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Design, synthesis, and biological evaluation of indole derivatives as novel nociceptin/orphanin FQ (N/OFQ) receptor antagonists

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Abstract—A novel series of 2-(1,2,4-oxadiazol-5-yl)-1*H*-indole derivatives as nociceptin/orphanin FQ (N/OFQ) receptor antagonists was discovered. Systematic modification of our original lead by changing the pendant functional groups, linker, heterocyclic core, and basic side chain revealed the structure–activity requirements for this novel template and resulted in the identification of more potent analog with improved potency as compared to the parent compound.

Opioid receptor-like 1 (ORL1) receptor (nociceptin/orphanin FQ (N/OFQ) receptor, NOP receptor)[1] was discovered as a fourth member of the opioid receptor family in 1994 through cDNA expression cloning techniques.[2] The endogenous ligand for its receptor, a novel heptadeca neuropeptide, was independently identified in 1995 by two groups.[3] Although ORL1 receptor is a member of the G-protein-coupled receptor (GPCR) superfamily with 47% overall identity to the classical opioid (μ, δ, and κ) receptors and 64% identity in the transmembrane domains, native opioid peptides and synthetic agonists selective for μ, δ, and κ receptors do not show significant affinity for ORL1 receptor.[4]

The ORL1 receptor and N/OFQ are mainly distributed in the brain and central nervous system (CNS).[4,5] It was observed that N/OFQ is involved in modulating pain mechanisms in the spinal cord and forebrain. Several in vivo studies with N/OFQ and its peptide analogs have demonstrated that N/OFQ modulates a variety of biological functions, such as feeding, learning, diuresis, drug addiction, cardiovascular functions, and locomotor activity, and that it controls the release of neurotransmitters including serotonin and dopamine at peripheral and central sites.[6] ORL1 receptor might also be relevant in the treatment of CNS disorders such as anxiety and drug abuse.[6,7] Therefore, identification of potent small molecule agonists and antagonists of nociceptin could provide new classes of drugs for several human disorders involving pain and anxiety.

Recently, several research groups have reported their efforts in the search for small molecule ORL1 agonists and antagonists, describing nonpeptide ligands such as benzimidazolinones,[8] benzimidazoles,[9] indolinones,[10] spiropiperidines,[11] aryl piperidines,[12] and 4-aminoquinolines.[13] Some of these ligands possess very high selectivity for the ORL1 receptor versus other opioid receptors.

Our effort toward identifying an ORL1 antagonist started with the high-throughput screening (HTS) of various compound libraries. Among hit compounds, we identified 2-(1,2,4-oxadiazol-5-yl)-1*H*-indole **8**[14] as a novel structure that showed antagonist activity in [^{35}S]GTPγS functional assay. With regard to drug candidates, indole scaffold is a well-known representative class of privileged structures[15] with high affinity for multiple biological targets in drug discovery. Herein, we describe design, synthesis, and structure–activity relationship (SAR) studies to improve the potency of this novel lead compound (**33**, 95 nM for ORL1).

The key reaction for the preparation of the indolyl-oxadiazole **8** is a cycloaddition reaction between indole derivative **4** or **7** and appropriately substituted amide

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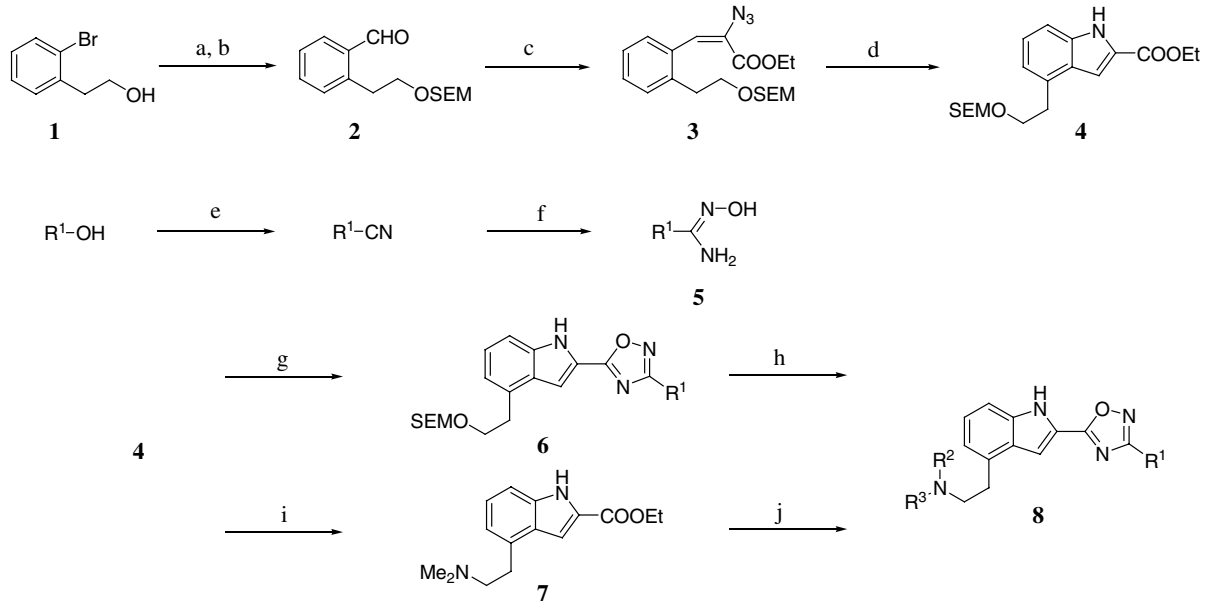
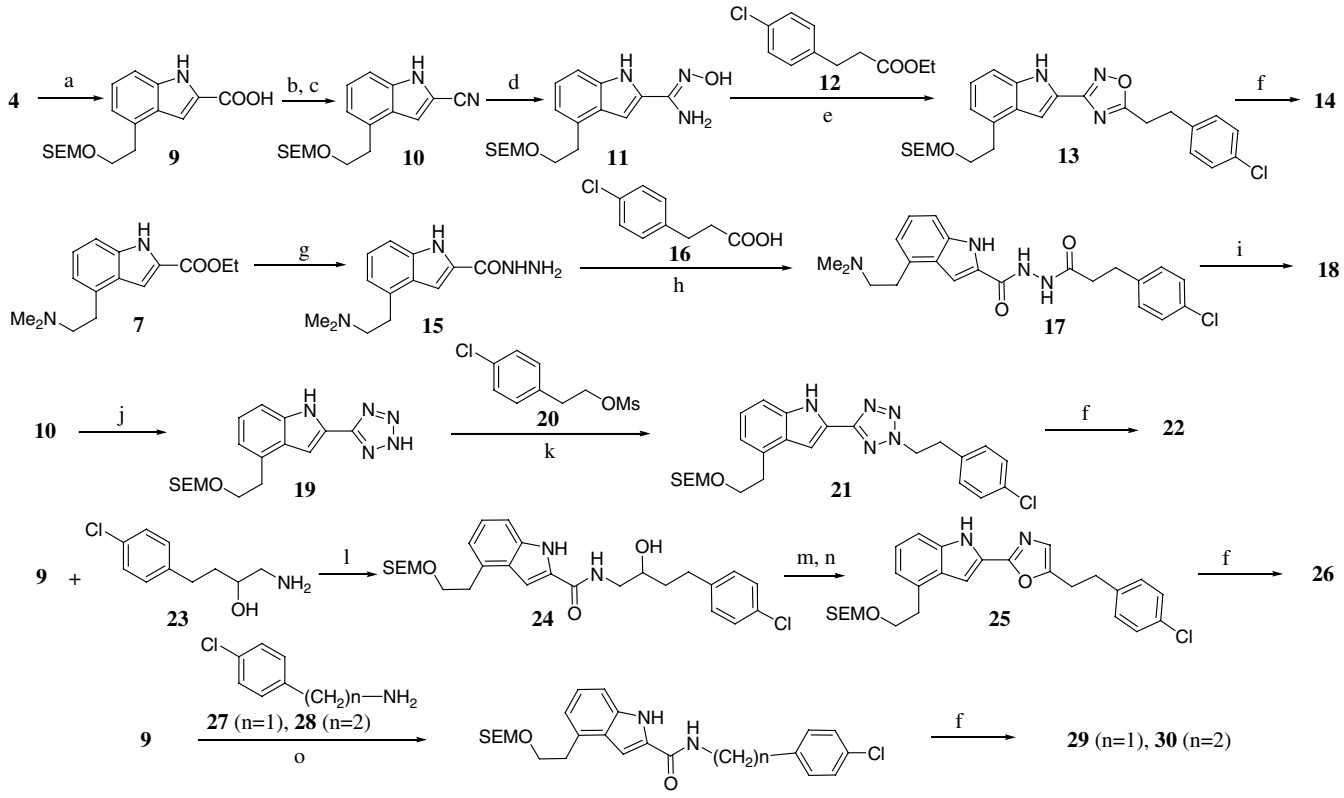
oxime **5**. Scheme 1 depicts the synthesis of indolyl-oxadiazole **8**. 2-(2-Bromophenyl)ethanol **1** was converted in two steps, SEM protection and aldehyde formation after halogen-lithium exchange, into 2-substituted benzaldehyde **2**. 2-Azido-3-phenylacrylate **3**,[16] prepared from the benzaldehyde **2** and ethyl azidoacetate in the



Scheme 1. Reagents: (a) SEMCl, *i*-Pr_2NEt, 100%; (b) *sec*-BuLi, then DMF; (c) N_3CH_2COOEt, NaOEt; (d) heat, 29% (3 steps); (e) MsCl, Et_3N, then NaCN or KCN, 21–90%; (f) H_2NOH–HCl, Na_2CO_3, 54–95%; (g) **5**, NaH, MS4A, 54–85%; (h) i—HCl, ii—MsCl, Et_3N, iii—amine, K_2CO_3, 3 steps 10–43%; (i) i—HCl, ii—MsCl, Et_3N, iii—Me_2NH–HCl, K_2CO_3, 3 steps 59%; (j) **5**, NaH, MS4A, 15–70%.



Scheme 2. Reagents: (a) aq NaOH, 83%; (b) WSC, HOBt, then NH_4OH, quant.; (c) TFAA, pyridine, 80%; (d) H_2NOH–HCl, $NaHCO_3$, 80%; (e) **11**, NaH, MS4A, 26%;(f) i—HCl, ii—MsCl, Et_3N, iii—Me_2NH–HCl, K_2CO_3, 3 steps 12–38%; (g) H_2NNH_2–H_2O, 82%; (h) DMC, Et_3N, 6%; (i) $POCl_3$, 27%; (j) NaN_3, Et_3N–HCl, quant.; (k) **20**, K_2CO_3, 30%; (l) WSC, HOBt, 58%; (m) Deoxo-Fluor, 84%; (n) DDQ, 73%; (o) WSC, HOBt, 42% (n = 1), 69% (n = 2).

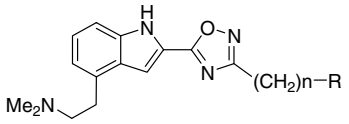
presence of NaOEt, was converted to ethyl 1*H*-indole-2-carboxylate **4** via thermolysis.[17] Addition of hydroxylamine to the appropriately substituted nitrile prepared from the corresponding alcohol afforded amide oxime **5**. Indole ester **4** was condensed with amide oxime **5** utilizing NaH to afford oxadiazole nucleus **6**. Deprotection of the SEM group and successive introduction of an amino group afforded **8**. Another synthetic route via **7** containing amino group was also possible.

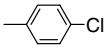
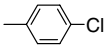
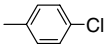
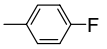
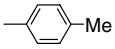
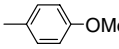
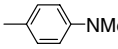
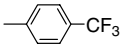
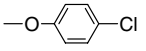
The synthesis of other heterocyclic ring systems other than 1,2,4-oxadiazole at the central core region is shown in Scheme 2. The reverse type of 1,2,4-oxadiazole **13** was obtained from indole-containing amide oxime **11**, prepared from 2-cyanoindole **10**, and ethyl 3-(4-chlorophenyl)propanoate **12**. Nitrile **10** was easily prepared from indole ester **4** in three steps via amide dehydration. Diacylhydrazide **17**, prepared from indole-2-carbohydrazide **15** and 3-(4-chlorophenyl)propanoic acid **16**, was converted to 1,3,4-oxadiazole **18** utilizing $POCl_3$. Cycloaddition of an azido group to **10** afforded 2-(2*H*-tetrazol-5-yl)-1*H*-indole **19**. Alkylation with 2-(4-chlorophenyl)ethyl mesylate **20** produced tetrazole analog **21**. β-Hydroxyamide **24**, prepared from indole carboxylic acid **9** and 2-hydroxyamine **23**,[18] was converted in two steps to 1,3-oxazole **25** utilizing Deoxo-Fluor® as a cyclodehydrating agent[19] and DDQ as an oxidant for conversion of oxazoline to an oxazole moiety. Linear analogs having amide linkages at the central regions were also prepared. Carboxylic acid **9** was condensed with amines **27** and **28**, and successive deprotection and conversion to amino groups afforded **29** and **30**, respectively.

These new indolyl-oxadiazole compounds were tested for competitive binding affinity for human ORL1 receptors transfected into Chinese hamster ovary (CHO) cells using [^{125}I][Tyr^{14}]N/OFQ as a radioligand, with the results given as an IC_{50}. Functional activity of potent compounds showing high binding affinity was determined by stimulation of [^{35}S]GTPγS binding to CHO-ORL1 membranes.[20]

Our initial efforts toward understanding the SAR of the lead molecule were focused on replacing the substituted phenylethyl group with other substituents and heteroaromatic nuclei. The results of this study are summarized in Table 1. Compounds **31**–**43** were prepared to evaluate the effects of the linker length and substituents. Optimal binding for ORL1 was achieved with our lead compound **33**. A shorter linker (**32**) or longer spacer (**34**) resulted in poor or loss of affinity for ORL1. Attempts to introduce heteroatom (**43**) on the linker leading the ether linkage also resulted in inactive compound. Removing the substituent from the phenyl group to give the unsubstituted derivative **31** or replacing the phenyl group with pyridine, as in examples **40**–**42**, led to complete loss of activity. In addition, replacement of the substituent on the phenyl ring with other functional groups, 4-fluoro (**35**), 4-methyl (**36**), and 4-methoxy (**37**), gave compounds 2- to 6-fold less potent. However, 4-trifluoromethyl analog **39** was approximately 2-fold more potent than the 4-chloro compound **33**.

Table 1. Effect of aromatic substituent on right part

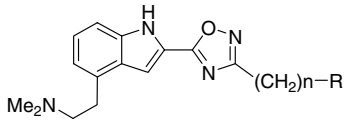


Compound	*n*	R	Binding IC_{50}[a] (nM)	GTPγS IC_{50}[b] (nM)
31	2		>1000 (46%)	
32	1		450	
33	2		95	632
34	3		>1000 (49%)	
35	2		450	
36	2		230	
37	2		560	
38	2		>1000 (44%)	
39	2		49	421
40	2		>1000 (11%)	
41	2		>1000 (1.7%)	
42	2		>1000 (12%)	
43	1		410	

[a] Binding affinities for the ORL1 receptor. Numbers in parentheses indicate % inhibition at 1 μM.
[b] Antagonist activities in the GTPγS functional assay.

The phenyl group was then replaced with alkyl or cycloalkyl moieties to confirm whether aromatic substituents are essential. The results for the alkyl analogs are shown in Table 2. Small and branched alkyl groups, as in compounds **44**–**47**, rendered the molecule inactive, whereas cycloalkyl groups, like cyclopentyl **49**, cyclohexyl **51**, and bicyclo[2.2.1]heptane **53**, displayed sub-micromolar potency. Interestingly, longer spacers increased the potency by 6-fold in the case of the cyclohexyl series (**50** vs **52**). There is clearly an increase in potency with linker ($n = 3$) and the size of the cycloalkyl substituent (six-membered ring) and it translates with an increase in C log *P* (analog **52**). Attempts to introduce hydrophilic heteroatoms on the alkyl region leading ether or amino analogs (C log *P* 2.06–2.50) resulted in inactive compounds (**54**–**56**).

Table 2. Effect of alkyl substituent on right part



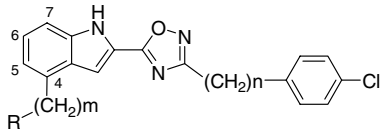
Compound	*n*	R	Binding IC_{50}[a] (nM)	GTPγS IC50[b] (nM)	C log *P*[c]
44	2	–Me	>1000 (14%)		2.50
45	2	–Et	>1000 (42%)		3.03
46	2		>1000 (29%)		3.37
47	2		>1000 (20%)		3.72
48	2		>1000 (29%)		2.86
49	2		580		3.99
50	1		440		4.02
51	2		140		4.56
52	3		71	294	5.09
53[d]	2		440		4.41
54	2		>1000 (4%)		2.36
55	3		>1000 (0%)		2.50
56	2		>1000 (11%)		2.06

[a] Binding affinities for the ORL1 receptor. Numbers in parentheses indicate % inhibition at 1 μM.
[b] Antagonist activities in the GTPγS functional assay.
[c] Calculated using ACD/log *P* software supplied by Advanced Chemical Development.
[d] Racemate of exo form.

The effects of the regiochemistry of the aminoalkyl chain on activity were examined. As shown in Table 3, the C-5, C-6, and C-7 substituted analogs **59**–**61**, respectively, were inactive when compared with C-4 analog **33**.

The aminoalkyl side chain was modified in two ways: by changing the length of the side chain and by varying the nature of the amine. From the limited number of chain lengths examined, the one- (**57**) and two-carbon lengths (**33**) were equipotent. Three-carbon analog **58** with the dimethylamino group was approximately 3-fold less potent than the one-carbon analog, possibly due to the higher pK_a of the amine region of the former. We then decided to incorporate cyclic amines in our linker modifications using a ring to fix the conformation. Such a design is embedded in fused cyclic amine **70**,[21] leading to complete loss of potency. Comparison of amines **62**–**69** indicated significant differences in activity as the amine varied; methylethylamine **62**, methylmethoxyethylamine **63**, and *N*-methylpiperidine **69**[22] analogs were tolerated, though five-membered cyclic amine seems to be unfavorable with **64** being less potent than **33**. On the other hand, morpholine **65** leads to complete loss of activity.

Table 3. Effect of amine side chain



Compound	Position	*m*	*n*	R	Binding IC_{50}[a] (nM)	GTPγS IC_{50}[b] (nM)
57	4	1	2	–NMe_2	62	233
33	4	2	2	–NMe_2	95	632
58	4	3	2	–NMe_2	210	
59	5	2	2	–NMe_2	>1000 (3%)	
60	6	2	2	–NMe_2	>1000 (22%)	
61	7	2	2	–NMe_2	>1000 (18%)	
62	4	2	2	–NMeEt	58	223
63	4	2	2		97	450
64	4	2	2		568	
65	4	2	2		>1000 (15%)	
66	4	0	2		120	
67[c]	4	0	2		40	199
68[d]	4	0	2		>1000 (27%)	
69	4	0	2		62	899
70	4, 5	2	2	–$NMeCH_2$–	>1000 (31%)	

[a] Binding affinities for the ORL1 receptor. Numbers in parentheses indicate % inhibition at 1 μM.
[b] Antagonist activities in the GTPγS functional assay.
[c] Position 5 is replaced by nitrogen.
[d] Position 7 is replaced by nitrogen.

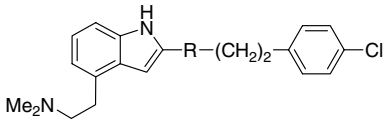
In addition, the indole skeleton was replaced with a pyrrolo[3,2-*c*]pyridine ring containing piperazine as the amine region leading to a 3-fold increase in potency (**66** vs **67**[23]), though pyrrolo[2,3-*b*]pyridine analog **68**[24] was inactive.

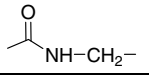
Most of the tested compounds (IC_{50} < 100 nM) behaved functionally as antagonists. In particular, compound **67** was discovered as a potent ORL1 antagonist. It showed a high affinity for ORL1 with an IC_{50} of 40 nM, full antagonistic activity (IC_{50} = 199 nM) in the GTPγS assay.

With respect to the central heteroaryl nucleus, the results for heterocyclic analogs are shown in Table 4. Replacing the 1,2,4-oxadiazole ring with a regioisomer of 1,2,4-oxadiazole **14** led to a 6-fold reduction in potency. 1,3,4-Oxadiazole **18**, tetrazole **22**, and oxazole analogs **26** decreased the potency significantly. A series of compounds with the amide-containing backbones **29**–**30** were also inactive.

In conclusion, we have identified potential nonpeptide ligands, indolyl-oxadiazole derivatives, as a novel class of nociceptin/orphanin FQ receptor antagonists. We have explored the structure–activity requirements for

Table 4. Effect of heterocycle and amide linkage on central part



Compound	R	IC_{50}[a] (nM)
14		600
18		>1000 (39%)
22		>1000 (46%)
26		>1000 (37%)
29		>1000 (18%)
30		>1000 (41%)

[a] Binding affinities for the ORL1 receptor. Numbers in parentheses indicate % inhibition at 1 μM.

this antagonist motif and the SAR resulted in a 2-fold increase (analog **67**) in potency for ORL1 compared to the prototype compound **33**.

Acknowledgment

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