Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 14 (2004) 377–381

Synthesis of novel melanocortin 4 receptor agonists and antagonists containing a succinamide core

Ning Xi,[a,*] Clarence Hale,[b] Michael G. Kelly,[a,†] Mark H. Norman,[a] Markian Stec,[a] Shimin Xu,[a] James W. Baumgartner[b] and Christopher Fotsch[a]

[a]*Department of Chemistry Research & Discovery, Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320, USA*
[b]*Department of Metabolic Disorders, Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320, USA*

Received 21 August 2003; revised 17 October 2003; accepted 31 October 2003

Abstract—A novel series of piperazines appended to a succinamide backbone were synthesized and found to have a high affinity for the melanocortin-4 receptor (IC_{50}s ranging from <0.1 to 200 nM). Both agonists and antagonists of MC4R were prepared by modifying the groups attached to the right-hand side of the succinamide. This series also exhibits a high level of selectivity (up to 7000-fold) over mouse MC1R and human MC3R.
© 2003 Elsevier Ltd. All rights reserved.

The five melanocortin receptor subtypes (MC1–MC5R) that have been identified and cloned[1] belong to the superfamily of seven transmembrane-spanning G-protein-coupled receptors (GPCRs). These receptors are activated by peptides derived from the prohormone proopiomelanocortin (POMC), such as α-, β-, and γ-melanocyte-stimulating hormones (MSH) and adrenocorticotropic hormome (ACTH).[2] The MCR subtypes differ in their tissue distribution and ligand binding specificity. One of the receptor subtypes, MC4R, is expressed in the hypothalamus, and is believed to play a role in controlling food intake and energy homeostasis.[3] MC4R has also been implicated in normal stimulation of sexual arousal.[4] Therefore, small molecule agonist of MC4R may be useful for the treatment of diseases and disorders, such as obesity and sexual dysfunction.[5]

Much of the research on MC4R agonists has focused on peptide analogues of the endogenous ligands (e.g., α-MSH), such as cyclic lactam MTII.[6] More recently, however, many groups have identified agonists containing the piperidine and piperazine cores[7,8] (see, for example, **1**[9] and **2**[10] in Fig. 1). A component that many of these agonists share is a central phenylalanine, which may mimic the same amino acid in the message sequence 'His-Phe-Arg-Trp' found in peptide ligands for the MCRs.[11] In the course of our work, we also identified a small molecule agonist of MC4R that contained the piperazine and a *p*-chloro-D-phenylalanine (e.g., compound **3** in Fig. 2).[12] We searched for alternatives to the D-phenylalanine subunit to expand the scope of these piperazine analogues. To that end, we considered compounds with the succinamide core (see **14a** in Fig. 2). Compound **14a** has the same connectivity as compound **3** except that the amide is reversed on the right side of the compound. The result of this change is that the NH on *p*-chloro-D-phenylalanine is replaced with a CH_2 group, but an amide carbonyl is still present. Herein, we describe the synthesis of these succinamides and their activities toward melanocortin receptors.

The construction of the chiral succinic acid was achieved using Evan's chiral oxazolidinone as outlined

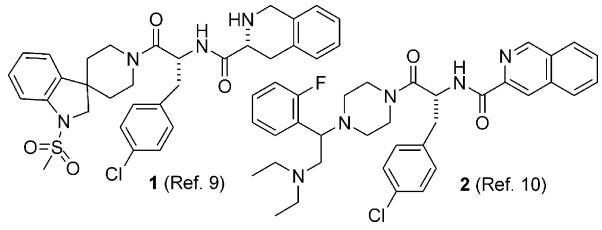


Figure 1. Examples of non-peptide MCR agonists.

* Corresponding author. Tel.: +1-805-447-2285; fax: +1-805-480-3016; e-mail: nxi@amgen.com
† Current address: Renovis, 270 Littlefield Avenue, South San Francisco, CA 94080, USA.

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.10.056

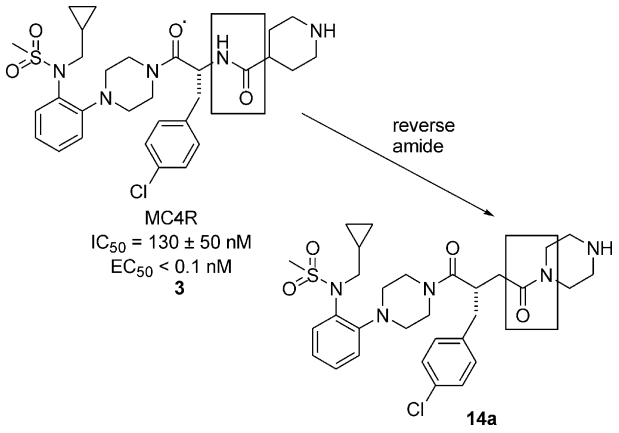
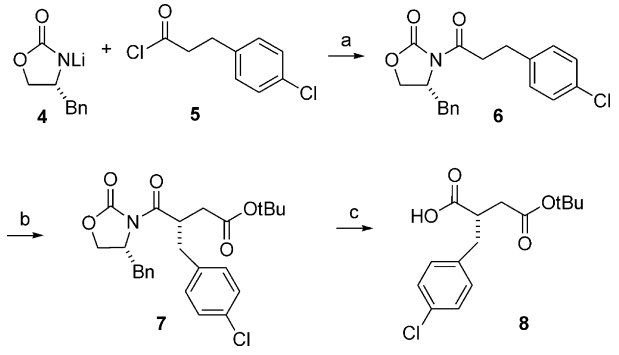


Figure 2. Concept for replacing the *p*-chloro-D-phenylalanine on compound **3** with a succinamide backbone.

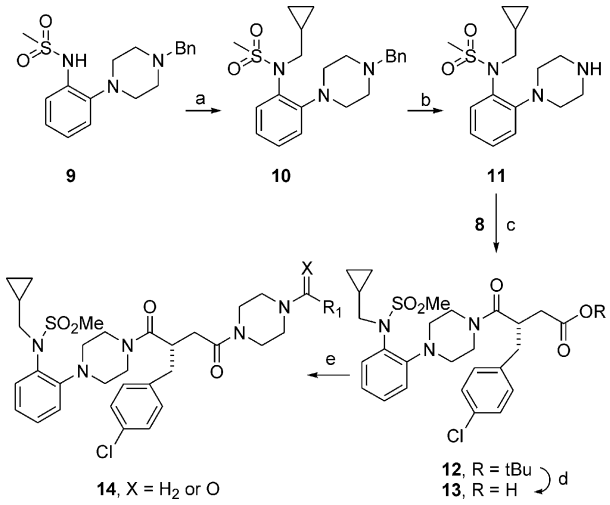


Scheme 1. (a) THF, −78 °C, 70%; (b) KHMDS, $BrCH_2CO_2t$-Bu, THF, −78 °C, 75%; (c) (i) LiOH–H_2O, H_2O_2, THF, 0 °C; (ii) 2 N HCl, 80%.

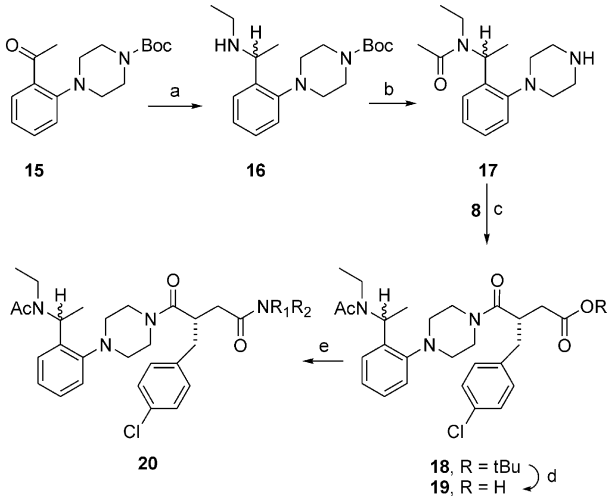
in Scheme 1.[13] The imide **6**, acquired through coupling of acid chloride **5** and lithium salt of (*R*)-4-benzyl-2-oxazolidinone (**4**), was alkylated with *t*-butyl bromoacetate to afford succinamide **7**. The chiral auxiliary was removed under standard condition to furnish acid **8**. The absolute configuration of this key intermediate was confirmed by X-ray crystallography.[14]

The preparation of the final products is illustrated in Schemes 2 and 3. Sulfonamide **9**[15] was alkylated with bromomethyl cyclopropane to furnish compound **10**. Removal of benzyl group under hydrogenation conditions led to piperazine **11**. Coupling of **11** with succinic acid **8** provided amide **12**, which was deprotected to furnish acid **13**. This compound was coupled with 4-Boc-piperazine, which following deprotection, was either substituted with aldehydes in a reductive amination step to give **14**, where X = H_2, or acylated with acid chlorides to give **14**, where X = O (see Table 1 for specific examples).

The acetamide analogues **20** were prepared in the similar fashion (Scheme 3). Reductive amination of compound **15**[16] with ethylamine gave compound **16**, which was acetylated and deprotected to afford compound **17**. Intermediate **19** was prepared from **17** using the same steps as described for the conversion of **11** to **13** in Scheme 2. Various amines were subsequently coupled



Scheme 2. (a) NaH, DMF, cyclopropyl methyl bromide, 90%; (b) H_2, Pd/C in EtOAc, 100%; (c) EDC, HOAt, Et_3N, CH_2Cl_2, 90%; (d) TFA/CH_2Cl_2, 100%; (e) (i) 4-Boc-piperazine, EDC, HOAt, CH_2Cl_2; (ii) TFA/CH_2Cl_2; (iii) when X = H_2, R_1CHO, $NaBH_3CN$, AcOH, MeOH; when X = O, $R_1(C{=}O)Cl$, Et_3N, $CHCl_3$.

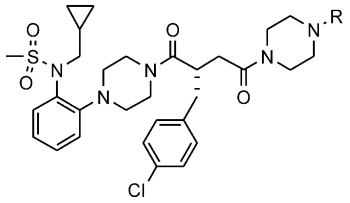


Scheme 3. (a) $EtNH_2$, $NaBH_4$, MeOH, 70%; (b) (i) Ac_2O, Et_3N, CH_2Cl_2, 99%; (ii) satd HCl in EtOAc, 100%; (c) EDC, HOAt, Et_3N, CH_2Cl_2; (d) TFA/CH_2Cl_2; (e) R_1R_2NH, EDC, HOAt, CH_2Cl_2.

with **19** to provide amides **20** (see Table 2 for specific examples).

All analogues were tested in binding and functional assays against the human MC4R by the method described previously,[17] and the results are summarized in Tables 1 and 2. We first examined the SAR of succinamides containing a six-membered ring that would mimic the isonipecotic acid found on the right-hand side of analogue **3**. Succinamde **14a** had an IC_{50} = 220 nM, which was comparable to the activity of **3** (IC_{50} = 130 nM). In the functional assay **14a** had an EC_{50} = 2000 nM, which is in sharp contrast to an EC_{50} < 0.1 nM (> 90% activation) measured for analogue **3**. The disparity in functional activities between **14a** and **3** suggests that the NH D-phenylalanine portion of **3** makes significant contribution in activating the receptor for this class of compounds.

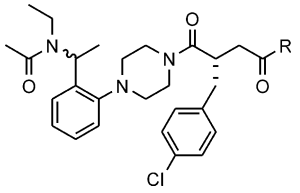
Table 1. Biological activity of **14**

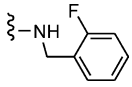
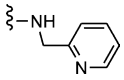
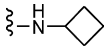
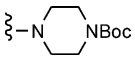


Compd	R	MC4R IC_{50} (nM)[a]	MC4R[b] EC_{50} (nM) (activation)[c]
MTII[d]	NA	1.4±0.3	<0.01
1[d]	NA	66±16	5.2±1.6 (100%)
14a	H	220±10	2000±1400 (70%)
14b	Ethyl	9.5±7.3	>10,000 (0%)
14c	*n*-Propyl	8.9±8.8	>10,000 (0%)
14d	*i*-Pentyl	4.8±4.3	>10,000 (0%)
14e	Cyclopropyl-methyl	3.0±2.9	>10,000 (0%)
14f	C(O)O*t*Bu	3.3±2.4	>10,000 (0%)
14g	C(O)OEt	1.4±1.3	>10,000 (0%)
14h	$C(O)CH_3$	11±3	>10,000 (0%)
14i	C(O)Ph	4.4±1.1	83±31 (55%)
14j	$C(O)CH_2tBu$	<0.1	74±48 (45%)

NA, not applicable.
[a] ^{125}I-NDP-α-MSH binding to the melanocortin-4 receptor, SEM of at least two IC_{50}s determined over six dilutions.
[b] Intracellular levels of cAMP in cells expressing the melanocortin-4 receptor, SEM of two EC_{50}s determined over six dilutions.
[c] Percentage of maximal response with respect to α-MSH.
[d] Cyclic lactam MTII and compound **1** were tested as benchmarks for the assays.

Table 2. Biological activity of **20**



Compd	R	MC4R[a] IC_{50} (nM)	MC4R[b] EC_{50} (nM) (activation)[c]
20a		9.4±6.4	530±510 (80%)
20b		19±2	340±240 (90%)
20c		13±1	89±44 (100%)
20d		32±22	37±4 (95%)
20e		5.8±3.1	15±6 (100%)
20f		2[d]	>10,000 (0%)

[a] ^{125}I-NDP-α-MSH binding to the melanocortin-4 receptor, SEM of at least two IC_{50}s determined over six dilutions.
[b] Intracellular levels of cAMP in cells expressing the melanocortin-4 receptor, SEM of two EC_{50}s determined over six dilutions.
[c] Percentage of maximal response with respect to α-MSH.
[d] One determination

We then examined derivatives of **14a** where the secondary amine was substituted with alkyl and acyl groups. Alkyl derivatives (**14b–e**) all had better binding affinity (IC_{50} <10 nM) than compound **14a**, but they did not activate MC4R at 10 μM in the functional assay. Carbamate and amide analogues were also prepared, and they were also more potent (IC_{50} ≤10 nM) than the parent compound **14a**. Apparently the basic nitrogen

found on **14a–e** is not required for high affinity ($IC_{50} < 15$ nM) since the amides and carbamates, **14f–j**, are just as active. Functional activities were >10,000 nM for analogues **14f–h**, but analogues **14i** and **14j** had EC_{50}s ≈80 nM with ca. 50% activation. The difference in functional activity is especially interesting for amide **14j** and the related carbamate **14f**. The only difference between these two compounds is a methylene and an oxygen atom, yet **14j** activates the receptor at 50% while **14f** does not activate the receptor at 10 μM.

In addition to the arylsulfonamide on the left side of the succinamide core, we used the tertiary acetamide moiety, which was also employed in our D-phenylalanine series.[12] Rather than trying to mimic the isonipecotic acid, we selected a more diverse set of amines as appendages to the succinamide core. (Table 2). Compounds **20a–e** have good binding affinities at the receptor ($IC_{50} < 30$ nM), but unlike the previously set of succinamides, **20a–e** have robust activation responses in the functional assay (80–100% of the response produced by α-MSH). Compounds **20a–e** have EC_{50}s between 15 and 530 nM and show little correlation with the binding activity. These results are consistent with the notion that binding does not necessarily correlate with functional efficacy.[18] To make a comparison between the two series, compound **20f** was prepared, which is an analogue of **14f**. Both compounds showed no activation at 10 μM, which suggests that the modifications on the right-hand side of **20a–e** were responsible for increasing the functional activity within this series of compounds. Apparently, the larger piperazine-Boc group of **20f** binds to MC4R in such a way that it no longer activates the receptor. Compound **20f** illustrates how a subtle change in structure can adversely affect functional activity.[19]

Selectivity for many of the succinamide analogues were determined at two receptor sub-types: MC1R (mouse) and MC3R (human) (Table 3). Of those compounds tested, they all showed a high degree of selectivity versus the other receptor sub-types whether they are from the sulfonamide series or the tertiary acetamide series.

In summary, we have shown that the succinamide analogues have a high affinity to the MC4R, and are a useful alternative to the D-phenylalanine found on many of the known MC4R agonists. We have prepared both potent agonists and antagonists for MC4R within this series by modifying the pendant groups. Compounds from series **14** have high affinity but are poor activators of the receptor in the functional assay. Series **20** analogues, on the other hand, have both a high affinity and high functional activity. Many of the most potent compounds at MC4R exhibited a high degree of selectivity over mouse MC1R and human MC3R, with selectivity ratios ranging from 40 to 7,000. Unlike many MC4R agonists or antagonists known in the current literature, our succinamides lack the central amino acid, which provides a novel alternative to the known MC4R agonists.

Table 3. MC1R and MC3R binding assay results for selected succinamides

Compd	Mouse MC1R IC_{50} (nM)	Human MC3R IC_{50} (nM)	Human MC4R IC_{50} (nM)
MTII	0.06±0.01	44±3	1.4±0.3
1	153±36	2900±400	66±16
14a	>8000	>8000	220±10
14c	>10,000	>10,000	8.9±8.8
14f	>10,000	>3500	3.3±2.4
14g	>10,000	>10,000	1.4±1.3
14h	>10,000	>10,000	11±3
14j	>1800	>10,000	<0.1
20b	>1400	>10,000	19±2
20c	>1600	>10,000	13±1
20e	>600	>10,000	5.8±3.1

References and notes

1. Wikberg, J. E. S.; Muceniece, R.; Mandrika, I.; Prusis, P.; Lindblom, J.; Post, C.; Skottner, A. *Pharmacol. Res.* **2000**, *42*, 393.
2. Pritchard, L. E.; Turnbull, A. V.; White, A. *J. Endocrinol.* **2002**, *172*, 411.
3. (a) Chen, A. S.; Metzger, J. M.; Trumbauer, M. E.; Guan, X. M.; Yu, H.; Frazier, E. G.; Marsh, D. J.; Forrest, M. J.; Gopal-Truter, S.; Fisher, J.; Camacho, R. E.; Strack, A. M.; Mellin, T. N.; MacIntyre, D. E.; Chen, H. Y.; Van der Ploeg, L. H. *Transgenic Res.* **2000**, *9*, 145. (b) Fan, W.; Boston, B. A.; Kesterson, R. A.; Hruby, V. J.; Cone, R. D. *Nature* **1997**, *385*, 165.
4. Wessells, H.; Fuciarelli, K.; Hansen, J.; Hadley, M. E.; Hruby, V. J.; Dorr, R.; Levine, N. *J. Urol.* **1998**, *160*, 389.
5. Wikberg, J. E. S. *Eur. J. Pharmacol.* **1999**, *375*, 295.
6. Hruby, V. J.; Slate, C. A. *Adv. Amino Acid Mimetics Peptidomimetics* **1999**, *2*, 191.
7. The piperidine and piperazine cores appear in several other patent applications on melanocortin receptor agonists. See: Yu, G.; Macor, J.; Herpin, T.; Lawrence, R. M.; Morton, G. C.; Ruel, R.; Poindexter, G. S.; Ruediger, E. H.; Thibault, C. PCT Int. Appl. 2002, WO 02/79146; *Chem. Abstr.* **2002**, *137*, 295252. Yu, G.; Macor, J.; Herpin, T.; Lawrence, R. M.; Morton, G. C.; Ruel, R.; Poindexter, G. S.; Ruediger, E. H.; Thibault, C. PCT Int. Appl. 2002, WO 02/70511; *Chem. Abstr.* **2002**, *137*, 232913. Bakshi, R. K.; Barakat, K. J.; Lai, Y.; Nargund, R. P.; Palucki, B. L.; Park, M. K.; Patchett, A. A.; Sebhat, I.; Ye, Z. PCT Int. Appl. 2002, WO 02/15909; *Chem. Abstr.* **2002**, *136*, 216648. Bakshi, R. K.; Barakat, K. J.; Nargund, R. P.; Palucki, B. L.; Patchett, A. A.; Sebhat, I.; Ye, Z.; Van, Der Ploeg L. H. T. PCT Int. Appl. 2000, WO 00/74679; *Chem. Abstr.* **2000**, *134*, 42445. Briner, K.; Doecke, C.r W.; Mancoso, V.; Martinelli, M. J.; Richardson, T. I.; Rothhaar, R. R.; Shi, Q.; Xie, C. PCT Int. Appl. 2002, WO 02/59117; *Chem. Abstr.* **2002**, *137*, 140779. Backer, R. T.; Briner, K.; Doecke, C. W.; Fisher, M. J.; Kuklish, S. L.; Mancuso, V.; Martinelli, M. J.; Mullaney, J. T.; Xie, C. PCT Int. Appl. 2002, WO 02/59107; *Chem. Abstr.* **2002**, *137*, 140776. Biggers, C. K.; Briner, K.; Doecke, C. W.; Fisher, M. J.; Hertel, L. W.; Mancoso; Martinelli, M. J.; Mayer, J. P.; Ornstein, P. L.; Richardson, T. I.; Shah, J. A.; Shi, Q.; Wu, Z.; Xie, C. PCT Int. Appl. 2002, WO 02/59108; *Chem. Abstr.* **2002**, *137*, 140777. Dyck, B. P.; Goodfellow, V.; Phillips, T.; Parker, J.; Zhang, X.; Chen, C.; Tran, J. A.; Pontillo, J.; Tucci, F. C. PCT Int. Appl. 2003, WO 03/31410; *Chem. Abstr.* **2003**, *138*, 321578.

8. A recent publication described piperidine analogues as selective MC4R agonists: Sebhat, I. K.; Martin, W. J.; Ye, Z.; Barakat, K.; Mosley, R. T.; Johnston, D. B. R.; Bakshi, R.; Palucki, B.; Weinberg, D. H.; MacNeil, T.; Kalyani, R. N.; Tang, R.; Stearns, R. A.; Miller, R. R.; Tamvakopoulos, C.; Strack, A. M.; McGowan, E.; Cashen, D. E.; Drisko, J. E.; Hom, G. J.; Howard, A. D.; MacIntyre, D. E.; van der Ploeg, L. H. T.; Patchett, A. A.; Nargund, R. P. *J. Med. Chem.* **2002**, *45*, 4589.
9. Nargund, R. P.; Ye, Z.; Palucki, B. L.; Bakshi, R. K.; Patchett, A. A.; Van Der Ploeg, L. H. T. PCT Int. Appl. 1999, WO 99/64002; *Chem. Abstr.* **1999**, *132*, 22957.
10. Backer, R. T.; Briner, K.; Collado C. I.; De Frutos-Garica, O.; Doecke, C. W.; Fisher, M. J.; Garcia-Paredes, C.; Kuklish, S. L.; Mancoso, V.; Martinelli, M. J.; Mateo H., Ana I.; Mullaney, J. T.; Ornstein, P. L.; Wu, Z.; Xie, C. PCT Int. Appl., WO 02/059095, 2002; *Chem. Abstr.* **2002**, *137*, 140775.
11. Al-Obeidi, F. A; Castrucci, M. L.; Hadley, M. E.; Hruby, V. J. *J. Med. Chem.* **1989**, *32*, 2555.
12. (a) Fotsch, C. H.; Arasasingham, P.; Bo, Y.; Chen, N.; Goldberg, M. H.; Han, N.; Hsieh, F.-Y.; Kelly, M. G.; Liu, Q.; Norman, M. H.; Smith, D. M.; Stec, M.; Tamayo, N.; Xi, N.; Xu, S. PCT Int. Appl., WO 03/09850 (2003); *Chem. Abstr.* **2003**, *138*, 137330. (b) Fotsch, C. H.; Croghan, M.; Doherty, E. M.; Kelly, M. G.; Norman, M. H.; Smith, D. M.; Tamayo, N.; Xi, N.; Xu, S. PCT Int. Appl. 2003, WO 03/09847; *Chem. Abstr.* **2003**, *138*, 137597.
13. Evans, D. A. *Asymmetric Synth.* **1984**, *3*, 1.
14. The single crystal was obtained from 50% EtOAc in hexane and its structure was determined by a Rigaku AFC7R diffractometer with graphite monochromated Cu-K_α radiation and a rotating anode generator: crystal system, monoclinic; lattice type, primitive; lattice parameters: $a = 12.429(1)$ Å; $b = 8.947(1)$ Å; $c = 14.910(1)$ Å; $\beta = 96.137(7)$; $V = 1648.4(3)$ Å^3; space group, $P2_1$ (#4); residuals: R, 0.118; Rw, 0.109.
15. For the preparation of compound **9**, see: Elliott, J. M.; Broughton, H.; Cascieri, M. A.; Chicchi, G.; Huscroft, I. T.; Kurtz, M.; MacLeod, A. M.; Sadowski, S.; Stevenson, G. I. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1851.
16. For the preparation of compound **15**, see: Kikuchi, C.; Ando, T.; Watanabe, T.; Nagaso, H.; Okuno, M.; Hiranuma, T.; Koyama, M. *J. Med. Chem.* **2002**, *45*, 2197.
17. Ligand Binding Assay and Functional (cAMP) Assay were previously described in: Fotsch, C.; Smith, D. M.; Adams, J. A.; Cheetham, J.; Croghan, M.; Doherty, E. M.; Hale, C.; Jarosinski, M. A.; Kelly, M. G.; Norman, M. H.; Tamayo, N. A.; Xi, N.; Baumgartner, J. W. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2337.
18. For a recent review discussing GPCR binding and functional phenomena see: Kenakin, T. *Nat. Rev. Drug Discov.* **2002**, *1*, 103.
19. Hruby and co-workers also showed that a subtle change in the structure of their MCR agonist, MTII, lead to loss in functional activity. The D-phenylalanine on MTII was replaced with the bulkier D-1-napthylalanine, which resulted in a peptide that was an antagonist (SHU9119): Hruby, V. J.; Lu, D.; Sharma, S. D.; Castrucci, A. M. L.; Kesterson, R. A.; Al-Obeidi, F. A.; Hadley, M. E.; Cone, R. D. *J. Med. Chem.* **1995**, *38*, 3545.