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Authors: Xudong Cao, Zhongyuan Yao, Fei Dou, Yifang Zhang, Yinli Qiu, Song Zhao, Xiangqing Xu, Xin Liu, Bi-Feng Liu, Yin Chen, and Guisen Zhang

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Chem. Biodiversity

Synthesis and Biological Evaluation of σ_1 Receptor Ligands Based on Phenyl-1,2,4-oxadiazole Derivatives

Xudong Cao,[a] Zhongyuan,Yao,[b] Fei, Dou,[b] Yifang Zhang,[a] Yinli Qiu,[b] Song Zhao,[b] Xiangqing Xu,[b] Xin Liu,[a] Bi-Feng Liu,[a] Yin Chen,[b,c,*] and Guisen Zhang [a,b,*]

[a] Systems Biology Theme, Department of Biomedical Engineering, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, China, e-Mail: gszhang@mail.hust.edu.cn

[b] Jiangsu Nhwa Pharmaceutical Co., Ltd. 69 Democratic South Road, Xuzhou, Jiangsu 221116, China

[c] Jiangsu Marine Resources Development Research Institute, Lianyungang 222005, China, e-Mail: chenyin0914001@126.com

Dedication ((optional))

Abstract.

In this study, a series of phenyl-1,2,4-oxadiazole derivatives was synthesized and evaluated for anti-allodynic activity. Structure activity relationship studies identified compound **39** with excellent affinity for the σ_1 receptor and selectivity for the σ_2 receptor, with poor activity to other central nervous system neurotransmitter receptors and transporters associated with pain. Compound **39** exhibited dose-dependent efficacy in suppressing the formalin-induced flinching and attenuating mechanical allodynia in chronic constriction injury-induced neuropathic rats. These results suggest that compound **39** exerts potent antihyperalgesic activity, suggesting it as a promising candidate for treating neuropathic pain.

Keywords: *antiallodynic effects; formalin-induced flinching; mechanical allodynia in CCI; phenyl-1,2,4-oxadiazole derivatives; σ receptor.*

Introduction

The sigma (σ) receptor was initially recognized as a new subtype of receptor in the opioid receptor family, but later molecular cloning of the σ_1 receptor confirmed definitively that it is dissimilar in sequence from true opioid receptors.[1,2] Pharmacological studies have suggested that the σ receptor presents at least two different subtypes, such as σ_1 and σ_2 receptors, which have distinct functions and are related to different potential therapeutic indications when they are exposed to disease-related stress or mutations.[3] The σ_1 receptor is widely distributed in peripheral organs and in different areas of the central nervous system (CNS), where it plays a key role in human physiology, modulating a variety of diseases of the cardiovascular and nervous systems.[4] After the σ_1 receptor was first cloned from the guinea pig liver, and the crystalline structure of the human σ_1 receptor in complex with σ_1 receptor ligands was reported, it offered a solid foundation for developing future biochemical and biophysical studies to investigate the σ_1 receptor at the molecular level.[5] Several σ_1 receptor ligands have been studied in the past for their increasingly clear importance in human physiology and a variety of diseases related to the cardiovascular system and CNS.[4,6] Although the structure of the σ_2 receptor has remained elusive, it is highly expressed in many different types of tumor cells.[7] Current evidence suggests that the σ_2 receptor may be a useful target to develop antineoplastic agents that would be effective against drug-resistant tumors.[8–10] Ligands acting on the σ_2 receptor have played a potential role in cancer imaging and treatment.[11,12] Neuropathic pain is an important health problem.[13] Until now, tricyclic anti-depressants, opioids, and anticonvulsants have been used to treat chronic pain; however, these agents are not effective in all cases. Moreover, some severe side effects (such as addiction or abuse) are caused by their chronic use.[14] All of these factors make treatment of neuropathic pain an unmet clinical need.[15,16] Recent research has suggested that the σ_1 receptor is a unique 'ligand-operated receptor chaperone' that interacts with other functional proteins in the plasma membrane, endoplasmic reticulum, mitochondria, and cytosol.[17] Increasing clinical and preclinical findings have indicated involvement of the σ_1 receptor in the pathophysiology of CNS disorders.[18] Interestingly, some research suggests that σ_1 receptor agonists may alter the mechanisms frequently associated with neurodegeneration to preserve or restore neuronal function.[19] These findings suggest a potential therapeutic effect to treat various neurodegenerative disorders.[20] Moreover, a wide body of clinical and preclinical research suggests that various σ_1 receptor antagonists possess beneficial effects in cases of drug addiction and pain.[21] Of particular interest, some studies lend support to ligands with antagonism to the σ_1 receptor resulting in an antinociceptive effect. The most clinically advanced selective σ_1 receptor antagonist based on a morpholinyl pyrazole scaffold S1RA (E-52862, **1**) is currently in phase II trials for treating different pain conditions (Figure 1).[22]

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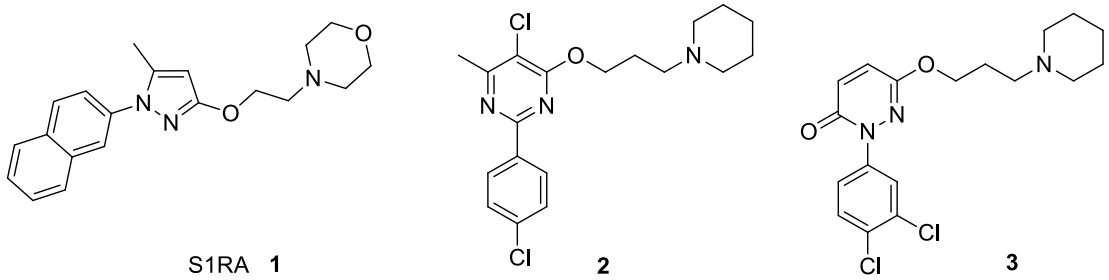


Figure 1. Representative σ_1 receptor ligands

Although the reported three-dimensional crystalline structure of the human σ_1 ($h\sigma_1$) receptor revealed a trimeric structure with a single transmembrane domain for each protomer and offered a solid foundation for the development of σ_1 receptor ligands, Glennon's σ_1 receptor pharmacophore is an elegant and simple model for designing new σ_1 receptor ligands.[23–25] The pharmacophore model for the σ_1 receptor ligands contains: (i) the "primary hydrophobic region" connected by a linker (mostly an alkyl) to the basic amino site at distances of 6–10 Å, (ii) the "secondary hydrophobic region" introduced by a secondary or tertiary amine with distances of 2.4–3.9 Å from the basic amino site (Figure 2A). Considering the pharmacophore requirements, a ligand based on the six-membered heterocyclic rings of pyrimidine (**2**) and pyridazinone (**3**) scaffold was designed during our previous studies on potent and selective σ_1 receptor antagonists, which exhibited dose-dependent anti-allodynic properties in pain models (Figure 1).[26,27] Moreover, the novel ligands contained the five-membered heterocyclic scaffolds triazole and isoxazole, which are selective σ_1 receptor antagonists with potent *in vivo* antinociceptive properties.[28,29]

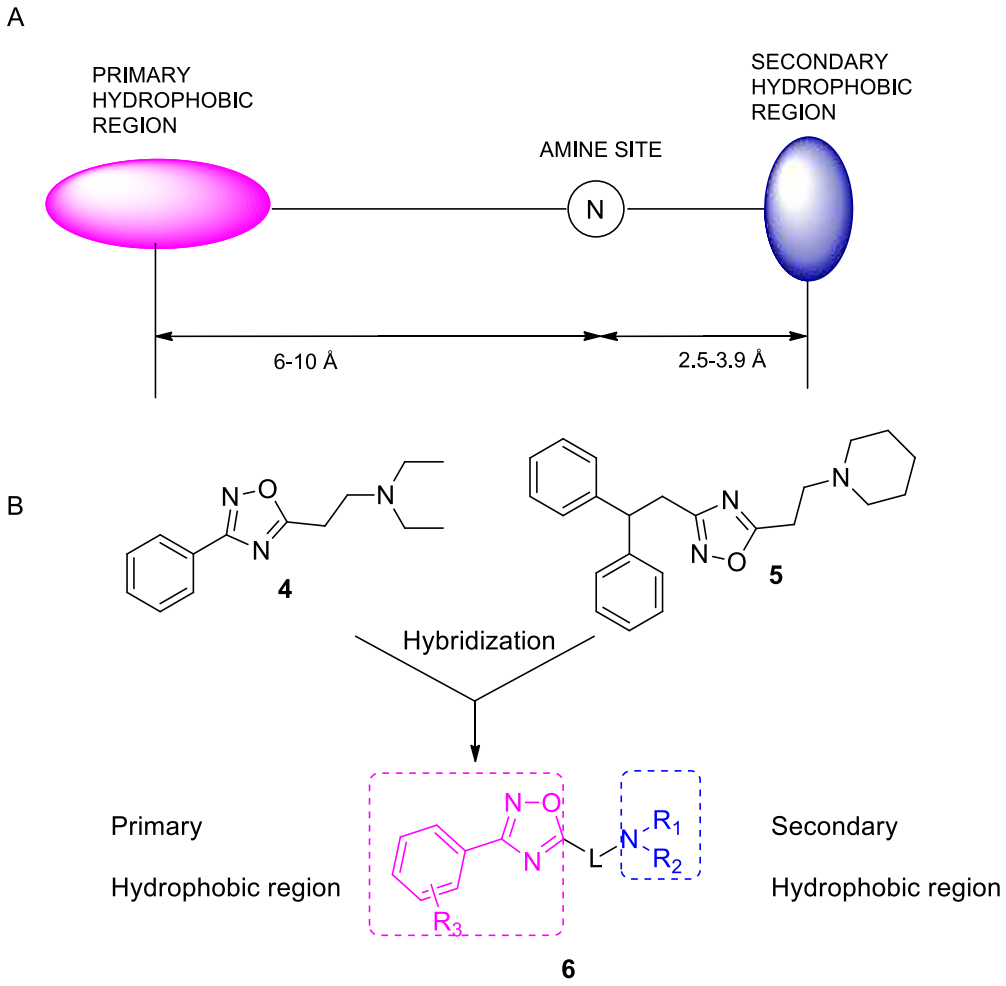


Figure 2. Glennon's pharmacophoric σ_1 receptor model (A) and general structure of phenyl-1,2,4-oxadiazole Derivatives (B)

In a previous study, compounds containing the oxazole scaffold were developed as clinical drugs or candidates, and these have been widely used to treat various types of diseases.[30,31] Oxazole-based derivatives, including isoxazole and oxadiazole exhibit anti-inflammatory, analgesic, antibacterial, and anti-neuropathic properties.[32] Specifically, drugs containing 1,2,4-oxadiazole have various activities, such as oxolamine (**4**) with antitussive and anti-

inflammatory activities, and libexin (**5**) with antitussive activity.[33,34] These findings attracted our attention and prompted us to develop phenyl-1,2,4-oxadiazoles as useful therapeutic agents for treating chronic pain.

A new series of phenyl-1,2,4-oxadiazole derivatives (**6**) was afforded based on the hybridization approach of privileged fragments from compounds **4** and **5.** According to Glennon's σ_1 receptor pharmacophore, the linker elongation between the basic nitrogen atom and the 1,2,4-oxadiazole, as well as the different substituents on the phenyl group of the 1,2,4-oxadiazole, the size of the basic amine core for the "secondary hydrophobic region", were modified and a new series of σ_1 receptor ligands with high σ_1 receptor affinity and selectivity over the σ_2 receptor subtype was obtained (Figure 2B).

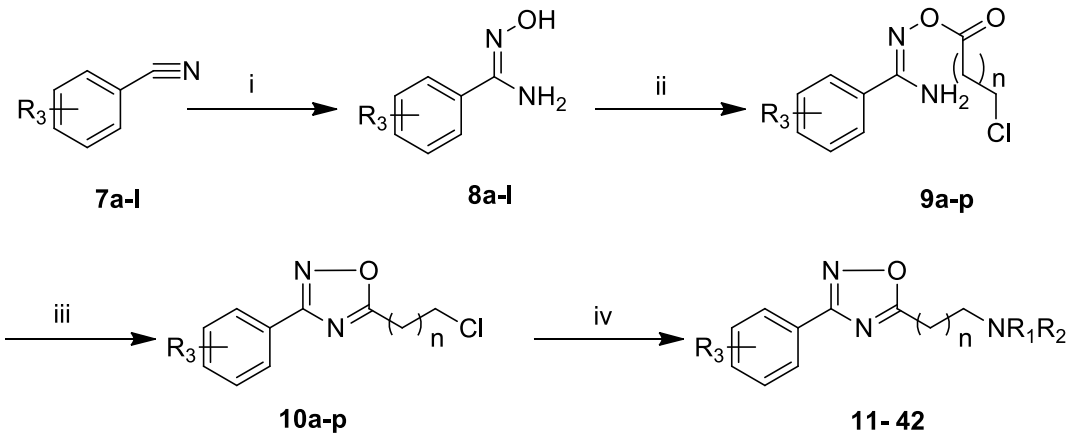
This strategy yielded a series of phenyl-1,2,4-oxadiazole derivatives (Tables 1–4), which were used in competitive receptor-binding assays to determine their relative affinities for the σ_1 and σ_2 receptors and to evaluate their pharmacological efficacy. Among the derivatives prepared, compound **39** exhibited high affinity for the σ_1 receptor and low affinity for the σ_2 receptor. Furthermore, compound **39** exerted dose-dependent antiallodynic effects in a rat formalin-induced pain model and mechanical allodynia in chronic constriction injury (CCI)-induced neuropathic rats. Thus, compound **39** is a σ_1 receptor antagonist with great potential as an analgesic agent for treating neuropathic pain.

Results and Discussion

Chemistry

Synthesis of the final compounds was quite straightforward, as it involved a four-step process starting from commercially available nitrile. [35] As depicted in Scheme **1**, the oxadiazole portion of compound **6** was synthesized from readily available nitrile building block **7a-l**. The nitrile building block was reacted with hydroxylamine hydrochloride and sodium carbonate during reflux in water and EtOH to yield hydroxyamidine **8a-l**. The intermediate hydroxyamidine library **8a-l** was reacted with chloroacyl chloride to provide library **9a-p**, which was cyclized in refluxing toluene to provide the oxadiazole portion of pharmacophore **10a-p**. Library **10a-p** was subsequently reacted with corresponding alkylamines in the presence of potassium carbonate during reflux in acetonitrile to afford final compounds **11**–**42.**

Scheme 1



[a]**Reagents and conditions**: (i) NH_2OH HCl, Na_2CO_3, water and EtOH, 70 °C; (ii) appropriate chloroacyl chloride, acetone, room temperature; (iii) Toluene, reflux; (iv) HNR_1R_2, K_2CO_3, KI, acetonitrile, reflux. Main Text Paragraph.

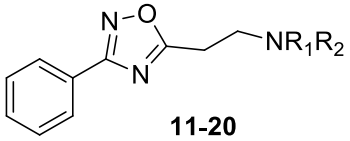
Structure-Activity Relationships

The SAR study around this family, which had been developed based on Glennon's σ_1 receptor pharmacophore,[24,25] indicated that a hydrophobic group was necessary, while an alkylene chain of 2–5 atoms between the basic nitrogen atom and the phenyl-1,2,4-oxadiazole group was optimum to achieve good potency and selectivity. Based on these previous findings, the SARs around the new phenyl-1,2,4-oxadiazoles **6** were developed. All synthesized compounds were evaluated in a primary σ_1 receptor binding assay using [^{3}H]-(+)-pentazocine as the radioligand, while [^{3}H]-di-o-tolylguanidine was used for the σ_2 receptor binding assays (Tables 1–4).

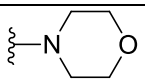
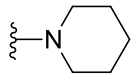
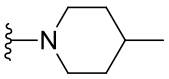
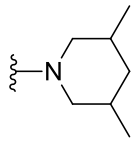
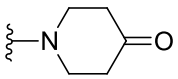
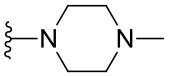
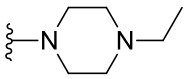
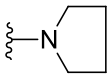
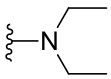
As indicated in Table 1, by maintaining a two carbon alkylene chain in the new σ_1 receptor ligand, our initial investigation focused on the effect of various basic amino moieties (Table 1, compounds **11**–**20**). Because S1RA contained a morpholine group and exhibited excellent affinity to the σ_1 receptor with high selectivity for the σ_2 receptor, the morpholine group was introduced and yielded compound **11** with moderate affinity to the σ_1 receptor (K_i σ_1 = 18.5 ± 2.1 nM) but weak affinity to the σ_2 receptor (K_i σ_2 = 112.5 ± 20.1 nM), compared to S1RA. Changing the morpholine to a piperidine group provided compound **12** (K_i σ_1 = 8.8 ± 1.5 nM, σ_2 = 187.4 ± 19.5 nM), which had better affinity than **11,** and was more selective (21.2 vs > 6.7 ratio). Affinity for the σ_1 receptor and selectivity to the σ_2 receptor was maintained when 4-methylpiperidine (**13**) was introduced, compared to compound **12**. Activity was impaired when 4-methylpiperidine was replaced with 3,5-dimethylpiperidine (**14**). The introduction of the 4-oxopiperidine (**15**) group resulted in significantly reduced affinity to both receptors (K_i σ_1 = 78.9 ± 11.3 nM, σ_2 = 121.4 ± 14.2 nM). Piperazine substituted with a methyl group (**16**) maintained

the affinities to both receptors, while an ethyl group (**17**) failed to improve either receptor, compared with compound **11**. Other amino-containing groups were introduced, the smaller pyrolidine (**18**) derivative or open-chain amines (**19** and **20**), but these failed to provide active and selective compounds.

Table 1. Binding Affinities for the σ_1 and σ_2 Receptor of Compounds **11 – 20**



11-20

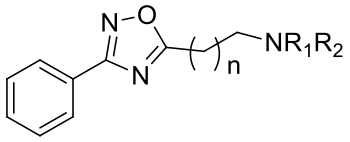
Compd	NR_1R_2	K_i (nM)		Selectivity
		σ_1 [a]	σ_2 [b]	(σ_2/σ_1)
11		18.5±2.1	112.5±20.1	6.1
12		8.8±1.5	187.4±19.5	21.2
13		9.9±1.7	168.2±15.6	16.9
14		23.4±3.2	182.1±24.8	7.8
15		78.9±11.3	121.4±14.2	1.5
16		18.3±2.2	176.2±14.5	9.6
17		34.6±3.4	262.0±36.7	7.6
18		53.3±6.8	250.8±34.2	4.7
19		102.3±13.3	890.1±90.5	8.7
20		88.2±12.6	458.2±54.3	5.2
S1RA		11.3 ±0.7	>2000	----

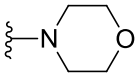
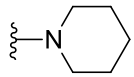
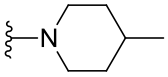
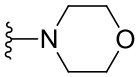
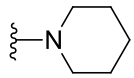
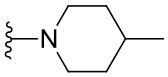
[a]Affinities were determined in guinea pig brain using [^{3}H]-(+)-pentazocine. [b]Affinities were determined in guinea pig brain using [^{3}H]-DTG in the presence of (+)-SKF-10047 to block sigma-1 receptors. [c]The values are means K_i ± SEM of three experiments performed in duplicate.

Different affinities and selectivity for the two receptors were observed when longer linkers were introduced. Compound **21** contained the morpholine moiety for the three-unit linker, which was detrimental to activity, while the compounds with piperidine (**22**) or 4-methylpiperidine (**23**) displayed good affinity for the σ_1 receptor (K_i < 5 nM) and high selectivity to the σ_2 receptor (σ_2/σ_1 > 30). Activity and selectivity were reduced in all cases when the linker chain was extended to four carbon atoms, regardless of the basic amine (**24–26**). As shown in Table 2, compound **22** bearing piperidine with three-unit linkers had the best affinity for the σ_1 receptor (K_i = 4.3 ± 0.3 nM) and the highest selectivity to the σ_2 receptor (σ_2/σ_1 = 45.6). The piperidine group with a three-unit linker emerged as the best moiety in terms of affinity and selectivity and was selected for further exploration of the SARs around **6** (Table 3). Different aromatic groups were modified on the oxadiazole ring. Compounds with naphthyl and methylphenyl groups decreased σ_1 receptor affinity with no improvement in selectivity. Halogen atoms were introduced on the phenyl ring, and both activity and selectivity were greatly improved with the trend: 4-chloro (**29**) < 4-fluoro (**30**) < 2,3-dichloro (**31**) < 2,4-dichloro (**32**). Compound **32** with a 2,4-dichoro substitution exhibited good activity (K_i = 1. 1 ± 0.1 nM) and selectivity (146-fold), whereas the other chlorine substitution patterns (**33**–**35**) led to a decrease in activity to the σ_1 receptor and selectivity to

the σ_2 receptor; only compound **35** retained selectivity (84.3-fold), albeit less than compound **32**. Changing the 2,4-dichloro for 3-chloro-4-fluoro or 3,4-difluoro substituents afforded compounds **36** and **37**, which impaired activity and selectivity.

Table 2. Binding Affinities for the σ_1 and σ_2 Receptor of Compounds **21 - 26**

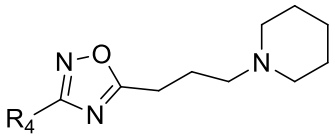


Compd	n	NR_1R_2	K_i (nM)		Selectivity (σ_2/σ_1)
			σ_1 [a]	σ_2 [b]	
21	2		25.3±2.7[c]	174.3±10.9	6.9
22	2		4.3±0.3	196.2±21.1	45.6
23	2		4.6±0.2	156.7±12.8	34.1
24	3		46.3±5.8	522.6±54.2	11.3
25	3		39.8±4.5	712.1±81.7	17.9
26	3		76.4±11.4	635.3±75.2	8.3

[a]Affinities were determined in guinea pig brain using [^{3}H]-(+)-pentazocine. [b]Affinities were determined in guinea pig brain using [^{3}H]-DTG in the presence of (+)-SKF-10047 to block sigma-1 receptors. [c]The values are means K_i ± SEM of three experiments performed in duplicate.

In the next SAR investigation, we retained 2,4-dichlorophenyl-1,2,4-oxadiazole and the basic amino group, and the effects of different linkers were determined (Table **4**). Introduction of the four-carbon chain compound **38** bearing a morpholine group produced poorly activity and selectivity, while the piperidinyl derivatives **39** and **40** exhibited more activity and selectivity than morpholinyl counterpart **38**. The piperidinyl counterpart **39** with four methylene groups provided the highest activity to the σ_1 receptor (K_i = 0.28 ± 0.4 nM) and excellent selectivity to the σ_2 receptor (587.1-fold). Increasing the distance between the 2,4-dichlorophenyl-1,2,4-oxadiazole scaffold and the basic amino group to five methylene groups provided low-activity derivatives (**41** and **42**), with less selectivity for the σ_2 receptor.

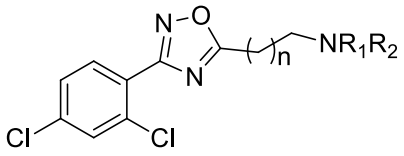
Table 3. Binding Affinities for the σ_1 and σ_2 Receptor of Compounds **27** - **37**

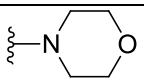
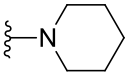
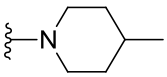
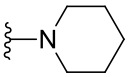
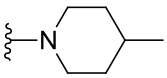


Compd	R_4	K_i (nM)		Selectivity
		σ_1 [a]	σ_2 [b]	(σ_2/σ_1)
27	2-naphthyl	14.2±1.1[c]	212.4±21.0	14.9
28	4-methylphenyl	46.3±15.2	142.9±15.3	3.1
29	4-chlorophenyl	11.2±1.3	91.4±12.1	8.2
30	4-fluorophenyl	9.8±1.4	114.2±15.2	11.7
31	2,3-dichlorophenyl	2.5±0.3	135.5±21.8	54.2
32	2,4-dichlorophenyl	1.1±0.1	175.2±20.2	146
33	2,5-dichlorophenyl	3.4±0.4	198.1±22.5	58.3
34	3,5-dichlorophenyl	3.6±0.3	186.9±20.1	51.9
35	3,4-dichlorophenyl	2.6±0.2	219.2±31.3	84.3
36	3-chloro-4-fluorophenyl	10.5±1.1	198.8±11.2	18.9
37	3,4-difluorophenyl	9.9±1.3	102.3±13.6	10.3

[a]Affinities were determined in guinea pig brain using [^{3}H]-(+)-pentazocine. [b]Affinities were determined in guinea pig brain using [^{3}H]-DTG in the presence of (+)-SKF-10047 to block sigma-1 receptors. [c]The values are means K_i ± SEM of three experiments performed in duplicate.

Table 4. Binding Affinities for the σ_1 and σ_2 Receptor of Compounds **38** - **42**



Compd	n	NR_1R_2	K_i (nM)		Selectivity
			σ_1 [a]	σ_2 [b]	(σ_2/σ_1)
38	3		11.4±1.2[c]	174.1±19.5	15.3
39	3		0.28±0.04	164.4±18.2	587.1
40	3		1.2±0.2	156.8±16.8	130.7
41	4		33.7±4.5	232.7±26.9	6.9
42	4		48.3±7.4	227.5±25.2	4.7

[a]Affinities were determined in guinea pig brain using [^{3}H]-(+)-pentazocine. [b]Affinities were determined in guinea pig brain using [^{3}H]-DTG in the presence of (+)-SKF-10047 to block sigma-1 receptors. [c]The values are means K_i ± SEM of three experiments performed in duplicate.

The SARs in Tables 1–4 indicate that the halogen substituents on the phenyl group of the phenyl-1,2,4-oxadiazoles are crucial for activity and affinity to the σ_1 receptor over the σ_2 receptor. The piperidinyl derivative **39,** with 2,4-dichlorophenyl-1,2,4-oxadiazole, exhibited higher σ_1 receptor affinity (K_i = 0.28 ± 0.04 nM) with more selectivity for the σ_1 receptor (587.1-fold). This compound was chosen to evaluate the histamine receptors (H_3 and H_1 receptors), which play a potential role processing pain.[36] As Table **5** shows, compound **39** had moderate activity to the H_3 receptor (K_i = 23.2 ± 2.4 nM) with poor

H_1 receptor affinity (K_i > 1000 nM). Furthermore, a broad-range screening study indicated that compound **39** had low activity toward other CNS neurotransmitter receptors and transporters associated with pain.

Table 5 Binding affinities for the additional receptors of compound **39**

Receptor	K_i (nM)
H_3	23.2±2.4[a]
H_1	> 1000
D_1	> 1000
5-HT_7	> 1000
5-HT_{1A}	> 1000
5-HT_{2A}	> 1000
5-HT transporter	> 1000
Noradrenaline transporter	> 1000
Dopamine transporter	> 1000
μ-opiod	> 1000
NMDA	> 1000

[a]The values are means K_i ± SEM of three experiments performed in duplicate.

Next, based on its excellent *in vitro* profile, compound **39** was chosen for administration in rats to determine the acute toxicity. Compound **39** displayed a good safety profile at the dose of 250-1000 mg/kg, with the LD_{50} values of 956.92 mg/kg (749.3-1151.2, 95% Confidence limits).

In a previous report, oxazole-based compounds displayed versatile biological activities, and 1,2,4-oxadiazole compounds exhibited good anti-inflammatory and analgesic activities.[30–33] Compound **39,** bearing the 1,2,4-oxadiazole scaffold, exhibited promising affinity toward the σ_1 receptor with an excellent selectivity profile over other receptors and transporters. This compound has been selected to evaluate the anti-allodynic activity in the rat formalin test and the CCI pain model.[37,38] In the formalin model, compound **39** was inactive during the early phase (phase I) of pain elicited by an intraplantar injection of formalin (results not shown). A 50 mg/kg dose of the compound had potency similar to **S1RA** in reducing phase II flinching behavior from 478 ± 54 s to 404 ± 57 s. Compound **39** dose-dependently reduced the number of phase II flinches at doses of 25–100 mg/kg, with the 100 mg/kg dose producing a 60.2% decrease in flinching. These results indicate that compound **39** produced potential antinociception during phase II of the formalin test, with an ED_{50} value of 52.5 ± 6.2 mg/kg.

Compound **39** was also active in the CCI rat model, which is a widely used rodent model of neuropathic pain.[39,40] In this model, the sciatic nerve is loosely ligated between the ischial notch and the popliteal fossa, and the hind limbs can be readily probed with appropriate stimuli. In this study, hyperalgesia to mechanical stimuli was used as an outcome measure of neuropathic pain in the von Frey test. As **Figure 4** shows, rats that underwent the sham-operation exhibited no significant changes compared with normal rats. The withdrawal threshold in the control group (16.0 ± 2.6) was significantly reduced 14 days following CCI, suggesting mechanical allodynia, compared with the normal and sham groups (43.2 ± 6.9 and 45.7 ± 4.1, respectively). Mechanical hypersensitivity in response to von Frey hair stimulation was significantly attenuated following administration of compound **39** at doses of 50 and 100 mg/kg, respectively. Compound **39** led to a dose-dependent decrease in the withdrawal threshold, and the values of % maximal possible effect increased to 44.7% at a dose of 100 mg/kg, indicating that compound **39** exhibits a potential anti-nociceptive effect for the neuropathic pain induced by CCI.

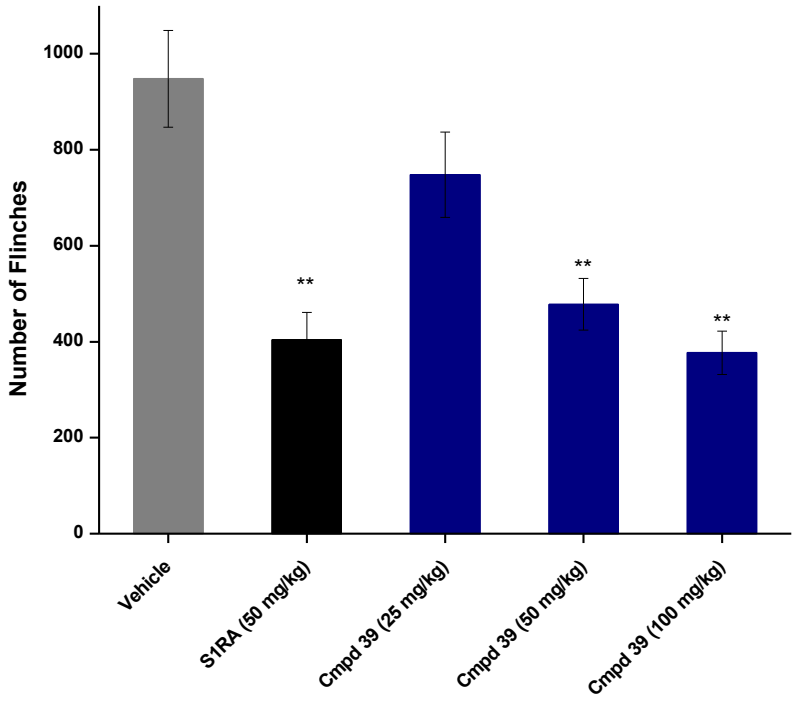


Figure 3. S1RA and compound **39** reduces formalin-induced flinching behavior during phase II of the formalin response. Each column and vertical line represents mean ±SEM of the values obtained in at least 8 animals. Statistically significant differences: * $p < 0.05$, ** $p < 0.01$ *vs* vehicle (Two-Way ANOVA followed by Newman–Keuls test)

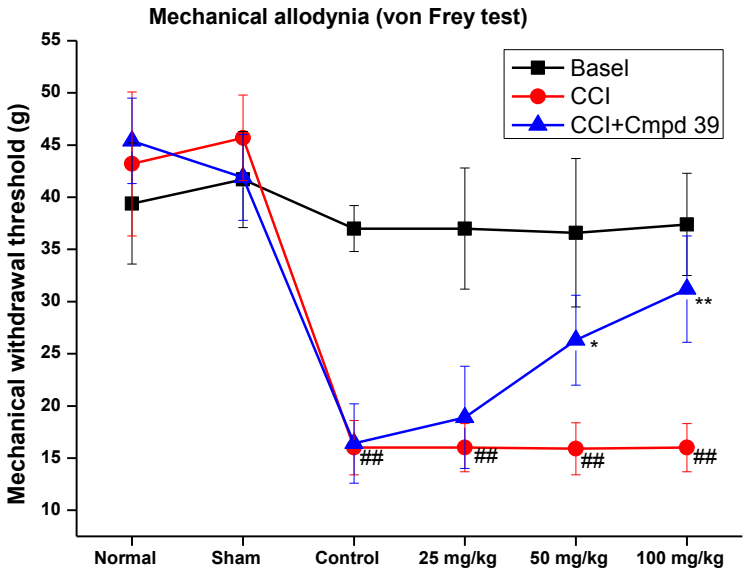


Figure 4. Data represent mean ± standard deviation. n = 8; normal: normal group, no CCI, no Cmpd **39**; Sham: operation to explore the sciatic nerve without any ligation; control: control group, CCI without Cmpd **39**; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with sham; $^{*}p < 0.05$, $^{**}p < 0.01$ compared with control on day 15. MPE% = $(Value_{CCI+Cmpd\ 39} - Value_{CCI}) * 100 / (50 - Value_{CCI})$.

Conclusions

In the present study, a series of phenyl-1,2,4-oxadiazole deviates were synthesized based on the Glennon's σ_1 receptor pharmacophore. The *in vitro* results indicated that these structural modifications had little impact on activity of the σ_1 receptor in contrast to the σ_2 receptor. The most potent compound 39 exhibited its highest σ_1 receptor affinity with the best selectivity profile for the σ_2 receptor. Furthermore, the selective profile over a panel of receptors and transporters indicated that compound 39 exhibited good H_3 receptor affinity, while showing low affinity toward other receptors and transporters. The compound 39 derivative exhibited potent antinociceptive properties during both the formalin flinch and mechanical allodynia in the rat CCI model. Compound 39 may be a promising candidate and useful to develop drugs for chronic pain.

Experimental Section

Chemistry

Reagents, starting materials, and anhydrous solvents were purchased from commercial suppliers and were used as received. Reaction courses were monitored by thin-layer chromatography (TLC) on pre-coated silica GF254 glass sheets, and the spots were visualized under UV light. ^{1}H-NMR spectra were recorded on a Bruker instrument in $CDCl_3$, using tetramethylsilane as the internal reference, operating at 400 MHz. ESI-MS spectra were obtained with a Agilent 6120 Quadrupole LC/MS mass spectrometer with mobile phases as methanol and water containing 0.2% formic acid. HPLC analysis was performed using an SHIMADU CBM-20A system with Agilent Eclipse Plus C18 column and detected at 254 nm wavelength, and all final test compounds were ≥ 95% purity. Column chromatography was performed using silica gel (200-300 mesh). All yields are unoptimized and generally represent.

General Procedures for the Preparation of Intermediates **8**. [35]

TN'-hydroxybenzimidamide (**8a**). Benzonitrile (10.3 g, 100 mmol) and hydroxylamine hydrochloride (13.8 g, 200 mmol) were dissolved in water (50 mL) and EtOH 150 mL. A solution of sodium carbonate (21.2 g, 200 mmol) was cautiously added, and the resulting solution was stirred and heated at 70 °C for 8 h. The solution was cooled and concentrated, and then added 200 mL saturated sodium chloride and extracted with EtOAc (3 x 100 ml). The organic phase was dried by MgSO4 and evaporated. The residue product was recrystallized from ethyl acetate to give the compound **8a** as a white solid (11.2 g, 82.3%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (t, *J* = 8.5 Hz, 2H), 7.46 – 7.40 (m, 3H), 5.00 (s, 2H), a signal for the OH-proton is not visible. MS (ESI) m/z 137.2 (calcd 137.1 for $C_7H_9N_2O^+$ $[M+H]^+$).

N'-hydroxy-2-naphthimidamide (**8b**). White solid (83.1%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.96 (d, J = 8.6 Hz, 1H), 7.90 (s, 1H), 7.74 (d, J = 6.4 Hz, 1H), 7.60 – 7.52 (m, 2H), 7.46 (s, 1H), 5.02 (s, 2H), a signal for the OH-proton is not visible. MS (ESI) m/z 187.2 (calcd 187.1 for $C_{11}H_{11}N_2O^+$ $[M+H]^+$).

N'-hydroxy-4-methylbenzimidamide (**8c**). White solid (82.0%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, J = 8.2 Hz, 2H), 7.28 (d, J = 8.2 Hz, 2H), 5.01 (s, 2H), 2.41 (s, 3H), a signal for the OH-proton is not visible. MS (ESI) m/z 151.2 (calcd 151.1 for $C_8H_{11}N_2O^+$ $[M+H]^+$).

4-chloro-N'-hydroxybenzimidamide (**8d**). White solid (84.1%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, J = 8.0 Hz, 2H), 7.50 – 7.44 (m, 2H), 5.00 (s, 2H), a signal for the OH-proton is not visible. MS (ESI) m/z 171.1 (calcd 171.0 for $C_7H_8ClN_2O^+$ $[M+H]^+$)

4-fluoro-N'-hydroxybenzimidamide (**8e**). White solid (84.1%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, J=7.80 Hz, 2H), 7.54 (d, J=8.40 Hz, 2H), 4.98 (s, 2H), a signal for the OH-proton is not visible. MS (ESI) m/z 155.2 (calcd 155.1 for $C_7H_8FN_2O^+$ $[M+H]^+$)

2,3-dichloro-N'-hydroxybenzimidamide (**8f**). White solid (82.3%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (d, *J* = 7.8 Hz, 1H), 7.66 (d, *J* = 8.0 Hz, 1H), 7.38 (t, *J* = 7.9 Hz, 1H), 5.00 (s, 2H), a signal for the OH-proton is not visible. MS (ESI) m/z 205.1 (calcd 205.0 for $C_7H_7Cl_2N_2O^+$ $[M+H]^+$)

2,4-dichloro-N'-hydroxybenzimidamide (**8g**). White solid (84.2%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 – 7.48 (m, 2H), 7.34 (dd, *J* = 8.4, 1.9 Hz, 1H), 4.98 (s, 2H), a signal for the OH-proton is not visible. MS (ESI) m/z 205.1 (calcd 205.0 for $C_7H_7Cl_2N_2O^+$ $[M+H]^+$)

2,5-dichloro-N'-hydroxybenzimidamide (**8h**). White solid (83.7%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 2.4 Hz, 1H), 7.56 (d, *J* = 8.6 Hz, 1H), 7.42 (dd, *J* = 8.6, 2.5 Hz, 1H), 5.02 (s, 2H), a signal for the OH-proton is not visible. MS (ESI) m/z 205.1 (calcd 205.0 for $C_7H_7Cl_2N_2O^+$ $[M+H]^+$)

3,5-dichloro-N'-hydroxybenzimidamide (**8i**). White solid (85.1%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (s, 2H), 7.54 (s, 1H), 5.01 (s, 2H), a signal for the OH-proton is not visible. MS (ESI) m/z 205.1 (calcd 205.0 for $C_7H_7Cl_2N_2O^+$ $[M+H]^+$)

3,4-dichloro-N'-hydroxybenzimidamide (**8j**). White solid (80.4%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, J = 2.4 Hz, 1H), 7.68 (dd, J = 8.0, 2.4 Hz, 1H), 7.56 (d, J = 8.8 Hz, 1H), 5.00 (s, 2H), a signal for the OH-proton is not visible. MS (ESI) m/z 205.1 (calcd 205.0 for $C_7H_7Cl_2N_2O^+$ $[M+H]^+$)

3-chloro-4-fluoro-N'-hydroxybenzimidamide (**8k**). White solid (82.5%). 1H NMR (400 MHz, $CDCl_3$) δ 7.80 (dd, *J* = 6.9, 1.9 Hz, 1H), 7.61 (ddd, *J* = 7.9, 4.3, 2.1 Hz, 1H), 7.18 (t, *J* = 8.6 Hz, 1H), 4.98 (s, 2H), a signal for the OH-proton is not visible. MS (ESI) m/z 189.1 (calcd 189.0 for $C_7H_7ClFN_2O^+$ $[M+H]^+$)

3,4-difluoro-N'-hydroxybenzimidamide (**8l**). White solid (81.8%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 – 7.54 (m, 1H), 7.50 – 7.40 (m, 1H), 7.24 (dd, *J* = 17.7, 8.6 Hz, 1H), 5.00 (s, 2H), a signal for the OH-proton is not visible. MS (ESI) m/z 173.2 (calcd 173.1 for $C_7H_7F_2N_2O^+$ $[M+H]^+$)

General Procedures for the Preparation of Intermediates **9**. [35]

N'-((3-chloropropanoyl)oxy)benzimidamide (**9a**). To a solution of N-hydroxybenzamidine (5.0 g, 36.5 mmol) in acetone (200 ml), 3-chloropropanoyl chloride (4.7 g, 37.0 mmol) was added slowly and the mixture was stirred for 4 h at room temperature. Acetone was evaporated and the residue was washed with 50 mL sat. $NaHCO_3$ and then 100 mL water. The compound **9a** was dried and obtained as a white solid (6.9 g, 83.6 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (t, *J* = 8.5 Hz, 2H), 7.46 – 7.40 (m, 3H), 5.24 (s, 2H), 3.80 (t, *J* = 6.0 Hz, 2H), 2.90 (t, *J* = 6.0 Hz, 2H). MS (ESI) m/z 227.2 (calcd 227.1 for $C_{10}H_{12}ClN_2O_2^+$ $[M+H]^+$)

N'-((4-chlorobutanoyl)oxy)benzimidamide (**9b**). White solid (82.0 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (t, *J* = 8.5 Hz, 2H), 7.43 (ddd, *J* = 14.3, 12.1, 7.6 Hz, 3H), 5.23 (s, 2H), 3.65 (dd, *J* = 10.6, 4.6 Hz, 2H), 2.79 – 2.55 (m, 2H), 2.27 – 2.07 (m, 2H). MS (ESI) m/z 241.2 (calcd 241.1 for $C_{10}H_{14}ClN_2O_2^+$ $[M+H]^+$)

N'-((5-chloropentanoyl)oxy)benzimidamide (**9c**). White solid (80.1 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (t, *J* = 8.5 Hz, 2H), 7.45 – 7.38 (m, 3H), 5.20 (s, 2H), 3.68 – 3.58 (m, 2H), 2.82 – 2.66 (m, 2H), 2.27 – 2.11 (m, 2H), 1.83 – 1.67 (m, 2H). MS (ESI) m/z 255.2 (calcd 255.1 for $C_{10}H_{16}ClN_2O_2^+$ $[M+H]^+$)

N'-((4-chlorobutanoyl)oxy)-2-naphthimidamide (**9d**). White solid (82.4 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.94 (d, J = 8.6 Hz, 1H), 7.89 (s, 1H), 7.73 (d, J = 6.4 Hz, 1H), 7.57 – 7.52 (m, 2H), 7.46 (s, 1H), 5.20 (s, 2H), 3.67 (t, *J* = 6.3 Hz, 2H), 2.90 (t, *J* = 7.4 Hz, 2H), 2.24 – 2.00 (m, 2H) MS (ESI) m/z 291.2 (calcd 291.1 for $C_{15}H_{16}ClN_2O_2^+$ $[M+H]^+$)

N'-((4-chlorobutanoyl)oxy)-4-methylbenzimidamide (**9e**). White solid (81.2 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 8.2 Hz, 2H), 7.28 (d, J = 8.2 Hz, 2H), 5.23 (s, 2H), 3.66 – 3.40 (m, 2H), 2.78 – 2.54 (m, 2H), 2.41 (s, 3H), 2.28 – 2.10 (m, 2H). MS (ESI) m/z 255.2 (calcd 255.1 for $C_{12}H_{16}ClN_2O_2^+$ $[M+H]^+$)

4-chloro-N'-((4-chlorobutanoyl)oxy)benzimidamide (**9f**) White solid (81.2 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, J = 8.0 Hz, 2H), 7.47 – 7.43 (m, 2H), 5.20 (s, 2H), 3.70 (t, *J* = 6.0 Hz, 2H), 2.88 (t, *J* = 7.2 Hz, 2H), 2.48 – 2.24 (m, 2H). MS (ESI) m/z 275.1 (calcd 275.0 for $C_{11}H_{13}Cl_2N_2O_2^+$ $[M+H]^+$)

N'-((4-chlorobutanoyl)oxy)-4-fluorobenzimidamide (**9g**). White solid (80.2 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, J = 8.0 Hz, 2H), 7.47 – 7.43 (m, 2H), 5.23 (s, 2H), 3.68 (t, *J* = 6.0 Hz, 2H), 2.84 (t, *J* = 7.2 Hz, 2H), 2.36 – 2.20 (m, 2H). MS (ESI) m/z 259.2 (calcd 259.1 for $C_{11}H_{13}ClFN_2O_2^+$ $[M+H]^+$)

2,3-dichloro-N'-((4-chlorobutanoyl)oxy)benzimidamide (**9h**). White solid (82.1 %). 1H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J* = 7.8 Hz, 1H), 7.62 (d, *J* = 8.0 Hz, 1H), 7.34 (t, *J* = 7.9 Hz, 1H), 5.23 (s, 2H), 3.70 (t, *J* = 6.2 Hz, 2H), 3.22 (t, *J* = 7.3 Hz, 2H), 2.44 – 2.26 (m, 2H). MS (ESI) m/z 309.1 (calcd 309.0 for $C_{11}H_{12}Cl_3N_2O_2^+$ $[M+H]^+$)

2,4-dichloro-N'-((4-chlorobutanoyl)oxy)benzimidamide (**9i**). White solid (83.2 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, *J* = 8.3 Hz, 1H), 7.48 (s, 1H), 7.35 (t, *J* = 10.1 Hz, 1H), 5.19 (s, 2H), 3.69 (t, *J* = 6.2 Hz, 2H), 2.73 (t, *J* = 7.1 Hz, 2H), 2.22 (p, *J* = 6.7 Hz, 2H).MS (ESI) m/z 309.1 (calcd 309.0 for $C_{11}H_{12}Cl_3N_2O_2^+$ $[M+H]^+$)

2,5-dichloro-N'-((4-chlorobutanoyl)oxy)benzimidamide (**9j**). White solid (84.3%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, *J* = 2.4 Hz, 1H), 7.50 (d, *J* = 8.6 Hz, 1H), 7.40 (dd, *J* = 8.6, 2.5 Hz, 1H), 5.22 (s, 2H), 3.70 (t, *J* = 6.2 Hz, 2H), 3.20 (t, *J* = 7.3 Hz, 2H), 2.48 – 2.26 (m, 2H). MS (ESI) m/z 309.1 (calcd 309.0 for $C_{11}H_{12}Cl_3N_2O_2^+$ [M+H]$^+$)

3,5-dichloro-N'-((4-chlorobutanoyl)oxy)benzimidamide (**9k**). White solid (83.1%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (s, 2H), 7.50 (s, 1H), 5.21 (s, 2H), 3.73 (t, *J* = 6.2 Hz, 2H), 3.68 (t, *J* = 7.3 Hz, 2H), 2.46 – 2.20 (m, 2H). MS (ESI) m/z 309.1 (calcd 309.0 for $C_{11}H_{12}Cl_3N_2O_2^+$ [M+H]$^+$)

3,4-dichloro-N'-((4-chlorobutanoyl)oxy)benzimidamide (**9l**). White solid (82.3%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, J = 2.4 Hz, 1H), 7.66 (dd, J = 8.0, 2.4 Hz, 1H), 7.54 (d, J = 8.8 Hz, 1H), 5.20 (s, 2H), 3.68 (t, J = 7.2 Hz, 2H), 3.16 (t, J = 6.4 Hz, 2H), 2.52–2.34 (m, 2H). MS (ESI) m/z 309.1 (calcd 309.0 for $C_{11}H_{12}Cl_3N_2O_2^+$ [M+H]$^+$)

3-chloro-N'-((4-chlorobutanoyl)oxy)-4-fluorobenzimidamide (**9m**). White solid (84.0%).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (dd, *J* = 6.9, 1.9 Hz, 1H), 7.58 (ddd, *J* = 7.9, 4.3, 2.1 Hz, 1H), 7.17 (t, *J* = 8.6 Hz, 1H), 5.23 (s, 2H), 3.68 (t, *J* = 6.2 Hz, 2H), 2.70 (t, *J* = 7.1 Hz, 2H), 2.20 (p, *J* = 6.7 Hz, 2H). MS (ESI) m/z 293.1 (calcd 293.0 for $C_{11}H_{12}Cl_2FN_2O_2^+$ [M+H]$^+$)

N'-((4-chlorobutanoyl)oxy)-3,4-difluorobenzimidamide (**9n**). White solid (83.6%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 – 7.51 (m, 1H), 7.50 – 7.39 (m, 1H), 7.23 (dd, *J* = 17.7, 8.6 Hz, 1H), 5.16 (s, 2H), 3.69 (t, *J* = 6.2 Hz, 2H), 2.72 (t, *J* = 7.1 Hz, 2H), 2.21 (p, *J* = 6.7 Hz, 2H). MS (ESI) m/z 277.2 (calcd 277.1 for $C_{11}H_{12}ClF_2N_2O_2^+$ [M+H]$^+$)

2,4-dichloro-N'-((5-chloropentanoyl)oxy)benzimidamide (**9o**). White solid (82.9%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (d, *J* = 8.3 Hz, 1H), 7.50 (d, *J* = 2.0 Hz, 1H), 7.35 (dd, *J* = 8.3, 2.0 Hz, 1H), 5.22 (s, 2H), 3.63 (ddd, *J* = 6.1, 3.8, 2.0 Hz, 2H), 2.64 – 2.50 (m, 2H), 1.94 (dt, *J* = 4.3, 3.3 Hz, 4H). MS (ESI) m/z 323.1 (calcd 323.0 for $C_{12}H_{14}Cl_3N_2O_2^+$ [M+H]$^+$)

2,4-dichloro-N'-((6-chlorohexanoyl)oxy)benzimidamide (**9p**). White solid (82.9%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, *J* = 8.0 Hz, 1H), 7.48 (s, 1H), 7.36 – 7.18 (m, 1H), 5.22（s, 2H), 3.62 (t, *J* = 5.2 Hz, 2H), 2.60 (t, *J* = 6.0 Hz, 2H), 1.98 – 1.80 (m, 4H), 1.66 – 1.50 (m, 2H). MS (ESI) m/z 337.1 (calcd 337.0 for $C_{13}H_{16}Cl_3N_2O_2^+$ [M+H]$^+$)

General Procedures for the Preparation of Intermediates **10**. [35]

5-(2-chloroethyl)-3-phenyl-1,2,4-oxadiazole (**10a**). N'-((3-chloropropanoyl)oxy) benzimidamide (4.5 g, 20 mmol) in toluene (150 ml) was refluxed for 12 h with water of reaction being removed by means of a Dean-Stark water trap. Upon cooling to room temperature, the reaction mixture was concentrated under vacuum to provide a crude residue. The crude residue was purified by chromatography (petroleum ether: EtOAc = 4: 1) to afford 10 as a pale yellow oil (3.5 g, 83.7 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, J = 7.2 Hz, 2H), 7.52 (t, J = 6.0 Hz, 3H), 3.86 (t, J = 6.0 Hz, 2H), 3.30 (t, J = 7.2 Hz, 2H). MS (ESI) m/z 209.1 (calcd 209.0 for $C_{10}H_{10}ClN_2O^+$ [M+H]$^+$)

5-(3-chloropropyl)-3-phenyl-1,2,4-oxadiazole (**10b**). Pale yellow oil (83.0 %).^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, J = 7.5 Hz, 2H), 7.52 (t, J = 6.2 Hz, 3H), 3.74 (t, J = 6.2 Hz, 2H), 3.17 (t, J = 7.3 Hz, 2H), 2.56 – 2.19 (m, 2H). MS (ESI) m/z 223.2 (calcd 223.1 for $C_{11}H_{12}ClN_2O^+$ [M+H]$^+$)

5-(4-chlorobutyl)-3-phenyl-1,2,4-oxadiazole (**10c**). Pale yellow oil (81.3 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 – 8.01 (m, 2H), 7.51 (d, *J* = 6.7 Hz, 3H), 3.63 (t, *J* = 6.3 Hz, 2H), 3.02 (t, *J* = 7.4 Hz, 2H), 2.23 – 2.02 (m, 2H), 2.02 – 1.88 (m, 2H). MS (ESI) m/z 237.2 (calcd 237.1 for $C_{12}H_{14}ClN_2O^+$ [M+H]$^+$)

5-(3-chloropropyl)-3-(naphthalen-2-yl)-1,2,4-oxadiazole (**10d**). Pale yellow oil (82.1 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.94 (d, J = 8.6 Hz, 1H), 7.89 (s, 1H), 7.73 (d, J = 6.4 Hz, 1H), 7.57 – 7.52 (m, 2H), 7.46 (s, 1H), 3.64 (t, *J* = 6.3 Hz, 2H), 2.92 (t, *J* = 7.4 Hz, 2H), 2.21 – 2.00 (m, 2H). MS (ESI) m/z 273.2 (calcd 273.1 for $C_{15}H_{14}ClN_2O^+$ [M+H]$^+$)

5-(3-chloropropyl)-3-(p-tolyl)-1,2,4-oxadiazole (**10e**). Pale yellow oil (84.0 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, J = 8.0 Hz, 2H), 7.27 (d, J = 8.0 Hz, 2H), 3.63 (t, *J* = 6.3 Hz, 2H), 2.90 (t, *J* = 7.4 Hz, 2H), 2.42 (s, 3H), 2.24 – 2.02 (m, 2H). MS (ESI) m/z 237.2 (calcd 237.1 for $C_{12}H_{14}ClN_2O^+$ [M+H]$^+$)

3-(4-chlorophenyl)-5-(3-chloropropyl)-1,2,4-oxadiazole (**10f**). Pale yellow oil (84.5 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 8.0 Hz, 2H), 7.47 – 7.43 (m, 2H), 3.68 (t, *J* = 6.0 Hz, 2H), 2.84 (t, *J* = 7.2 Hz, 2H), 2.45 – 2.21 (m, 2H). MS (ESI) m/z 257.1 (calcd 257.0 for $C_{11}H_{11}Cl_2N_2O^+$ [M+H]

5-(3-chloropropyl)-3-(4-fluorophenyl)-1,2,4-oxadiazole (**10g**). Pale yellow oil (84.2 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (t, *J* = 8.4 Hz, 2H), 7.13 (t, *J* = 8.5 Hz, 2H), 3.70 (t, *J* = 6.2 Hz, 2H), 2.74 (t, *J* = 7.1 Hz, 2H), 2.35 – 2.11 (m, 2H). MS (ESI) m/z 241.2 (calcd 241.1 for $C_{11}H_{11}ClFN_2O^+$ [M+H]

5-(3-chloropropyl)-3-(2,3-dichlorophenyl)-1,2,4-oxadiazole (**10h**). Pale yellow oil (83.5 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J* = 7.8 Hz, 1H), 7.62 (d, *J* = 8.0 Hz, 1H), 7.34 (t, *J* = 7.9 Hz, 1H), 3.73 (t, *J* = 6.2 Hz, 2H), 3.20 (t, *J* = 7.3 Hz, 2H), 2.46 – 2.28 (m, 2H). MS (ESI) m/z 291.1 (calcd 291.0 for $C_{11}H_{10}Cl_3N_2O^+$ [M+H]

5-(3-chloropropyl)-3-(2,4-dichlorophenyl)-1,2,4-oxadiazole (**10i**). Pale yellow oil (84.7 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, *J* = 8.4 Hz, 1H), 7.58 (d, *J* = 1.8 Hz, 1H), 7.40 (dd, *J* = 8.4, 1.8 Hz, 1H), 3.74 (t, *J* = 6.2 Hz, 2H), 3.20 (t, *J* = 7.3 Hz, 2H), 2.44 – 2.30 (m, 2H). MS (ESI) m/z 291.1 (calcd 291.0 for $C_{11}H_{10}Cl_3N_2O^+$ [M+H]

5-(3-chloropropyl)-3-(2,5-dichlorophenyl)-1,2,4-oxadiazole (**10j**). Pale yellow oil (85.2 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, *J* = 2.4 Hz, 1H), 7.49 (d, *J* = 8.6 Hz, 1H), 7.42 (dd, *J* = 8.6, 2.5 Hz, 1H), 3.74 (t, *J* = 6.2 Hz, 2H), 3.21 (t, *J* = 7.3 Hz, 2H), 2.49 – 2.29 (m, 2H). MS (ESI) m/z 291.1 (calcd 291.0 for $C_{11}H_{10}Cl_3N_2O^+$ [M+H]

5-(3-chloropropyl)-3-(3,5-dichlorophenyl)-1,2,4-oxadiazole (**10k**). Pale yellow oil (84.9 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (s, 2H), 7.50 (s, 1H), 3.73 (t, *J* = 6.2 Hz, 2H), 3.18 (t, *J* = 7.3 Hz, 2H), 2.49 – 2.25 (m, 2H). MS (ESI) m/z 291.1 (calcd 291.0 for $C_{11}H_{10}Cl_3N_2O^+$ [M+H]

5-(3-chloropropyl)-3-(3,4-dichlorophenyl)-1,2,4-oxadiazole (**10l**). Pale yellow oil (84.0 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, J = 2.4 Hz, 1H), 7.65 (dd, J = 8.0, 2.4 Hz, 1H), 7.52 (d, J = 8.8 Hz, 1H), 3.25 (t, J = 7.2 Hz, 2H), 3.15 (t, J = 6.4 Hz, 2H), 2.50–2.30 (m, 2H). MS (ESI) m/z 291.1 (calcd 291.0 for $C_{11}H_{10}Cl_3N_2O^+$ [M+H]

3-(3-chloro-4-fluorophenyl)-5-(3-chloropropyl)-1,2,4-oxadiazole (**10m**). Pale yellow oil (85.1 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (d, *J* = 7.0 Hz, 1H), 8.05 – 7.90 (m, 1H), 7.26 (t, *J* = 8.5 Hz, 1H), 3.73 (t, *J* = 6.2 Hz, 2H), 3.17 (t, *J* = 7.3 Hz, 2H), 2.49 – 2.27 (m, 2H). MS (ESI) m/z 275.1 (calcd 275.0 for $C_{11}H_{10}Cl_2FN_2O^+$ [M+H]

5-(3-chloropropyl)-3-(3,4-difluorophenyl)-1,2,4-oxadiazole (**10n**). Pale yellow oil (84.3 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 – 7.87 (m, 1H), 7.87 – 7.82 (m, 1H), 7.28 (dd, *J* = 17.9, 8.5 Hz, 1H), 3.73 (t, *J* = 6.2 Hz, 2H), 3.16 (t, *J* = 7.3 Hz, 2H), 2.48 – 2.29 (m, 2H). MS (ESI) m/z 259.1 (calcd 259.0 for $C_{11}H_{10}ClF_2N_2O^+$ [M+H]

5-(4-chlorobutyl)-3-(2,4-dichlorophenyl)-1,2,4-oxadiazole (**10o**). Pale yellow oil (80.3 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, *J* = 8.4 Hz, 1H), 7.59 (d, *J* = 2.0 Hz, 1H), 7.41 (dd, *J* = 8.4, 2.1 Hz, 1H), 3.64 (t, *J* = 6.3 Hz, 2H), 3.06 (t, *J* = 7.4 Hz, 2H), 2.19 – 2.05 (m, 2H), 2.02 – 1.93 (m, 2H). MS (ESI) m/z 305.1 (calcd 305.0 for $C_{12}H_{12}Cl_3N_2O^+$ [M+H]

5-(5-chloropentyl)-3-(2,4-dichlorophenyl)-1,2,4-oxadiazole (**10p**). Pale yellow oil (80.1 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, *J* = 8.4 Hz, 1H), 7.57 (d, *J* = 1.7 Hz, 1H), 7.39 (dd, *J* = 8.4, 1.8 Hz, 1H), 3.66 (t, *J* = 6.3 Hz, 2H), 3.10 (t, *J* = 7.4 Hz, 2H), 2.12 – 2.00 (m, 2H), 1.94 – 1.82 (m, 2H), 1.70 – 1.62 (m, 2H). MS (ESI) m/z 319.1 (calcd 319.0 for $C_{13}H_{14}Cl_3N_2O^+$ [M+H].

General Procedures for the Preparation of Final Compounds **11**-**42**.

4-(2-(3-phenyl-1,2,4-oxadiazol-5-yl)ethyl)morpholine (**11**). 5-(2-chloroethyl)-3-phenyl-1,2,4-oxadiazole (**10a**) (0.63 g, 3 mmol), morpholine (0.29 g, 3.3 mmol), K_2CO_3 (0.46, 3.3 mmol) and catalytic amount of KI in CH_3CN (100 ml) was refluxed refluxed for 8 h. Upon cooling to room temperature, the reaction mixture was concentrated under vacuum to provide a crude residue. The crude residue was purified by chromatography (EtOAc: MeOH = 20: 1) to afford 4-(2-(3-phenyl-1,2,4-oxadiazol-5-yl)ethyl)morpholine (**11**) as a pale yellow oil (0.66 g, 84.6 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (dd, *J* = 7.4, 1.6 Hz, 2H), 7.53 – 7.45 (m, 3H), 3.74 – 3.66 (m, 4H), 3.15 (t, *J* = 7.4 Hz, 2H), 2.92 (t, *J* = 7.4 Hz, 2H), 2.58 – 2.49 (m, 4H). MS (ESI) m/z 260.2 (calcd 260.1 for $C_{14}H_{18}N_3O^+$ $[M+H]^+$)

3-phenyl-5-(2-(piperidin-1-yl)ethyl)-1,2,4-oxadiazole (**12**). Pale yellow oil (83.2 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (dd, *J* = 7.4, 1.6 Hz, 2H), 7.53 – 7.45 (m, 3H), 3.74 – 3.66 (m, 4H), 3.15 (t, *J* = 7.4 Hz, 2H), 2.92 (t, *J* = 7.4 Hz, 2H), 2.58 – 2.49 (m, 4H), 2.12 – 1.99 (m, 2H) . MS (ESI) m/z 258.2 (calcd 258.2 for $C_{15}H_{20}N_3O^+$ $[M+H]^+$)

5-(2-(4-methylpiperidin-1-yl)ethyl)-3-phenyl-1,2,4-oxadiazole (**13**). Pale yellow oil (83.4 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 – 8.03 (m, 2H), 7.57 – 7.43 (m, 3H), 3.16 (t, *J* = 7.7 Hz, 2H), 2.93 (dd, *J* = 14.5, 6.6 Hz, 4H), 2.08 (dd, *J* = 16.5, 6.7 Hz, 2H), 1.65 (d, *J* = 13.1 Hz, 2H), 1.46 – 1.32 (m, 1H), 1.25 (ddd, *J* = 15.4, 12.1, 3.7 Hz, 2H), 0.94 (d, *J* = 6.4 Hz, 3H). MS (ESI) m/z 272.2 (calcd 272.2 for $C_{16}H_{22}N_3O^+$ $[M+H]^+$)

5-(2-(3,5-dimethylpiperidin-1-yl)ethyl)-3-phenyl-1,2,4-oxadiazole (**14**). Pale yellow oil (83.4 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (dd, *J* = 7.3, 1.9 Hz, 2H), 7.52 (t, *J* = 5.2 Hz, 3H), 3.15 (t, *J* = 7.7 Hz, 2H), 2.94 (dd, *J* = 14.5, 6.6 Hz, 2H), 2.08 (dd, *J* = 16.5, 6.7 Hz, 2H), 1.65 (d, *J* = 13.1 Hz, 2H), 1.29 – 1.20 (m, 2H), 0.84 – 72 (d, *J* = 6.4 Hz, 6H), 0.64 – 0.52 (m, 2H). MS (ESI) m/z 286.3 (calcd 286.2 for $C_{17}H_{24}N_3O^+$ $[M+H]^+$)

1-(2-(3-phenyl-1,2,4-oxadiazol-5-yl)ethyl)piperidin-4-one (**15**). Pale yellow oil (84.2 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (dd, *J* = 7.4, 1.6 Hz, 2H), 7.53 – 7.45 (m, 3H), 4.40 – 4.20 (m, 4H), 3.15 (t, *J* = 7.4 Hz, 2H), 2.82 (t, *J* = 7.4 Hz, 2H), 2.52 – 2.46 (m, 4H). MS (ESI) m/z 272.2 (calcd 272.1 for $C_{15}H_{18}N_3O_2^+$ $[M+H]^+$)

5-(2-(4-methylpiperazin-1-yl)ethyl)-3-phenyl-1,2,4-oxadiazole (**16**). Pale yellow oil (81.3 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (dd, *J* = 7.4, 1.6 Hz, 2H), 7.53 – 7.45 (m, 3H), 3.74 – 3.66 (m, 4H), 3.15 (t, *J* = 7.4 Hz, 2H), 2.92 (t, *J* = 7.4 Hz, 2H), 2.64 (s, 3H), 2.58 – 2.49 (m, 4H). MS (ESI) m/z 273.3 (calcd 273.2 for $C_{15}H_{21}N_4O^+$ $[M+H]^+$)

5-(2-(4-ethylpiperazin-1-yl)ethyl)-3-phenyl-1,2,4-oxadiazole (**17**). Pale yellow oil (82.6 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (dd, *J* = 7.4, 1.6 Hz, 2H), 7.54 – 7.48 (m, 3H), 3.76 – 3.68 (m, 4H), 3.18 (t, *J* = 7.4 Hz, 2H), 2.92 (t, *J* = 7.4 Hz, 2H), 2.58 – 2.49 (m, 4H), 2.12–1.90 (m, 2H), 1.14 (t, J = 7.3 Hz, 3H). MS (ESI) m/z 287.3 (calcd 287.2 for $C_{16}H_{23}N_4O^+$ $[M+H]^+$)

3-phenyl-5-(2-(pyrrolidin-1-yl)ethyl)-1,2,4-oxadiazole (**18**). Pale yellow oil (80.1 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (dd, *J* = 7.3, 1.8 Hz, 2H), 7.50 (d, *J* = 6.8 Hz, 3H), 3.19 (t, *J* = 7.6 Hz, 2H), 3.03 (t, *J* = 7.6 Hz, 2H), 2.61 (t, *J* = 5.9 Hz, 4H), 1.82 (dt, *J* = 6.4, 3.0 Hz, 4H). MS (ESI) m/z 244.2 (calcd 244.1 for $C_{14}H_{18}N_3O^+$ $[M+H]^+$)

N,N-dimethyl-2-(3-phenyl-1,2,4-oxadiazol-5-yl)ethanamine (**19**). Pale yellow oil (74.2 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (dd, *J* = 7.4, 1.6 Hz, 2H), 7.53 – 7.45 (m, 3H), 3.15 (t, *J* = 7.4 Hz, 2H), 2.92 (t, *J* = 7.4 Hz, 2H), 2.58 – 2.49 (m, 6H). MS (ESI) m/z 218.2 (calcd 218.1 for $C_{14}H_{16}N_3O^+$ $[M+H]^+$)

N,N-diethyl-2-(3-phenyl-1,2,4-oxadiazol-5-yl)ethanamine (**20**). Pale yellow oil (74.2 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 – 7.94 (m, 2H), 7.58 – 7.38 (m, 3H), 3.11 (t, *J* = 7.5 Hz, 2H), 2.86 (t, *J* = 8.8, 5.3 Hz, 2H), 2.10 – 1.97 (m, 4H), 1.03 (t, *J* = 7.1 Hz, 6H). MS (ESI) m/z 246.3 (calcd 246.2 for $C_{14}H_{20}N_3O^+$ $[M+H]^+$)

4-(3-(3-phenyl-1,2,4-oxadiazol-5-yl)propyl)morpholine (**21**). Pale yellow oil (81.3 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (dd, *J* = 7.3, 1.8 Hz, 2H), 7.50 (d, *J* = 6.8 Hz, 3H), 4.34 (t, *J* = 12.3 Hz, 2H), 4.02 (d, *J* = 11.6 Hz, 2H), 3.53 (d, *J* = 11.7 Hz, 2H), 3.21 (dd, *J* = 16.2, 9.4 Hz, 4H), 2.93 (d, *J* = 10.5 Hz, 2H), 2.73 – 2.52 (m, 2H). MS (ESI) m/z 274.3 (calcd 274.2 for $C_{15}H_{20}N_3O_2^+$ $[M+H]^+$)

3-phenyl-5-(3-(piperidin-1-yl)propyl)-1,2,4-oxadiazole (**22**). Pale yellow oil (82.1 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (dd, *J* = 6.9, 1.6 Hz, 2H), 7.48 (dd, *J* = 9.9, 5.4 Hz, 3H), 3.00 (t, *J* = 7.5 Hz, 2H), 2.54 – 2.19 (m, 6H), 2.07 (p, *J* = 7.3 Hz, 2H), 1.62 – 1.50 (m, 4H), 1.42 (d, *J* = 5.2 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.04, 168.23, 131.06, 128.84, 127.41, 127.02, 58.14, 54.55, 25.99, 24.88, 24.44, 24.03. MS (ESI) m/z 272.2 (calcd 272.2 for $C_{16}H_{22}N_3O^+$ $[M+H]^+$)

5-(3-(4-methylpiperidin-1-yl)propyl)-3-phenyl-1,2,4-oxadiazole (**23**). Pale yellow oil (82.1 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (dd, *J* = 7.3, 1.8 Hz, 2H), 7.52 (d, *J* = 6.8 Hz, 3H), 3.64 (d, *J* = 11.9 Hz, 2H), 3.14 (t, *J* = 6.9 Hz, 4H), 2.77 – 2.63 (m, 2H), 2.59 (dd, *J* = 15.4, 7.6 Hz, 2H), 2.14 – 1.98 (m, 2H), 1.86 (d, *J* = 14.3 Hz, 2H), 1.58 (d, *J* = 73.3 Hz, 4H), 1.06 (t, *J* = 8.7 Hz, 3H). MS (ESI) m/z 286.2 (calcd 286.2 for $C_{17}H_{24}N_3O^+$ $[M+H]^+$)

4-(4-(3-phenyl-1,2,4-oxadiazol-5-yl)butyl)morpholine (**24**). Pale yellow oil (79.3 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (dd, *J* = 7.2, 1.3 Hz, 2H), 7.62 – 7.40 (m, 3H), 3.81 – 3.65 (m, 4H), 3.01 (t, *J* = 7.5 Hz, 2H), 2.43 (dd, *J* = 15.3, 7.9 Hz, 6H), 2.03 – 1.84 (m, 2H), 1.73 – 1.54 (m, 2H). MS (ESI) m/z 288.3 (calcd 288.2 for $C_{16}H_{22}N_3O_2^+$ $[M+H]^+$)

3-phenyl-5-(4-(piperidin-1-yl)butyl)-1,2,4-oxadiazole (**25**). Pale yellow oil (78.1 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (dd, *J* = 7.2, 1.3 Hz, 2H), 7.60 – 7.38 (m, 3H), 3.57 (d, *J* = 11.6 Hz, 2H), 3.02 (dt, *J* = 11.5, 6.1 Hz, 4H), 2.64 (dd, *J* = 21.0, 9.9 Hz, 2H), 2.32 (q, *J* = 13.0 Hz, 2H), 2.18 – 2.05 (m, 2H), 2.02 – 1.85 (m, 5H), 1.42 (dd, *J* = 25.8, 12.8 Hz, 1H). MS (ESI) m/z 286.2 (calcd 286.2 for $C_{17}H_{24}N_3O^+$ $[M+H]^+$)

5-(4-(4-methylpiperidin-1-yl)butyl)-3-phenyl-1,2,4-oxadiazole (**26**). Pale yellow oil (79.5 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (dd, *J* = 7.3, 1.9 Hz, 2H), 7.50 (t, *J* = 5.2 Hz, 3H), 3.16 (t, *J* = 7.7 Hz, 2H), 2.93 (dd, *J* = 14.5, 6.6 Hz, 4H), 2.08 (dd, *J* = 16.5, 6.7 Hz, 2H), 1.92 – 1.80 (m, 2H),1.65 (d, *J* = 13.1 Hz, 2H), 1.46 – 1.31 (m, 3H), 1.25 (ddd, *J* = 15.4, 12.1, 3.7 Hz, 2H), 0.94 (d, *J* = 6.4 Hz, 3H). MS (ESI) m/z 300.3 (calcd 300.2 for $C_{18}H_{26}N_3O^+$ $[M+H]^+$)

3-(naphthalen-2-yl)-5-(3-(piperidin-1-yl)propyl)-1,2,4-oxadiazole (**27**). Pale yellow oil (80.1 %). ^{1}H NMR (400 MHz, DMSO) δ 8.14 (s, 1H), 7.94 (d, J = 8.6 Hz, 1H), 7.89 (s, 1H), 7.73 (d, J = 6.4 Hz, 1H), 7.57–7.52 (m, 2H), 7.46 (s, 1H), 3.64 (t, *J* = 6.3 Hz, 2H), 3.13 (t, *J* = 6.8 Hz, 4H), 2.92 (t, *J* = 7.4 Hz, 2H), 2.632.53 (m, 2H), 2.21 – 2.00 (m, 2H), 1.94 (t, *J* = 18.4 Hz, 2H), 1.50 – 1.42 (m, 2H). MS (ESI) m/z 322.3 (calcd 322.2 for $C_{20}H_{24}N_3O^+$ $[M+H]^+$)

5-(3-(piperidin-1-yl)propyl)-3-(p-tolyl)-1,2,4-oxadiazole (**28**). Pale yellow oil (82.3 %). ^{1}H NMR (400 MHz, DMSO) δ 7.47 (d, J = 8.0 Hz, 2H), 7.27 (d, J = 8.0 Hz, 2H), 3.63 (t, *J* = 6.3 Hz, 2H), 3.13 (t, *J* = 6.8 Hz, 4H), 2.90 (t, *J* = 7.4 Hz, 2H), 2.63 – 2.53 (m, 2H), 2.43 (s, 3H), 2.24 – 2.02 (m, 2H), 1.96 (t, *J* = 18.4 Hz, 2H), 1.48 – 1.42 (m, 2H). MS (ESI) m/z 286.3 (calcd 286.2 for $C_{17}H_{24}N_3O^+$ $[M+H]^+$)

3-(4-chlorophenyl)-5-(3-(piperidin-1-yl)propyl)-1,2,4-oxadiazole (**29**). Pale yellow oil (81.5 %). ^{1}H NMR (400 MHz, DMSO) δ 7.62 (d, J = 8.0 Hz, 2H), 7.47 – 7.43 (m, 2H), 3.68 (t, *J* = 6.0 Hz, 2H), 3.13 (t, *J* = 6.8 Hz, 4H), 2.84 (t, *J* = 7.2 Hz, 2H), 2.63 – 2.53 (m, 2H), 2.45 – 2.21 (m, 2H), 1.94 (t, *J* = 18.4 Hz, 2H), 1.54 – 1.48 (dd, *J* = 25.5, 12.6 Hz, 2H). MS (ESI) m/z 306.2 (calcd 306.1 for $C_{16}H_{21}ClN_3O^+$ $[M+H]^+$)

3-(4-fluorophenyl)-5-(3-(piperidin-1-yl)propyl)-1,2,4-oxadiazole (**30**). Pale yellow oil (82.0 %). ^{1}H NMR (400 MHz, DMSO) δ 8.06 (dd, *J* = 8.5, 5.5 Hz, 2H), 7.19 (t, *J* = 8.6 Hz, 2H), 3.62 (d, *J* = 11.5 Hz, 2H), 3.13 (t, *J* = 6.8 Hz, 4H), 2.69 (dd, *J* = 21.0, 10.2 Hz, 2H), 2.63 – 2.53 (m, 2H), 2.34 (dd, *J* = 26.7, 12.8 Hz, 2H), 1.92 (t, *J* = 18.4 Hz, 2H), 1.44 (dd, *J* = 25.5, 12.6 Hz, 2H). MS (ESI) m/z 290.3 (calcd 290.2 for $C_{16}H_{21}FN_3O^+$ $[M+H]^+$)

3-(2,3-dichlorophenyl)-5-(3-(piperidin-1-yl)propyl)-1,2,4-oxadiazole (**31**). Pale yellow oil (82.0 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J* = 7.8 Hz, 1H), 7.64 (d, *J* = 8.0 Hz, 1H), 7.35 (t, *J* = 7.9 Hz, 1H), 3.58 (d, *J* = 11.5 Hz, 2H), 3.07 (q, *J* = 7.6 Hz, 2H), 3.04 – 2.93 (m, 2H), 2.63 (q, *J* = 10.2 Hz, 2H), 2.15 (ddd, *J* = 11.5, 10.1, 6.2 Hz, 2H), 2.10 – 1.91 (m, 4H), 1.82 (d, *J* = 13.3 Hz, 2H). MS (ESI) m/z 340.2 (calcd 340.1 for $C_{16}H_{20}Cl_2N_3O^+$ $[M+H]^+$)

3-(2,4-dichlorophenyl)-5-(3-(piperidin-1-yl)propyl)-1,2,4-oxadiazole (**32**). Pale yellow oil (83.1 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, *J* = 8.4 Hz, 1H), 7.56 (d, *J* = 2.0 Hz, 1H), 7.39 (dd, *J* = 8.4, 2.1 Hz, 1H), 3.60 (d, *J* = 11.7 Hz, 2H), 3.24 – 3.11 (m, 4H), 2.79 – 2.50 (m, 4H), 2.39 – 2.20 (m, 2H), 1.90 (dd, *J* = 23.7, 10.0 Hz, 3H), 1.50 – 1.36 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.52, 166.49, 137.50, 134.20, 132.50, 130.91, 127.48, 124.33, 56.17, 53.44, 23.95, 22.54, 22.11, 20.44. MS (ESI) m/z 340.2 (calcd 340.1 for $C_{16}H_{20}Cl_2N_3O^+$ $[M+H]^+$)

3-(2,5-dichlorophenyl)-5-(3-(piperidin-1-yl)propyl)-1,2,4-oxadiazole (**33**). Pale yellow oil (82.4 %). ^{1}H NMR (400 MHz, DMSO) δ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 2.4 Hz, 1H), 7.50 (d, *J* = 8.6 Hz, 1H), 7.43 (dd, *J* = 8.6, 2.3 Hz, 1H), 3.59 (d, *J* = 11.7 Hz, 2H), 3.11 – 3.00 (m, 4H), 2.69 – 2.53 (m, 2H), 2.24 – 2.08 (m, 4H), 2.08 – 1.94 (m, 2H), 1.83 (d, *J* = 14.1 Hz, 2H). MS (ESI) m/z 340.2 (calcd 340.1 for $C_{16}H_{20}Cl_2N_3O^+$ $[M+H]^+$)

3-(3,5-dichlorophenyl)-5-(3-(piperidin-1-yl)propyl)-1,2,4-oxadiazole (**34**). Pale yellow oil (83.1 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 2H), 7.52 (s, 1H), 3.64 (d, *J* = 11.7 Hz, 2H), 3.15 (dd, *J* = 13.6, 6.7 Hz, 4H), 2.69 (dd, *J* = 21.7, 10.4 Hz, 2H), 2.64 – 2.51 (m, 2H), 2.24 – 2.06 (m, 4H), 1.86 (d, *J* = 14.2 Hz, 2H). MS (ESI) m/z 340.2 (calcd 340.1 for $C_{16}H_{20}Cl_2N_3O^+$ $[M+H]^+$)

3-(3,4-dichlorophenyl)-5-(3-(piperidin-1-yl)propyl)-1,2,4-oxadiazole (**35**). Pale yellow oil (82.3 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, *J* = 9.4 Hz, 1H), 7.77 – 7.60 (m, 1H), 7.44 – 7.35 (m, 1H), 3.62 (d, *J* = 11.5 Hz, 2H), 3.14 (t, *J* = 6.9 Hz, 4H), 2.74 – 2.66 (m, 2H), 2.63 – 2.52 (m, 2H), 2.34 (dd, *J* = 26.1, 12.8 Hz, 2H), 2.00 – 1.73 (m, 4H). MS (ESI) m/z 340.2 (calcd 340.1 for $C_{16}H_{20}Cl_2N_3O^+$ [M+H]$^+$)

3-(3-chloro-4-fluorophenyl)-5-(3-(piperidin-1-yl)propyl)-1,2,4-oxadiazole (**36**). Pale yellow oil (82.3 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (dd, *J* = 7.0, 1.9 Hz, 1H), 8.01 – 7.93 (m, 1H), 7.32 – 7.23 (m, 1H), 3.56 (d, *J* = 11.4 Hz, 2H), 3.08 (t, *J* = 7.2 Hz, 2H), 3.00 (dt, *J* = 9.3, 5.3 Hz, 2H), 2.64 (dd, *J* = 21.7, 9.9 Hz, 2H), 2.32 (dd, *J* = 26.8, 12.9 Hz, 2H), 2.22 – 2.08 (m, 2H), 2.01 (dd, *J* = 15.1, 7.5 Hz, 2H), 1.97 – 1.81 (m, 2H). MS (ESI) m/z 324.2 (calcd 324.1 for $C_{16}H_{20}ClFN_3O^+$ [M+H]$^+$)

3-(3,4-difluorophenyl)-5-(3-(piperidin-1-yl)propyl)-1,2,4-oxadiazole (**37**). Pale yellow oil (82.3 %). ^{1}H NMR (400 MHz, DMSO) δ 7.90 (d, *J* = 9.4 Hz, 1H), 7.87 – 7.80 (m, 1H), 7.34 – 7.25 (m, 1H), 3.62 (d, *J* = 11.5 Hz, 2H), 3.14 (t, *J* = 6.9 Hz, 4H), 2.70 (dd, *J* = 20.0, 10.0 Hz, 2H), 2.63 – 2.52 (m, 2H), 2.34 (dd, *J* = 26.1, 12.8 Hz, 2H), 2.00 – 1.73 (m, 4H). MS (ESI) m/z 308.2 (calcd 308.2 for $C_{16}H_{20}F_2N_3O^+$ [M+H]$^+$)

4-(4-(3-(2,4-dichlorophenyl)-1,2,4-oxadiazol-5-yl)butyl)morpholine (**38**). Pale yellow oil (80.0 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, *J* = 8.4 Hz, 1H), 7.57 (d, *J* = 1.7 Hz, 1H), 7.40 (dd, *J* = 8.4, 1.8 Hz, 1H), 4.32 (t, *J* = 12.1 Hz, 2H), 4.00 (d, *J* = 12.0 Hz, 2H), 3.49 (d, *J* = 11.4 Hz, 2H), 3.08 (dd, *J* = 16.4, 9.3 Hz, 4H), 2.89 (s, 2H), 2.23 – 2.09 (m, 2H), 2.03 (dd, *J* = 15.1, 7.5 Hz, 2H). MS (ESI) m/z 356.2 (calcd 356.1 for $C_{16}H_{20}Cl_2N_3O_2^+$ [M+H]$^+$)

3-(2,4-dichlorophenyl)-5-(4-(piperidin-1-yl)butyl)-1,2,4-oxadiazole (**39**). Pale yellow oil (81.5 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, *J* = 8.4 Hz, 1H), 7.58 (d, *J* = 2.0 Hz, 1H), 7.40 (dd, *J* = 8.4, 2.1 Hz, 1H), 3.03 (t, *J* = 7.6 Hz, 2H), 2.37 (dd, *J* = 8.3, 6.9 Hz, 6H), 1.93 (dt, *J* = 20.9, 7.6 Hz, 2H), 1.72 – 1.55 (m, 6H), 1.52 – 1.36 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.55, 166.35, 137.15, 134.24, 132.49, 130.83, 127.32, 124.82, 58.73, 54.67, 26.48, 26.35, 26.02, 24.79, 24.48. MS (ESI) m/z 354.2 (calcd 354.1 for $C_{17}H_{22}Cl_2N_3O^+$ [M+H]$^+$)

3-(2,4-dichlorophenyl)-5-(4-(4-methylpiperidin-1-yl)butyl)-1,2,4-oxadiazole (**40**). Pale yellow oil (81.5 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, *J* = 8.4 Hz, 1H), 7.58 (d, *J* = 1.6 Hz, 1H), 7.40 (dd, *J* = 8.4, 1.7 Hz, 1H), 3.58 (d, *J* = 11.8 Hz, 2H), 3.08 (t, *J* = 7.2 Hz, 2H), 3.03 – 2.94 (m, 2H), 2.62 (dd, *J* = 21.1, 10.4 Hz, 2H), 2.22 – 1.93 (m, 6H), 1.83 (d, *J* = 14.4 Hz, 2H), 1.66 – 1.52 (m, 1H), 1.06 (d, *J* = 6.4 Hz, 3H). MS (ESI) m/z 368.2 (calcd 368.1 for $C_{18}H_{24}Cl_2N_3O^+$ [M+H]$^+$)

3-(2,4-dichlorophenyl)-5-(5-(piperidin-1-yl)pentyl)-1,2,4-oxadiazole (**41**). Pale yellow oil (81.5 %). ^{1}H NMR (400 MHz, DMSO) δ 7.91 (d, *J* = 8.4 Hz, 1H), 7.58 (d, *J* = 1.8 Hz, 1H), 7.40 (dd, *J* = 8.4, 1.9 Hz, 1H), 3.57 (d, *J* = 11.7 Hz, 2H), 3.08 (t, *J* = 7.2 Hz, 2H), 3.04 – 2.93 (m, 2H), 2.58 (dd, *J* = 21.5, 9.7 Hz, 2H), 2.23 (dd, *J* = 27.0, 12.9 Hz, 2H), 2.12 – 2.00 (m, 2H), 1.96 – 1.84 (m, 6H), 1.03 – 0.95 (m, 2H). MS (ESI) m/z 368.2 (calcd 368.1 for $C_{18}H_{24}Cl_2N_3O^+$ [M+H]$^+$)

3-(2,4-dichlorophenyl)-5-(5-(4-methylpiperidin-1-yl)pentyl)-1,2,4-oxadiazole (**42**). Pale yellow oil (81.5 %). ^{1}H NMR (400 MHz, DMSO) δ 7.90 (d, *J* = 8.4 Hz, 1H), 7.60 (d, *J* = 1.6 Hz, 1H), 7.42 (dd, *J* = 8.4, 1.7 Hz, 1H), 3.56 (d, *J* = 11.8 Hz, 2H), 3.10 (t, *J* = 7.2 Hz, 2H), 3.03 – 2.94 (m, 2H), 2.60 – 2.50 (m, 2H), 2.20 – 1.93 (m, 6H), 1.84 – 1.80 (m, 4H), 1.06 – 0.98 (m, 1H), 0.80 (d, *J* = 6.4 Hz, 3H). MS (ESI) m/z 382.2 (calcd 382.1 for $C_{19}H_{26}Cl_2N_3O^+$ [M+H]$^+$)

Receptor Binding Studies.

Materials. The following specific radioligands and tissue sources were used: (a) σ1 receptor, [^{3}H]-(+)-pentazocine (250 μCi, PerkinElmer, NET-1056250UC), and male Dunkin Hartley guinea pig brain membrane; (b) σ2 receptor, [^{3}H]-dio-tolylguanidine ([3H]-DTG, 250 μCi, PerkinElmer, NET-986250UC), (+)-SKF-10047 (Sigma-Aldrich), and male Dunkin Hartley guinea pig brain membrane. Chemicals and reagents were purchased from different commercial sources and of analytical grade. Membrane preparation was performed by previously reported method. [26, 27] General Procedures for the Binding Assays.

All the test compounds were prepared by dissolving in DMSO with compound concentration of 2×10^{-2} M. The filter mats were presoaked in 0.5% polyethylenimine solution for 2 h at room temperature before use.

σ_1 Receptor Binding Assays.

The binding properties of the test compounds to guinea pig σ_1 receptor were studied in guinea pig brain membranes using [^{3}H]-(+)-pentazocine as the radioligand. To each total binding assay tube were added 900 μL of the tissue suspension, 50 μL of 4.0 nM [^{3}H]-(+)-pentazocine, 50 μL Tris-HCl buffer, pH 8.0. To nonspecific binding each assay tube were added 900 μL of the tissue suspension, 50 μL of [^{3}H]-(+)-pentazocine, 50 μL of 10 μM haloperidol. To each specific binding assay tube were added 900 μL of the tissue suspension, 50 μL of [^{3}H]-(+)-pentazocine, 50 μL of reference drug or test compounds solution in various concentrations(10^{-5} mol to 10^{-10} mol). The tubes were incubated at 25 °C for 180 min. The incubation was followed by a rapid vacuum filtration through Whatman GF/B glass filters, and the filtrates were washed twice with 5 mL of cold buffer and transferred to scintillation vials. Scintillation fluid (2.0 mL) was added, and the radioactivity bound was measured using a Beckman LS 6500 liquid scintillation counter.

σ_2 Receptor Binding Assays.

The binding properties of the test compounds to guinea pig σ_2 receptor were studied in guinea pig brain membranes using [^{3}H]-DTG. The membranes were incubated with 3 nM [^{3}H]-DTG in the presence of 400 nM (+)-SKF-10047 to block σ1 sites. To each total binding assay tube were added 850 μL of the tissue suspension, 50 μL of 3.0 nM. [^{3}H]-DTG, 50 μL of 400 nM (+)-SKF-10047, 50 μL Tris-HCl buffer, pH 8.0. To each nonspecific binding assay tube were added 850 μL of the tissue suspension, 50 μL of [^{3}H]-DTG, 50 μL of 400 nM (+)-SKF-10047, 50 μL of 10 μM DTG. To each specific binding assay tube were added 850 μL of the tissue suspension, 50 μL of [^{3}H]-DTG, 50 μL of 400 nM (+)-SKF-10047, 50 μL of reference drug or test compounds solution in various concentrations (10^{-5} mol to 10^{-10} mol). The tubes were incubated at 25 °C for 120 min. The incubation was followed by a rapid vacuum filtration through Whatman GF/B glass filters, and the filtrates were washed twice with 5 mL of cold buffer and transferred to scintillation vials. Scintillation fluid (2.0 mL) was added, and the radioactivity bound was measured using a Beckman LS 6500 liquid scintillation counter.

In Vivo Test.

Animals. SD rats (250 ± 50 g) were used as experimental animals in this study. All the animals were housed under standardized conditions for light, temperature, and humidity and received standard rat chow and tap water and libitum. Animals were assigned to different experimental groups randomly, each kept in a separate cage. All studies involving animals in this research follow the guidelines of the bylaw of experiments on animals and have been approved by the Ethics and Experimental Animal Committee of Jiangsu Nhwa Pharmaceutical Co., Ltd.

Formalin Test.

To examine the effects of compound **39** on formalin induced flinching behavior, **S1RA** (50 mg/kg), compound **39** (25, 50 and 100 mg/kg) and saline in a volume of 10 ml was administered (i.g.) spinally 15 min before the intraplantar injection of formalin. Formalin was diluted to 5% from a stock solution of 100% (formaldehyde solution 37% w/w, Sigma-Aldrich) and was injected subcutaneously into the right hindpaw in a volume of 75 μl. Immediately after the formalin injection, the rat was placed in a test chamber and was observed continuously by automated nociceptive test equipment for the next 60 min. The number of flinches, defined as quick shakes of the injected hindpaw, were recorded. The formalin injection resulted in a biphasic reaction of flinching behaviors (phase 1, 0–10 min; phase 2, 10–60 min).

CCI Model.

The CCI of the sciatic nerve as an animal model was used to study the efficacy of treatments in neuropathic pain. Rats were randomly separated into several groups: sham control and vehicle- and drug-treated groups, and each group contained 8 rats. Pain threshold base values of each group were measured 1–2 days before surgery, and those with the value of 2 days were picked. The pain thresholds were measured again 14 days after surgery to check whether the model was successful. Drugs were dose orally with administration (i.g.) of a 10 mL/kg volume of vehicle 0.5% methylcellulose (Sigma-Aldrich). Each group was measured after first administration on the 15th day in the single administration.

The rats with sciatic nerve exposure without ligation served as the sham group. The test was performed: the von Frey test (electronic von Frey rigid tips with 50 g range (ugo basile)) on the touch stimulator (dynamic plantar aesthesiometer, No. 37400). Allodynia to mechanical

stimuli was used as outcome measures of neuropathic pain by using the von Frey. In the von Frey test, briefly, animals were placed in a transparent test chamber with a wire-mesh grid floor through which von Frey monofilaments were applied. The monofilaments were applied in increasing force until the rats withdrew the ipsilateral, nerve injury paw using an up–down paradigm. Clear paw withdrawal was considered as nociceptive-lick response to determine the mechanical withdrawal threshold (MWT). Animals were adapted to the testing situation for at least 30 min before the sessions started. For each measurement, the paw was sampled three times and a mean calculated. At least 10 min elapsed between.

Statistics

To estimate the potency of test and reference compounds, the ED_{50} values and their 95% confidence limits were calculated by using the program SPSS (Statistical Package for the Social Science).

Supplementary Material

Acute toxicity of compound **39**, additional receptors binding affinities of compound **39**; ^{1}H NMR, ^{13}C NMR, HRMS and HPLC of compound **22**, **32** and **39**.

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Author Contribution Statement

Zhongyuan,Yao, Fei, Dou and Yifang Zhang performed the synthesis of compounds; Yinli Qiu, Song Zhao,and Xiangqing Xu performed biological stuidies; Xin Liu and Bi-Feng Liu collected and analyzed the data, Xudong Cao and Yin Chen wrote the article; Xudong Cao and Guisen Zhang designed the experiments and supervised the study.

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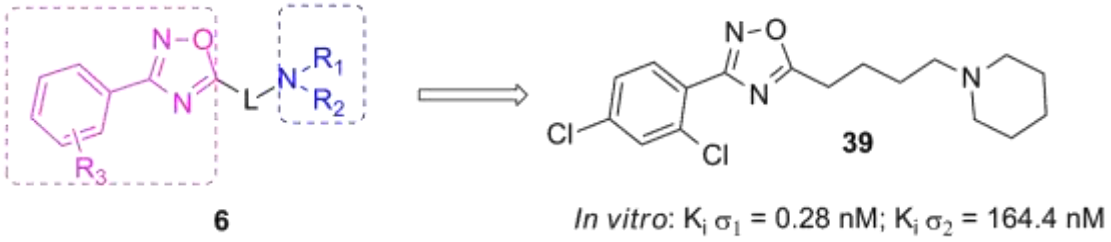

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In this study, compound **39** showed excellent affinity for the σ_1 receptor and selectivity for the σ_2 receptor or other CNS targets associated with pain. Compound **39** exerts as a promising candidate for treating neuropathic pain based on its potent antihyperalgesic activity in animal model.