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Synthesis and biological evaluation of imidazole derivatives as novel NOP/ORL1 receptor antagonists: Exploration and optimization of alternative pyrazole structure

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ABSTRACT

Nonpeptidic small-molecule NOP/ORL1 receptor antagonists with an imidazole scaffold were designed and synthesized to investigate alternatives to the pyrazole analog. Systematic modification of the original pyrazole lead [Kobayashi et al., *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3627; Kobayashi et al., *Bioorg. Med. Chem. Lett.*, in press] to change the heterocyclic core, substituted side chain, and pendant functional group demonstrated that examining the structure–activity relationship for novel templates allowed the identification of potent, fully substituted 4-aminomethyl-1*H*-imidazole and 2-aminomethyl-1*H*-imidazole. These compounds exhibited excellent potency for ORL1 receptor with minimal P-gp efflux and/or reduced hERG affinity.

The fourth member of the opioid receptor family was discovered in 1994 and was called opioid receptor-like 1 (ORL1),[2] also known as nociceptin (NOP) receptor, using cDNA expression cloning techniques.[3] Its endogenous ligand, nociceptin (Orphanin FQ), is a novel heptadecapeptide and was isolated from brain extracts in 1995.[4] Although ORL1 receptor is a member of the G-protein coupled receptor (GPCR) superfamily with close homology to classical opioid (μ, δ, and κ) receptors, native opioid peptides and synthetic agonists selective for μ, δ, and κ receptors do not show significant affinity for ORL1 receptor.[5]

The ORL1 receptor and NOP are mainly distributed in the brain and central nervous system (CNS).[5,6] It has been observed that NOP is involved in modulating pain mechanisms in the spinal cord and forebrain. Several in vivo studies with NOP and its peptide analogs have demonstrated that NOP modulates a variety of biological functions, such as feeding, learning, diuresis, drug addiction, cardiovascular function, and locomotor activity and that it controls the release of neurotransmitters including serotonin and dopamine at peripheral and central sites.[7] ORL1 receptor may also be relevant in the treatment of CNS disorders including anxiety and drug abuse.[7,8] Therefore, the identification of potent, small-molecule agonists and antagonists of nociceptin could provide new classes of drugs for some human disorders involving pain and anxiety or for the treatment of Parkinson's disease.[9]

Recently, several research groups have reported findings related to the search for small-molecule ORL1 antagonists, describing nonpeptide ligands such as benzimidazolones[10] and related compounds,[11] benzimidazoles,[12] indolinones,[13] spiropiperidines,[14] aryl piperidines,[15] 4-aminoquinolines,[16] and indolyl-oxadiazole.[17] Some of these ligands possess very high selectivity for the ORL1 receptor versus other opioid receptors.

In the course of our search for an ORL1 antagonist, 1,5-diaryl-3-aminomethyl-1*H*-pyrazole **1** (Fig. 1) was confirmed as a primary hit compound via high-throughput screening (HTS) of in-house compound libraries. In previous structure–activity relationship (SAR) investigations, we found novel pyrazole derivatives which showed potent antagonistic activity against ORL1 receptor.[1] We initiated a core structure optimization study with the aim of exploring an alternative pyrazole moiety. As a result of preliminary modification of the core part, we identified imidazole analogs **2** and **3** as active prototypes and which deserved further derivation. Herein, we describe synthesis, SAR investigation and evaluation of novel imidazole scaffolds as ORL1 antagonists.

Our initial efforts toward understanding the SAR of the lead molecule were focused on replacing the core part with other five-membered heterocycles. The results of this study are summarized in Table 1. Pyrazole compounds **4** and **5** were prepared to evaluate the effect of a substituent at C-4 position. A methyl group

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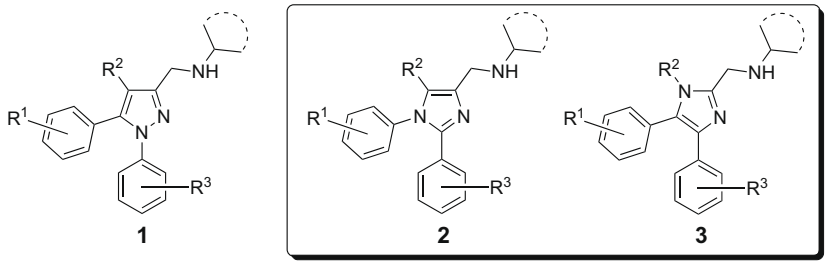
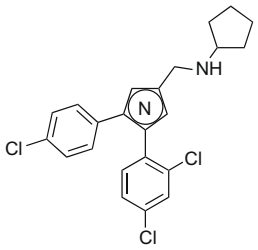


Figure 1. Structures of pyrazole (**1**) and imidazole (**2**, **3**) compounds.

Table 1
Effect of heterocycle on central part



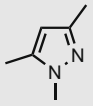
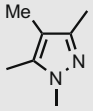
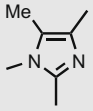
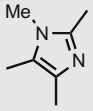
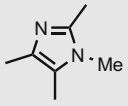
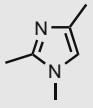
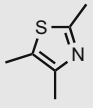
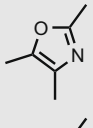
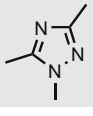
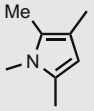
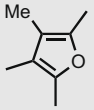
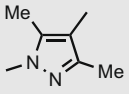
Compound		ORL1 IC_{50}[a] (nM)
4		130
5		28
6		28
7		520
8		850
9		620
10		>1000 (7%)
11		>1000 (28%)
12		>1000 (48%)

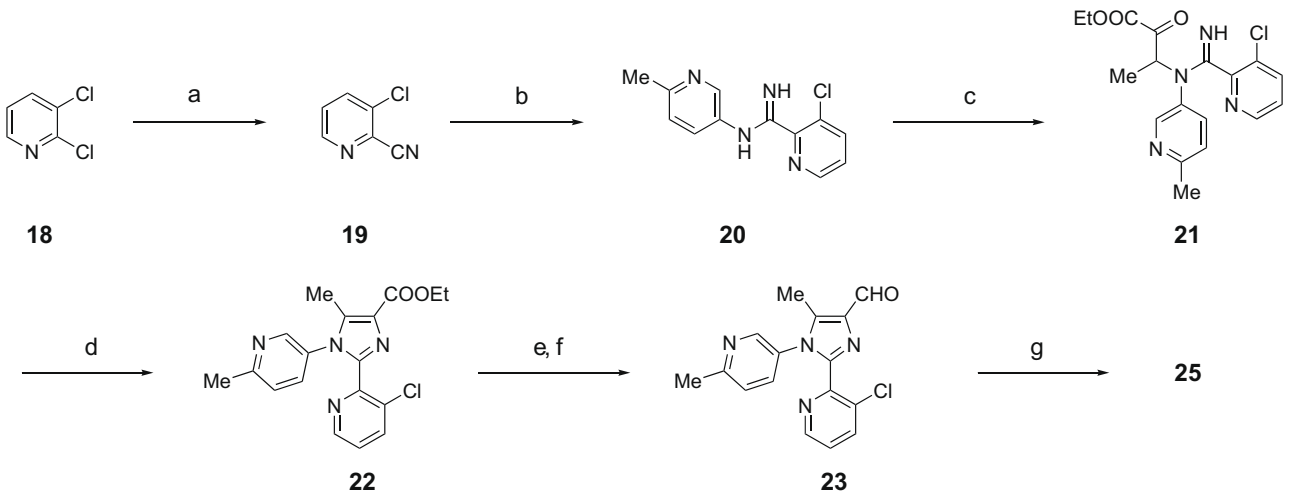
Table 1 (*continued*)

Compound		ORL1 IC_{50}[a] (nM)
13		200
14		>1000 (25%)
15[b]		>1000 (44%)
16[c]		>1000 (40%)
17[d]		>1000 (19%)

[a] Binding affinities for the ORL1 receptor. Numbers in parentheses indicate % inhibition at 1 μM.
[b] 4-Chlorophenyl part is replaced by phenyl group.
[c] 4-Chlorophenyl part is replaced by 3,4-difluorophenyl group.
[d] 4-Chlorophenyl part is replaced by 3,5-difluorophenyl group.

increased the ORL1 affinity fourfold. Compounds **6**–**9** were prepared to evaluate the effect of imidazole nuclei. Optimal binding affinity for ORL1 was achieved with 1,2-diaryl-5-methylimidazole **6**. As for 4,5-diarylimidazole compounds, a substitution pattern similar to that of compound **7** was preferable to regioisomer **8**. Another 1,2-diarylimidazole **9** had activity comparable to **7**. Thiazole **10**, oxazole **11**, triazole **12**, and furan **14** resulted in poor affinity or loss of affinity for ORL1. However, pyrrole analog **13** possessed moderate activity. Incorporation of other heterocycles without substitution at the bottom also had a detrimental effect on activity.

In order to reduce the lipophilicity of imidazole **6**, we modified the phenyl group into a more hydrophilic ring similar to that of pyridine. The synthesis of representative analog **25** is outlined in Scheme 1. Functionalization of the 2-position of commercially available 2,3-dichloropyridine **18** followed by cyanidation using $Zn(CN)_2$ and addition of sodium 6-methyl-3-pyridineamide gave the pyridinecarboxamidine derivative **20**. A cyclization reaction of **21,** obtained by alkylation of the amidine moiety in **20,** provided ethyl 1,2-dipyridinyl-imidazole-4-carboxylate **22**. Ethyl ester was converted in two steps, DIBAL-H reduction and MnO_2 oxidation,



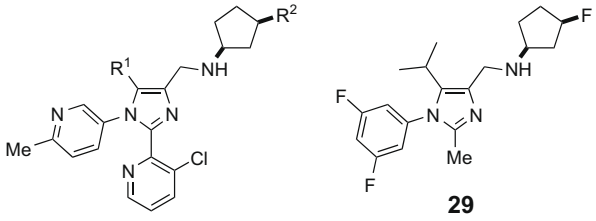
Scheme 1. Reagents: (a) $Zn(CN)_2$, $Pd(PPh_3)_4$, 64%; (b) 6-methyl-3-pyridineamine, NaHMDS, 57%; (c) ethyl 3-bromo-2-oxobutanoate, 75%; (d) 4 N HCl/dioxane, 38%; (e) DIBAL-H, 56%; (f) MnO_2, 88%; (g) (1*S*, 3*R*)-3-fluorocyclopentanamine hydrochloride, Et_3N, $NaBH_3CN$-1/2$ZnCl_2$, 28%.

into imidazole-4-carbaldehyde **23**. Finally, compound **23** was subjected to a reductive amination reaction with (1*S*,3*R*)-3-fluorocyclopentanamine[18] to produce the desired compound **25**. Close analogs were also prepared by a similar reaction sequence employing corresponding congeners. Several pyridine analogs were prepared and the selected derivatives (**24**, **26**–**28**) are summarized in Table 2.

These new imidazole compounds were tested for competitive binding affinity for human ORL1 receptors transfected into Chinese hamster ovary (CHO) cells using [^{125}I][Tyr^{14}] nociceptin as a radioligand, with the results expressed as IC_{50}. Functional antagonism was also determined by stimulation of [^{35}S] GTPγS binding to CHO-ORL1 membranes.[19] We carried out the human ether-a-go-go related gene (hERG) K^+ channel assay[20] using [^{35}S]MK-499[21] as a radioligand to evaluate hERG liability, for example QT prolongation followed by lethal cardiac arrhythmia. A P-glycoprotein (P-gp) transporter efflux assay was used to determine if a compound is a P-gp substrate.[22] Performance in this assay proved to be decisive in selecting potential CNS drug candidates for in vivo assessments of brain penetration. Additionally, the accuracy of efflux ratio (B–A/A–B) measurements may be compromised for compounds displaying apparent permeability of P_{app} <10.

The first phase of our SAR investigation involved the evaluation of simple derivatives related to prototypical compound **6** in order to define the optimal substituent. Although methyl analog **24** had activity comparable to **6**, the isopropyl derivative **27** exhibited a fivefold increase in potency against ORL1 relative to **6**. To find a synergistic effect on potency without significant affinity for the

Table 2
Profiles of 1,2-diarylimidazole (**24–28**) and 1-arylimidazole (**29**) compounds



Compound	R^1	R^2	Binding IC_{50}[a] (nM)	GTPγS IC_{50}[b] (nM)	hERG IC_{50}[c] (nM)	Human P-gp		Log P[f]	pK_a[g]
						B–A/A–B[d]	P_{app}[e]		
24	Me–	H	26	NT[h]	19,000	NT	NT	3.99	8.20
25	Me–	F	9.1	3.7	31,000	5.1	16.8	3.38	7.36
26	Et–	F	3.2	2.3	15,000	19.7	20.6	3.91	7.38
27	^{i}Pr–	H	4.7	9.6	9900	22.9	25.0	4.87	8.12
28	^{i}Pr–	F	1.4	2.3	15,000	21.8	24.1	4.26	7.28
29			8.6	4.5	34,000	3.2	34.0	3.90	7.74

[a] Binding affinities for the ORL1 receptor.
[b] Antagonist activities in the GTPγS functional assay.
[c] Displacement binding assay of [^{35}S]MK-499 in membranes derived from HEK293 cells stably transfected with the hERG gene and expressing the Ikr channel protein. See Ref. 20.
[d] Efflux ratio. See Ref. 22.
[e] Apparent permeability (10^{-6} cm/s).
[f] Calculated using ACD/log *P* DB supplied by Advanced Chemical Development Inc.
[g] Calculated using ACD/pK_a DB supplied by Advanced Chemical Development Inc.
[h] Not tested.

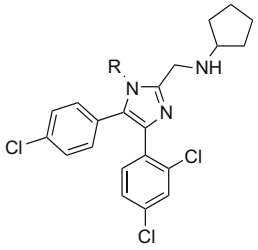
Table 3
Microsomal stabilities and rat pharmacokinetic property for compound **26**

Microsomal stability (% remaining)			Rat PK[a]	
Human	Rat	Cl (ml/min/kg)	$T_{1/2}$ (h)	F (%)
85	82	114	0.5	10

[a] Oral dose 3 mg/kg and IV dose 1 mg/kg.

hERG K^+ channel, we also prepared (1*S*,3*R*)-3-fluorocyclopentylamine[23] derivatives (**25**, **26**, and **28**). As expected, the intrinsic potency improved appreciably and this modification led to a threefold increase in potency (**24** vs **25** and **27** vs **28**). In addition, F-containing derivatives had even less affinity for the hERG K^+ channel than parent compounds due to a reduction in basicity. It is a general trend that more basic substances tend to have an increased affinity for the potassium channel (**24** vs **25** and **27** vs **28**). Another significant concern for CNS activity is a compound's susceptibility to P-gp mediated transport. Although all tested compounds (**25**–**28**) displayed good permeability (P_{app} = 17–25), most of the potent analogs (**26**–**28**) were highly susceptible substrates for P-gp (B–A/A–B ratio = 20–23). In contrast, a small alkyl group had a positive effect in reducing susceptibility to P-gp efflux and **25** was only moderately susceptible for P-gp (B–A/A–B ratio = 5).

Table 4
Effect of alkyl substituent on imidazole ring



Compound	R	ORL1 IC_{50}[a] (nM)
7	Me	520
30	Et	160
31	^{i}Pr	33
32	^{t}Bu	>1000 (26%)
33		190
34		530
35		>1000 (43%)
36		>1000 (48%)
37		160
38		540
39		160
40		500
41		>1000 (32%)
42		96

[a] Binding affinities for the ORL1 receptor. Numbers in parentheses indicate % inhibition at 1 μM.

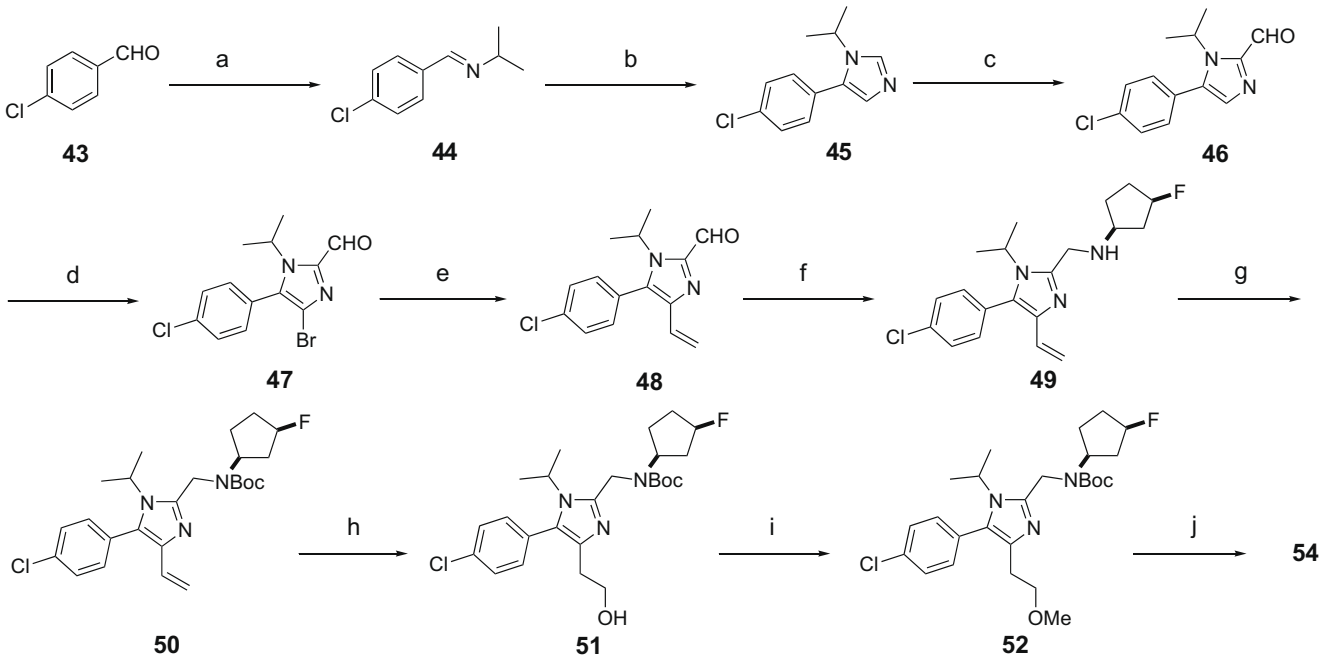
Next, we performed a drug metabolism study for representative derivative **26** (Table 3). In vitro metabolic stabilities using human and rat liver microsomes[24] were relatively high (82–85%); however, the rat pharmacokinetic (PK) profile needs improvement due to its short half-life and high clearance followed by low oral bioavailability (F 10%). With regard to substitution pattern, the aryl group on the bottom was replaced with alkyl moieties to confirm whether aromatic substituents are essential. On the basis of previous research,[17] we examined several alkyl groups together with the other aryl part at the C-1 position. Of the alkyl derivatives examined, a methyl group was accepted as the optimal C-2 substituent and benchmark compound **29** had an activity comparable to **25** with negligible hERG inhibitory activity and acceptable P-gp susceptibility (Table 2).

As the second lead optimization attempt for finding an alternative imidazole template, we carried out replacement of the methyl group on imidazole ring **7** with alkyl, cycloalkyl or fluoroalkyl moieties to confirm which alkyl substituent was better suited as a skeleton. The results for the alkyl analogs are shown in Table 4. As observed for compounds **32**, **35**, and **36**, *tert*-butyl and cycloalkyl groups rendered the molecule inactive, whereas cyclobutyl **34** and isobutyl **38** retained potency. Interestingly, relatively small and branched groups increased the potency by threefold in the case of ethyl **30** and 3-pentyl **37** and 15-fold in the case of isopropyl **31** versus parent compound **7**. Attempts to introduce an F atom in the alkyl region to reduce imidazole basicity resulted in moderate compounds **39**–**42**. ORL1 binding affinity was observed to decrease with an increase in the number of F atoms.

Successive lead optimization efforts were employed in conformity with a similar strategy to affect replacement of the aryl group with an alkyl side chain. Scheme 2 depicts the preparation of 2-methoxyethyl analog **54** as a typical example. Isopropyl aldimine **44**, prepared from 4-chlorobenzaldehyde **43** and isopropylamine, was converted to 5-(4-chlorophenyl)-1-isopropylimidazole **45** utilizing tosylmethyl isocyanine (TosMIC). Formylation at C-2 position as aldehyde **46** followed by bromination provided fully substituted imidazole **47**. Stille cross-coupling of bromide **47** with tributyl(vinyl)tin gave the corresponding vinyl intermediate **48**. Reductive amination, as mentioned in Scheme 1, generated the secondary amine **49**, which was protected as *tert*-butyl carbamate **50**. Imidazolylethanol **51** was then prepared from **50** by hydroboration and oxidation with alkaline H_2O_2. Alkylation with MeI produced methylether, which upon deprotection of the Boc group with TFA, afforded the desired amine **54**.

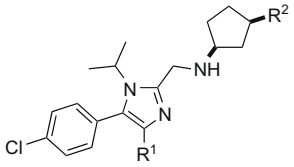
As observed in the previous imidazole series (Table 2), incorporation of small alkyl groups into this imidazole series is also acceptable in terms of their potency. Introduction of (1*S*,3*R*)-3-fluorocyclopentylamine resulted in compound **54** which was five times weaker in affinity than **53** in the hERG assay with decreased basicity and lipophilicity.[25] Both compounds revealed good permeability (P_{app} = 18 and 29, respectively) and P-gp efflux susceptibility (B–A/A–B ratio = 1.0 and 1.4, respectively) (Table 5).

In conclusion, based on the former pharmacophore model,[1] we designed novel ORL1 receptor antagonists by replacing a pyrazole ring with the imidazole motif. Two different types of imidazole scaffold showed excellent antagonistic activity toward ORL1. In particular, compounds **28** and **54** displayed high affinity (ORL1



Scheme 2. Reagents: (a) $^{i}PrNH_2$, $MgSO_4$; (b) TosMIC, $^{i}PrNH2$, 68% (2 steps); (c) $^{n}BuLi$ then DMF, 87%; (d) NBS, 97%; (e) tributyl(vinyl)tin, $Pd(PPhh_3)_4$, 55%; (f) (1*S*, 3*R*)-3-fluorocyclopentanamine hydrochloride, Et_3N, $NaBH_3CN$-1/2$ZnCl_2$, 74%; (g) Boc_2O, 1 N NaOH, 91%; (h) $NaBH_4$, BF_3OEt_2 then 30% H_2O_2, 5 N NaOH, 24%; (i) MeI, NaH, 72%; (j) TFA, 48%.

Table 5
Profiles of 5-arylimidazole compounds



Compound	R^1	R^2	Binding IC_{50}[a] (nM)	GTPγS IC_{50}[b] (nM)	hERG IC_{50}[c] (nM)	Human P-gp		Log P[f]	pK_a[g]
						B–A/A–B[d]	P_{app}[e]		
53	Me–	H	11	NT[h]	3200	1.0	18.2	5.46	8.98
54	$MeO(CH_2)_2$–	F	7.6	5.6	16,000	1.4	28.6	4.32	8.13

[a–h] See footnotes of Table 2.

IC_{50} = 1.4 and 7.6 nM, respectively) without hERG liability (IC_{50} >15 μM). Furthermore, improvement of the physicochemical property allows freedom from P-gp susceptibility. As a result, we have identified an unprecedented imidazole antagonist **54** with a CNS drug-like profile.

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References and notes

1. (a) Kobayashi, K.; Uchiyama, M.; Ito, H.; Takahashi, H.; Yoshizumi, T.; Sakoh, H.; Nagatomi, Y.; Asai, M.; Miyazoe, H.; Tsujita, T.; Hirayama, M.; Ozaki, S.; Tani, T.; Ishii, Y.; Ohta, H.; Okamoto, O. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3627; (b) Kobayashi, K.; Tsujita, T.; Ito, H.; Ozaki, S.; Tani, T.; Ishii, Y.; Okuda, S.; Tadano, K.; Fukuroda, T.; Ohta, H.; Okamoto, O. *Bioorg. Med. Chem. Lett.*, in press.
2. (a) Barlocco, D.; Cignarella, G.; Giardina, G. A. M.; Toma, L. *Eur. J. Med. Chem.* **2000**, *35*, 275; (b) Zaveri, N. *Life Sci.* **2003**, *73*, 663; (c) Bignan, G. C.; Connolly, P. J.; Middleton, S. A. *Expert Opin. Ther. Pat.* **2005**, *15*, 357; (d) Chiou, L. C.; Liao, Y. Y.; Fan, P. C.; Kuo, P. H.; Wang, C. H.; Riemer, C.; Prinssen, E. P. *Curr. Drug Targets* **2007**, *8*, 117.
3. (a) Mollereau, C.; Parmentier, M.; Mailleux, P.; Butour, J.; Moisand, C.; Chalon, P.; Caput, D.; Vassart, G.; Meunier, J.-C. *FEBS Lett.* **1994**, *341*, 33; (b) Fukuda, K.; Kato, S.; Mori, K.; Nishi, M.; Takeshima, H.; Iwabe, N.; Miyata, T.; Houtani, T.; Sugimoto, T. *FEBS Lett.* **1994**, *343*, 42; (c) Chen, Y.; Fan, Y.; Liu, J.; Mestek, A.; Tian, M.; Kozak, C. A.; Yu, L. *FEBS Lett.* **1994**, *347*, 279.
4. (a) Meunier, J.-C.; Mollereau, C.; Toll, L.; Suaudeau, C.; Moisand, C.; Alvinerie, P.; Butour, J.-L.; Guillemot, J.-C.; Ferrara, P.; Monsarrat, B.; Mazargil, H.; Vassart, G.; Parmentier, M.; Costentin, J. *Nature* **1995**, *377*, 532; (b) Reinscheid, R. K.; Nothacher, H.-P.; Bourson, A.; Ardati, A.; Henningsen, R. A.; Bunzow, J. R.; Grady, D. K.; Langen, H.; Monsma, F. J., Jr.; Civelli, O. *Science* **1995**, 270.
5. Meunier, J.-C. *Eur. J. Pharmacol.* **1997**, *340*, 1.
6. (a) Bigoni, R.; Giuliani, S.; Calo', G.; Rizzi, A.; Guerrini, R.; Salvadori, S.; Regoli, D.; Maggi, C. A. *Naunyn Schmiedeberg's Arch. Pharmacol.* **1999**, *359*, 160; (b) Mollereau, C.; Mouledous, L. *Peptides* **2000**, *21*, 907.
7. (a) Mogil, J. S.; Grisel, J. E.; Reinscheid, R. K.; Civelli, O.; Belknap, J. K.; Grandy, D. K. *Neuroscience* **1996**, *75*, 333; (b) Calo', G.; Guerrini, R.; Rozzo, A.; Salvadori, S.; Regoli, D. *Br. J. Pharmacol.* **2000**, *129*, 1261; (c) Calo', G.; Rizzi, A.; Bigoni, R.; Guerrini, R.; Salvadori, S.; Regoli, D. *Clin. Exp. Pharmacol. Physiol.* **2002**, *29*, 223.
8. Ueda, H.; Yamaguchi, T.; Tokuyama, S.; Inoue, M.; Nishi, M.; Takeshima, H. *Neurosci. Lett.* **1997**, *237*, 136.
9. Morari, M.; Marti, M. WO 2005/004896 A1, 2005.

10. Kawamoto, H.; Ozaki, S.; Itoh, Y.; Miyaji, M.; Arai, S.; Nakashima, H.; Kato, T.; Ohta, H.; Iwasawa, Y. *J. Med. Chem.* **1999**, *42*, 5061.
11. (a) Chen, Z.; Goehring, R. R.; Valenzano, K. J.; Kyle, D. J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1347; (b) Trapella, C.; Guerrini, R.; Piccagli, L.; Calo', G.; Carra', G.; Spagnolo, B.; Rubini, S.; Fanton, G.; Hebbes, C.; McDonald, J.; Lambert, D. G.; Regoli, D.; Salvadori, S. *Bioorg. Med. Chem.* **2006**, *14*, 692.
12. (a) Okamoto, O.; Kobayashi, K.; Kawamoto, H.; Ito, S.; Satoh, A.; Kato, T.; Yamamoto, I.; Mizutani, S.; Hashimoto, M.; Shimizu, A.; Sakoh, H.; Nagatomi, Y.; Iwasawa, Y.; Takahashi, H.; Ishii, Y.; Ozaki, S.; Ohta, H. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3278; (b) Okamoto, O.; Kobayashi, K.; Kawamoto, H.; Ito, S.; Yoshizumi, T.; Yamamoto, I.; Hashimoto, M.; Shimizu, A.; Takahashi, H.; Ishii, Y.; Ozaki, S.; Ohta, H. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3282.
13. (a) Zaveri, N. T.; Jiang, F.; Olsen, C. M.; Deschamps, J. R.; Parrish, D.; Polgar, W.; Toll, L. *J. Med. Chem.* **2004**, *47*, 2973; (b) Bignan, G. C.; Battista, K.; Connolly, P. J.; Orsini, M. J.; Liu, J.; Middleton, S. A.; Reitz, A. B. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5022.
14. a Arai, T.; Nishikimi, Y.; Imamura, S.; Kamiyama, K.; Kobayashi, M. WO 02/26714 A1, 2002.; (b) Goto, Y.; Arai-Otsuki, S.; Tachibana, Y.; Ichikawa, D.; Ozaki, S.; Takahashi, H.; Iwasawa, Y.; Okamoto, O.; Okuda, S.; Ohta, H.; Sagara, T. *J. Med. Chem.* **2006**, *49*, 847; (c) Yoshizumi, T.; Miyazoe, H.; Ito, H.; Tsujita, T.; Takahashi, H.; Asai, M.; Ozaki, S.; Ohta, H.; Okamoto, O. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3778.
15. (a) Zaratin, P. F.; Petrone, G.; Sbacchi, M.; Garnier, M.; Fossati, C.; Petrillo, P.; Ronzoni, S.; Giardina, G. A. M.; Scheideler, M. A. *J. Pharmacol. Exp. Ther.* **2004**, *308*, 454; (b) Yoshizumi, T.; Takahashi, H.; Miyazoe, H.; Sugimoto, Y.; Tsujita, T.; Kato, T.; Ito, H.; Kawamoto, H.; Hirayama, M.; Ichikawa, D.; Azuma-Kanoh, T.; Ozaki, S.; Shibata, Y.; Tani, T.; Chiba, M.; Ishii, Y.; Okuda, S.; Tadano, K.; Fukuroda, T.; Okamoto, O.; Ohta, H. *J. Med. Chem.* **2008**, *51*, 4021.
16. (a) Shinkai, H.; Ito, T.; Iida, T.; Kitao, Y.; Yamada, H.; Uchida, I. *J. Med. Chem.* **2000**, *43*, 4667; (b) Sestili, I.; Borioni, A.; Mustazza, C.; Rodomonte, A.; Turchetto, L.; Sbraccia, M.; Riitano, D.; Giudice, M. R. D. *Eur. J. Med. Chem.* **2004**, *39*, 1047.
17. Sugimoto, Y.; Shimizu, A.; Kato, T.; Satoh, A.; Ozaki, S.; Ohta, H.; Okamoto, O. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3569.
18. Synthesized from (1*R*,4*S*)-(+)-2-cyclopentene-1,4-diol 1-acetate. For details, see the following patent: Okamoto, O.; Kobayashi, K.; Ito, H.; Tsujita, T.; Ozaki, S.; Ohta, H. WO 2007/037513 A1, 2007.
19. [^{35}S]GTPγS binding assay was conducted as described previously Ozaki, S.; Kawamoto, H.; Itoh, Y.; Miyaji, M.; Azuma, T.; Ichikawa, D.; Nambu, H.; Iguchi, T.; Iwasawa, Y.; Ohta, H. *Eur. J. Pharmacol.* **2000**, *402*, 45.
20. Butcher, J. W.; Claremon, D. A.; Connolly, T. M.; Dean, D. C.; Karczewski, J.; Koblan, K. S.; Kostura, M. J.; Liverton, N. J.; Melillo, D. G. WO 02/05860 A1, 2002.
21. The synthesis of [^{35}S]MK-499 has been reported by C. E. Raab et al. Raab, C. E.; Butcher, J. W.; Connolly, T. M.; Karczewski, J.; Yu, N. X.; Staskiewicz, S. J.; Liverton, N.; Dean, D. C.; Melillo, D. G. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1692.
22. P-gp efflux is determined by a transcellular transport study using MDR1 cells. For details, see the following literatures: (a) Hochman, J. H.; Pudvah, Q.; Yamazaki, M.; Tang, C.; Lin, J. H.; Prueksaritanont, T. *Pharm. Res.* **2004**, *21*, 1686; (b) Hochman, J. H.; Yamazaki, M.; Ohe, T.; Lin, J. H. *Curr. Drug Metab.* **2002**, *3*, 257; (c) Yamazaki, M.; Neway, W. E.; Ohe, T.; Chen, I.-W.; Rowe, J. F.; Hochman, J. H.; Chiba, M.; Lin, J. H. *Pharmacol. Exp. Ther.* **2001**, *296*, 723.
23. The preferable effect of F atom on cyclopentylamine has been already reported. For details, see Ref. 1.
24. Human and rat microsomal stabilities were determined by percentage parent compound (1 μM) remaining after 30 min incubation at 37 °C with human/rat liver microsomes (0.25 mg protein/ml).
25. Recently, AstraZeneca's researchers have been reported that lipophilicity is a significant contributor to hERG potency and is of similar importance to ionization class by conducting in-house dataset analysis: Waring, M. J.; Johnstone, C. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1759.