Bioorganic & Medicinal Chemistry Letters 19 (2009) 3100–3103

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Optimization of benzimidazole series as opioid receptor-like 1 (ORL1) antagonists: SAR study directed toward improvement of selectivity over hERG activity

Kensuke Kobayashi, Tetsuya Kato, Izumi Yamamoto, Atsushi Shimizu, Sayaka Mizutani, Masanori Asai, Hiroshi Kawamoto, Satoru Ito, Takashi Yoshizumi, Mioko Hirayama, Satoshi Ozaki, Hisashi Ohta, Osamu Okamoto *

Tsukuba Research Institute, Banyu Pharmaceutical Co., Ltd, Okubo-3, Tsukuba, Ibaraki 300-2611, Japan

ARTICLE INFO

Article history:
Received 4 February 2009
Revised 27 March 2009
Accepted 1 April 2009
Available online 11 April 2009

Keywords:
ORL1 antagonist
Nociceptin/orphanin FQ
Benzimidazole
hERG channel

ABSTRACT

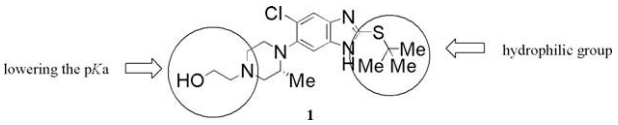
A structure–activity relationship (SAR) study on the benzimidazole series of opioid receptor-like 1 (ORL1) antagonists related to **1** is described. Optimization of **1** by introduction of a hydrophilic substituent into the thioether part resulted in identification of potent ORL1 antagonists with high selectivity over binding affinity for hERG and other opioid receptors.

Opioid receptor-like 1 (ORL1), was discovered in 1994 based on its high amino acid sequence homology to the classical opioid receptors.[1] Despite this homology, this receptor was found not to bind classical opioids with appreciable affinity. Soon after, its endogenous agonist, a 17-amino acid peptide known as nociceptin or orphanin FQ (NC/OFQ), was identified.[2] Subsequently, a number of reports have demonstrated the possible involvement of the NC/OFQ-ORL1 system in pain regulation,[3] morphine tolerance,[4] learning and memory,[5–7] food intake,[8] anxiety,[9] the cardiovascular system,[10,11] locomotor activity,[12] etc.[13] To date, however, the development of non-peptidic ORL1 antagonists with good selectivity towards opioid receptors has resulted in only a few classes[14–16]: benzimidazolyl piperidines,[17–19] 4-aminoquinolines,[20] and spiropiperidines and 4-aryl piperidines.[21–28] The biological role of the ORL1 receptor and therapeutic roles for ORL1 antagonists will be established through the development of drug-like molecules with good bioavailability and good brain penetration.

In a previous communication,[29] we reported the discovery of orally active and brain-penetrable ORL1 antagonist **1**. However, compound **1** showed relatively potent human ether-a-go-go related gene (hERG) K^+ channel binding affinity (IC_{50} = 1600 nM). Therefore, our efforts focused on removal of hERG inhibitory activity. Herein, we report an extensive structure–activity relationship (SAR) investigation on the benzimidazole series to overcome this issue. Our strategy to attenuate hERG channel binding in compounds related to **1** consisted of reducing the basicity of the piperazine nitrogen[30] and introducing a hydrophilic group to the thioether part, as shown Figure 1.

The synthesis of benzimidazole ORL1 antagonists reported herein is outlined in Scheme 1–3.

For SAR studies on the basicity of the piperazine ring, compounds **5a–f** were prepared according in Scheme 1. A chiral nitroaniline **2**[29] was reduced with Fe followed by condensation with carbon disulfide gave a benzimidazole 2-thione **3**. Coupling **3** with t-butyl alcohol and deprotection of the Boc group on the piperazine ring in TFA provided N-unsubstituted piperazine intermediate **4**. Next, **4** was converted to compounds **5a–f** by reductive amination with the corresponding aldehyde, acylation with the related acid anhydride followed by reduction with borane-methyl sulfide complex (BMS), or alkylation with bromoacetonitrile or 3-hydroxypropionitrile, respectively.

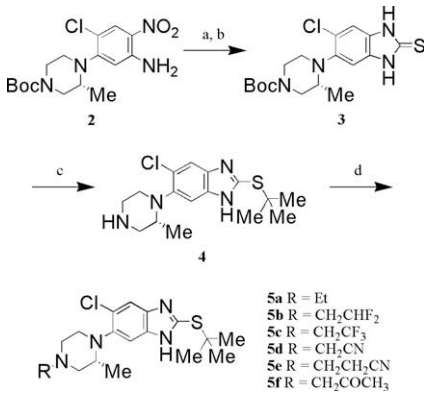


1

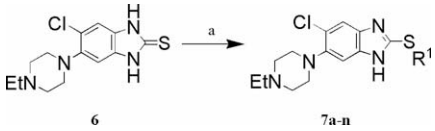
Figure 1. Strategy to improve the selectivity over the hERG binding affinity.

* Corresponding author. Tel.: +81 29 877 2000; fax: +81 29 877 2029.
E-mail address: osamu_okamoto@merck.com (O. Okamoto).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.04.022



Scheme 1. Reagents and conditions: (a) Fe, NH_4Cl, THF–MeOH–H_2O, reflux; (b) CS_2, aq NaOH, EtOH, 80 °C, 92% (2 steps); (c) *t*-BuOH, TFA, rt, 94%; (d) **5a**, **5f**: MeCHO or AcCHO, $Zn(BH_3CN)_2$, MeOH, 70% (for **5a**), 26% (for **5f**); **5b, 5c**: $(CF_2HCO)_2O$ or TFAA, NEt_3, THF; then BMS, THF, 23% (for **5b**), 23% (for **5c**); **5d**: $BrCH_2CN$, K_2CO_3, DMF, 39%; **5e**: $HOCH_2CH_2CN$, DIAD, PPh_3, THF, 19%.



Scheme 2. Reagents and conditions: (a) tertiary alcohols, TFA, rt-80 °C, then separation by Chiral HPLC (for **7d**–**n**).

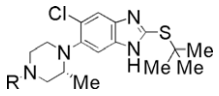
Compounds **7a–n**, which have hydrophilic functionality in the thioether part, were prepared according to Scheme 2. Analogues were derived from key intermediate **6**[31] via a coupling reaction with the corresponding tertiary alcohol in TFA. The stereoisomers of cyclohexane or cyclopentane derivatives (**7d–n**) were separated using an appropriate chiral HPLC.

Analogues **7o–q** were prepared according to Scheme 3; cyclization of ester **8**, which was prepared by the procedure described in Scheme 2, with N-hydroxyacetamidine gave 1,2,4-oxadiazole derivative **7o**. Preparation of hydrazide from **8** with hydrazine hydrate followed by cyclization with the corresponding ortho ester gave 1,3,4-oxadiazole derivatives **7p** and **7q**.

Analogues were tested for their inhibitory effects on ligands to the human ORL1 receptor, and on GTPγS binding to proteins using membrane fractions of CHO cells expressing ORL1. Binding affinities for ORL1 were determined by displacement of [^{125}I]Tyr^{14}-NC/OFQ, and agonist/antagonist activities were measured by the [^{35}S]GTPγS binding method.[32] Binding affinity to the hERG K^+ channel was measured by displacement of [^{35}S]-radiolabeled MK499 in membranes derived from HEK 293 cells stably transfected with the hERG gene and expressing the I_{Kr} channel protein.[33,34]

First, the effects of substituents at the piperazine nitrogen were investigated. In the previous SAR study,[31] it was found that introduction of bulky substituents at the piperizine nitrogen in our benzimidazole class decreased the binding affinity for ORL1. *N*-Hydroxyethyl and *N*-ethyl analogues showed potent binding affin-

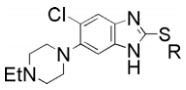
Table 1
Binding affinity and functional activity to ORL1, binding affinity to hERG, and calculated pK_a of **5a–f**

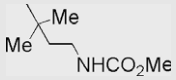
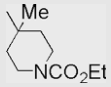
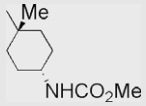
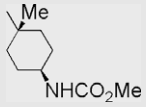
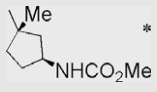


Compd	R	ORLl binding[a] IC_{50} (nM)	ORL1 antagonism[a] IC_{50} (nM)	hERG binding IC_{50} (nM)	pK_a[b]
1	Hydroxyethyl	2.6	0.65	1600	7.24
5a	Et	9.2	2.9	990	7.74
5b	CH_2CHF_2	23		>10,000	4.64
5c	CH_2CF_3	630			3.62
5d	CH_2CN	61		>10,000	2.48
5e	CH_2CH_2CN	27	6.4	>10,000	4.97
5f	CH_2COCH_3*	38	41	>10,000	4.04

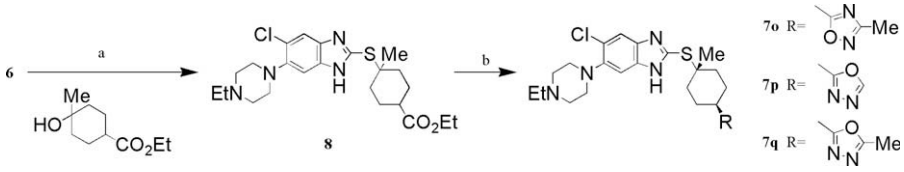
* Racemate.
[a] $n = 1$ (Ref. 35).

Table 2
Binding affinity and functional activity to ORL1 and binding affinity to hERG of **7a–f**



Compd	R	Binding[a] IC_{50} (nM)	Antagonism[a] IC_{50} (nM)	hERG binding IC_{50} (nM)
7a		22		
7b		21	8.6	
7c		4.3	1.1	3900
7d		9.5	2.1	1500
7e		2.4	0.72	3500
7f		16	46	2000

* Racemate.
[a] $n = 1$ (Ref. 35).



Scheme 3. Reagents and conditions: (a) TFA, 80 °C; (b) **7o**: *N*-hydroxyacetamidine, NaH, THF, reflux, 11%; **7p, 7q**: hydrazine hydrate, EtOH, reflux, 83%, $HC(OEt)_3$ or $MeC(OEt)_3$, reflux, 33% (for **7p**), 45% (for **7q**).

ity. Therefore, we selected relatively small substituents with the ability to lower the pK_a of the basic nitrogen for our initial approach (Table 1).[35] Introduction of a difluoroethyl group resulted in a significant decrease in hERG activity; however, the binding affinity for ORL1 also decreased by 10-fold (compound **5b**). The trifluoroethyl analogue **5c**, whose calculated pK_a is lower than **5b**, showed very low ORL1 affinity. The other derivatives (**5d–f**) also showed>10-fold decreased intrinsic potency, despite their attenuated hERG binding. As extensive precedent for the circumvention of hERG activity, the reduction of basicity of the molecule worked well in this series, but this approach impaired ORL1 binding affinity.

We then shifted our attention to another approach, the introduction of a hydrophilic substituent onto the thioether portion. We selected *N*-ethyl piperazine as a template, as shown in Table 2.

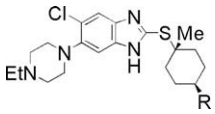
Compound **7b** with a carbamate group at the acyclic thioether part was equipotent to a *t*-butyl analogue **7a**. Encouraged by the observation that introduction of the hydrophilic substituent has little impact on the intrinsic potency, further exploration of the thioether part was performed. As a result, piperidine analogue **7c** showed increased ORL1 activity. Moreover, when a carbamate group was installed at the 4-position of the 1-methylcyclohexylthioether, trans-isomer **7e** exhibited significantly enhanced binding affinity and antagonistic activity for ORL1. On the other hand, ring contraction of the cyclohexane ring to a cyclopentane ring resulted in a decrease in ORL1 affinity (**7f**).

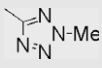
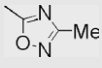
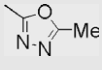
By modifying the thioether substituent, ORL1 activity was enhanced. Interestingly, however, the hERG inhibitory activity remained high. We next focused on optimization of the 4-substituent on the 1-methylcyclohexane ring. Table 3 summarizes the results from SAR studies on the potency of ORL1 and hERG binding affinity. Replacement of the carbamate group with the other nitrogen substituents, such as amide or sulfonamide (**7g–j**), resulted in negligible hERG inhibitory activity (IC_{50} > 10,000 nM) while retaining ORL1 potency. This result can be attributed to the increased hydrophilicity of **7g–j** (log $D_{7.4}$ = 2.5, 2.7, 2.5 and 2.9, respectively) in comparison to **7e** (log $D_{7.4}$ = 3.4).[30] Analogues with carbamoyl (**7k**) and sulfone (**7l**) also showed good selectivity over hERG binding. We then incorporated heterocycles into this position. Derivatives having five-membered heterocycles (**7m–q**) exhibited good ORL1 antagonistic activity, with 2-methyl-1,3,4-oxadiazole **7q** showing negligible binding affinity for hERG K^+ channels.

Representative compound **7h**[36] from this series was evaluated for selectivity over other opioid receptors (Table 4). This compound had good selectivity over μ and κ receptors, similarly to lead compound **1**.

In conclusion, we optimized the benzimidazole lead **1** for the removal of hERG activity. The results of the SAR study led to the identification of potent ORL1 antagonists, which exhibited high selectivity over binding affinity for hERG. In addition, the selected compound **7h** showed good selectivity over μ and κ receptors. Further SAR studies of this series are currently underway.

Table 3
Effects of 4-substitution on the 1-methylcyclohexane ring



Compd	R	ORLl binding[a] IC_{50} (nM)	Antagonism[a] IC_{50} (nM)	hERG binding IC_{50} (nM)	Log $D_{7.4}$[b]
7e	$NHCO_2Me$	2.4	0.72	3500	3.4
7g	NHAc	1.9	1.1	>10,000	2.5
7h	N(Me)Ac	0.81	1.3	>10,000	2.7
7i	$NHSO_2Me$	2.1	1.8	>10,000	2.5
7j		1.4	0.55	>10,000	2.9
7k	CONHMe	1.0	1.1	>10,000	
71	SO_2Me	6.9	4.0	>10,000	
7m		2.2	0.90	4400	3.1
7n		7.4	3.7	6300	2.3
7o		1.7	0.91	380	3.8
7p		1.1	0.59	3400	
7q		1.8	7.1	>10,000	

[a] n = 1 (Ref. 35).
[b] Measured by shake-flask method.

Table 4
Off-target activities (μ-, κ-opipid receptors) of **1** and **7h**

Compd	Binding IC_{50} (nM)		
	ORL1	μ[a]	κ[a]
1	2.6	1900	120
7h	0.81	840	160

[a] Displacement of a [^{3}H]diprenorphin (μ) and [^{3}H]U69593 (κ) binding to CHO cells stably expressing cloned human μ-, and κ-opioid receptors, respectively.

Acknowledgments

We would like to acknowledge the contributions of the following scientists to this work: T. Azuma-Kanoh, H. Nambu, N. Sakai, T. Inoue, D. Ichikawa, S. Okuda, N. Ami, M. Fukushima and M. Nishino.

References and notes

1. Meunier, J. C.; Mollereau, C.; Toll, L.; Suaudeau, C.; Moisand, C.; Alvinerie, P.; Butour, J. L.; Guillemot, J. C.; Ferrara, P.; Monsarrat, B.; Mazarguil, H.; Vassart, G.; Parmentier, M.; Costentin, J. *Nature* **1995**, *377*, 532.
2. Reinscheid, R. K.; Nothacker, H. P.; Bourson, A.; Ardati, A.; Henningsen, R. A.; Bunzow, J. R.; Grandy, D. K.; Langen, H.; Monsma, F. J.; Civelli, O. *Science* **1995**, *270*, 792.
3. Mogil, J. S.; Grisel, J. E.; Reinscheid, K. K.; Civelli, O.; Belknap, J. K.; Grandy, D. K. *Neuroscience* **1996**, *75*, 333.
4. Ueda, H.; Yamaguchi, T.; Tokuyama, S.; Inoue, M.; Nishi, M.; Takeshima, H. *Neurosci. Lett.* **1997**, *237*, 136.
5. Manabe, T.; Noda, Y.; Mamiya, T.; Katagiri, H.; Houtani, T.; Nishi, M.; Noda, T.; Takahashi, T.; Sugimoto, T.; Nabeshima, T.; Takeshima, H. *Nature* **1998**, *394*, 577.
6. Sandin, J.; Georgieva, J.; Schott, P. A.; Ogren, S. O.; Terenius, L. *Eur. J. Neurosci.* **1997**, *9*, 194.
7. Yu, T. P.; Fein, J.; Phan, T.; Evans, C. J.; Xie, C. W. *Hippocampus* **1997**, *7*, 88.
8. Pomonis, J. D.; Billington, C. J.; Levine, A. S. *Neuroreport* **1996**, *8*, 369.

9. Jenck, F.; Moreau, J. L.; Martin, J. R.; Kilpatrick, G. J.; Reinscheid, R. K.; Monsma, F. J.; Nothacker, H. P.; Civelli, O. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 14854.
10. Champion, H. C.; Kadowitz, P. J. *Life Sci.* **1997**, *60*, L241.
11. Gumusel, B.; Hao, Q. Z.; Hyman, A.; Chang, J. K.; Kapusta, D. R.; Lippton, H. *Life Sci.* **1997**, *60*, L141.
12. Florin, S.; Suaudeau, C.; Meunier, J. C.; Costentin, J. *Eur. J. Pharmacol.* **1996**, *317*, 9.
13. Champion, H. C.; Wang, R.; Hellstrom, W. J. G.; Kadowitz, P. J. *Am. J. Physiol.* **1997**, *36*, E214.
14. Ronzoni, S.; Peretto, L.; Giardina, G. A. M. *Exp. Opin. Ther. Patents* **2001**, *11*, 525.
15. Zaveri, N. *Life Sci.* **2003**, *73*, 663.
16. Bignan, G. C.; Connolly, P. J.; Middleton, S. A. *Exp. Opin. Ther. Patents* **2005**, *15*, 357.
17. Kyle, D. WO 2001039775, **2001**; *Chem. Abstr.* **2001**, *135*, 33477.
18. Kawamoto, H.; Ozaki, S.; Itoh, Y.; Miyaji, M.; Arai, S.; Nakashima, H.; Kato, T.; Ohta, H.; Iwasawa, Y. *J. Med. Chem.* **1999**, *42*, 5061.
19. Ito, F.; Kondo, H.; Noguchi, H.; Ohashi, Y.; Yamagishi, T. WO 9936421, **1999**; *Chem. Abstr.* **1999**, *131*, 102281.
20. Shinkai, H.; Ito, T.; Iida, T.; Kitao, Y.; Yamada, H.; Uchida, I. *J. Med. Chem.* **2000**, *43*, 4667.
21. Arai, T.; Nishikimi, Y.; Imamura, S.; Kamiyama, K.; Kobayashi, M. WO 2002026714, **2002**; *Chem. Abstr.* **2002**, *136*, 294733.
22. 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.
23. Ito, F.; Koike, H.; Sudo, M.; Yamagishi, T.; Ando, K. WO 2003000677, **2003**; *Chem. Abstr.* **2003**, *138*, 73181.
24. Kolczewski, S.; Adam, G.; Cesura, A. M.; Jenck, F.; Hennig, M.; Oberhauser, T.; Poli, S. M.; Rossler, F.; Rover, S.; Wichmann, J.; Dautzenberg, F. M. *J. Med. Chem.* **2003**, *46*, 255.
25. Rover, S.; Adam, G.; Cesura, A. M.; Galley, G.; Jenck, F.; Monsma, F. J.; Wichmann, J.; Dautzenberg, F. M. *J. Med. Chem.* **2000**, *43*, 1329.
26. 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.
27. 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.
28. 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.
29. 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.
30. Jamieson, C.; Moir, E. M.; Rankovic, Z.; Wishart, G. *J. Med. Chem.* **2006**, *49*, 5029.
31. 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.
32. 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.
33. Lynch, J. J.; Wallace, A. A.; Stupienski, R. F.; Baskin, E. P.; Beare, C. M.; Appleby, S. D.; Salata, J. J.; Jurkiewicz, N. K.; Sanguinetti, M. C.; Stein, R. B.; Gehret, J. R.; Kothstein, T.; Claremon, D. A.; Elliott, J. M.; Butcher, J. W.; Remy, D. C.; Baldwin, J. J. *J. Pharmacol. Exp. Ther.* **1994**, *269*, 541.
34. Butcher, J. W. WO 2002005860, **2002**; *Chem. Abstr.* **2002**, *136*, 112612.
35. J-113,397 (Ref. 18) was used as an internal control across all assay plates for data validation.
36. *Analytical data*: **7h** HCl salt: ^{1}H NMR (400 MHz, CD_3OD) δ [ppm] 1.43 (3H, t, J = 7.2 Hz), 1.57 (3H, s), 1.61–2.13 (11H, m), 2.80 (1H, s), 2.91 (2H, s), 3.20–3.40 (6H, m), 3.58–4.38 (5H, m), 7.60–7.62 (1H, m), 7.94 (1H, s). MS (ESI): *m/z* 464.2 $[M+H]^+$.