishihara, A.; Ihara, M.; Kanatani, A.; Van der Ploeg, L. H. T.; Howard, A. D.; Liu, Q. Y. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 7564.
6. Tan, C. P.; Sano, H.; Iwaasa, H.; Pan, J.; Sailer, A. W.; Hreniuk, D. L.; Feighner, S. D.; Palyha, O. C.; Pong, S. S.; Figueroa, D. J.; Austin, C. P.; Jiang, M. M.; Yu, H.; Ito, J.; Ito, M.; Ito, M.; Guan, X. M.; MacNeil, D. J.; Kanatani, A.; Van der Ploeg, L. H. T.; Howard, A. D. *Genomics* **2002**, *79*, 785.
7. Saito, Y.; Cheng, M.; Leslie, F. M.; Civelli, O. *J. Comp. Neurol.* **2001**, *435*, 26.
8. Handlon, A. L.; Zhou, H. Q. *J. Med. Chem.* **2006**, *49*, 4017.
9. Shimazaki, T.; Yoshimizu, T.; Chaki, S. *CNS Drugs* **2006**, *20*, 801.
10. Rivera, G.; Bocanegra-Garcia, V.; Galiano, S.; Cirauqui, N.; Ceras, J.; Perez, S.; Aldana, I.; Monge, A. *Curr. Med. Chem.* **2008**, *15*, 1025.
11. Kowalski, T. J.; Sasikumar, T. *BioDrugs* **2007**, *21*, 311.
12. Qu, D. Q.; Ludwig, D. S.; Gammeltoft, S.; Piper, M.; Pelleymounter, M. A.; Cullen, M. J.; Mathes, W. F.; Przypek, J.; Kanarek, R.; MaratosFlier, E. *Nature* **1996**, *380*, 243.
13. Ito, M.; Gomori, A.; Ishihara, A.; Oda, Z.; Mashiko, S.; Matsushita, H.; Yumoto, M.; Ito, M.; Sano, H.; Tokita, S.; Moriya, M.; Iwaasa, H.; Kanatani, A. *Am. J. Physiol. Endocrinol. Metab.* **2003**, *284*, E940.
14. Gomori, A.; Ishihara, A.; Ito, M.; Mashiko, S.; Matsushita, H.; Yumoto, M.; Ito, M.; Tanaka, T.; Tokita, S.; Moriya, M.; Iwaasa, H.; Kanatani, A. *Am. J. Physiol. Endocrinol. Metab.* **2003**, *284*, E583.
15. Shearman, L. P.; Camacho, R. E.; Stribling, D. S.; Zhou, D.; Bednarek, M. A.; Hreniuk, D. L.; Feighner, S. D.; Tan, C. P.; Howard, A. D.; Van der Ploeg, L. H. T.; MacIntyre, D. E.; Hickey, G. J.; Strack, A. M. *Eur. J. Pharmacol.* **2003**, *475*, 37.
16. Marsh, D. J.; Weingarth, D. T.; Novi, D. E.; Chen, H. Y.; Trumbauer, M. E.; Chen, A. S.; Guan, X. M.; Jiang, M. M.; Feng, Y.; Camacho, R. E.; Shen, Z.; Frazier, E. G.; Yu, H.; Metzger, J. M.; Kuca, S. J.; Shearman, L. P.; Gopal-Truter, S.; MacNeil, D. J.; Strack, A. M.; MacIntyre, D. E.; Van der Ploeg, L. H. T.; Qian, S. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 3240.
17. Bjursell, M.; Gerdin, A. K.; Ploj, K.; Svensson, D.; Svensson, L.; Oscarsson, J.; Snaith, M.; Tornell, J.; Bohlooly, Y. *Diabetes* **2006**, *55*, 725.
18. Kowalski, T. J.; Farley, C.; Cohen-Williams, M. E.; Varty, G.; Spar, B. D. *Eur. J. Pharmacol.* **2004**, *497*, 41.
19. Takekawa, S.; Asami, A.; Ishihara, Y.; Terauchi, J.; Kato, K.; Shimomura, Y.; Mori, M.; Murakoshi, H.; Kato, K.; Suzuki, N.; Nishimura, O.; Fujino, M. *Eur. J. Pharmacol.* **2002**, *438*, 129.
20. Borowsky, B.; Durkin, M. M.; Ogozalek, K.; Marzabadi, M. R.; DeLeon, J.; Heurich, R.; Lichtblau, H.; Shaposhnik, Z.; Daniewska, I.; Blackburn, T. P.; Branchek, T. A.; Gerald, C.; Vaysse, P. J.; Forray, C. *Nat. Med.* **2002**, *8*, 825.
21. Marzabadi, M. R.; DeLeon, J.; Daniewska, I.; Jiang, Y.; Lagu, B.; Li, B. S.; Wang, Z. H.; Forray, C.; Borowsky, B.; Ogozalek, K.; Lichtblau, H.; Lim, K.; Tian, D.; Gluchowski, C.; Noble, S. A.; Wetzel, J. M. *Abstr. Papers Am. Chem. Soc.* **2002**, *224*, 340.
22. Mendez-Andino, J. L.; Wos, J. A. *Drug Discovery Today* **2007**, *12*, 972.
23. Andersen, D.; Storz, T.; Liu, P. L.; Wang, X.; Li, L. P.; Fan, P. C.; Chen, X. Q.; Allgeier, A.; Burgos, A.; Tedrow, J.; Baum, J.; Chen, Y.; Crockett, R.; Huang, L.; Syed, R.; Larsen, R. D.; Martinelli, M. *J. Org. Chem.* **2007**, *72*, 9648.
24. Rokosz, L. L. *Exp. Opin. Drug Discovery* **2007**, *2*, 1301.
25. Ishihara, A.; Kanatani, A.; Mashiko, S.; Tanaka, T.; Hidaka, M.; Gomori, A.; Iwaasa, H.; Murai, N.; Egashira, S.; Murai, T.; Mitobe, Y.; Matsushita, H.; Okamoto, O.; Sato, N.; Jitsuoka, M.; Fukuroda, T.; Ohe, T.; Guan, X. M.; MacNeil, D. J.; Van der Ploegn, L. H. T.; Nishikibe, M.; Ishii, Y.; Ihara, M.; Fukami, T. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 7154.
26. McBriar, M. D.; Guzik, H.; Shapiro, S.; Paruchova, J.; Xu, R.; Palani, A.; Clader, J. W.; Cox, K.; Greenlee, W. J.; Hawes, B. E.; Kowalski, T. J.; O'Neill, K.; Spar, B. D.; Weig, B.; Weston, D. J.; Farley, C.; Cook, J. *J. Med. Chem.* **2006**, *49*, 2294.
27. Audinot, V.; Lahaye, C.; Suply, T.; Beauverger, P.; Rodriguez, M.; Galizzi, J. P.; Fauchere, J. L.; Boutin, J. A. *Br. J. Pharmacol.* **2001**, *133*, 371.
28. Smith, D. G.; Hegde, L. G.; Wolinsky, T. D.; Miller, S.; Papp, M.; Ping, X.; Edwards, T.; Gerald, C. P.; Craig, D. A. *Behav. Brain Res.* **2009**, *197*, 284.
29. Suzuki, T.; Moriya, M.; Sakamoto, T.; Suga, T.; Kishino, H.; Takahashi, H.; Ishikawa, M.; Nagai, K.; Imai, Y.; Sekino, E.; Ito, M.; Iwaasa, H.; Ishihara, A.; Tokita, S.; Kanatani, A.; Sato, N.; Fukami, T. *Bioorg. Med. Chem. Lett.*, in press.
30. Compounds **4** and **5** were resolved by Chiralpak AD. Compound **4** was eluted with hexanes:*i*-PrOH:TFA = 70:30:0.1 to afford **4a** as a second-eluted enantiomer. Compound **5** was eluted with hexanes:*i*-PrOH:TFA = 80:20:0.1 to afford **5a** as a second-eluted enantiomer.