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Bioorganic & Medicinal Chemistry Letters 18 (2008) 3278–3281
Bioorganic & Medicinal Chemistry Letters

Identification of novel benzimidazole series of potent and selective ORL1 antagonists

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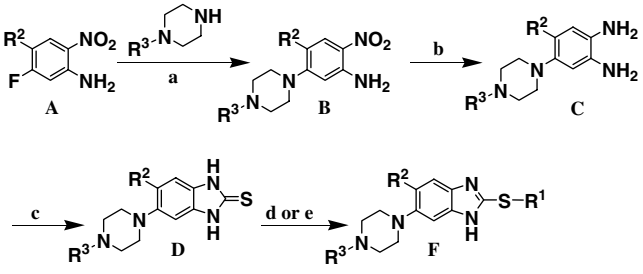
Received 12 December 2007; revised 19 March 2008; accepted 19 April 2008
Available online 25 April 2008

Abstract—Structure–activity studies on benzimidazole lead **1** obtained from library screening led to the discovery of potent and selective ORL1 antagonist **28**, 5-chloro-2-[(1-ethyl-1-methylpropyl)thio]-6-[4-(2-hydroxyethyl)piperazin-1-yl]-1*H*-benzimidazole, which is structurally distinct from conventional non-peptide antagonists known to date.

Opioid receptor like 1 (ORL1) is a G-protein-coupled receptor with a high degree of amino acid sequence homology to the classical opioid receptors. Since the identification of nociceptin (orphanin FQ or NC/OFQ) as the endogenous ligand of ORL1,[1,2] a number of reports have demonstrated the possible involvement of the NC/OFQ-ORL1 system in pain regulation,[3] cognition,[4–6] anxiety[7] and cardiovascular function.[8,9] Until now, few classes of non-peptidic ORL1 antagonists with fairly good selectivity towards opioid receptors have been developed,[10–12] namely benzimidazolyl piperidines,[13,14] spiropiperidines[15] and 4-aminoquinolines.[16] 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. Here, we report the discovery and SAR study of a novel series of selective ORL1 antagonists. A sample collection screening directed at identifying new and structurally diverse leads provided 6-(piperazin-1-yl) benzimidazole **1** as a sub-micromolar receptor antagonist. Modification of the 6-piperazinyl benzimidazole lead and their effects on in vitro measures of ORL1 antagonism are also addressed.

6-(Piperazin-1-yl)-benzimidazole derivatives (F) were synthesized according to the general procedure illustrated in Scheme 1. N-substituted piperazines were condensed with 5-fluoro-2-nitroaniline (A) in the presence of *N*,*N*-diisopropylethylamine at 140 °C to afford the desired *N*-phenylpiperazines (B). Reduction of the nitro function with Fe followed by condensation with carbon disulfide gave the key intermediate benzimidazole 2-thione (D). Alkylation of D with appropriate alkyl and arylalkyl halides in the presence of potassium carbonate provided the corresponding thioether analogs (F). Compounds derived from *N*-Boc piperazine were deprotected under acidic conditions. Analogs incorporating a ter-



Scheme 1. Synthesis of 6-(piperazin-1-yl)-benzimidazole derivatives. Reagents and conditions: (a) iPr_2NEt, DMSO; (b) Fe, NH_4Cl, THF–MeOH–H_2O; (c) CS_2, 1 N NaOH, EtOH; (d) R^1-X, K_2CO_3, DMF; (e) R^1-OH, TFA.

Keywords: Nociceptin/orphanin FQ; Nociceptin receptor (NOP); Opioid receptor-like 1 (ORL1) antagonist.
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doi:10.1016/j.bmcl.2008.04.054

tiary carbon adjacent to the sulfur atom were synthesized by coupling the benzimidazole 2-thione with the corresponding tertiary alcohol in TFA at room temperature.

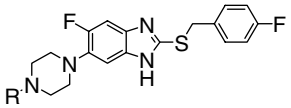
Analogs were tested for their inhibitory effects on ligand binding to the human ORL1 receptor, and on GTPγS binding to proteins using membrane fractions of CHO cells expressing ORL1. Binding affinities for ORL1 were determined by displacement of [^{125}I]Tyr^{14}-NC/OFQ, and agonist/antagonist activities were measured by the [^{35}S]GTPγS binding method.[17] Cross reactivity to other opioid receptors was also tested. Affinities for human μ-, κ- and δ-receptors were assayed similarly to ORL1 using membrane fractions of CHO cells expressing each receptor.

Structural modification was performed at three individual sites of the benzimidazole core of compound **1**. Our initial interest was to examine the effects of substituents at the piperazine nitrogen atom (Table 1).[18] It is known that all the reported small-molecule ORL1 ligands incorporating a piperidine framework have a bulky substituent at the nitrogen, such as cyclooctylmethyl, naphthylmethyl and acenaphthenyl groups, which confers high affinity for the ORL1 receptor.[10–12] In contrast to these reports, introduction of a bulky substituent at the piperazine nitrogen in our benzimidazole core resulted in a drastic loss of the binding affinity for ORL1 (**2** and **3** vs **1**). N-unsubstituted piperazine analogue **4** showed the best binding affinity. This suggested that the new series represents a different type of ORL1 antagonist.

We then directed our SAR efforts towards the modification of the thioether portion using the N-unsubstituted piperazine analogue **4** as a template (Table 2).[18] Replacement of *p*-fluorobenzylthioether with methylthioether resulted in a significant decrease in potency for ORL1 (Compound **5**); further exploration of the SAR around the *p*-fluorobenzylthioether **4** was conducted using commercially available benzyl halides for exploration of preliminary SAR (**6**–**12**). We found that 2,3-dichlorobenzyl derivative **12** appeared to be the most potent antagonist among the derivatives synthesized. It is likely that some structural bulkiness in the thioether part is essential for the antagonistic activity. Conversion of the thioether to sulfone or sulfoxide was found to markedly decrease the potency for ORL1 (**13** and **14**).

We then incorporated bulky branched alkyl groups into this region in order to examine its steric effect. The cyclohexylmethyl analogue **15** and (1-ethylpropyl)thioether analogue **16** showed good binding affinity for ORL1 but a loss in antagonist activity. Next, we focused on the effects of 5-substitution (X) of the benzimidazole ring (Table 3).[18] Replacement of the

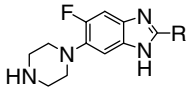
Table 1. Effects of N-substitution of the piperazine ring on binding affinity at human ORL1 receptor

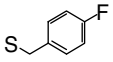
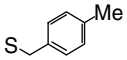
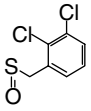
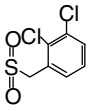


Compound	R	Binding* IC_{50} (nM)
1	Me	910
2	Benzyl	8200
3	Cyclooctylmethyl	>10000
4	H	63

* $n = 1$ (Ref. 18).

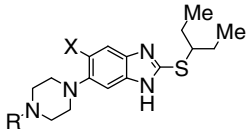
Table 2. Binding affinity and functional activity of **4** and its analogs at human ORL1 receptor



Compound	R	Binding* IC_{50} (nM)	Antagonism* IC_{50} (nM)
4		63	
5		2900	
6		140	
7		78	1700
8		180	
9		31	
10		23	380
11		55	2700
12		6.0	140
13		2900	
14		240	
15		17	930
16		4.7	690

* $n = 1$ (Ref. 18).

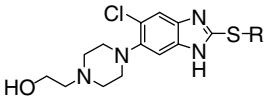
Table 3. Effects of 5-substitution of the benzimidazole ring on binding affinity and functional activity at human ORL1 receptor



Compound	R	X	Binding* IC_{50} (nM)	Antagonism* IC_{50} (nM)
16	H	F	4.7	690
17	H	H	320	
18	H	Ac	3100	
19	H	CN	15	78
20	H	Cl	2.1	9.1
21	Et	Cl	10	3.9
22	Hydroxyethyl	Cl	9.7	3.3

* $n = 1$ (Ref. 18).

Table 4. Binding affinity and functional activity of **22** and its analogs at human ORL1 receptor



Compound	R	Binding* IC_{50} (nM)	Antagonism* IC_{50} (nM)	Binding IC_{50} (nM)**		
				μ	κ	δ
22		9.7	3.3	3100	60	>1000
23		120		3100	60	NT
24		17	21	4400	120	NT
25		5.6	2.5	3500	160	NT
26		20	20	520	31	NT
27		1.8	1.8	1400	88	NT
28		1.2	0.66	2800	170	>1000

* $n = 1$ (Ref. 18).

** Displacement of [^{3}H]diprenorphin (μ), [^{3}H]U-69593 (κ and [^{3}H]naltrindole for (δ) binding in CHO cells stably expressing cloned human opioid μ-, opioid κ- and opioid δ-receptors, respectively. $n = 1$.

fluorine atom with a hydrogen (**17**) or an acetyl (**18**) resulted in a loss of potency for ORL1. Binding affinity for ORL1 was recovered with a cyano (**19**) or a chloride (**20**–**22**), resulting in excellent functional activity for ORL1 (ORL1 IC50 = 3–9 nM). The increase in potency can be explained by the torsion angle between the benzimidazole core ring and the piperazine ring produced by the bulky Cl atom or by the electronic factor of the Cl atom.

At this stage, we again examined the effects of the substituents at the piperazine nitrogen atom. Although the binding affinity of *N*-alkyl analogs (**21** and **22**) decreased slightly when compared to N–H analogue **20**, as observed in the initial modification (Table 1), their functional activity was unexpectedly enhanced. The reason for this difference was not clear. Further evaluation of this series showed better metabolic stability for the *N*-hydroxyethyl analogue when compared to the *N*-ethyl

analogue (e.g. 51% for **22** vs 31% for **21**, as measured by the percentage of parent compound remaining after 30 min of 37 °C incubation with human liver microsomes). Therefore, we selected the 2-hydroxyethyl group as the preferred substituent at the piperazine nitrogen and revisited the SAR at the thioether position (Table 4).[18]

It was found that structural hindrance around the sulfur atom significantly enhanced the potency for ORL1. This steric factor was confirmed with the introduction of a methyl group (**23** vs **24**, **25** and **26** vs **27**). Lead optimization of the benzimidazole series resulted in the identification of analogue **28**;[19] the compound had single digit nanomolar binding affinity for ORL1, excellent antagonist activity in the functional assay and very good selectivity over other opioid receptors.

In conclusion, we optimized the novel benzimidazole structure of potent ORL1 antagonists. The results of SAR study in this class led to the identification of ORL1-selective antagonist **28**, which exhibited high affinity for the human ORL1 receptor. Further SAR studies of this series are currently underway.

Acknowledgments

We 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. The authors are also grateful to Dr. J. Sakaki and Dr. N. Ohtake for critically reading the manuscript.

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18. J-113,397 (Ref. 13) was used as an internal control across all assay plates for data validation.
19. Analytical data for HCl salt of compound **28**. ^{1}H NMR(400 MHz, CD_3OD) δ ppm] 1.26 (6H, t, J = 7.2 Hz), 1.41 (3H, s), 1.65–1.85 (4H, m), 3.20–3.50 (6H, m), 3.50–3.68 (2H, m), 3.70–3.84 (2H, m), 3.90–4.00 (2H, m), 7.60 (1H, s), 7.92 (1H, s). MS (ESI+): *m/z* 397.2 $(M+H)^+$.