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Discovery of novel arylpyrazole series as potent and selective opioid receptor-like 1 (ORL1) antagonists

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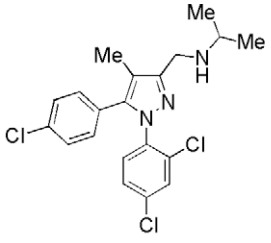
ABSTRACT

The synthesis and biological evaluation of new potent opioid receptor-like 1 antagonists are presented. A structure–activity relationship (SAR) study of arylpyrazole lead compound **1** obtained from library screening identified compound **31**, (1*S*,3*R*)-*N*-{[1-(3-chloropyridin-2-yl)-5-(5-fluoro-6-methylpyridin-3-yl)-4-methyl-1*H*-pyrazol-3-yl]methyl}-3-fluorocyclopentanamine, which exhibits high intrinsic potency and selectivity against other opioid receptors and hERG potassium channel.

Opioid receptor-like 1 (ORL1), was discovered in 1994 based on its high degree of amino acid sequence homology to the classical opioid receptors.[1] Despite this homology, it was shown that this receptor did not bind to classical opioids with appreciable affinity. Subsequently, its endogenous agonist, a 17-amino acid peptide known as nociceptin or orphanin FQ (NC/OFQ), was identified.[2] A number of reports have since demonstrated the possible involvement of the NC/OFQ-ORL1 system in pain reguration,[3] morphine tolerance,[4] learning and memory,[5–7] food intake,[8] anxiety,[9] the cardiovascular system,[10,11] locomotor activity,[12] and so on.[13]

Several small-molecule antagonists with high affinity and selectivity for ORL1 have recently been reported.[14–19] We also reported novel classes of orally active and brain penetrable ORL1 antagonists that exhibit a wide safety window with regard to adverse cardiovascular effects.[20,21] However, additional structurally diverse ORL1 antagonists are required for better understanding of the physiological roles of ORL1 receptor and the therapeutic potential of its antagonists. We report here a series of structurally novel arylpyrazole ORL1 antagonists that demonstrate high affinity for the human ORL1 receptor, while possessing desirable in vitro features.

Screening of the sample collection identified arylpyrazole derivative **1** (Fig. 1), which has a structure distinct from various published ORL1 antagonists. This derivative showed sub-micromolar ORL1 binding affinity and antagonistic activity, as well as potent human ether-a-go-go related gene (hERG) K^+ channel binding affinity. Thus, we attempted to enhance its intrinsic potency while removing its hERG inhibitory activity.



1

ORL1 binding IC_{50}	350 nM
GTPγS antagonism IC_{50}	480 nM
hERG binding IC_{50}	23 nM

Figure 1. Biological profiles of lead compound **1**.

Synthesis of arylpyrazole ORL1 antagonists followed the general procedure shown in Scheme 1. Aryl ethyl ketone **2** was coupled with 2-(1*H*-benzotriazol-1-yl)-2-oxoethyl acetate **3** to afford the desired β-diketone **4**. Treatment of **4** with the corresponding arylhydrazine in acetic acid followed by saponification gave 3-hydroxymethylpyrazole **5**. The key intermediate 3-formylpyrazole **6** was prepared via oxidation of alcohol function with MnO_2. Reductive

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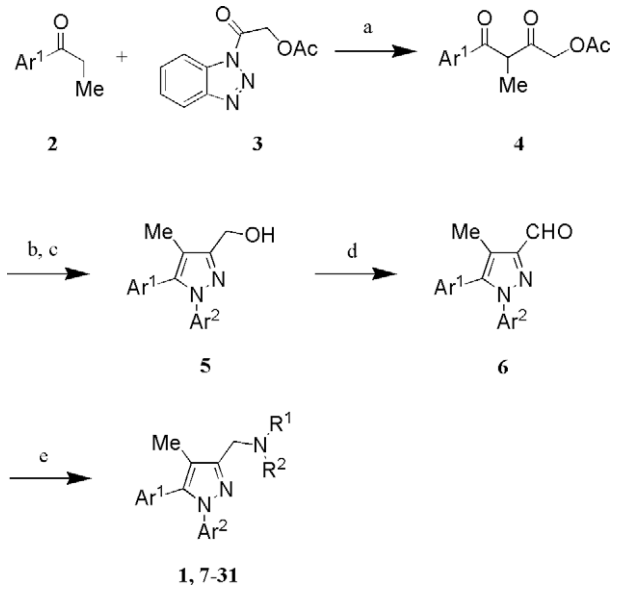


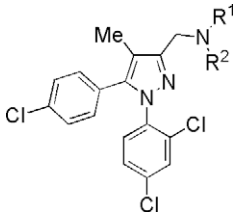
Scheme 1. Reagents and conditions: (a) LHMDS, THF, −78 °C to rt; (b) Ar^2NHNH_2, AcOH, 80 °C; (c) NaOH aq, MeOH, rt; (d) MnO_2, $CHCl_3$, rt; (e) R^1R^2NH, $Zn(BH_3CN)_2$, MeOH, rt.

amination of **6** with the related amine provided arylpyrazole derivatives **1**, **7**–**31**.

Analogues were tested for their inhibitory effects on ligands for 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.[22] Binding affinity to hERG K^+ channel was measured by the 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.[23,24]

Our first objective was to examine the effects of substituents at the pyrazolylmethylamine region. A rapid analogue synthesis was carried out in order to prepare a series of arylpyrazoles with a variety of secondary or tertiary amines. The ORL1 binding affinity of selected compounds from the approximately 40 pyrazoles prepared (data not shown) are listed in Table 1.[25] Replacement of an isopropyl (**1**) with an isobutyl (**7**) resulted in a twofold increase in binding activity; however, further prolongation of the alkyl group (**8**) showed no effect on ORL1 activity. Significant enhancement was observed when a cyclohexane was installed at the amine region (**9**). Further introduction of the substituent at the amine nitrogen in **9** resulted in a dramatic loss of potency (**10** and **11**). Piperidine analogue **12** also showed weak ORL1 activity, suggesting that the tertiary amine was unfavourable in terms of binding affinity. Thus, this series has an extremely different feature from an ORL1 antagonist, because, to our knowledge, a tertiary amine is essential for ORL1 affinity among most of the reported small-molecule ORL1 ligands.[26] Among the synthesized compounds, cyclopentylamine analogue **13** exhibited the best intrinsic potency.

Table 1
Effects of N-substitution on binding affinity at human ORL1 receptor



Compounds	R^1	R^2	ORL1 binding[a] IC_{50} (nM)
1	^{i}Pr	H	350
7	$CH_2{}^i$Pr	H	150
8	$CH_2CH_2{}^i$Pr	H	150
9	Cyclohexyl	H	75
10	Cyclohexyl	Me	>1000
11	Cyclohexyl	^{i}Pr	>1000
12	$-(CH_2)_5-$		560
13	Cyclopentyl	H	28

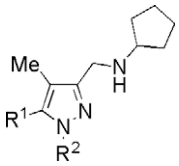
[a] $n = 1$ (Ref. 25).

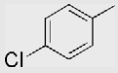
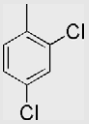
By modifying the amine part, ORL1 activity was enhanced; however, hERG inhibitory activity of **13** remained high (Table 2). We then directed our SAR efforts toward the modification of two aryl moieties using cyclopentylamine derivative **13** as a template. As reduction of the lipophilicity of the molecule was essential in removing affinity for hERG,[27] SAR studies were carried out using analogues that incorporated hydrophilic pyridine ring(s). Although the 2- and 4-pyridine derivatives at the 5-position of the pyrazole ring (**14** and **16**, respectively) showed significantly impaired ORL1 binding affinity, 3-pyridine analogue **15** was equipotent to compound **13**. In addition, binding affinity of **15** for hERG was 100-fold less than that of **13**, which can be attributed to the increased hydrophilicity of **15** ($\log D_{7.4} = 2.9$) in comparison to **13** ($\log D_{7.4} = >4$). Introduction of an additional pyridine ring at the 1-position of the pyrazole ring (**17**) resulted in negligible hERG inhibitory activity while retaining the desired intrinsic potency.

Next, the effects of substituents on the two pyridine rings were investigated (Table 3). Cl-substitution at the 6-position of the left pyridine ring (**18**) resulted in increased ORL1 binding affinity, but hERG binding affinity was also enhanced. In contrast, 6-methylpyridine analogue **19** displayed potent ORL1 binding affinity and antagonistic activity, while retaining good selectivity over hERG. Introduction of a bulkier ethyl (**20**) or trifluoromethyl (**21**) group at the 6-position, or a methyl group (**22**) at the 5-position impaired binding affinity. We thus examined the effect of substituents on the bottom pyridine ring using 6-methylpyridine **19**. Replacement of 3′-chlorine with a methyl (**23**), trifluoromethyl (**24**) or methanesulfonyl (**25**) group resulted in a decrease in intrinsic potency. For compound **26**, which has two chlorine atoms at the 3′- and 5′-positions, binding affinity was good, while affinity for hERG binding was enhanced. The SAR results (Table 3) also demonstrated a good correlation between lipophilicity and hERG affinity.

Extensive SAR studies resulted in **19**, which demonstrated potent ORL1 activity and good selectivity over hERG binding; however, further evaluation revealed that this compound was subject to human P-glycoprotein (P-gp) efflux (transport ratio, 4.1) (Table 4).[28] Therefore, we focused our efforts on overcoming this issue. Based on a report by Cox et al.,[29] we speculated that lowering the pK_a of two amine moieties, cyclopentylamine (N^1) and/or methylpyridine (N^2), would remove P-gp susceptibility. To confirm this hypothesis, analogues of **19** with fluorine installed directly on the cyclopentane or methylpyridine rings were prepared and evaluated. Consequently, 2-fluoro- (**27**) and 3,3-difluoro- (**29**) cyclopentylamine analogues (calculated pK_a of 6.9 and 6.5, respectively) were not subject to P-gp efflux, although the intrinsic potency decreased two- to threefold. In contrast, incorporation of a fluorine at the 3-position on the cyclopentane ring (pK_a 7.4) resulted in enhanced ORL1 binding affinity; however, the fluorine atom was ineffective against P-gp efflux (**28**). With regard to the basicity of the left pyridine, 5-fluorination resulted in **30** with reduced pK_a (from 4.5 to 2.1) and susceptibility to P-gp efflux without any loss of potency toward ORL1. Combination of **30** with the 3-fluorocyclopentylamine moiety led to identification of com-

Table 2
Binding affinities of **13**–**17** toward ORL1 and hERG



Compounds	R^1	R^2	ORL1 binding[a] IC_{50} (nM)	hERG binding IC_{50} (nM)	Log $D_{7.4}$[b]
13			28	23	>4
14		↑	530		
15		↑	37	2900	2.9
16		↑	>1000		
17			61	23,000	1.3

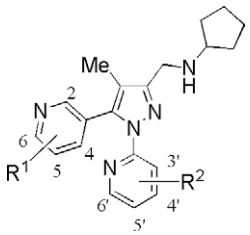
[a] $n = 1$ (Ref. 25).
[b] Measured by shake-flask method.

pound **31**, which was not a human P-gp substrate while possessing sub-nanomolar ORL1 activity.

Further evaluation revealed that compound **31**[30] was a highly selective ORL1 antagonist against μ- and κ-opioid receptors (Table 5).

In conclusion, SAR investigation of a novel class of arylpyrazoles as ORL1 antagonists identified analogue **31**, which exhibits high affinity for human ORL1 receptor and good selectivity over binding affinity for hERG and other opioid receptors. In addition, **31** is not subject to human P-gp efflux, suggesting that its brain penetrability may be suitable for humans. Further evaluation of this compound is currently underway.

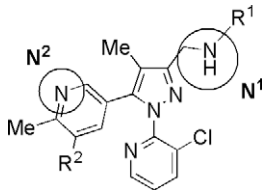
Table 3
SAR of substituents on the left and bottom pyridine rings

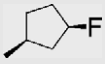
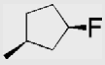


Compounds	R^1	R^2	ORL1 binding[a] IC_{50} (nM)	Antagonism[a] IC_{50} (nM)	hERG binding IC_{50} (nM)	Log $D_{7.4}$[b]
17	—	3′-Cl	61		23,000	1.3
18	6-Cl	3′-Cl	11	12	2700	2.1
19	6-Me	3′-Cl	4.0	4.7	16,000	1.7
20	6-Et	3′-Cl	67			2.0
21	6-CF_3	3′-Cl	270			2.2
22	5-Me	3′-Cl	13		9000	1.6
23	6-Me	3′-Me	33			1.2
24	6-Me	3′-CF_3	36			1.9
25	6-Me	3′-SO_2Me	72			
26	6-Me	3′-Cl, 5′-Cl	8.2		1200	2.5

[a] $n = 1$ (Ref. 25).
[b] Measured by shake-flask method.

Table 4
Effects of fluorine atom on in vitro profiles



Compounds	R^1	R^2	ORL1 binding[a] IC_{50} (nM)	Antagonism[a] IC_{50} (nM)	hERG binding IC_{50} (nM)	log $D_{7.4}$[b]	human P-gp transport ratio[c]	pK_a[d]	
								N^1	N^2
19		H	4.0	4.7	16,000	1.7	4.1	8.2	4.5
27[e]		H	11	7.1	30,000	2.0	1.9	6.9	4.5
28[e]		H	2.5	1.0	29,000	1.3	4.4	7.4	4.5
29[e]		H	9.3	4.5	21,000	2.1	1.4	6.5	4.5
30		F	1.1	1.2	23,000	2.1	2.3	8.2	2.1
31		F	0.52	0.31	41,000	1.8	1.7	7.4	2.1

[e]More potent enantiomers.
[a] $n = 1$ (Ref. 25).
[b] Measured by shake-flask method.
[c] Transport ratio: B − A/A − B (Ref. 28).
[d] Calculated pK_a values.

Table 5
Off-target activities (μ- and κ-opipid receptors) of **31**

Compounds	Binding IC_{50} (nM)		
	ORL1	μ[a]	κ[a]
31	0.52	5600	530

[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.

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30. *Analytical data of* ***31***: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.62–2.32 (9H, m), 2.49 (3H, d, J = 2.9 Hz), 3.31 (1H, quinted, J = 6.3 Hz), 3.93 (2H, s), 5.03–5.22 (1H, m), 7.18 (1H, dd, J = 1.5, 9.8 Hz), 7.31 (1H, dd, J = 4.9, 7.8 Hz), 7.78 (1H, dd, J = 1.5, 7.8 Hz), 8.11 (1H, t, J = 1.5 Hz), 8.43 (1H, dd, J = 1.5, 4.9 Hz).