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Novel ORL1-selective antagonists with oral bioavailability and brain penetrability

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Abstract—Following the discovery of 5-chloro-6-[piperazin-1-yl]-1*H*-benzimidazole as a novel pharmacophore for potent and selective ORL1 antagonist activity, optimization of this new lead by introduction of a methyl substitution on the piperazine ring resulted in a highly potent and selective, orally available, and brain penetrable ORL1 antagonist, 2-(*tert*-butylthio)-5-chloro-6-[(2*R*)-4-(2-hydroxyethyl)-2-methylpiperazin-1-yl]-1 *H*-benzimidazole. Stereochemistry of the methyl substituent on the piperazine ring to control the functional activity of other opioid receptors is also described.

In a previous communication,[1] we reported the preparation and biological evaluation of a series of 6-(piperazin-1-yl)-1*H*-benzimidazoles as nociceptin receptor antagonists. The fourth opioid receptor, opioid receptor-like 1 (ORL1), was discovered in 1994 by homology cloning.[2–5] Subsequently, its endogenous agonist, a 17-amino acid peptide termed nociceptin or orphanin FQ (NC/OFQ), was identified.[6,7] Pharmacological studies using NC/OFQ and ORL1-deficient mice showed that the NC/OFQ-ORL1 system may have important roles in the regulation of pain response,[8] morphine tolerance,[9] learning and memory,[10–12] food intake,[13] anxiety,[14] the cardiovascular system,[15,16] locomotor activity,[17] etc.[18]

These results prompted many pharmaceutical companies to identify potent and selective ORL1 agonists and antagonists. However, to elucidate the biological role of the ORL1 receptor and investigate the therapeutic potential of ORL1 antagonists, there remains a need to develop orally available and brain penetrable ORL1 antagonists.

In this communication, we report an extensive SAR investigation on a benzimidazole series to elaborate ORL1 antagonists, which exhibit enhanced affinity and improved metabolic stability. In addition, we report an interesting finding regarding the opioid κ functional activity of this class. The SAR study culminated in the identification of orally available, brain penetrable, and selective ORL1 antagonist **8**.

Benzimidazole analogs were prepared as described previously.[1] The preparation of representative analog **8** is depicted in Scheme 1.

In the previous SAR study,[1] we found that structural bulkiness around the sulfur atom significantly enhanced in vitro potency (Table 1).[19–21] However, these modifications resulted in decreased metabolic stability. Metabolic stability decreases with increasing lipophilicity of the *S*-alkyl substituent. To determine potent antagonists with good metabolic stability, we expanded the SAR study on the hitherto-unmodified region, the piperazine ring. A series of compounds with various substituted piperazines were prepared while retaining a *t*-Bu or 3-pentyl group at the thioether moiety due to the relatively good metabolic stability and in vitro potency. Table 2[19–21] summarizes the results from SAR studies. Introduction of methyl or dimethyl groups on the 3-position of the piperazine resulted in a significant decrease in metabolic stability (compounds **4** and **5**). A bridged piperazine structure, **6**, showed better metabolic stability without a significant loss of potency.

Keywords: Nociceptin/orphanin FQ; Nociceptin receptor; Opioid receptor-like 1(ORL1) antagonist.
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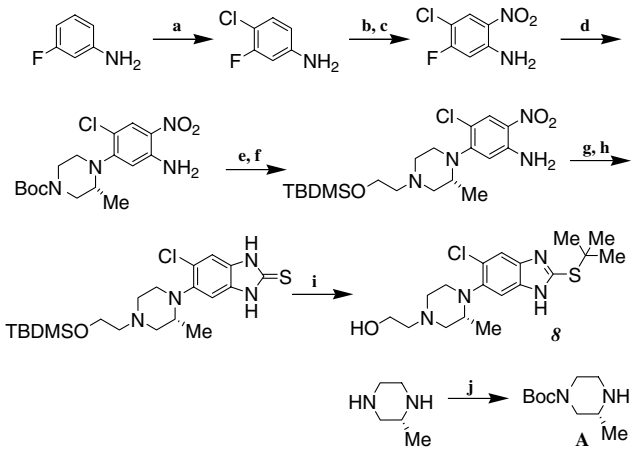


Scheme 1. Synthesis of representative derivative **8**. Reagents and conditions: (a) NCS, $CHCl_3$, 0 °C, 12 h (29%); (b) KNO_3, TFAA, rt, 12 h; (c) K_2CO_3, MeOH, rt, 0.5 h (90%, 2 steps); (d) **A**, *i*-Pr_2NEt, DMSO, 140 °C, 18 h (52%); (e) HCl, dioxane, rt, 16 h; (f) TBDMS-OCH_2CH_2CHO, $NaBH(OAc)_3$, MeOH, rt, 1 h (85%, 2 steps); (g) Fe, aq NH_4Cl, MeOH, THF, reflux, 3 h; (h) CS_2, aq NaOH, MeOH, 50 °C, 2 h (quant., 2 steps); (i) *t*-BuOH, TFA, rt, 16 h (78%); (j) Boc_2O, THF, rt, 12 h (48%).

We then shifted our attention to the introduction of a substituent on the 2-position of the piperazine ring. In this modification, we utilized the *t*-Bu group at the thioether part due to its good metabolic stability. A finding to be noted is that introduction of a methyl group at the 2-position of the piperazine ring leads to an approximately 10-fold improvement in potency and the retention of good metabolic stability. In addition, there is no difference in potency or metabolic stability between the enantiomers (Compounds **7** and **8**). Hence, we conducted further evaluation of compounds **7** and **8** and made observations with regard to selectivity against other opioids (Table 3).[19]

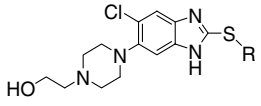
Compound **7** having an (*S*)-methyl configuration on the piperazine ring showed moderate affinity for other opioid receptors, and exerted agonistic activity at the κ and δ receptors, respectively. On the other hand, compound **8** with an (*R*)-methyl configuration showed 100-fold higher selectivity for κ receptors. It has been reported that κ agonists have adverse effects such as psychotomimetic effects and aversion.[22,23] Therefore, we selected compound **8** for further evaluation.

In a standard panel for off-target activity, compound **8** did not display significant affinity for the other 163 receptors and ion channels, indicating that compound **8** is a selective ORL1 antagonist (the receptors and channels that reached over 50% at 10 μM were the monoamine transporter (52%), potassium channel-HERG (86%), serotonin 5-HT_{2B} (52%), and sodium channel site 2 (68%)). Pharmacokinetic (PK) studies of compound **8** were carried out in mice, rats and monkeys and showed moderate PK profiles (Table 4). Despite high clearance and short half-life in rodents, relatively low clearance and good plasma half-life were observed in monkeys.

With regard to brain penetrability in ICR mice, the brain–plasma ratio was 0.47, which represents modest penetrability; at 60 min after po administration (30 mg/kg) of **8**, brain concentration was 1.04 nmol/kg brain and plasma level was 2.38 μM. Further study revealed that compound **8** was subject to mouse P-gp mediated efflux (mdr1a directional transport ratio (B/A)/(A/B) is 6.2; passive permeability is 25.3×10^{-6} cm/s). It is likely that the limited brain penetrability in mice is due to susceptibility to P-gp efflux. On the other hand, compound **8** was not a substrate for human P-gp efflux ((B/A)/(A/B) is 2.2). Hence, compound **8** should be a good brain penetrant in humans.

In conclusion, we have optimized a novel benzimidazole structure in a series of potent ORL1 antagonists. The result of the SAR study in this class led to the identification of antagonist **8**, which exhibited high affinity for the human ORL1 receptor and a selectivity over 100-fold higher than other opioid receptors. Compound **8** presents fair pharmacokinetic properties in mice, rats, and monkeys and moderate brain penetrability in mice.

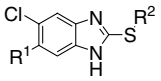
Table 1. Binding affinity, functional activity, and metabolic stability of benzimidazole analogs

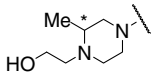
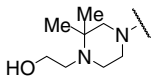
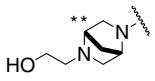
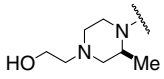
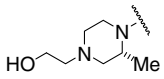


Compound	R	Binding[a] IC_{50} (nM)	Antagonism[a] IC_{50} (nM)	HM stability[b] % remaining	*c*log *P*
1		9.7	3.3	51	3.94
2		17	21	71	3.19
3		1.2	0.66	36	4.16

[a] See Ref. 19 for detailed description. $n = 1$ (Ref. 21).
[b] See Ref. 20 for detailed description.

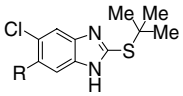
Table 2. Binding affinity, functional activity, and metabolic stability of benzimidazole analogs modified at the piperazine ring

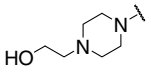
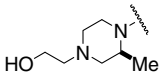
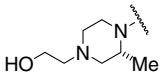


Compound	R^1	R^2	Binding[a] IC_{50} (nM)	Antagonism[a] IC_{50} (nM)	HM stability[b] % remaining
4			8.0	22	17
5			73	59	7
6			18	27	63
7			1.1	2.2	70
8			2.6	0.65	87

*Racemate.
**Chiral isomer.
[a] See Ref. 19 for detailed description. $n = 1$ (Ref. 21).
[b] See Ref. 20 for detailed description.

Table 3. Effects of Me substitution on selectivity over other opioid receptors



Compound	R	Binding IC_{50}[a] (nM)				Functional profiles in [^{35}S]GTPγS binding assay[a]			
		ORL1	μ	κ	δ	ORL1	μ	κ	δ
2		17	4400	120	>1000	IC_{50} 21 nM	NT	NT	NT
7		1.1	67	5.3	238	IC_{50} 2.2 nM	IC_{50} 25 nM	EC_{50} 18 nM E_{max} 80%	EC_{50} 14 nM E_{max} 94%
8		2.6	1900	120	>1000	IC_{50} 0.65 nM	IC_{50} 97 nM	IC_{50} 180 nM	IC_{50} 1200 nM

[a] See Ref. 19 for detailed description. $n = 1$.

Table 4. Pharmocokinetics of compound **8** in mouse, rat, and monkey

	F (%)	$T_{1/2}$ (h)	Cl (ml/min/kg)
ICR mouse	33	0.81	121
SD rat	29	0.92	81
Rhesus monkey	37	3.1	20

Oral dose 3 mg/kg and IV dose 1 mg/kg.

The overall profiles of **8**[24] are complementary and appropriate for pharmacological evaluation of ORL1 antagonism. Further pharmacological studies of this antagonist are currently underway.

Acknowledgments

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19. Binding affinities to 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. Cross reactivity to other opioid receptors was also tested. Affinities and agonist/antagonist activities for human μ-, κ-, and δ-receptors were assayed similarly to ORL1 using membrane fractions of CHO cells expressed in each receptor (Affinities were measured by displacement of a [^{3}H]diprenorphin for μ, [^{3}H]U-69593 for κ and [^{3}H]naltrindole for δ binding, respectively. Antagonist activities were measured by DAMGO for μ, U-69593 for κ and DADLE for δ, respectively).
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24. Analytical data for compound **8**. ^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm] 0.91 (3H, d, J = 6.4 Hz), 1.50 (9H, s), 2.25–2.96 (8H, m), 3.14–3.22 (1H, m), 3.35–3.45 (1H, m), 3.69 (2H, t, J = 5.2 Hz), 7.20–7.90 (2H, m), 9.50 (1H, br s). MS (ESI+): m/z 383.2 $(M+H)^+$.