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Identification of MK-1925: A selective, orally active and brain-penetrable opioid receptor-like 1 (ORL1) antagonist

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ABSTRACT

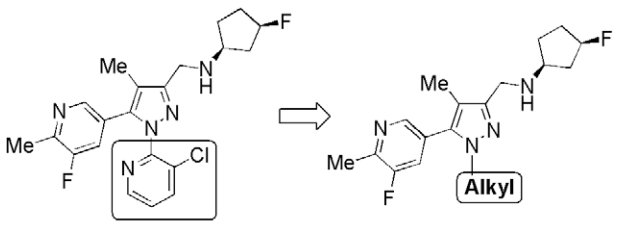
Structure–activity relationship studies directed toward improving the metabolic stability of compound **1** resulted in the identification of 3-[5-(3,5-difluorophenyl)-3-({[(1*S*,3*R*)-3-fluorocyclopentyl]amino}-methyl)-4-methyl-1*H*-pyrazol-1-yl]propanenitrile **39** (MK-1925) as a selective, orally available and brain-penetrable opioid receptor-like 1 (ORL1) antagonist. The compound also showed in vivo efficacy after oral dosing. Therefore, compound **39** was selected to undergo further studies as a clinical candidate.

In 1994, opioid receptor-like 1 (ORL1) was identified as a fourth opioid receptor using cloning techniques.[1–4] Subsequently, its endogenous agonist, a 17-amino acid peptide termed nociceptin or orphanin FQ (NC/OFQ), was identified.[5,6] Nociceptin and ORL1 receptors are widely distributed in the central nervous system, and the physiological roles of the NC/OFQ–ORL1 system have been the focus of intense research. In addition to investigations using NC/OFQ, studies involving ORL1-deficient mice showed that this system may play important roles in pain regulation,[7] learning and memory,[8–10] food intake,[11] anxiety[12] and the cardiovascular system,[13,14] as well as other areas,[15] thus prompting pharmaceutical companies to identify small molecules as potent and selective ORL1 agonists and antagonists. However, additional structurally diverse ORL1 antagonists are required for better understanding of the physiological roles of the ORL1 receptor and the therapeutic potential of its antagonists.[16–19]

In previous communications,[20] we described the identification of potent and selective ORL1 antagonist **1** (Fig. 1). However, further evaluation showed that **1** had a poor pharmacokinetic (PK) profile in rats ($F = 3.2\%$, $t_{1/2} = 0.5$ h, CLp = 68 ml/min/kg) with unacceptable metabolic stability in human hepatocytes (39% remaining),[21] thus suggesting that its oral availability in humans was insufficient. Herein, we report structure–activity relationship (SAR) studies that we conducted to address this issue in the arylpyrazole series of ORL1 antagonists. As the first step, we replaced the lower aryl moiety with smaller alkyl groups, aiming to simplify the molecular structure, as illustrated in Figure 1.

Analogues **25**–**33**, **36** and **39** were prepared as described previously.[20] The synthesis of representative compound **39** is depicted in Scheme 1. 3,5-Difluorophenyl ethyl ketone **2** was coupled with 2-(1*H*-benzotriazol-1-yl)-2-oxoethyl acetate **3** to afford β-diketone **4**. Cyclization of **4** with 2-cyanoethylhydrazine followed by saponification provided 3-hydroxymethylpyrazole **5**. Finally, reductive amination of (1S,3R)-3-fluorocyclopentanamine **7** and the aldehyde **6**, obtained through oxidation of the alcohol **5**, gave the desired compound **39**.

4-Chloro and 4-methoxymetyl analogues, **34** and **35** were prepared according to Scheme 2. 4-Unsubstituted pyrazole **9** prepared by coupling of starting material **8** with $(CO_2Et)_2$ followed by cyclization was halogenated with *N*-bromosuccinimide (NBS) or *N*-chlorosuccinimide (NCS) to afford 4-chloro (**10**) and 4-bromopyrazole (**11**), respectively, in almost quantitative yield. Cross-cou-



1

Figure 1. Compound **1** and modification design.

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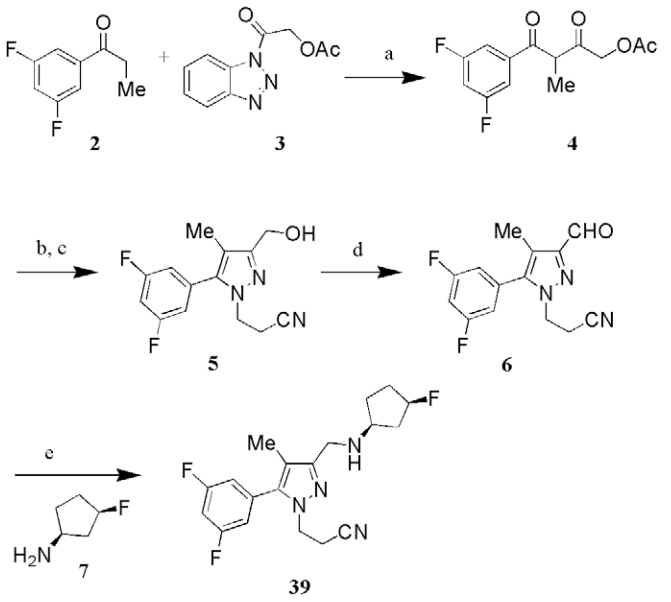
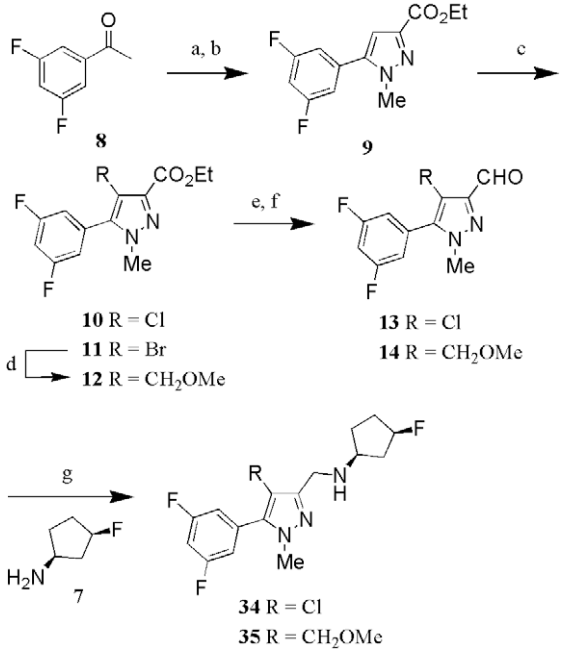


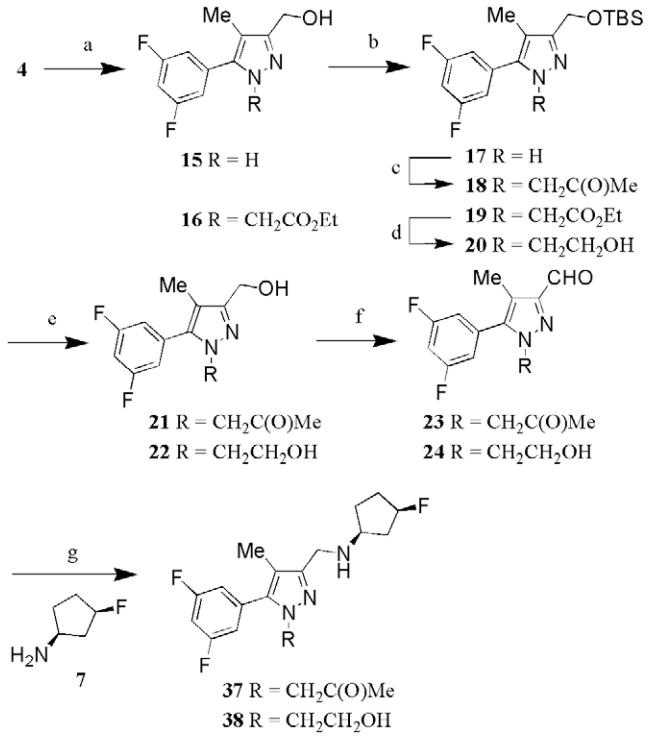
Scheme 1. Reagents and conditions: (a) LHMDS, THF, −78 °C to rt, 61%; (b) 2-cyanoethylhydrazine, AcOH, 90 °C; (c) aq. NaOH, MeOH, rt, 77% (2 steps); (d) Dess–Martin periodinane, $CHCl_3$, rt, 75%; (e) **7**, $Zn(BH_3CN)_2$, MeOH, rt, 85%.



Scheme 2. Reagents and conditions: (a) LHMDS, $(CO_2Et)_2$, THF, −78 °C to rt; (b) $MeNHNH_2$, EtOH, rt, 58% (2 steps); (c) NCS or NBS, MeCN, 80 °C, 98%–quant.; (d) (methoxymethyl)tributyltin, $PdCl_2(PPh_3)_2$, DMF, 90 °C, 18%; (e) $LiAlH_4$, THF, 0 °C; (f) MnO_2, $CHCl_3$, rt, 60–70% (2 steps); (g) **7**, $Zn(BH_3CN)_2$, MeOH, rt, 65–67%.

pling[22] of **11** with (methoxymethyl)tributyltin gave **12**. Ethyl ester of **10** and **12** was converted in two steps, $LiAlH_4$ reduction and MnO_2 oxidation, into the corresponding pyrazole-4-carbaldehydes **13** and **14**. Finally, **13** and **14** underwent reductive amination with (1*S*,3*R*)-3-fluorocyclopentanamine **7** to produce the desired chiral analogues.

Synthesis of analogues **37** and **38** are outlined in Scheme 3. Alcohols **15** and **16** obtained in a similar manner as shown in Scheme 1 were protected using *tert*-butyldimethylsilyl (TBS) groups to afford **17** and **19**, which were converted to **18** and **20**

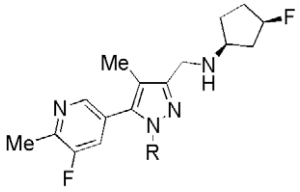


Scheme 3. Reagents and conditions: (a) hydrazine hydrate or ethyl hydrazinoacetate, 4 N HCl–dioxane, EtOH, reflux, 74–80%; (b) TBSCl, imidazole, DMF, 80 °C; (c) bromoacetone, Et_3N, toluene–DMF, reflux, 6% (2 steps); (d) $LiAlH_4$, THF, rt, 83% (2 steps); (e) TBAF, THF, rt; (f) Dess–Martin periodinane (for **18**) or MnO_2 (for **20**), $CHCl_3$, rt, 27–38% (2 steps); (g) **7**, $Zn(BH_3CN)_2$, MeOH, rt, 80–90%.

by alkylation with bromoacetone or reduction with $LiAlH_4$, respectively. The yield of the alkylation with bromoacetone was low due to the major formation of the *N*-alkyl regioisomer. After deprotection of the TBS group, the final products **37** and **38** were prepared via the same procedure for the compound **39**.

Firstly, we explored the effect of alkyl substituents upon the intrinsic potency and the metabolic stability (Table 1). Replacement of the chloropyridine moiety with an isopropyl group (**25**) resulted in no significant decrease in potency toward ORL1. Smaller ethyl (**26**) or methyl (**27**) analogues were tolerable in terms of

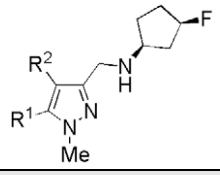
Table 1
SAR of alkyl substituents at 1 position on pyrazole ring

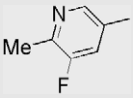
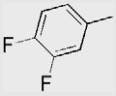
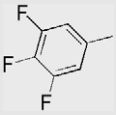
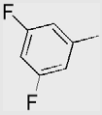
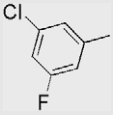
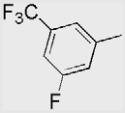
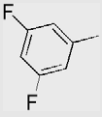
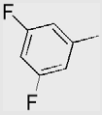


Compds	R	ORL1 binding[a] IC_{50} (nM)	Antagonism[a] IC_{50} (nM)	HH stability[b] % remaining
1		0.52	0.31	39
25	*i*-Pr	0.92	0.61	
26	Et	2.0	0.61	
27	Me	4.3	2.2	25
28	H	9.9	7.8	21

[a] See Ref. 23 for detailed description. $n = 1$ (Ref. 24).
[b] See Ref. 21 for detailed description.

Table 2
SAR of 4- and 5-substituents on pyrazole ring

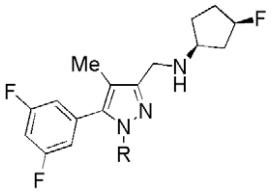


Compds	R^1	R^2	ORL1 binding[a] IC_{50} (nM)	Antagonism[a] IC_{50} (nM)	HH stability[b] % remaining
27		Me	4.3	2.2	25
29		Me	6.4	6.5	
30		Me	15	13	
31		Me	3.1	2.5	56
32		Me	17		
33		Me	210		
34		Cl	9.3	2.5	40
35		CH_2OMe	1.4	1.0	39

[a] See Ref. 23 for detailed description. $n = 1$ (Ref. 24).
[b] See Ref. 21 for detailed description.

ORL1 affinity. Surprisingly, analogue **28** without any substituents at the 1 position also exhibited nanomolar binding affinity. However, analogues **27** and **28** still had poor metabolic stability in human hepatocytes. Because the metabolic stability was not improved by only this approach, we shifted our efforts toward the modification of other sites using **27** as a template.

Table 3
SAR of hydrophilic substituents at 1 position on pyrazole ring



Compds	R	ORL1 binding[a] IC_{50} (nM)	Antagonism[a] IC_{50} (nM)	HH stability[b] % remaining	Log $D_{7.4}$[c]
31	Me	3.1	2.5	56	1.9
36	$CH_2CH_2SO_2Me$	33			<1
37	$CH_2C(O)Me$	12			1.4
38	CH_2CH_2OH	15			1.3
39	CH_2CH_2CN	8.2	4.6	77	1.5

[a] See Ref. 23 for detailed description. $n = 1$ (Ref. 24).
[b] See Ref. 21 for detailed description.
[c] Measured by shake-flask method.

Metabolite identification study of **27** revealed that the major metabolic pathway in human hepatocytes was hydroxylation on the left pyridine moiety or the methyl group at the 4 position of the pyrazoles moiety. Therefore, modification of these parts was performed (Table 2). We first replaced the metabolically labile methylpyridine with a fluorobenzene ring. As a result, 3,5-difluorobenzene analogue **31** exhibited potent ORL1 activity in comparison with 3,4-difluoro (**29**) or 3,4,5-trifluoro (**30**) analogues. This modification led to the improvement of metabolic stability in human hepatocytes as expected. Further optimization of 3,5-disubstituted benzene moiety resulted in the impaired intrinsic potency (**32** and **33**). We thus examined the effects of 4-substituents on the pyrazole ring. Although 4-chloro (**34**) and 4-methoxymethyl (**35**) analogues showed equipotent ORL1 affinity as compound **31**, the metabolic stability decreased slightly.

At this stage, we again examined the effects of the lower alkyl region. SAR studies were carried out using analogues that incorporated hydrophilic alkyl groups (Table 3). Introduction of the substituent with a sulfone (**36**), a ketone (**37**), and an alcohol (**38**) reduced the ORL1 binding affinity. In contrast, when a cyanoethyl group was introduced, compound **39** displayed better metabolic stability than **31** while retaining good ORL1 binding affinity and antagonistic activity, which can be attributed to the increased hydrophilicity of **39** ($\log D_{7.4} = 1.5$) in comparison to **31** ($\log D_{7.4} = 1.9$). Consequently, we selected compound **39** for further evaluation.

As shown in Table 4, compound **39**[25] showed excellent selectivity over other opioid receptors and hERG potassium channel.[26,27] In a standard panel for off-target activity, compound **39** did not display significant affinity for the other 163 receptors and ion

Table 4
Biological profiles of **39**

Binding IC_{50} (nM)					Human P-gp transport ratio[c]	Pharmacokinetic parameters[d]						
ORL1	μ[a]	κ[a]	δ[a]	hERG[b]		Species	iv CLp (ml/min/Kg)	V_{dss} (L/Kg)	PO $AUC_{0\text{-}inf}$ (μM h)	C_{max} (μM)	$t_{1/2}$ (h)	F (%)
8.2	>10,000	9800	>10,000	9500	1.2	Rat	83	5.2	0.3	0.40	0.7	21
						Dog	37	3.5	1.3	2.6	2.8	62

[a] Displacement of [^{3}H]diprenorphin (μ), [^{3}H]U69593 (κ) and [^{3}H]naltrindole binding in CHO cells stably expressing cloned human μ-, κ- and δ-opioid receptors, respectively.
[b] Displacement of [^{35}S]-radiolabeled MK499 in membranes derived from HEK 293 cells stably transfected with hERG gene and expressing the I_{Kr} channel protein (Ref. 26,27).
[c] Transport ratio: B–A/A–B (Ref. 28).
[d] Oral dose 3 mg/kg, and IV dose 1 mg/kg. The values represent the mean ($n = 3$).

Table 5
In vivo antagonistic activity of **39**

po dose (mg/kg)	In vivo antagonism[a] (% reversal)	Brain penetrability[b]		
		Plasma (μM)	Brain (nmol/g brain)	b/p ratio
3	17	0.016	0.035	3.42
10	81	0.315	0.516	1.62
30	112	1.627	3.017	1.84

[a] Data shows antagonistic activity of **39** against the reduction in locomotor activity produced by ORL1 agonist for 60 min in mice. Values are expressed as % reversal of the agonist response.
[b] At 60 min after oral administration in mice.

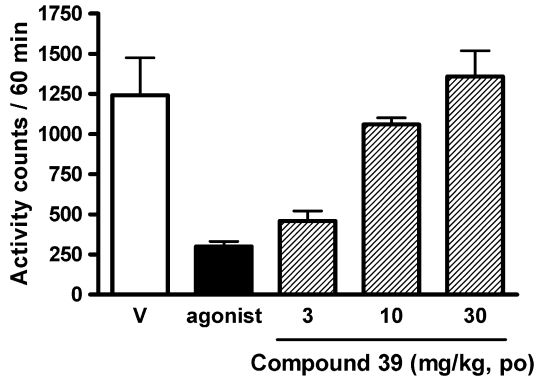


Figure 2. In vivo locomotor test by oral treatment of compound **39** at 3, 10, and 30 mg/kg.

channels, indicating that compound **39** is a selective ORL1 antagonist (the receptor that reached over 50% at 10 μM was the choline transporter (IC_{50} of 3.7 μM)). Compound **39** was not subject to human P-gp efflux,[28] suggesting that its brain penetrability is suitable for humans. The potential cardiovascular effects of **39** were evaluated in anesthetized dogs, and no adverse effects was observed up to 10 mg/kg (C_{max} = 9.7 μM). PK studies were carried out, and **39** displayed fair PK profiles in rats and dogs. Our modification to increase metabolic stability resulted in improved oral bioavailability in rats (F = 21%) in comparison with the lead compound **1** (F = 3.2%).

Furthermore, in vivo antagonist activity against the reduction in locomotor activity induced by ORL1 agonist[29] was determined. As shown in Table 5 and Figure 2, when dosed in mice orally, **39** exhibited in vivo antagonistic activity in a dose-dependent manner, and complete reversal was observed at 30 mg/kg po, thus reflecting its good brain penetrability.

In conclusion, SAR studies performed in the arylpyrazole series related to **1** with the aim of reducing metabolism led to the identification of compound **39**, which exhibited good ORL1 activity and improved metabolic stability. This was achieved by replacing the lower pyridine moiety with a cyanoethyl group and substituting the left 3-fluoro-2-methylpyridine with 3,5-difluorobenzene. Compound **39** showed good selectivity over other opioid receptors and the hERG channel. In addition, **39** was orally active in the in vivo locomotor test as well as good brain penetrability in mice. Based on the profiles reported in this communication, this compound was moved forward as a clinical development candidate, labeled as MK-1925.

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25. *Analytical data of* **39** *L-tartaric acid salt*: ^{1}H NMR (400 MHz, CD_3OD) δ [ppm] 1.99 (m, 3H), 2.07 (s, 3H), 2.20 (m, 2H), 2.34 (m, 1H), 2.51 (m, 1H), 3.04 (t, 2H, J = 6.3 Hz), 3.87 (m, 1H), 4.32 (s, 2H), 4.35 (t, 2H, J = 6.3 Hz), 5.21 (m, 1H), 7.11 (m, 2H), 7.18 (m, 1H). MS (ESI+): m/z 363.2 $[M+H]^+$.
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