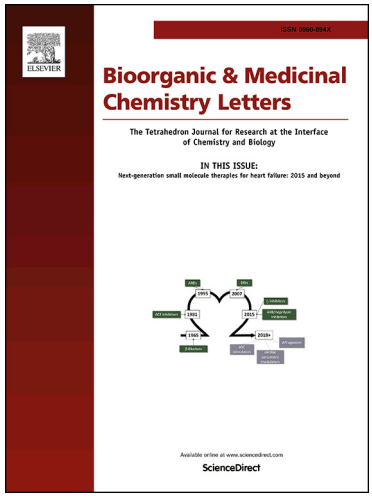
Accepted Manuscript



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PII: S0960-894X(19)30224-0
DOI: https://doi.org/10.1016/j.bmcl.2019.04.015
Reference: BMCL 26382

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 28 January 2019
Accepted Date: 7 April 2019

Please cite this article as: Dou, F., Cao, X., Jing, P., Wu, C., Zhang, Y., Chen, Y., Zhang, G., Synthesis and evaluation of histamine H_3 receptor ligand based on lactam scaffold as agents for treating neuropathic pain, *Bioorganic & Medicinal Chemistry Letters* (2019), doi: https://doi.org/10.1016/j.bmcl.2019.04.015

Synthesis and evaluation of histamine H_3 receptor ligand based on lactam scaffold as agents for treating neuropathic pain

Fei Dou[a,c 1], Xudong Cao[b,1], Peng Jing [c,] Chunyan Wu [c], Yuxin Zhang[d] , Yin Chen [a,c] * and Guisen Zhang[b,]*

[a]Jiangsu Marine Resources Development Research Institute, Lianyungang 222005, China

[b] Systems Biology Theme, Department of Biomedical Engineering, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074

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

[d] College of life science, Nanjing Agricultural University, Nanjin, 210095

[1]These authors contributed equally to this work

[*]Corresponding authors.

E-mail addresses: chenyin0914001@126.com (Y. Chen), gszhang@hust.edu.cn (G. Zhang)

Abstract

The synthesis and H_3 receptor ligand of a new series of lactam derivatives are reported. The new compounds were evaluated in vitro in H_3 and H_1 receptor-binding assays. The structure-activity relationship led us to the promising derivative 2-methyl-7-(3-morpholinopropoxy)-3,4-dihydroisoquinolin-1(2H)-one (**11**). The compound with highest affinity and greatest selectivity were further profiled, In addition, compound **11** exerted dose-dependent anti-nociceptive effects in the formalin test. These characteristics suggested that the potent and selective compound **11** could be a potent candidate for pain treatment.

Keywords: Synthesis； H_3 receptors； anti-nociceptive

Pain, as a fundamental and central life experience, limits our exposure to potentially damaging or life-threatening events[1,2]. Current analgesic medications include opioids, non-steroidal anti-inflammatory drugs (NSAIDs) and analgesic adjuvants[3,4]. Opioids induce a wide variety of well-known side effects, including tolerance and physical dependence. Meanwhile, NSAIDs cause adverse gastrointestinal reactions and renal toxicity and inhibit platelet aggregation [3]. Antidepressants, anticonvulsants, and anesthetics, have demonstrated some efficacy in the treatment of neuropathic pain, earning the nickname "analgesic adjuvants". However, they have limited independent efficacy in treating pain[4]. The so-called "analgesic adjuvants", e.g., antidepressants, anticonvulsants and anesthetics [5], show some efficacy in the treatment of neuropathic pain [6]. However, these analgesic adjuvants have also shown limited effectiveness for neuropathic pain [7]. Therefore, there is a continuing unmet medical need for analgesics with new mechanisms of action to improve the efficacy of existing therapies and reduce unwanted effects.

The histamine H_3 receptor is largely expressed in the anterior part of the cortex, in the hippocampus, striatum, and to a lesser extent in the hypothalamus and spinal cord. H_3 receptor have invoked more complexity in vivo H_3R biology and interesting therapeutic applications, such as Alzheimer's disease, attention deficit hyperactivity disorder, schizophrenia, sleep disorder.[8] Moreover, the H_3 receptors are expressed in the tissues known to be involved in nociception (specific thalamic areas, dorsal root ganglia, spinal cord, and skin tissues) and therefore, might offer treatment opportunities for different modalities of pain.[9]

Our interest has been focused on the development of novel ligands with high affinity towards the H_3 receptor and selectivity over the H_1 subtype. Pitolisant is an inverse agonist of the histamine H_3 receptor that is being developed by Bioproject[10]. Oral pitolisant is approved in the European for the treatment of narcolepsy with or without cataplexy in adults. Bavisant (JNJ-31001074) is a highly selective, orally active antagonist of the human H3 receptor with a novel mechanism of action, involving wakefulness and cognition, with potential as a treatment for ADHD.[11] **S**

38093 is an inverse agonist/antagonist of H_3 receptor, which has displayed antiallodynic and antihyperalgesic effect in chronic pain especially in a context of neuropathic pain after chronic administration.[12] In this paper, on the basis of S 38093, by way of replacement of octahydrocyclopenta[c]pyrrole moiety with introduction of a basicamine center and followed by the rigidization of amidic group to form lactam derivatives **9-23** (**Fig. 2**). Evaluating their pharmacological efficacy and in competitive receptor binding assays to determine their relative affinity for H_3 and H_1 receptors. Among the derivatives prepared, compound **11** exhibited high affinity for the H_3 receptor and low affinity for the H_1 receptor. Further, compound **11** inhibited formalin-induced pain in mice.

First of all, we synthesized all the lactam derivatives 9-23 and 27as described in Scheme 1. 2-(4-methoxy-phenyl)-ethylamine (**1**) in chloroform was reacted with chloro ethyl formate and triethylamine to afford the product **3** . [13] Phosphorus pentoxide was added to methanesulfonic acid in portions and heated at 130°C for 2 h. Intermediate 3 was added and to afford the title compound **4**. Compound **4** in DMF was reacted with methyl iodide under the sodium hydride to give brown solid **5**. Under a nitrogen atmosphere, intermediate **5** was dissolved in HBr and refluxed 4 h to afford the compound **6**. Intermediate 6 reacted with 1,3-dibromopropane or 1,4-dibromobutane to give compounds **7a-c**, and then reaction with the piperidine or pyrrodine afforded the final compounds **9-22**.Intermediate 6 reacted with epichlorohydrin and potassium carbonate to afford the product **8**, and then reaction with the morpholine to afford the compound **23**.

Intermediate **4** in DMF was reacted with Iodoethane under the sodium hydride to give compound **24**. Compound **24** was dissolved in HBr and refluxed 4 h to afford the compound **25**. Intermediate 25 reacted with 1,3-dibromopropane o to give intermediate **26**, and then reaction with the morpholine to give compound **27**.

In this work, our initial design focus was to investigate the effect of different amine moieties for H_1 and H_3 receptors (**Table 1**, compounds **9-20**). As shown in **Table 1**, compound **9** (piperidine) and **10** (4-methylpiperidine) showed moderate affinities for the H_3 receptor, and weakly Histamine1/3 selectivity (8.2 and 8.7-fold). It should be

noted that the morpholine derivative **11** displayed high affinities for the H_3 receptors (K_i = 6.5 nM). Moreover, compound **11** showed good Histamine 1/3 selectivity (158-fold) than **S38093** (118-fold). Compounds **12** (2-methylpyrrolidin) and **13** (pyrrolidine) exhibited moderate affinities for the H_3 receptor, and weakly Histamine1/3 selectivity. The amine moieties 1-ethylpiperazine, 1-methylpiperazine and diethylamine (compounds 14 -16) showed low affinities for H_3 receptors. When amine moieties were piperidin-4-one, 2-methylpiperidine, 3-hydroxypyrrolidine and 4-hydroxypiperidine compounds **17-20** exhibited affinities for the H_3 receptor.

We determined the effect of the length of the linker between the phenyl group and morpholine the ring. As shown in **Table 1**, chain lengths of two (**21**，K_i = 1208 nM) or four (**22**，K_i = 856 nM) carbon atoms resulted in significantly reduced H_3 receptor binding. Introducing OH to the carbon chain of compound **23 (**K_i > 2000 nM**)** resulted in inactivation of H_3 receptor. The length of the alkyl chain appeared to have a direct impact on affinity for the H_3 receptor. Taken together, the data indicate that the three-carbon chain length (compound **11**) was the most active. Compound **27 (**K_i = 252 nM**)** is structurally identical to compound **11** except that the methyl is replaced with an ethyl group. This replacement resulted in reduced the affinity for H_3 receptor.

Together, the data indicated that compound **11** had high affinities for H_3 receptor, with good selectivity (compared to H_1 receptor). The compound were considered promising and were subjected to in vivo activity testing.

The above results led to the conclusion that compound **11** exhibited good affinities for dopamine H_3 receptor and with a low affinity for the H_1 receptor. We then assayed the acute toxicity of the compounds by determining their LD_{50}. Compound **11** showed good safety profiles even at the highest dose tested (LD_{50} > 1000 mg/kg).

The classical model of acute and chronic pain, the formalin test, was performed to evaluate the antinociceptive effects. Intraplantar (ipl) injection of formalin elicits a biphasic pain response: early (phase I) and delayed (phase II), characterized by paw licking, biting, and other behaviors. Phase I pain is predominantly caused by direct activation of C-fibers, whereas phase II pain appears to be dependent on a

combination of peripheral tissue inflammation and functional changes in the spinal cord, involving both peripheral and central sensitization.

In the formalin test, pregabalin (40 mg/kg) and S38093 (1mg/kg) shown antinociception in both phases. Compound **11** (1−10 mg/kg) produced dose-dependent antinociception in the formalin test and was (slightly) more efficacious against delayed-phase pain (**Figure 3**). The ED_{50} value was mg/kg for 1.3mg/kg and 2.4mg/kg in the phase I and phase II pain, respectively.

In this study, a series of lactam derivatives were synthesized *via* an efficient method, several of which were shown to be potent and selective H_3 receptor ligands. Compound **11** appears safe in preliminary tests and exerts clear dose-dependent antiallodynic effects against formalin induced pain in mice. Moreover, compound **11** showed significant anti-nociceptive effects in phases I and II, identifying compound **11** as a candidate agent for the treatment of pain. Further studies of this compound are currently underway in our laboratory.

Acknowledgement

Financial support for this research provided by the National Natural Science Foundation of China for youth (Nos. 81602971).

Conflict of Interest

The authors declare that there is no financial/commercial conflict of interest.

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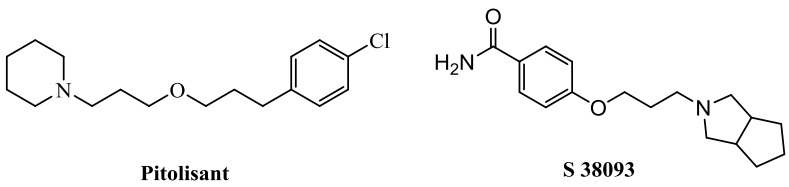


Pitolisant **S 38093**

Figure.1 Representative H_3 receptor ligand

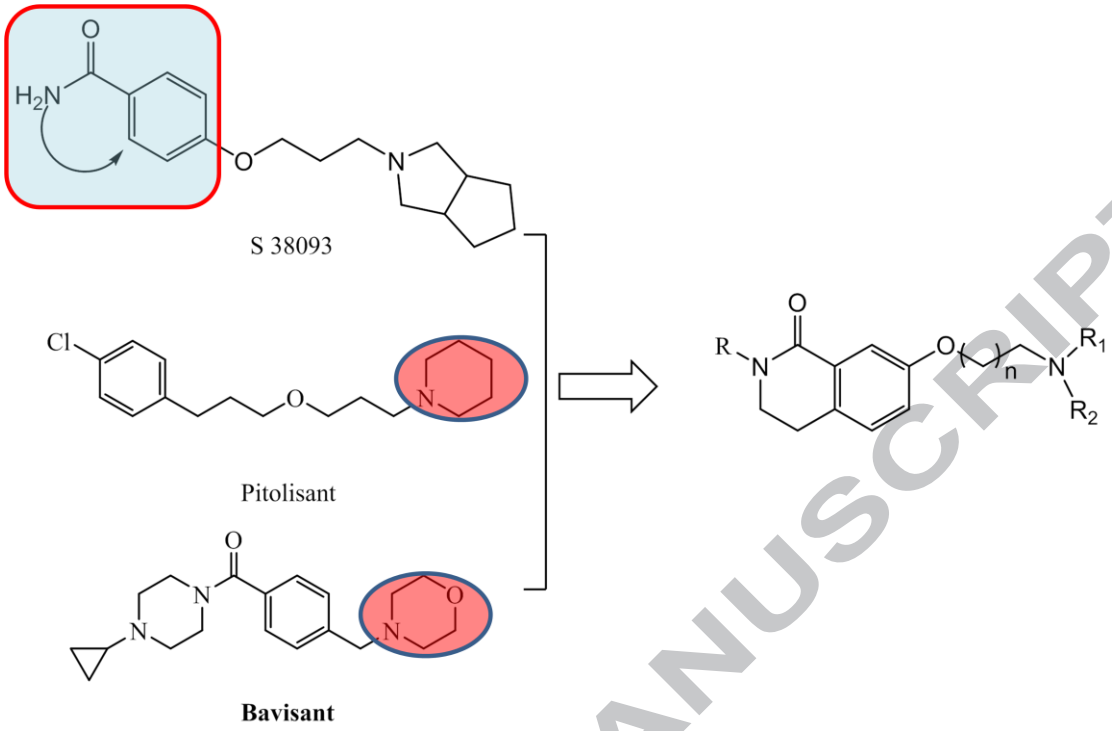
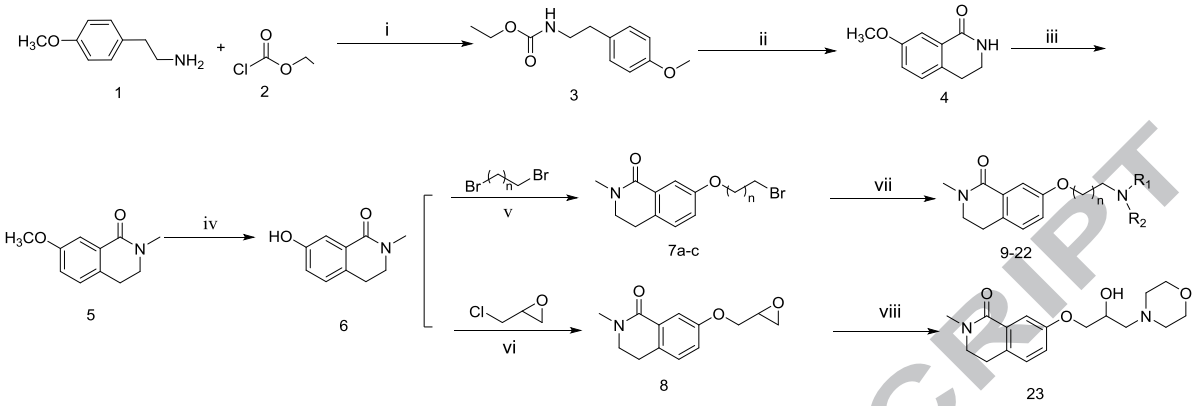


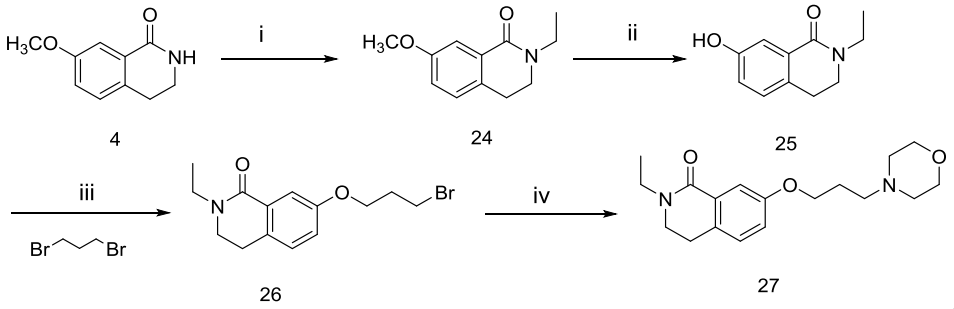
Figure. 2 Design of new lactam derivatives

Scheme 1[a]



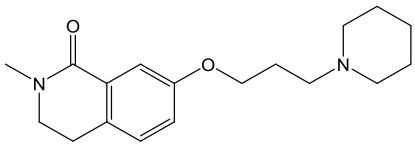
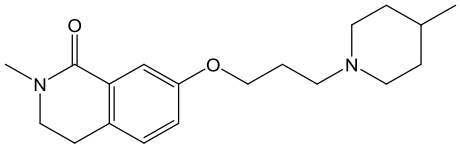
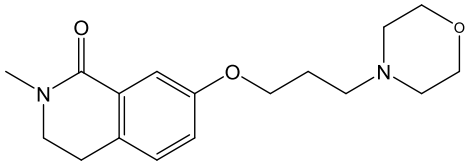
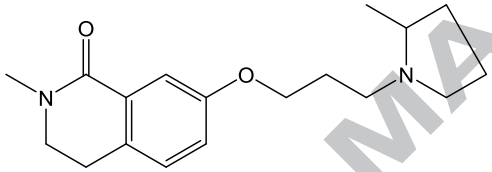
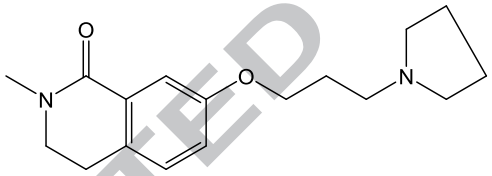
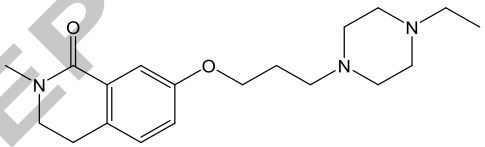
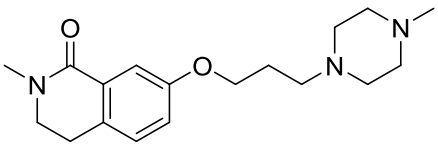
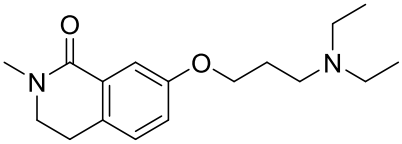
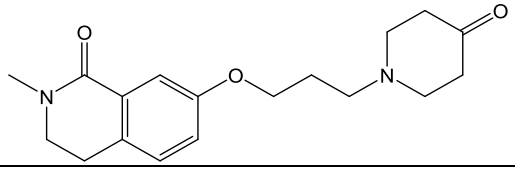
[a]Reagents and conditions: (i) DCM, Triethylamine , 0^{o}C-r.t.; (ii) P_2O_5, Sulphonethane, 160^{o}C; (iii) CH_3I,NaH, DMF; (iv) HBr/H_2O; (v) Acetone, K_2CO_3, reflux; (vi) CH_3CN, K_2CO_3, reflux; (vii) Acetone, K_2CO_3, reflux; (viii) CH_3OH, reflux.

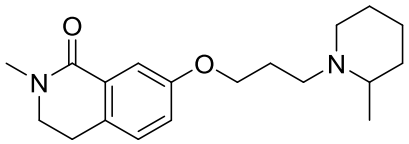
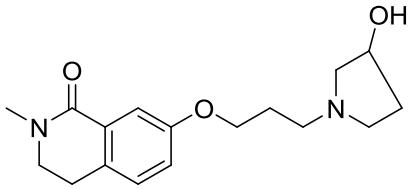
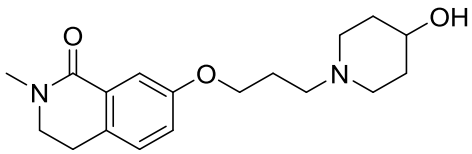
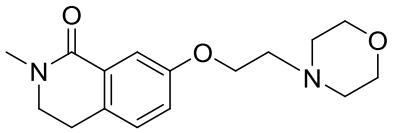
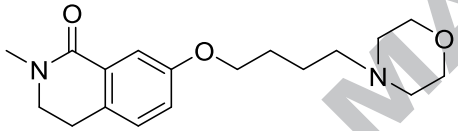
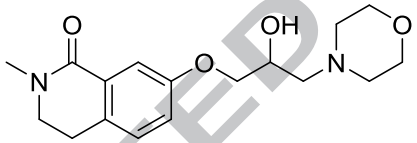
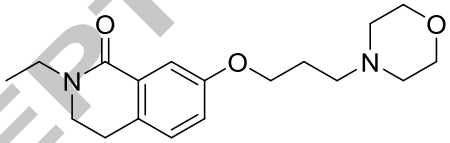
Scheme 2[a]



[a]Reagents and conditions: (i) CH_3CH_2Br,NaH, DMF; (ii) HBr/H_2O; (iii) Acetone, K_2CO_3, reflux; (iv) CH_3CN, K_2CO_3, reflux

Table.1. Binding Affinities for the H_1 and H_3 Receptors of Compounds 9-23 and 27.

Compound	Structure	*Ki*（nM）		Selectivity
		H_3	H_1	(H_1/H_3)
9		23	189	8.2
10		32	279	8.7
11		6.5	1027	158
12		26	68	2.6
13		39	169	4.3
14		1098	-	-
15		706		
16		542	-	
17		176	-	-

18		165	-	-
19		112	-	-
20		35	908	25.9
21		1208	-	-
22		856	-	-
23		>2000	-	-
27		252	-	-
S 38093	-	3.6	426	118

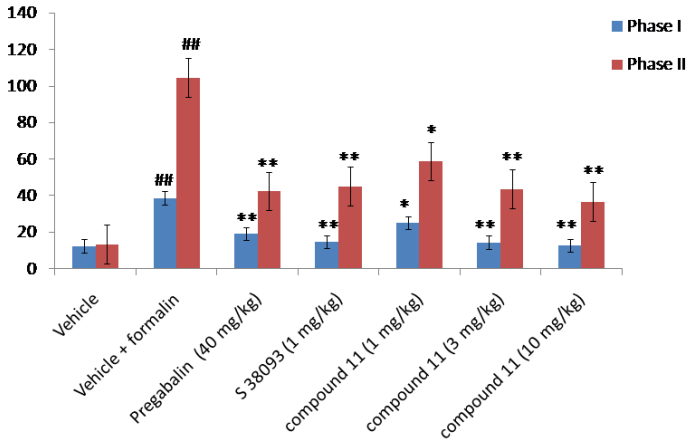
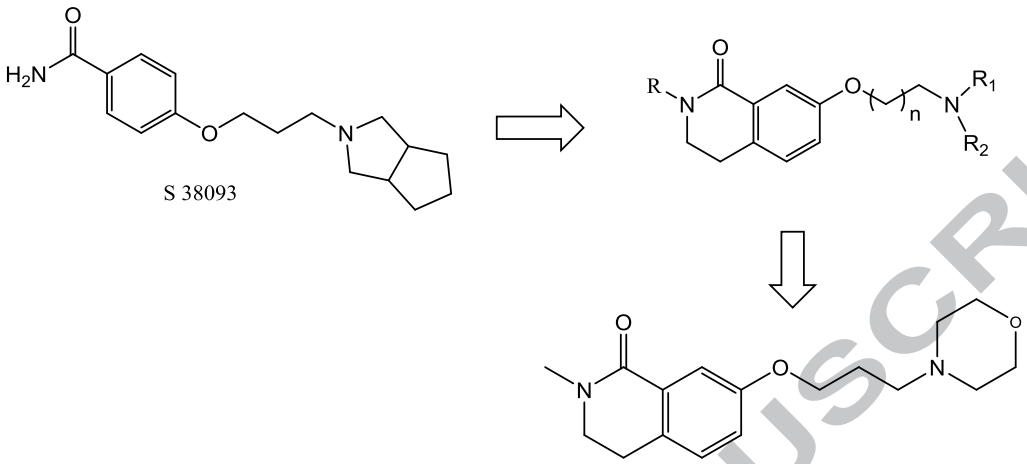


Fig. 3. Anti-nociceptive effect of pregabalin, **S38093** and compound **11** in phase I (0-5 min) and phase II (15-45 min) of the formalin test in rats (10/group). Each column and vertical line represents mean ± SEM of the values obtained in at least ten animals. Statistically significant differences: $^{\#}p<0.05$; $^{\#\#}p<0.01$ vs. vehicle; *p<0.05; **p<0.01 vs. vehicle+formalin (two-way ANOVA followed by Newman-Keuls test).

Graphical abstract



Ki: H_3 = 6.5 nM; H_1 = 1027 nM;
selectivity = 158

Highlights

- 1. A a series of lactam derivatives were designed and synthesized.
- 2. Compound 11 shown to be potent and selective H_3 receptor ligands.
- 3. Compound 11 exerted dose-dependent anti-nociceptive effects in the formalin test.