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Synthesis and biological evaluation of novel 6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidine and 5,6,7,8-tetrahydroquinazoline derivatives as sigma-1 (σ_1) receptor antagonists for the treatment of pain

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Abstract

The synthesis and biological evaluation of new series of 6,7-dihydro-5H-cyclopenta[*d*]pyrimidine and 5,6,7,8-tetrahydroquinazoline derivatives as selective sigma-1 receptor (σ_1R) antagonists are reported. The receptor affinities of new compounds were evaluated in *vitro* in σ_1 and σ_2 receptor binding assays. The structure-activity relationship study leads us to the most promising compound:

2-(4-chlorophenyl)-4-(3-(4-methylpiperidin-1-yl)propoxy)-5,6,7,8-tetra-hydroquinazo line (**33**). Compound **33** has exerted nanomolar affinity for σ_1R ($K_i\sigma_1$ = 15.6 nM) and high σ_1/σ_2 selectivity ($K_i\sigma_2$ > 2000 nM), and identified to be a σ_1R antagonist. In animal model, compound **33** exhibited dose dependent anti-nociceptive effeccts in the formalin test. These results suggest that compound **33** could be a potent analgesic for pain treatment.

Keywords

6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidine; 5,6,7,8-tetrahydroquinazoline; sigma-1 (σ_1) receptor antagonists; analgesic

The sigma (σ) receptor was first discoverd in 1976, it was originally mischaracterized as a noval class of opioid receptor and later confused with phencyclidine/*N*-methyl-D-aspartate (NMDA) glutamate receptor complex.[1, 2] Currently, sigma receptors are considered as unique proteins and classified into two subtypes by pharmacological studies and biochemical analyses, which were named as sigma-1 (σ_1) receptor and sigma-2 (σ_2) receptor.[3] The σ_1 receptor has been cloned, encodes a protein of 223 amino acids with no homology to any other known mammalian receptor protein, and shares a 90% amino acid identity and a 95% similarity across various species.[4] The σ_1R is widely distributed in the central nervous system (CNS) and peripheral nervous system (PNS) in voloved in memory, emotions, and sensory and motor functions.[5] Moreover, the σ_1R is highly expressed in the key areas of pain processing such as the superficial dorsal horn, periaqueductal gray, rostral ventromedial medulla and also in the astrocytes and microglia.[6-8] The σ_1R modulates the activity of various neurotransmitter receptors and ion channels, including NMDA recepter, potassium channels, calcium channals and sodium channels.[6, 9] The σ_2R has not been cloned, but numerous works report that the σ_2R have been involved in tumor cell proliferation and death, suggesting a promising role in cancer imaging and treatment.[10, 11] Due to their different pharmacological functions, selectivity between the σ_1R and σ_2R is desirable.

Currently, selective σ_1R ligands have been not introduced to the market, but many of the compounds have been tested in clinical studies as antidepressants,[12] antipsychotics,[13] treatments for drug abuse,[14] and learning/memory enhancers.[15] Moreover, numerous groups have studied the potential role of the σ_1R in pain management.[16] The σ_1R has been known to modulate opioid analgesia since the 1990s. σ_1R agonists inhibit antinociception induced by morphine but σ_1R antagonists and σ_1R antisense oligodeoxynucleotides enhance other μ-opioid receptor agonists in the acute nociceptive test.[17, 18] The pharmacological studies using genetic σ_1R knockout (KO) mice supported a role for σ_1R in modulating pain behaviors in the absence of opioids. Intraplantar administration of formalin or capsaicin elicited pain behaviours in those of wild-type (WT) mice, but the phenotype of pain was reduced in σ_1R KO mice.[19, 20]

Pharmacological antagonism of the σ_1R produced similar results. Haloperidol (**1**), with an affinity and antagonism effect to the σ_1R, exhibited the inhibition in formalin-induced pain and capsaicin-induced sensitization in WT mice.[21] The σ_1R antagonists BD-1063 (**2**) and NE-100 (**3**) inhibited the mechanical allodynia induced by capsaicin.[20] The leading compound in the field of σ_1R antagonists, S1RA (**4**), showed high affinity to σ_1 receptor (K_i = 17 nM) and excellent selectivity ratio (σ_1 /σ_2 > 550) (**Figure 1**). S1RA is currently undergoing phase II clinical trials in several neuropathic pain conditions and the results are eagerly awatied.[22]

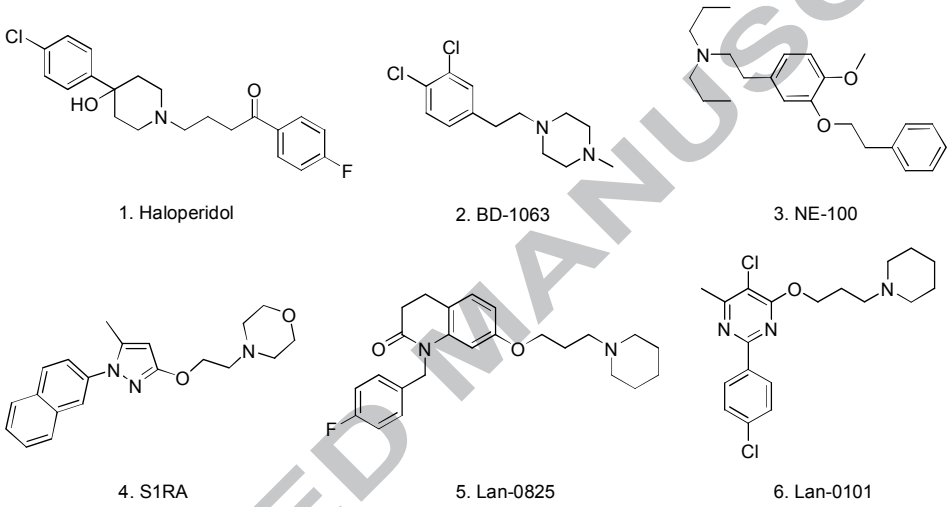


Figure 1. Representative σ_1 receptor ligands.

In our previous study, we have identified two new series of compounds based on 3,4-dihydro-2(1*H*)-quinolinone and pyrimidine scaffolds through a three-dimensional (3D) pharmacophore model of σ_1R antagonists (**Figure 2**).[23] The candidated compounds, Lan-0825 (**5**) and Lan-0101 (**6**), exhibited the most potent *in vivo* antinociceptive properties in animal models.[23, 24] Forthermore, the cyclizing derivatives of pyrimidine scaffold also possesses moderate affinities for σ_1 receptor and low affinity for σ_2 receptor.

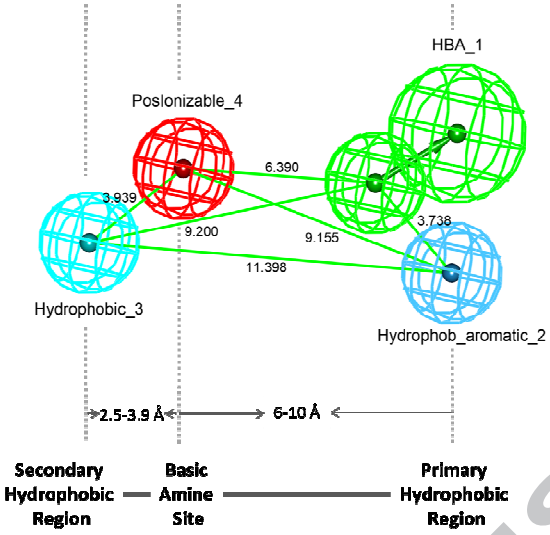


Figure 2. Main features of Lan`s $\sigma_1$R pharmacophore in comparison to the distances described by Glennon`s σ_1R pharmacophore model.

This finding was from the study conducted by our research group in which two novel classses of σ_1R antagonists, compounds **10-34**, were developed by connecting 4-position and 5-position of pyrimidine scaffold of **6** into cycloalkanes (**Figure 3**). Target compounds **10-34** have been subjected to preliminary biological evaluation to determine their affinities for the σ_1 and σ_2 receptor. Among the derivatives synthesized, compound **33** exerted nanomolar affinity for σ_1R ($K_i\sigma_1$ = 15.6 nM), high σ_1/σ_2 selectivity ($K_i\sigma_2$ > 2000 nM), and identified to be a σ_1R antagonist. In animal model, compound **33** exhibited dose dependent anti-nociceptive effeccts in the formalin test.

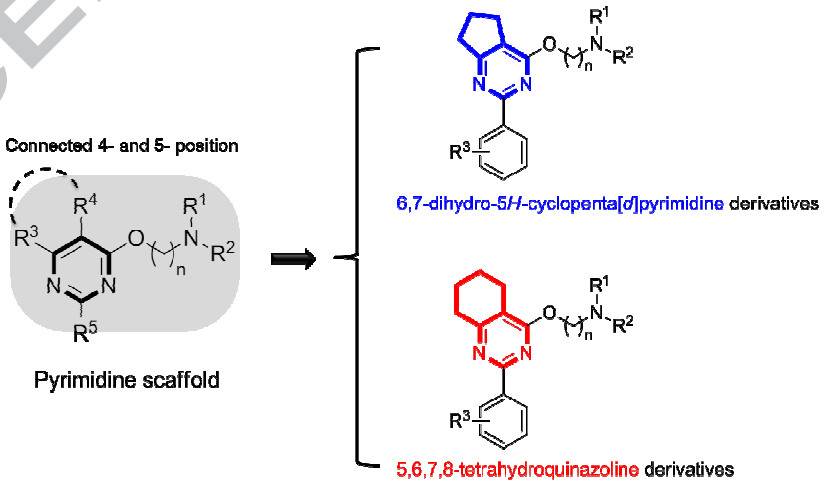
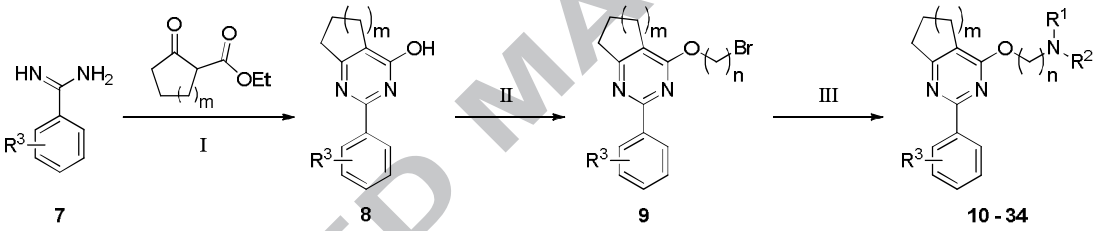


Figure 3. Design of new 6,7-dihydro-5H-cyclopenta[*d*]pyrimidine and 5,6,7,8-tetrahydroquinazoline derivatives.

The general strategy used to synthesize compounds **10-34** was performed in a three step process, according to a previously reported method with slightly modification. As shown in **Sheme 1**, the key intermediates 2-aryl-6,7-dihydro-5H-cyclopenta[*d*]pyrimidin-4-ol or 2-aryl-5,6,7,8-tetrahydroquinazolin-4-ol derivatives **8** were prepared through cyclization reactions from 4-substituted benzimidamide **7**, which reacted with ethyl 2-oxocyclopentanecarboxylate and 2-oxocyclohexanecarboxylate in methanol, respectively. The standard alkylation procedure of **8** with 1,3-dibromopropane, 1,4-dibromobutane, 1,5-dibromopentane or 1,6-dibromohexane in acetone led to compounds **9**. Compounds **9** were then reacted with the various amines in acetonitrile under the basic conditions. The crude products were purified by means of chromatography to yield the target compounds **10-34** (**Table 1-3**).



Scheme 1. Reagents and conditions: (I) *t*-BuOK, MeOH, reflux; (II) $Br(CH_2)_nBr$, K_2CO_3, acetone, reflux; (III) HNR^1R^2, Cs_2CO_3, acetonitrile, reflux

The design of the new 6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidine and 5,6,7,8-tetrahydroquinazoline derivatives was based on the represented σ_1 receptor ligand pharmacophore model summarized by Glennon (**Figure 2**).[25, 26] On the basis of our previous findings, a hydrophobic group (aromatic hydrophobic group was better) was necessary in position 2 of pyrimidine ring, and a three-atom carbon chain was the most suitable distance to achieve good potency and selectivity. All the derivatives synthesized were evaluated in primary σ_1 receptor and σ_2 receptor binding assays, using [^{3}H]-(+)-pentazocine and [^{3}H]-di-*o*-tolylguanidine ([^{3}H]-DTG) as radioligands, respectively.[23, 24]

In this work, our initial focus was to investigate the effect of different amine moieties for the binding affinities to σ_1 and σ_2 receptors. In

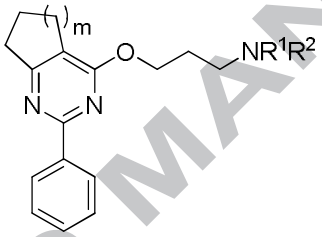
6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidine derivatives, the piperidine and pyrrolidine derivatives **10** (CAS number: 1639219-80-9) and **11** (CAS number: 1639219-81-0) showed a moderate affinities to σ_1 receptor.[23] After induced a methyl group in the 4-position of piperidine moiety (**12**), the σ_1R binding affinity was improved ($K_i\sigma_1$ = 32.3 nM and $K_i\sigma_2$ = 1774 nM). This improvement is possibly due to the hydrophobicity of methyl group in piperidine moiety, which is more fitable to the requirement of the second hydrophobic region in Glennon`s pharmacophore model. The 1-methylpiperazine derivative (**13**) was also potent and selective ($K_i\sigma_1$ = 4.3 nM and $K_i\sigma_2$ = 161 nM), similar to 4-methylpiperidine derivative **12**, that proved this hypothesis. However, substitution of the methyl in 1-methylpiperazine with a large group such as phenyl (**14**, $K_i\sigma_1$ > 2000 nM and $K_i\sigma_2$ > 2000 nM) was not tolerated by either the σ_1 or σ_2 receptor. The morpholine derivative was synthesized and evaluated because its frequent appearance in σ_1R ligands (such as S1RA and its derivatives),[22, 27] but the receptor affinity of compound **15** was not as good as 4-methylpiperidine derivative ($K_i\sigma_1$ = 102.8 nM and $K_i\sigma_2$ = 1889 nM). Substituting the oxygen atom of the morpholino with a carbonyl group (**16**, $K_i\sigma_1$ = 188.7 nM and $K_i\sigma_2$ > 2000 nM)) decreased greatly the affinity to both the σ_1R and σ_2R, which suggested the polar groups were not conducive to σ_1R or σ_2R binding in this position. Open chain amines (**17, 18**) retained some activity, though not as much as compound **12**, and had lower selectivity.

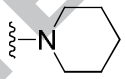
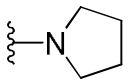
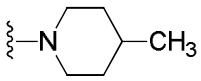
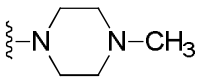
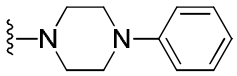
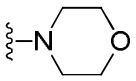
5,6,7,8-tetrahydroquinazoline derivatives demonstrated a similar results with 6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidine derivatives with a few differences. The 4-methylpiperidine moiety produced a higher affinity for σ_1 receptor (**21**, $K_i\sigma_1$ = 25.7 nM and $K_i\sigma_2$ > 2000 nM) than compound **19** (CAS number: 1639219-85-4) or **20** (CAS number: 1639219-87-6),[23] and the improvement of binding affinity was even greater than compound **12**, while 6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidine derivatives were thought to be more active due to the smaller ring size in our previous work.[23] The reasons for these interesting results were partially because the basic amine center played a key role in σ_1R binding affinity, and the secondary hydrophobic region which close to center amine site might produce more influences in receptor binding. The

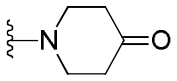
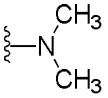
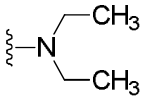
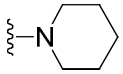
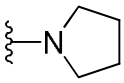
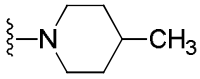
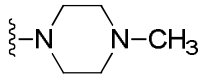
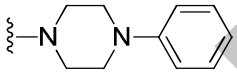
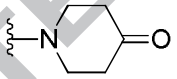
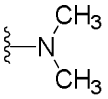
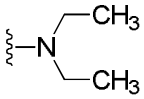
1-methylpiperazine derivative exerted some potent and selective (**22**, $K_i\sigma_1$ = 27.3 nM and $K_i\sigma_2$ = 1922 nM), but 1-phenylpiperazine derivative still lost the actives in both receptors. Amino Groups with oxygen atom or polar group (morpholine and piperidin-4-one) decreased the σ_1R binding, and open chain amines derivatives (**26, 27**) were less active than compound **21**.

Together the receptor binding affinity data in **Table 1** and our structure-active relationship (SAR) studies summerized, it revealed that 5,6,7,8-tetrahydroquinazoline derivatives have greater potential than 6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidine derivatives. Above of these derivatives, compound **21** exerted the highest σ_1R binding affinity and good selectivity to σ_2R, which encouraged further exploration.

Table 1. Binding affinities for the σ_1 and σ_2 Receptor of Compounds **10-27**.



Compound	m	NR^1R^2	K_i σ_1 (nM)[a]	K_i σ_2 (nM)[b]	Selectivity (σ_2/σ_1)
10	1		44.2 ± 8.2 [c]	1626 ± 187	36.7
11	1		58.9 ± 7.6	1896 ± 227	32.1
12	1		32.3 ± 6.4	1774 ± 193	54.9
13	1		31.9 ± 6.1	1821 ± 184	57.1
14	1		>2000	>2000	– –
15	1		102.8 ± 13	1889 ± 203	18.5

16	1		188.7 ± 46	>2000	– –
17	1		120.8 ± 21	>2000	– –
18	1		62.4 ± 5.6	1493 ± 155	23.9
19	2		138.5 ± 20	>2000	– –
20	2		111.8 ± 16	1916 ± 227	17.1
21	2		25.7 ± 5.6	>2000	– –
22	2		27.3 ± 6.2	1922 ± 243	70.4
23	2		>2000	>2000	– –
24	2		119.6 ± 17	>2000	– –
25	2		166.2 ± 39	>2000	– –
26	2		137.8 ± 26	>2000	– –
27	2		66.3 ± 5.7	1546 ± 168	23.3

[a] Affinities were determined in guinea pig brain using [^{3}H]-(+)-pentazocine.

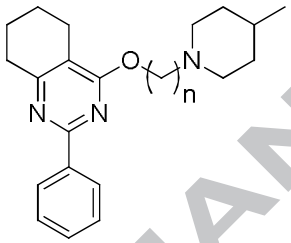
[b] Affinities were determined in guinea pig brain using [^{3}H]-DTG in the presence of (+)-SKF-10047 to block sigma-1 receptors.

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

After identification 4-methylpiperidine as the most potent amino substituent, several analogs with different chain lengths between the 5,6,7,8-tetrahydroquinazoline scaffold and 4-methylpiperidine moiety were prepared. Compound **28**, which

contained only one additional carbon atom in the linker compared to compound **21**, decreased binding affinity and selectivity for the σ_1R over the σ_2R ($K_i\sigma_1$ = 238.2 nM and $K_i\sigma_2$ = 1856 nM). Further elongation of the straight carbon linker 5,6,7,8-tetrahydroquinazoline scaffold and 4-methylpiperidine moiety provided derivatives **29-30**, which had lower σ_1R and σ_2R affinities than propylene counterparts. The results showed in **Table 2** supported our previous finding that a three-atom carbon chain between basic amino groups and primary hydrophobic region offered the most fitable distance for receptor binding.

Table 2. Binding affinities for the σ_1 and σ_2 Receptor of Compounds **21**, **28–30**.



Compound	n	K_i σ_1 (nM)[a]	K_i σ_2 (nM)[b]	Selectivity (σ_2/σ_1)
21	3	25.7 ± 7.6 [c]	>2000	– –
28	4	238.2 ± 77	1856 ± 219	7.79
29	5	353.9 ± 85	>2000	– –
30	6	>2000	>2000	– –

[a] Affinities were determined in guinea pig brain using [^{3}H]-(+)-pentazocine.

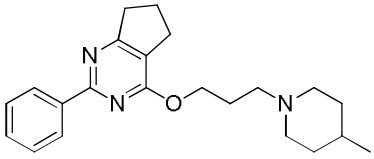
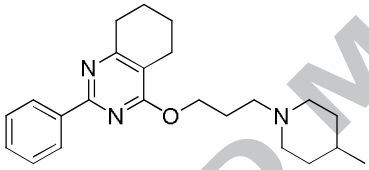
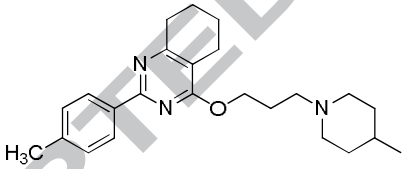
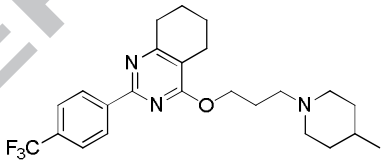
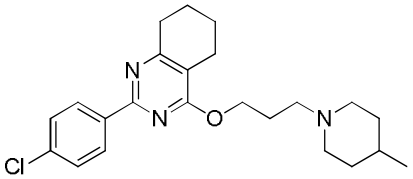
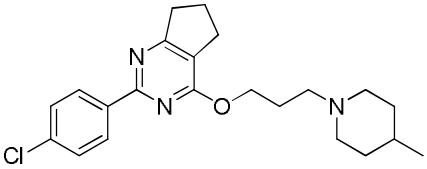
[b] Affinities were determined in guinea pig brain using [^{3}H]-DTG in the presence of (+)-SKF-10047 to block sigma-1 receptors.

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

Compound **21** exhibited significant affinity to the σ_1R and σ_1/σ_2 selectivity and were thus used for further investigation of the effect of various substituted phenyl groups. As shown in **Table 3**, the aromatic group on the 2-position of 5,6,7,8-tetrahydroquinazoline was improtent for activity and selection. Either an electron-donating group (methyl) or electron-withdrawing group (trifluoromethyl) could change slightly (increase or decrese) in receptor binding affinities, but after

induced chlorine atom in 4-position of phenyl group in compound **21**, it showed higher affinity to σ_1R and better σ_1/σ_2 selectivity (**33**, $K_i\sigma_1$ = 15.6 nM and $K_i\sigma_2$ > 2000 nM). 6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidine derivatives with 1-methylpiperidine and 4-chlorophenyl groups were also synthesized and assessed, which maintained the activity of σ_1 receptors and selectivity to σ_2R (**34**, $K_i\sigma_1$ = 21.1 nM and $K_i\sigma_2$ > 2000 nM).

Table 3. Binding affinities for the σ_1 and σ_2 Receptor of Compounds **12**, **21**, **31-34**.

Compound	Structure	K_i σ_1 (nM)[a]	K_i σ_2 (nM)[b]	Selectivity (σ_2/σ_1)
12		32.3 ± 6.4 [c]	1774 ± 193	54.9
21		25.7 ± 7.6	>2000	– –
31		24.1 ± 6.5	1961 ± 235	81.3
32		29.4 ± 7.2	>2000	– –
33		15.6 ± 3.3	>2000	– –
34		21.1 ± 4.8	>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 ± SEM of three experiments performed in duplicate.

The phenytoin-media shift on σ_1R ligand affinity and fluorescence resonance energy transfer (FRET) based biosensor of σ_1R ligand were mainly used to categorize compounds into agonists and antagonists recently.[28, 29] Phenytoin, a low-potency allosteric modulator of the σ_1 receptor, shifts σ_1R agonists to significantly higher affinities (K_i ratios without phenytoin *vs.* with phenytoin > 1), while σ_1R antagonists show no effect or a very little effect on lowering the affinity values (K_i ratios without phenytoin *vs.* with phenytoin ≤ 1). In this work, compound **33** produced a small shift lowering the affinity when incubated in the presence of phenytoin (K_i ratios without phenytoin *vs.* with phenytoin = 0.92), which exhibited antagonist properties on the σ_1 receptor.

Due to the marked effects on the σ_1R and σ_2R with its antagonist property, compound **33** was selected as a promising candidate and subjected to further pharmacological evaluation. As a classic model of acute and chronic pain, the formalin test was representative and frequently used for the evaluation of the anti-nociceptive effect. Intraplantar injection of formalin solution into the hind paw produces a biphasic pain response, a brief, acute phase that caused by direct activation of C-fibers (phase I) followed by a longer-lasting tonic phase that reflects inflammation (phase II).[30] The time spent licking or biting the paw after injection is measured as an indicator of the pain response in mice.

Recent research have reported that σ_1R antagonists could reduce both phases of formalin-induced paw licking/biting behavior in mice, especially in the delayed phase II, which appears to be dependent on the combination of an inflammatory reaction in the peripheral tissue and functional changes in the spinal cord, involving both peripheral and central sensitization.[23, 24, 27] As shown in **Figure 4**, pretreatment with compound **33** (80 mg/kg, i.p.) inhibited pain responses to a similar degree as compound **4** (S1RA), reducing licking and biting time to 23.7 ± 5.2 s in phase I and 64.2 ± 8.3 s in phase II. Compound **4** at the same dose reduced licking and biting time

to 16.8 ± 3.4 s and 55.4 ± 7.7 s during phase I and II, respectively; the vehicle had no effect relative to treatment with formalin alone. To better characterize the antinociceptive effects of **33**, a wide range of doses was tested (20-160 mg/kg). Compound **33** produced dose dependent anti-nociception in both phases; the ED_{50} values were 51.8 ± 5.3 and 57.5 ± 4.6 mg/kg for phase I and II, respectively.

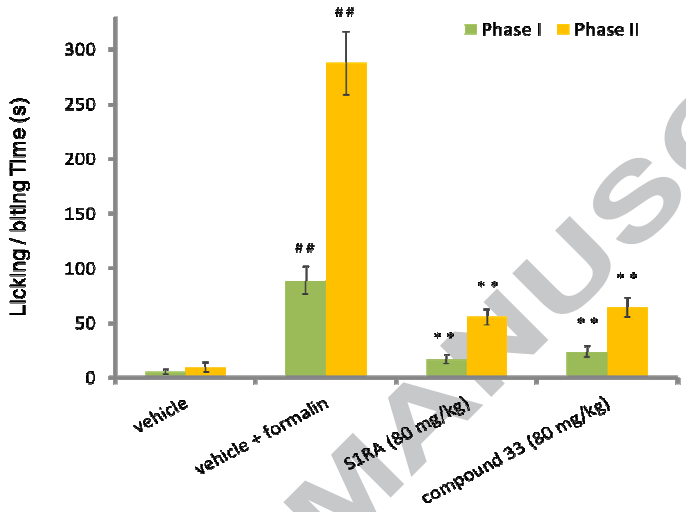


Figure 4. Anti-nociceptive effect of compound **S1RA** (**4**) and **33** in phase I (0 – 5 min) and phase II (15 – 45 min) of the mice formalin test at the dose of 80 mg/kg. Each column and vertical line represents mean ± SEM of the values obtained in at least ten animals. Statistically significant differences: ## $p<0.01$ VS vehicle; ** $p<0.01$ VS vehicle+formalin (two-way ANOVA followed by Newman-Keuls test).

A detailed SAR investigation of 6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidine and 5,6,7,8-tetrahydroquinazoline derivatives has shown that several factors influence the binding affinity of these compounds to σ_1 and σ_2 receptors: (1) a basic amine was necessary matching the known σ_1R pharmacophoric model, piperidine and 4-methylpiperidine were favored; (2) a straight three-carbon chain alkyl between the pyrimidine ring and the amino moiety was preferred over other linkers; and (3) introduction of a halogen (chloro) on the 5-position (R^3) showed moderate affinities to σ_1R and increased selectivity to σ_2R.

In summary, we described the synthesis and pharmacological evaluation of series of 6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidine and 5,6,7,8-tetrahydroquinazoline derivatives as novel selective σ_1 receptor antagonists for pain treatment. The most promising compound, compound **33**, exhibited the high affinity for σ_1R ($K_i\sigma_1$= 15.6

nM) and good σ_1/σ_2 selectivity ($K_i\sigma_2$ = 1922 nM), and was identified to be a σ_1R antagonist. In the formalin test, compound **33** exerted clear dose-dependent antinociceptive effects, which suggested that compound **33** may facilitate the development of a novel class of drugs for pain treatment.

Conflict of Interest

There is no conflict of interests among the authors.

Supplementary data

Supplementary data (experimental procedures and analytical data of new compounds) associated with this article can be found, in the online version, at _____

Abbreviations

σ_1R, sigma-1 receptor; σ_2R, sigma-2 receptor; NMDA, *N*-methyl-D-aspartate; CNS, central nervous system; PNS, peripheral nervous system; KO, knockout; WT, wild-type; [^{3}H]-DTG, [^{3}H]-di-*o*-tolylguanidine; SAR, structure-active relationship; FRET, fluorescence resonance energy transfer; i.p., intraperitoneal injection.

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Graphical abstract