**ABSTRACT**

Histamine H3 receptors (H3R) antagonists/inverse agonists are becoming a promising therapeutic approach for epilepsy. In this article, novel nonimidazole H3R antagonists/inverse agonists have been designed and synthesised via hybriding the H3R pharmacophore (aliphatic amine with propyloxy chain) with the 1,2,4-triazole moiety as anticonvulsant drugs. The majority of antagonists/inverse agonists prepared here exerted moderate to robust activities in cAMP-response element (CRE) luciferase screening assay. 1-(3-(4-(3-Phenyl-4H-1,2,4-triazol-4-yl)phenoxy)propyl)piperidine (3I) and 1-(3-(4-(3-(4-chlorophenyl)-4H-1,2,4-triazol-4-yl)phenoxy)propyl)piperidine (3m) displayed the highest H3R antagonistic activities, with IC50 values of 7.81 and 5.92 nM, respectively. Meanwhile, the compounds with higher H3R antagonistic activities exhibited protection for mice in maximal electroshock seizure (MES)-induced convulsant model. Moreover, the protection of 3m against the MES induced seizures was fully abrogated when mice were co-treated with RAMH, a CNS-penetrant H3R agonist, which suggested that the potential therapeutic effect of 3m was through H3R. These results indicate that the attempt to find new anticonvulsant among H3R antagonists/inverse agonists is practicable.

**1. Introduction**

Epilepsy, a very common neurologic disorder, affects about 1% of world population. Presently, antiepileptic drugs (AEDs) are the main strategy of therapy. However, the AEDs available in the clinic such as phenytoin, carbamazepine, sodium valproate, topiramate, and oxcarbazepine are only effective in approximately 70% of the patients with epilepsy. Moreover, their use is long-term and often accompanied with severely side effects, including naupathia, headache, and ataxia, even threaten the life of patients.

The role of central histaminergic system being concerned in epilepsy have been demonstrated in many experimental and epidemiological studies, in which histamine regulated seizure susceptibility as an anticonvulsant neurotransmitter. For example, H1-antagonists such as pyrilamine, ketotifen that decrease brain histamine levels increased the duration of convulsive phase in electrically-induced convulsions model. Histidine, as the precursor of histamine, showed protection against chemically-induced convulsions in rats, via activating the histamine H1 receptors.

Histamine H3 receptors (H3R) as a G-protein coupled receptor (GPCR) binding to histamine like other histamine receptors, is expressed mainly in the central nervous system, where it acts as an autoreceptor in histaminergic neurons, and negatively regulates the synthesis and release of histamine. What is more, as an inhibitory heteroreceptor, H3R also regulates the release of other neurotransmitters including dopamine, acetylcholine, serotonin, norepinephrine, γ-aminobutyric acid, and glutamate. These neurotransmitters, especially γ-aminobutyric acid and glutamate, are related to epilepsy inextricably. Therefore, more attention has been focussed on H3R as an attractive therapeutic target for epilepsy treatment.

A large number of experimental studies involved in acute and chronic models of epilepsy confirmed the anticonvulsant potential of H3R antagonists/inverse agonists. They showed the protection against experimental seizures by feedback increase of histamine release and binding with H1 receptors. Besides, other mechanisms might be involved in their anticonvulsive action, such as facilitating of GABA release, increasing histidine decarboxylase (HDC) activity and synergism with AEDs.

Early, anticonvulsant activity of some imidazole H3R antagonists such as thioperamide and clobenpropit was confirmed in models of epilepsy. Recently, a large number of non-imidazole H3R antagonists such as DL77 prepared by a group/team of Kiec-Kononowicz exhibited excellent anticonvulsant activity in the electrically-induced seizures model and subcutaneously pentylenetetrazole (PTZ)-induced seizure model at dose-dependent, and the therapeutic action was proved through H3R antagonists/inverse agonists hybrid; 1,2,4-triazole.
Pitolisant (PIT), a H3R antagonist/inverse agonist, has been subjected into clinical Phase III for the treatment of epilepsy. When used alone or in combination with other AEDs in the human photosensitivity model at dose ranges of 30–60 mg, PIT showed a favourable EEG profile in a dose-dependent manner.

Supported by the above results, in this work, we designed and synthesised some novel H3R antagonists/inverse agonists by hybriding the H3R pharmacophore (aliphatic amine with propyloxy chain) with the 1,2,4-triazole, the latter have been identified as an important and effective anticonvulsive fragment in recent years.

According to Quan's reports, the 1,2,4-triazole derivatives were likely to have several mechanisms of action such as inhibiting voltage-gated sodium ions channel and modulating GABAergic activity. And a group of Plech illustrated the anticonvulsive effects of 4-alkyl-5-aryl-1,2,4-triazole-3-thione derivatives and suggested that the influence on the voltage-gated Na⁺ channels was involved in them at least. Therefore, in this work, our strategy was to design molecules combining pharmacophores of H3R antagonists and another anticonvulsant active pharmacophore (e.g. 1,2,4-triazole moiety) into one skeleton, and then produced a synergism for anticonvulsant active.

2. Results and discussion

2.1. Chemistry

According to Schemes 1 and 2, the target compounds (3a-3q) were synthesised smoothly. In brief, formyl hydrazine reacted with...
4-aminophenol in dimethoxyl-N,N-dimethylformamide (DMF-DMA) to give the 4-(4H-1,2,4-triazol-4-yl)phenol (1a). Compound 1a underwent a nucleophilic substitution with 1-bromo-3-chloropropane to get 4-(4-(3-chloropropoxy)phenyl)-4H-1,2,4-triazole (2a). The reaction was conducted in the presence of potassium hydroxide in dimethyl sulfoxide (DMSO) at room temperature to ensure the formation of single-substituted derivatives. Finally, proper amines reacted with compound 2a in the presence of K2CO3 and KI in the solvent of CH3CN to give the desired compounds 3a-3j.

To enrich the structure–activity relationship, we also prepared the derivatives of 3h via introducing the substituents at the triazole ring and adjusting the length of the link. The reaction conditions used to prepare these compounds (3k-3q) were the same as above. Compounds (3a, 3b, 3c, 3d, 3e, 3g, 3k, 3o, and 3p) obtained as the form of oil were transformed to hydrochlorate. Their structures were characterised and confirmed by 1H-NMR, 13C-NMR, and HR-MS.

2.2. Biological activities

2.2.1. Evaluation of H3R antagonistic activity

cAMP-response element (CRE) reporter gene assay has been extensively used to evaluate the efficacy of GPCR antagonists or agonists. In this work, the H3R antagonistic activities of the prepared 3-(4-(4H-1,2,4-triazol-4-yl)phenoxy)-propylamine derivatives have been screened by CRE-driven luciferase assay, in which the HEK-293 cells expressing the human H3R and a reporter gene consisting of the firefly luciferase coding region were used43,44. Ciproxifan (CXP) and Pitolisant (PIT) were employed as the positive control.
controls. Initially, compounds and positive controls were tested at two concentrations (100 nM and 1 μM) to obtain the preliminary investigation of their H3R antagonistic activities. In the assays, the antagonistic activities was positively correlated with the rise of the fluorescence value and indicated by the % antagonism. For the prominent compounds IC50 values were determined at additional assays.

As seen in Table 1, majority of the synthesised compounds displayed gentle to robust H3R antagonistic activities and eight of them exhibited micromolar inhibitory activity. The antagonistic

| Compounds | R          | n | % Antagonism | 100 nM         | 1 μM          | H3R antagonistic activity (IC50, μM) |
|-----------|------------|---|--------------|----------------|--------------|-------------------------------------|
| 3a        |           |   | 49.85 ± 9.73 | 223.76 ± 7.56  | 2.99         |
| 3b        |           |   | 13.24 ± 4.83 | 15.02 ± 4.03   | NT           |
| 3c        |           |   | 32.23 ± 5.69 | 143.98 ± 16.16 | 0.553        |
| 3d        |           |   | 11.33 ± 5.86 | 79.4 ± 4.50    | NT           |
| 3e        |           |   | 9.86 ± 0.87  | 21.78 ± 3.04   | NT           |
| 3f        |           |   | 9.96 ± 10.9  | 24.41 ± 6.30   | NT           |
| 3g        |           |   | -0.76 ± 4.87 | 20.24 ± 7.56   | NT           |
| 3h        |           |   | 10.56 ± 7.94 | 239.79 ± 3.17  | 0.127        |
| 3i        |           |   | 5.19 ± 2.06  | 16.45 ± 1.90   | NT           |
| 3j        |           |   | -4.2 ± 3.11  | 6.12 ± 2.05    | NT           |
| 3k        | Me        | 1 | 133.91 ± 8.91| 183.54 ± 14.41 | 0.021        |
| 3l        | C6H5      | 1 | 146.62 ± 4.43| 169.04 ± 15.04 | 0.00781      |
| 3m        | C6H4(p-Cl)| 1 | 201.12 ± 12.63| 203.92 ± 8.44  | 0.00592      |
| 3n        | Biphenyl   | 1 | 1.03 ± 7.79  | 3.36 ± 1.27    | NT           |
| 3o        | H         | 0 | 45.04 ± 3.61 | 94.20 ± 2.60   | 0.25         |
| 3p        | H         | 2 | 25.00 ± 3.95 | 90.52 ± 5.71   | 3.44         |
| 3q        | H         | 3 | 1.18 ± 12.38 | 2.39 ± 13.85   | NT           |
| CPXc      |           |   | 33.96 ± 8.59 | 63.05 ± 9.33   | 0.082        |
| PItd      |           |   | 54.16 ± 7.33 | 246.29 ± 21.48 | 0.51         |

*a% Antagonism, value represented as mean ± standard deviation of three independent experiments.

bNT, IC50 was not tested.

cCPXc, an antagonist of H3R ciproxifan.

dPItd, an antagonist/inverse agonist of H3R pitolisant.
activities of all compounds depended on the concentration treated. It is worth mentioning that compounds 3\textit{l} (IC\textsubscript{50} = 7.81 nM) and 3\textit{m} (IC\textsubscript{50} = 5.92 nM) displayed the most potent H\textsubscript{3}R antagonistic activities, with the much stronger potency than that of CXP (IC\textsubscript{50} = 0.082 \mu M) and PIT (IC\textsubscript{50} = 0.5 \mu M) in the CRE reporter gene assay.

Surprisingly, antagonism percent of some compounds as well PIT were above 100%. It is well known that H\textsubscript{3}R is a GPCR coupled reporter gene assay. When cells were pre-treated by H\textsubscript{3}R antagonists, the levels of CXP (IC\textsubscript{50} = 0.082 \mu M) and PIT (IC\textsubscript{50} = 0.5 \mu M) in the CRE reporter gene assay.

The H\textsubscript{3}R inverse agonistic activity (EC\textsubscript{50}, \mu M) was calculated by seven experiments when pre-treated alone. As shown in Figure 2, forskolin (2 \mu M) treated group gave more than 200 times rise for the cAMP level when compared to the control group. While Histamine, ciprofloxin, and compounds 3\textit{a}, 3\textit{c}, 3\textit{h}, 3\textit{k}, 3\textit{l}, and 3\textit{m} have no significant effects on the level of cAMP when carried out comparisons by ANOVA followed by Dunnett’s test.

Another explanation is that these compounds may be inverse agonists when binding to H\textsubscript{3}R, which not only antagonise the function of histamine, but also give the inverse agonistic performance. Actually, PIT is a well-known H\textsubscript{3}R antagonist and reverse function of histamine, but also give the inverse agonistic performance. The H\textsubscript{3}R antagonistic activities, giving the two prominent compounds as well PIT being potent H\textsubscript{3}R antagonistic activities. The EC\textsubscript{50} value of 403 and 129 \mu M, respectively.

Simple structure–activity relationships (SARs) could be obtained from Table 1. In the series of 3\textit{a}-3\textit{j}, the different tertiary amines significantly influenced the H\textsubscript{3}R antagonistic activities. The N-ethyl derivative 3\textit{a} showed an IC\textsubscript{50} of 2.9 \mu M, while the activity declined sharply for the N-propyl derivative 3\textit{b}. Interestingly, compounds containing piperazine or morpholine (3\textit{d}-3\textit{g}) exhibited weaker activities than those with piperidine or pyrrolidine (3\textit{c} and 3\textit{h}). This probably attributed to the increase of the molecular polarity. The introduction of phenyl or amide group on the piperidine ring of compound 3\textit{h}, gave the compounds 3\textit{i} and 3\textit{j}, which also decreased the H\textsubscript{3}R antagonistic activities when compared to compound 3\textit{h}. Based on the facts above, it could be concluded that the N,N-diethyl group, pyrrolidine and piperidine were more of benefit to the H\textsubscript{3}R antagonistic activities of the 3-(4-(4H-1,2,4-triazol-4-yl)phenoxy)-propylamine skeleton, and piperidine derivative 3\textit{h} was the best one with the IC\textsubscript{50} of 0.127 \mu M.

To enrich the structure–activity relationships, we prepared the derivatives of 3\textit{h} via introducing the substituents at the triazole ring and adjusting the length of the link.

Compounds 3\textit{k}, 3\textit{l}, 3\textit{m}, and 3\textit{n} were substituted on 3-position of 1,2,4-triazole ring with methyl, phenyl, para-chlorophenyl, and biphenyl, respectively. Encouragingly, the introduction of methyl, phenyl, and para-chlorophenyl groups significantly increased the H\textsubscript{3}R antagonistic activities, giving the two prominent compounds 3\textit{l} and 3\textit{m} with nanomolar IC\textsubscript{50} values. While the biphenyl substituted compound 3\textit{n} showed weaker activity when compared to 3\textit{h}. Replacing the three-carbon link in the compound 3\textit{h} with two-carbon, four-carbon and five-carbon links, gave the compounds 3\textit{o}, 3\textit{p}, and 3\textit{q}, respectively. It could be seen that the length of the link had a direct impact on H\textsubscript{3}R receptor antagonistic activities of the 3-(4-(4H-1,2,4-triazol-4-yl)phenoxy)-propylamine derivatives. The activity order of the link length of carbon was 3 \geq 2 \geq 4 \geq 5.

To investigate the molecular determinants that manage the antagonistic activities of the tested compounds, molecular docking studies of PIT, 3\textit{h}, and 3\textit{m} with the H\textsubscript{3}R homology model were carried out. The homology model was constructed from the crystal structure of the H\textsubscript{1} receptor (PDB ID: 3RZE)\textsuperscript{45}. The docking results are shown in Figure 4.

As shown in Figure 4(A), PIT bound to H\textsubscript{3}R through two critical H-bond interactions with Tyr115 and Glu206, and other interactions with amino acid residues Arg381, Phe193, Met378 and so on. Figure 4(B) revealed that compound 3\textit{h} had a similar binding pattern to PIT, interacting with the same amino acid residues Glu206, Tyr115, Arg381, and Met378. Surprisingly, the compound
With the highest H3R antagonistic activity showed a different binding pattern to PIT (as seen in Figure 4(C)). The overlying pattern of PIT, 3h, and 3m was shown in Figure 4(D). The piperidine group of 3m was not involved in the formation of the salt bridge or hydrogen-bond interactions with Glu206, which was generally considered as the critical residue of H3R. The unexpected binding pattern of 3m might be due to the phenyl group on the triazole ring, which did not fit into the hydrophobic cavity in TMs 3-5-6 region of H3R, even though the compound 3m showed a forceful binding with H3R via another mode. The triazole nitrogen established an ionic bond with Glu206, and a hydrogen bond was observed between piperidine nitrogen and Tyr115. π-π shaped, and alkyl interactions with Trp371, Tyr343, Arg381, His187, Leu199, and ALA202 were observed to support the forceful binding with H3R.

2.2.2. Anticonvulsant activity evaluation

To investigate the anticonvulsant effects, all the target compounds (3a-3q) were screened in the MES-induced and PTZ-induced convolution models in mice. Compounds were administered intraperitoneally (i.p.) to mice at dosage of 10 mg/kg in the both models. PIT and valproic sodium (VPA) were used as positive controls in the tests.

2.2.2.1. Protective effects of H3R antagonists/inverse agonists 3a-3q on MES-induced convulsions. Protection for the mice was defined as the reduction or abolition of the tonic hind limb extension (THLE) in the MES model in mice. As seen in Figure 5, compounds 3a, 3g, 3h, 3l, 3m, and 3o showed moderate protection for the electro-stimulated mice with significant difference from that of the control group (p<0.05, p<0.01, or p<0.001). Mice pre-treated with PIT (10 mg/kg, i.p.) and VPA (300 mg/kg, i.p.) were moderately or potently protected, respectively. Generally, the anticonvulsant activities of these compounds in MES model correlated directly to their H3R antagonistic activities. For example, the antiepileptic activity obtained of compound 3m was the highest, and in vitro H3R antagonistic activity measured for 3m with IC50 of 5.92 nM was also the highest. Compounds 3a, 3h, 3l, and 3o, showing anticonvulsant activity in the MES model, also showed good H3R antagonistic activities. Compound 3c, 3k, and 3p
The above findings suggested that H3R antagonism was the main contributor for the anticonvulsant activity of compound 3m in MES model. When the H3R was blocked by H3R antagonist PIT, at 20 mg/kg dose, fully abrogate the THLE for all the tested mice. Protective effects of compound 3m (10 mg/kg, i.p.) against MES-induced convulsions when pre-treatment of RAMH (10 mg/kg, i.p.). Protection in the test was defined as the reduction or abolition of the THLE in mice. Results are showed as mean ± SEM with seven animals in each group. Values are considered significant at *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 as compared to saline-treated group.

2.2.2.2. Protective effects of H3R antagonists/inverse agonists 3a-3q on PTZ-induced convulsions. Some experiments indicated that H3R antagonists/inverse agonists could protect animals in PTZ-induced convulsions model. So the compounds 3a-3q, PIT, and VPA were also screened in the PTZ model in mice. Unfortunately, all compounds tested at the dose of 10 mg/kg (i.p.) did not show any protection against the seizures induced by PTZ. PIT also failed to protect the PTZ-treated mice as well at the same conditions. By contrast, anticonvulsant agent VPA showed full protection against the PTZ-induced convulsions (Figure 6).

2.2.2.3. Effects of compound 3m on MES-induced convulsions in dose dependent manner. In a further experiment, compound 3m, as the most active one in the MES-induced seizure model, was chosen to verify its protective effect in different doses. Encouragingly, the 3m-provided protections were observed and were dose dependent. The standard antagonist PIT also displayed anticonvulsive activity dose-dependently at the same condition. Notably, when pre-treated with 20 mg/kg dose, PIT could fully abrogate the tonic hind limb extension induced by electro-stimulation, showing its potential anticonvulsant activity (Figure 7). To exclude the possibility that the anticonvulsant activity of 3m was connected with sedative effect, we carried out a rotarod test for 3m. The result showed that compound 3m had no neurotoxicity at the maximum dose of 10 and 20 mg/kg (the details could be seen in Support Table 1).

2.2.2.4. Effects of RAMH pre-treatment on the compound 3m-provided protection in MES-induced seizure model. To investigate the correlation between the anticonvulsant activity and H3R antagonistic activity of compound 3m, the protection provided by compound 3m against MES-induced seizure was reassessed after the administration of RAMH (10 mg/kg, i.p.), a CNS penetrant histamine H3R agonist. The results indicated that when co-administration with RAMH, compound 3m lost its original protective effect (Figure 8). Administration of RAMH alone also did not affect the duration of THLE of mice with p > 0.05 for saline versus RAMH. The above findings suggested that H3R antagonism was the main contributor for the anticonvulsant activity of compound 3m in MES model.
histamine or other neurotransmitter such as GABA in the CNS increased, finally leading to anticonvulsive effects.

3. Conclusion
To identify novel H3R antagonists/inverse agonists with potential anticonvulsant activities, a series of 3-(4-(4H-1,2,4-triazol-4-yl)phenoxo)-propylamine derivatives were designed through combining pharmacophore of H3R antagonists and another anticonvulsant active pharmacophore (1,2,4-triazole moiety) into one molecule. The majority of those prepared compounds displayed moderate to robust H3R antagonistic activities. The SAR analysis revealed that piperidine and triazolephenol linked by three-carbon chain was benefit for the H3R antagonistic activity, and substitution by aromatic nucleus on the 3-position of 1,2,4-triazole further increased the H3R antagonistic activities. The most potent H3R antagonists/inverse agonists 3l and 3m exhibited nanomolar H3R antagonistic activities with IC50 of 7.81 nM and 5.92 nM, respectively. Molecular docking analysis demonstrated that 3m strongly bound to H3R via interactions with Tyr115, Glu206, Trp371, Tyr343, and so on, although its binding mode was not similar to PIT. The anticonvulsant screens in vivo indicated that compounds with higher H3R antagonistic activities showed more protection in the MES-induced convulsant model in mice, while no one was observed protective effect in PTZ-induced convulsant model. Moreover, the protection of 3m in the seizure model was fully abrogated when mice were co-treated with a H3R agonist RAMH, which suggested that its potential therapeutic effect was through H3R.

4. Experimental section
4.1. Synthesis
All the chemical solvents and reagents were purchased from supplier and used as received. Unless otherwise specified, reactions were monitored by thin-layer chromatography (TLC). All NMR spectra were recorded on a JEOL instrument operating at 300 MHz. High resolution mass spectra were measured on an MALDI-TOF/TOF mass spectrometer.

4.1.1. Synthesis of compounds 1a-1e
Taking compound 1a as an example: dimethoxyl-N,N-dimethyl formamide (DMF-DMA, 1.31 g, 11 mmol) and formyl hydrazine (0.65 g, 11 mmol) were added into a flask containing 30 ml of formamide (DMF-DMA, 1.31 g, 11 mmol) and formyl hydrazine (0.65 g, 11 mmol) were added into a flask containing 30 ml of formamide (DMF-DMA, 1.31 g, 11 mmol) and formyl hydrazine (0.65 g, 11 mmol). The mixture was heated up to 60 °C, and 4-aminophenol (0.60 g, 5.5 mmol) and acetic acid (3 mL) were added. The mixture was stirred for 5 min at 20 °C. Then added 1-bromo-3-chloropropane (0.98 g, 6.2 mmol) into the mixture and continued the reaction. After completion of the reaction indicated by the TLC (developing agent ratio: CH2Cl2/CH3OH = 15/1), the mixture was poured into 30 ml of water. The solution was extracted with dichloromethane three times. The organic layers were washed with saturated salt water, dried over MgSO4, filtered, and concentrated. Purification by column chromatography (silica gel, 0–5% methanol in CH2Cl2) gave the compound 2a. Chemical formula: C11H12ClN3O (MW = 237.69), m.p. 102–104 °C, yield 81%. 1H-NMR (300 MHz, DMSO-d6): δ 2.15–2.23 (m, 2H, OCH2), 3.80 (t, 2H, J = 6.5 Hz, CH2CH2), 4.16 (t, 2H, J = 6.1 Hz, OCH2), 7.33 (d, 2H, J = 9.0 Hz, Ph-H). 7.60 (d, 2H, J = 9.0 Hz, Ph-H), 9.00 (s, 2H, N–CH3). 13C-NMR (75 MHz, DMSO-d6): δ 158.40, 143.02, 127.75, 123.40, 116.03, 65.22, 42.35, 32.05. As mentioned above, replacing the reactant 1a by the alternative 4-(3-substituted-4-H-1,2,4-triazol-4-yl)phenols (1b-1e) to give the compounds 2b-2e. Compounds 2f, 2g, 2h were obtained by the same method as above just replacing 1-bromo-3-chloropropane by 1-bromo-2-chloroethane, 1-bromo-4-chlorobutane, 1-bromo-2-chloropentane, respectively.

4.1.2. Synthesis of compounds 3a-3q
Taking compound 3a as an example: in a 100 ml round-bottom flask with 15 ml of acetonitrile, compound 2a (0.40 g, 1.68 mmol), diethylamine (0.245 g, 3.36 mmol), K2CO3 (0.46 g, 3.36 mmol) and potassium iodide (0.56 g, 3.36 mmol) were added one by one. The mixture was heated up to 110 °C for 12–16 h. After cooling the mixture to 40 °C, it was filtered and dried by vacuum to obtain a residue. Purification by column chromatography (silica gel, 0–20% methanol in CH2Cl2) gave the compound 3a. The same conditions were used to prepare the compounds 3b-3q. Compounds 3a, 3c, 3d, 3e, 3g, 3k, 3o, and 3p obtained as oils were transformed into the corresponding hydrochloride by hydrogen chloride in CH3Cl2.

4.1.3. Characterisation for the target compounds
4.1.3.1. 3-(4-(4H-1,2,4-triazol-4-yl)phenoxo)-N,N-diethylpropan-1-amine hydrochloride (3a).
Chemical formula: C15H27N4O·HCl (MW = 310.83), m.p. 105–106 °C, yield 82%. 1H-NMR (300 MHz, DMSO-d6): δ 1.25 (t, 6H, J = 7.2 Hz, CH3), 2.18 (t, 2H, J = 9.0 Hz, OCH2CH2), 3.08–3.16 (m, 6H, N(CH2)3), 4.17 (t, 2H, J = 6.0 Hz, OCH2), 7.19 (d, 2H, J = 8.8 Hz, Ph-H), 7.75 (d, 2H, J = 8.8 Hz, Ph-H), 9.84 (s, 2H, N–CH3), 10.96 (s, 1H, HCl). 13C-NMR (75 MHz, DMSO-d6): δ 159.34, 143.39, 126.44, 124.35, 116.08, 66.00, 48.04, 46.59, 23.40, 8.87. ESI-HRMS calculated for C15H27N4O·HCl: 275.1866; found: 275.1860.

4.1.3.2. 3-(4-(4H-1,2,4-triazol-4-yl)phenoxo)-N,N-dipropylpropan-1-amine hydrochloride (3b).
Chemical formula: C19H33N4O·HCl (MW = 338.88), m.p. 180–183 °C, yield 62%. 1H-NMR (300 MHz, CDCl3): δ 0.55 (t, 6H, J = 7.3 Hz, CH3), 1.77–1.90 (m, 4H, N(CH2CH2)3), 2.27–2.37 (m, 2H, OCH2CH2), 3.15 (t, 4H, J = 8.0 Hz, N(CH2CH2)3), 3.40 (t, 2H, J = 8.3 Hz, NCH2), 4.18 (t, 2H, J = 5.6 Hz, OCH2), 7.05 (d, 2H, J = 8.9 Hz, Ph-H), 7.43 (d, 2H, J = 8.9 Hz, Ph-H), 8.62 (s, 2H, CH=N=), 9.51 (s, 1H, HCl). 13C-NMR (75 MHz, CDCl3): δ 163.08, 146.48, 132.18, 128.41, 120.58, 69.93, 59.42, 55.13, 28.54, 21.98, 15.92. ESI-HRMS calculated for C19H33N4O·HCl: 303.2179; found: 303.2178.

4.1.3.3. 4-(4-(3-pyrrolidin-1-yl)propoxy)phenyl)-4H-1,2,4-triazole hydrochloride (3c).
Chemical formula: C17H19N3O·HCl (MW = 308.81), m.p. 120–122 °C, yield 56%. 1H-NMR (300 MHz, DMSO-d6): δ 1.92–2.00 (m, 4H, NCH2CH2), 2.19–2.22 (m, 2H, OCH2CH2), 3.00–3.53 (m, 6H, N(CH2CH2)3), 4.17 (t, 2H, J = 5.7 Hz, OCH2), 7.17 (d,
2H, J = 8.7 Hz, Ph-H), 7.72 (d, 2H, J = 8.7 Hz, Ph-H), 9.75 (s, 2H, N=CH). \(^1^3\)C-NMR (75 MHz, DMSO-d6): \(\delta\) 159.25, 149.30, 141.26, 126.57, 124.25, 116.07, 66.03, 53.21, 51.46, 25.53, 23.24. ESI-HRMS calculated for \(\text{C}_15\text{H}_{21}\text{N}_5\text{O}\) (\([M+H]^+\)): 363.2179; found: 363.2178.

1.1.4.10. 1-(3-(4H-1,2,4-triazol-4-yl)phenoxo)propyl)piperidine-4-carboxamide (3j). Chemical formula: \(\text{C}_{29}\text{H}_{27}\text{N}_5\text{O}_2\) (MW = 363.86). m.p. 240–242 °C, yield 61%. \(^1^H\)-NMR (300 MHz, CDCl3): \(\delta\) 1.68–2.43 (m, 8H, \text{NCH}_{2}\text{CH}_2\text{CH}_2\text{CH}_2\text{N}), 2.18–2.30 (m, 2H, \text{OCH}_2\text{CH}_2\text{N}), 3.18–3.25 (m, 6H, \text{NCH}_3\text{CH}_3\text{CH}_3), 4.13 (t, 2H, J = 6.0 Hz, OCH_3), 7.13 (d, 2H, J = 5.8 Hz, Ph-H), 7.55 (d, 2H, J = 5.8 Hz, Ph-H), 9.33 (s, 1H, HCl). \(^1^3\)C-NMR (75 MHz, CDCl3): \(\delta\) 164.95, 156.81, 149.03, 132.21, 129.15, 120.75, 70.66, 59.08, 57.74, 28.43, 27.48, 26.65. 15.04. ESI-HRMS calculated for \(\text{C}_{29}\text{H}_{27}\text{N}_5\text{O}_2\) (\([M+H]^+\)): 301.2023; found: 301.2025.

1.1.4.11. 1-(3-(4-(4H-1,2,4-triazol-4-yl)phenoxo)propyl)piperidine-4-carboxamide hydrochloride (3k). Chemical formula: \(\text{C}_{29}\text{H}_{27}\text{N}_5\text{O}_2\cdot\text{HCl}\) (MW = 363.86). m.p. 240–242 °C, yield 61%. \(^1^H\)-NMR (300 MHz, CDCl3): \(\delta\) 1.68–2.43 (m, 8H, \text{NCH}_{2}\text{CH}_2\text{CH}_2\text{CH}_2\text{N}), 2.18–2.30 (m, 2H, \text{OCH}_2\text{CH}_2\text{N}), 3.18–3.25 (m, 6H, \text{NCH}_3\text{CH}_3\text{CH}_3), 4.13 (t, 2H, J = 6.0 Hz, OCH_3), 7.13 (d, 2H, J = 5.8 Hz, Ph-H), 7.55 (d, 2H, J = 5.8 Hz, Ph-H), 9.33 (s, 1H, HCl). \(^1^3\)C-NMR (75 MHz, CDCl3): \(\delta\) 164.95, 156.81, 149.03, 132.21, 129.15, 120.75, 70.66, 59.08, 57.74, 28.43, 27.48, 26.65. 15.04. ESI-HRMS calculated for \(\text{C}_{29}\text{H}_{27}\text{N}_5\text{O}_2\cdot\text{HCl} (\([M+H]^+\)): 301.2023; found: 301.2025.
115.61, 65.70, 55.17, 53.69, 24.32, 23.08, 22.30, 22.30. ESI-HRMS calculated for 

\[ C_{20}H_{31}N_4O \] \( ^+ \): 349.2492; found: 349.2494.

4.1.4.15. 1-(2-(4-(4H-1,2,4-triazol-4-yl)phenoxy)ethyl)piperidine hydrochloride (3o). Chemical formula: \( C_{15}H_{20}N_4O \). 1H-NMR (300 MHz, CDCl3): \( \delta \) 1.63–1.96 (m, 6H, NCH(2)CH2CH2), 2.29–2.36 (m, 2H, OCH2CH2), 3.27 (t, 4H, J = 5.5 Hz, OCH2), 3.46 (t, 2H, J = 3.4 Hz, OCH2). m.p. 85–87°C, yield 72%. 1H-NMR (300 MHz, CDCl3): \( \delta \) 1.62–1.96 (m, 6H, NCH(2)CH2CH2), 2.28–2.36 (m, 2H, OCH2CH2), 3.39 (t, 4H, J = 3.7 Hz, OCH2), 3.46 (t, 2H, J = 3.4 Hz, OCH2). m.p. 85–87°C, yield 77%. 1H-NMR (300 MHz, CDCl3): \( \delta \) 1.63–1.96 (m, 6H, NCH(2)CH2CH2), 2.29–2.36 (m, 2H, OCH2CH2), 3.40 (t, 4H, J = 5.5 Hz, OCH2), 3.46 (t, 2H, J = 3.4 Hz, OCH2). m.p. 85–87°C, yield 72%.

4.1.4.16. 1-(4-(4H-1,2,4-triazol-4-yl)phenoxy)butyl)piperidine hydrochloride (3p). Chemical formula: \( C_{18}H_{26}N_4O \). 1H-NMR (300 MHz, CDCl3): \( \delta \) 1.96–2.21 (m, 8H, CH2), 2.93–3.04 (m, 2H, CH2), 3.25–3.39 (m, 4H, CH2). m.p. 80–82°C, yield 69%. 1H-NMR (300 MHz, CDCl3): \( \delta \) 1.88–2.21 (m, 8H, NCH2CH2), 2.22–2.36 (m, 2H, OCH2CH2), 2.82–3.08 (m, 6H, NCH2CH2), 4.10 (t, 2H, J = 8.8 Hz, Ph-H), 7.23 (d, 2H, J = 8.8 Hz, Ph-H), 9.69 (s, 2H, CH=N). 13C-NMR (75 MHz, CDCl3): \( \delta \) 158.38, 142.33, 127.14, 124.20, 116.33, 63.37, 54.89, 53.01, 22.70, 21.68. ESI-HRMS calculated for \( C_{18}H_{27}N_4O \) \( ^+ \): 273.1710; 273.1711.

4.1.4.17. 1-(5-(4-(4H-1,2,4-triazol-4-yl)phenoxy)pentyl)piperidine (3q). Chemical formula: \( C_{18}H_{27}N_4O \). 1H-NMR (300 MHz, CDCl3): \( \delta \) 1.88–2.21 (m, 8H, NCH2CH2), 2.93–3.04 (m, 2H, CH2), 3.25–3.39 (m, 4H, CH2). m.p. 80–82°C, yield 69%. 1H-NMR (300 MHz, CDCl3): \( \delta \) 1.88–2.21 (m, 8H, NCH2CH2), 2.22–2.36 (m, 2H, OCH2CH2), 2.82–3.08 (m, 6H, NCH2CH2), 4.10 (t, 2H, J = 8.8 Hz, Ph-H), 7.23 (d, 2H, J = 8.8 Hz, Ph-H), 9.69 (s, 2H, CH=N). 13C-NMR (75 MHz, CDCl3): \( \delta \) 158.93, 141.86, 126.87, 123.86, 115.89, 67.27, 56.93, 53.26, 26.37, 22.50, 21.88, 20.76. ESI-HRMS calculated for \( C_{18}H_{27}N_4O \) \( ^+ \): 301.2023; found: 301.2021.

4.2. Pharmacology

4.2.1. In vitro screening

4.2.1.1. Cell culture and transfection. Human thalamus poly-A RNA (Clontech, Palo Alto, CA, USA) was used to clone the hH3R gene by RT-PCR. DNA PCR primers were designed in the light of the reported human histamine receptor gene sequences (GenBank accession no.AF140538). HEK-293 cells were cultured and transfected for the luciferase assay. The detailed procedures were described in the previous publication.

4.2.1.2. CRE-driven reporter gene assay. Stable HEK-293 cells, which had been co-transfected with hH3R and pCRE-Luc, were seeded in a 96-well plate overnight, and were grown to 90–95% confluence. Then the cells were treated with various concentrations of tested compounds in serum-free DMEM and incubated for 20 min. Cells were then stimulated with 100 nM Histamine in serum-free DMEM containing 2 μM Forskolin and incubated for 4 h at 37°C. Firefly luciferase assay kits (Ken-real, Shanghai, China) were used to determine the luciferase activity.

4.2.2. In vivo pharmacology

4.2.2.1. Drugs and animals. Valproic acid (VPA) was obtained from melongpharma, Dalian, China. Ciprofloxin maleate was purchased from Shanghai Hanxiang Biotechnology Co., Ltd, China. R-(2)-methyl-histamine (RAMH), Pitolisant (PIT), and Pentylentetrazol (PTZ) were bought from Macklin Co. KunMing mice were purchased from Changsha tianqin Biotechnology Co., Ltd, China and used with the body weight 20–25 g. Procedures involving animals were performed according to the Guide for the Care and Use of Laboratory Animals (8th Edition, National Academies Press, Washington, DC), and was approved by the local animal ethics committee (Institutional Animal Ethics Committee of Jingshangshan University, approval number: 201906018).

4.2.2.2. MES-induced seizure. Ear stimulation with alternating current (0.2 s, 60 Hz, 50 mA) was used to induce the seizures in mice. The reduction or abolition of the hind limb tonic extension (THLE) of mice was considered protective against the MES-induced seizures.29,48 Test compounds and positive drugs VPA (300 mg/kg) and PIT (10 mg/kg) were i.p. administrated half an hour prior to the electric stimulation. To investigate the mechanism of action, the most promising one 3m was chose for a further test. In one group of animals of seven mice, compound 3m (10 mg/kg) was injected with RAMH 10 mg/kg with 5 min interval. The animals in other three groups were single treated with RAMH 10 mg/kg, compound 3m 10 mg/kg, and vehicle, respectively.

4.2.2.3. PTZ-induced seizures. PTZ (85 mg/kg) was injected subcutaneously to induce seizures. Firstly, vehicle, tested compounds 3a-3q (10 mg/kg), and positive controls (VPA 300 mg/kg, PIT 10 mg/kg) were administrated i.p. After 30 min, PTZ was injected to all the animals. Animals were observed for 30 min (experiment period) for any convulsion signs, and graded scores were used to assess the seizures severity. The following are the specific mean-
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Disclosure statement

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