Design, synthesis, and biological evaluation of novel iso-flavones derivatives as H₃R antagonists

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ABSTRACT
Histamine H₂ receptor (H₂R), a kind of G-protein coupled receptor (GPCR), is expressed mainly in the central nervous system (CNS) and plays a vital role in homeostatic control. This study describes the design and synthesis of a series of novel H₃R antagonists based on the iso-flavone scaffold. The results of the bioactivity evaluation show that four compounds (1c, 2c, 2h, and 2o) possess significant H₃R inhibitory activities. Molecular docking indicates that a salt bridge, π–π T-shape interactions, and hydrophobic interaction all contribute to the interaction between compound 2h and H₃R.

Introduction
Histamine, a distinctly important neurotransmitter, exerts as a modulator in the brain and dominates several homeostatic functions such as thermoregulation, fluid balance, and energy metabolism. Apart from that, histamine is also involved in numerous processes, for instance, circadian rhythms, the sleep–wake cycle, attention, memory, learning, and neuroendocrine regulation. According to recent studies, the biosynthesis and release of histamine in central nervous system (CNS) are modulated by four different G-protein coupled receptors (GPCRs) subtypes, namely histamine H₁ receptor (H₁R), histamine H₂ receptor (H₂R), histamine H₃ receptor (H₃R), histamine H₄ receptor (H₄R). Unlike H₁R and H₂R, H₃R shows higher homology to H₄R and is highly expressed in brain, such as basal ganglia and globus pallidus, which could couple with G i/o protein and then activate mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) pathways. Subsequently, the phospholipase A₂ (PLA₂) is induced to recruit Ca²⁺ from intracellular stores, reduces cAMP formation and enhances phosphorylation. Moreover, H₂R is recognised as an auto- and hetero-receptor on non-histaminergic neurons controlling the release of many other important neurotransmitters, such as acetylcholine, norepinephrine, dopamine, and serotonin. A clinical study revealed that neurotransmitters could trigger the postsynaptic signalling pathways bound to cognition which supported the hypothesis that H₂R is a drug target for cognitive disorders, especially for Alzheimer Disease (AD), schizophrenia and epilepsy. Because of the unique functions of H₂R, a wide variety of selective H₂R antagonists have been developed and some of them have shown promising effects. Flavone and iso-flavone, which are regarded as privileged structures, exhibit variety of pharmacological activities, such as anti-cancer, antimicrobial, anti-inflammatory, and also are used in neurodegenerative disorders, for example, Alzheimer’s disease. Our previous study had confirmed the iso-flavone and flavone compounds possessed moderate inhibitory activity against H₃R. Particularly, the optimization at the 8-position of the flavones and 7-position of iso-flavone provided satisfactory bioactivity (compound A, B, and C, Figure 1), which enlightened us to modify 8-position of iso-flavone to enhance the H₃R inhibitory effect. In addition, we also want to modify the 6-position of isoflavones to see whether compounds with better antagonistic activity can be obtained. In this current work, two series of novel iso-flavone derivatives were designed and synthesised based on our previous study. After screening the H₃R inhibitory activities at a fixed concentration, compounds that possessed good H₃R inhibitory activity were further tested to determine the IC₅₀ values. In addition, molecular docking studies were performed to investigate the interaction between H₃R and the most potent antagonist.

Materials and methods
Chemistry
Unless otherwise indicated, all solvents and organic reagents were obtained from commercially available sources and were used without further purification. The reaction process was monitored using thin layer chromatography (TLC) with silica gel plates (thickness = 0.20 mm, GF254) under UV light. Column chromatography was performed using a ZCZ-II (200–300 mesh), to purify the final products. All final products were found to have purities ≥95% analysed by HPLC. Melting points were determined using a YRT-3 apparatus (Tian Jin Optical Instrument Factory, Tianjin, China) and were presented as uncorrected values. ¹H NMR spectra were recorded on a Varian Mercury-300 MHz instrument, whereas ¹³C NMR was recorded at 300 MHz on a Varian Mercury using DMSO-d₆ as a solvent and tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained using a Waters Acquity UPLC-SQD mass spectrometer (Waters, Milford, MA). High-resolution mass spectra

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HRMS) were recorded on an Agilent Technologies LC/MSD TOF spectrometer (Agilent Technologies Co. Ltd., Santa Clara, CA).

The synthetic route of novel compounds is depicted in Scheme 1. All title compounds were synthesised through Mannich reactions using iso-flavone, 37% formalin, and aliphatic amines as starting materials. Compounds 1a–1g, 2a–2i, and 2j–2t were synthesised from genistein, daidzein, and formononetin, respectively. The use of DMF-methanol as a solvent for formononetin and daidzein never resulted in the formation of 6-substituted products, but only 8-position substituted products were obtained.

General procedure for the synthesis of compounds 1a–1g

Genistein (0.50 g, 1.85 mmol), 37% formalin (0.30 g, 3.70 mmol), aliphatic amines (0.225 g, 2.780 mmol), and methanol (30 ml) were added into a three-necked flask (100 ml) and stirred at 25 °C for 24 h. After reactions completed monitored by TLC (DCM:MeOH = 10:1), the solvent was removed under reduced pressure. The residue was purified by column chromatography using a mixture of dichloromethane and methanol (30:1) as the eluent to give the target compounds in yields ranging from 41% to 91%.

The similar procedure was followed for the synthesis of compounds 2a–2t.

Title compounds were characterised as follows:

5,7-dihydroxy-3-(4-hydroxyphenyl)-8-(pyrrolidin-1-ylmethyl)-4H-chromen-4-one (1a)

White solid, yield: 24%; mp 218–220 °C; 1H NMR (300 MHz, DMSO-d6) δ 8.15 (s, 1H), 7.35 (d, J = 8.7 Hz, 2H), 6.80 (d, J = 8.4 Hz, 2H), 6.10 (s, 1H), 3.96 (s, 2H), 2.83 (m, 4H), 1.83 (m, 4H). 13C NMR (100 MHz, DMSO-d6) δ 179.9, 170.4, 161.6, 159.5, 157.8, 153.5,
5.7-dihydroxy-3-(4-hydroxyphenyl)-8-(3-(methyl)piperidin-1-yl)methyl)-4H-chromen-4-one (1f). White solid, yield: 16%; mp 210–213 °C; 1H NMR (300 MHz, DMSO-d$_6$) δ 8.34 (s, 1H), 7.92 (d, J = 8.7 Hz, 1H), 7.38 (d, J = 8.7 Hz, 2H), 6.82 (d, J = 8.7 Hz, 2H), 6.18(s, 1H), 3.81(s, 2H), 2.95(s, 4H), 2.35 (m, 4H), 2.07(s, 3H). 13C NMR (100 MHz, DMSO-d$_6$) δ 180.7, 166.4, 161.5, 158.0, 155.6, 154.2, 130.7, 122.7, 121.7, 115.6, 104.7, 100.1, 99.5, 63.2, 53.2, 52.9, 51.7. HR-MS (ESI) Calcd for C$_{22}$H$_{22}$N$_2$O$_5$ [M + H]$^+$, 383.1607, found: 383.1609.

7-hydroxy-8-((4-(3-hydroxypiperidin-1-yl)methyl)phenyl)-3-(4-hydroxyphenyl)-4H-chromen-4-one (2b). White solid, yield: 14%; mp 250–252 °C; 1H NMR (300 MHz, DMSO-d$_6$) δ 8.28 (s, 1H), 7.89 (d, J = 8.7 Hz, 1H), 7.37 (d, J = 8.7 Hz, 2H), 6.84–6.77 (m, 3H), 3.98 (s, 2H), 3.26 (d, J = 6.3 Hz, 2H), 2.95 (d, J = 11.2 Hz, 1H), 2.21 (t, J = 11.2 Hz, 2H), 1.73 (d, J = 13.2 Hz, 2H), 1.43 (s, 1H), 1.19 (m, 2H). 13C NMR (100 MHz, DMSO-d$_6$) δ 175.3, 164.2, 157.7, 155.2, 152.9, 130.6, 129.1, 123.0, 116.6, 115.7, 115.5, 108.4, 66.0, 53.3, 52.9, 38.2, 28.9. HR-MS (ESI) Calcd for C$_{22}$H$_{23}$N$_2$O$_5$ [M + H]$^+$, 397.1763, found: 397.1767.

7-hydroxy-8-((4-(3-hydroxypiperidin-1-yl)methyl)phenyl)-3-(4-hydroxyphenyl)-4H-chromen-4-one (2c). White solid, yield: 27%; mp 230–232 °C; 1H NMR (300 MHz, DMSO-d$_6$) δ 8.32 (s, 1H), 7.92 (d, J = 9.9 Hz, 1H), 7.38 (d, J = 9.9 Hz, 2H), 6.93 (d, J = 9.9 Hz, 1H), 6.80 (d, J = 8.4 Hz, 2H), 3.88 (s, 2H), 3.56 (t, J = 8.4 Hz, 2H), 2.83 (d, J = 10.8 Hz, 2H), 1.90 (t, J = 11.2 Hz, 2H), 1.05 (d, J = 6.3 Hz, 6H). 13C NMR (100 MHz, DMSO-d$_6$) δ 175.4, 162.8, 157.8, 155.8, 153.1, 130.6, 126.4, 123.8, 116.9, 115.5, 115.5, 108.5, 53.1, 34.1, 30.2, 22.0. HR-MS (ESI) Calcd for C$_{22}$H$_{23}$N$_2$O$_5$ [M + H]$^+$, 366.1705, found: 366.1749.

8-((3RS,5S)-3,5-dimethylmorpholinomethyl)-7-hydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one (2d). White solid, yield: 27%; mp 230–232 °C; 1H NMR (300 MHz, DMSO-d$_6$) δ 8.32 (s, 1H), 7.92 (d, J = 9.9 Hz, 1H), 7.38 (d, J = 9.9 Hz, 2H), 6.93 (d, J = 9.9 Hz, 1H), 6.80 (d, J = 8.4 Hz, 2H), 3.88 (s, 2H), 3.56 (t, J = 8.4 Hz, 2H), 2.83 (d, J = 10.8 Hz, 2H), 1.90 (t, J = 11.2 Hz, 2H), 1.05 (d, J = 6.3 Hz, 6H). 13C NMR (100 MHz, DMSO-d$_6$) δ 175.4, 162.8, 157.8, 155.8, 153.1, 130.6, 126.4, 123.8, 116.9, 115.5, 115.5, 108.5, 53.1, 34.1, 30.2, 22.0. HR-MS (ESI) Calcd for C$_{22}$H$_{23}$N$_2$O$_5$ [M + H]$^+$, 366.1705, found: 366.1749.

5,7-dihydroxy-3-(4-hydroxyphenyl)-8-((4-methyl)piperidin-1-yl)methyl)-4H-chromen-4-one (1g). White solid, yield: 19%; mp 231–233 °C; 1H NMR (300 MHz, DMSO-d$_6$) δ 8.34 (s, 1H), 7.37 (d, J = 8.4 Hz, 2H), 6.82 (d, J = 8.7 Hz, 2H), 6.18(s, 1H), 3.80(s, 2H), 2.95(s, 4H), 2.35 (m, 4H), 2.07(s, 3H). 13C NMR (100 MHz, DMSO-d$_6$) δ 180.7, 166.4, 161.5, 158.0, 155.6, 154.2, 130.7, 122.7, 121.7, 115.6, 104.7, 100.1, 99.6, 54.9, 52.4, 51.7, 46.0. HR-MS (ESI) Calcd for C$_{22}$H$_{22}$N$_2$O$_5$ [M + H]$^+$, 383.1607, found: 383.1609.
7-hydroxy-3-(4-hydroxyphenyl)-8-(pyrrolidin-1-ylmethyl)-4H-chromen-4-one (2f).

White solid, yield: 23%; mp 177–179 °C; 1H NMR (300 MHz, DMSO-d6) δ 8.27 (s, 1H), 7.88 (d, J = 8.7 Hz, 1H), 7.37 (d, J = 8.4 Hz, 2H), 6.84–6.77 (m, 3H), 4.08 (s, 2H), 2.67 (m, 4H), 1.77 (m, 4H). 13C NMR (100 MHz, DMSO-d6) δ 175.3, 164.7, 157.7, 155.4, 152.8, 130.6, 126.2, 123.8, 123.1, 116.1, 115.9, 115.5, 109.1, 53.6, 50.0, 23.7. HR-MS (ESI) Calcd for C22H23NO4 [M + H]+, 338.1392, found: 338.1413.

(S)-7-hydroxy-8-[(2-hydroxymethyl)pyrrolidin-1-yl)methyl]-3-[(4-hydroxyphenyl)-4H-chromen-4-one (2g).

White solid, yield: 35%; mp 205–207 °C; 1H NMR (300 MHz, DMSO-d6) δ 8.27 (s, 1H), 7.88 (d, J = 8.7 Hz, 1H), 7.37 (d, J = 8.4 Hz, 2H), 6.85–6.77 (m, 3H), 4.34–4.01 (s, 2H), 3.51 (brs, 2H), 2.92–2.83 (d, J = 27.6 Hz, 2H), 2.40 (d, J = 8.1 Hz, 1H). 13C NMR (100 MHz, DMSO-d6) δ 175.3, 164.7, 157.7, 155.4, 152.8, 130.6, 126.2, 123.8, 123.1, 116.1, 115.9, 115.5, 109.6, 65.6, 62.8, 54.6, 49.5, 27.6, 23.1. HR-MS (ESI) Calcd for C22H23NO4 [M + H]+, 368.1498, found: 368.1482.

7-hydroxy-3-(4-hydroxyphenyl)-8-[(2-methylpiperidin-1-yl)methyl]-4H-chromen-4-one (2h).

White solid, yield: 12%; mp 228–230 °C; 1H NMR (300 MHz, DMSO-d6) δ 8.28 (s, 1H), 7.89 (d, J = 8.7 Hz, 1H), 7.37 (d, J = 8.4 Hz, 2H), 6.79–6.77 (m, 3H), 4.32–4.27 (d, J = 15, 1H), 3.9–3.85 (d, J = 15 Hz, 1H), 2.83 (d, J = 12 Hz, 1H), 2.66 (brs, 1H), 2.30 (t, J = 9.3 Hz, 1H), 1.62–1.35 (m, 6H), 1.15 (d, J = 6.3 Hz, 3H). 13C NMR (100 MHz, DMSO-d6) δ 175.3, 164.6, 157.7, 155.2, 152.9, 130.6, 125.9, 123.8, 123.1, 116.4, 115.9, 108.7, 57.8, 56.3, 50.4, 36.3, 33.8, 25.6, 22.5. HR-MS (ESI) Calcd for C22H23NO4 [M + H]+, 368.1498, found: 368.1482.

7-hydroxy-3-(4-hydroxyphenyl)-8-[(4-methylpiperazin-1-yl)methyl]-4H-chromen-4-one (2i).

White solid, yield: 23%; mp 202–204 °C; 1H NMR (300 MHz, DMSO-d6) δ 8.36 (s, 1H), 7.92 (d, J = 8.7 Hz, 1H), 7.50 (d, J = 9 Hz, 2H), 6.99 (d, J = 8.7 Hz, 2H), 6.87 (d, J = 9 Hz, 1H), 3.95 (s, 2H), 3.77 (s, 3H), 2.57 (m, 4H), 2.36 (m, 4H), 2.16 (s, 3H). 13C NMR (100 MHz, DMSO-d6) δ 175.3, 163.2, 159.5, 155.6, 153.4, 130.6, 126.3, 124.7, 123.5, 116.9, 115.9, 114.1, 109.0, 55.7, 55.0, 52.6, 52.3, 46.1. HR-MS (ESI) Calcd for C22H22N2O4 [M + H]+, 381.1814, found: 381.1814.
7-hydroxy-8-((3-hydroxypiperidin-1-yl)methyl)-3-(4-methoxyphenyl)-4H-chromen-4-one (2p)
White solid, yield: 25%; mp 188–190 °C; 1H NMR (300 MHz, DMSO-d6) δ 8.33 (s, 1H), 7.91 (d, J = 9.9 Hz, 1H), 7.50 (d, J = 8.7 Hz, 2H), 6.98 (d, J = 9 Hz, 2H), 6.84 (d, J = 8.7 Hz, 1H), 3.95 (s, 2H), 3.77 (s, 3H), 3.58 (brs, 1H), 2.85 (d, J = 9.3 Hz, 1H), 2.66 (d, J = 11.1 Hz, 1H), 2.27–2.13 (m, 2H), 1.71 (d, J = 10.5 Hz, 2H), 1.45 (m, 1H), 1.24 (m, 1H). 13C NMR (100 MHz, DMSO-d6) δ 175.2, 164.0, 159.5, 155.5, 153.2, 130.6, 126.2, 124.7, 123.5, 116.7, 115.7, 114.1, 108.6, 65.8, 60.4, 55.6, 53.0, 52.9, 32.6, 22.7. HR-MS (ESI) Calcd for C23H25NO5 [M + H]+, 380.1718, found: 380.1718.

8-((benzyl)piperazin-1-yl)-7-hydroxy-3-(4-methoxyphenyl)-4H-chromen-4-one (2q)
White solid, yield: 27%; mp 220–222 °C; 1H NMR (300 MHz, DMSO-d6) δ 8.36 (s, 1H), 7.92 (d, J = 8.7 Hz, 1H), 7.50 (d, J = 8.7 Hz, 2H), 7.29 (m, 5H), 6.98 (d, J = 8.7 Hz, 2H), 6.87 (d, J = 8.7 Hz, 1H), 3.96 (s, 2H), 3.77 (s, 3H), 3.47 (s, 2H), 2.59–2.49 (m, 8H). 13C NMR (100 MHz, DMSO-d6) δ 175.3, 159.7, 159.5, 157.4, 155.7, 142.5, 138.5, 130.6, 129.4, 128.7, 127.3, 124.4, 123.3, 116.7, 115.5, 114.1, 108.6, 69.1, 62.4, 55.8, 52.9, 52.6. HR-MS (ESI) Calcd for C28H28N2O4 [M + H]+, 451.2127, found: 457.2113.

7-hydroxy-8-((4-(2-hydroethy)lpiperazin-1-yl)methyl)-3-(4-methoxyphenyl)-4H-chromen-4-one (2s)
White solid, yield: 27%; mp 196–198 °C; 1H NMR (300 MHz, DMSO-d6) δ 8.35 (s, 1H), 7.91 (d, J = 8.7 Hz, 1H), 7.50 (d, J = 8.7 Hz, 2H), 6.98 (d, J = 9 Hz, 2H), 6.89 (d, J = 9 Hz, 1H), 3.94 (s, 2H), 3.77 (s, 3H), 3.49 (t, J = 6.3 Hz, 3H), 2.57–2.40 (m, 8H), 2.36 (t, J = 7.2 Hz, 2H). 13C NMR (100 MHz, DMSO-d6) δ 175.2, 163.4, 159.5, 155.6, 153.3, 130.6, 126.3, 124.7, 123.8, 116.9, 115.9, 115.9, 108.8, 60.5, 59.0, 55.7, 53.5, 52.7, 52.4. HR-MS (ESI) Calcd for C23H23N2O4 [M + H]+, 411.1920, found: 411.1904.

7-hydroxy-3-(4-methoxyphenyl)-8-((2-methylpiperidin-1-yl)methyl)-4H-chromen-4-one (2t)
White solid, yield: 17%; mp 141–143 °C; 1H NMR (300 MHz, DMSO-d6) δ 8.32 (s, 1H), 7.87 (d, J = 9.9 Hz, 1H), 7.50 (d, J = 9.9 Hz, 2H), 6.98 (d, J = 9 Hz, 2H), 6.80 (d, J = 9 Hz, 1H), 4.31–4.26 (d, J = 15 Hz, 1H), 3.90–3.85 (d, J = 15 Hz, 1H), 3.77 (s, 3H), 2.83 (d, J = 12.3 Hz, 1H), 2.66 (brs, 1H), 2.33 (t, J = 9.6 Hz, 1H), 1.48–1.35 (m, 6H), 1.15 (d, J = 6.3 Hz, 3H). 13C NMR (100 MHz, DMSO-d6) δ 175.1, 164.8, 159.5, 155.2, 153.1, 130.6, 125.9, 124.8, 123.5, 116.4, 115.9, 114.1, 108.6, 56.6, 55.6, 51.7, 50.4, 33.8, 26.9, 25.7, 22.6. HR-MS (ESI) Calcd for C23H23N2O4 [M + H]+, 380.1862, found: 380.2130.
Bioassay studies

Cell lines and cell culture

The cell-based histamine receptor 3 (H3R) assay was carried out based on β-lactamase complementation technology. The H3-bla U2OS cells (Invitrogen, Invitrogen, Waltham, Massachusetts) stably expressed two fusion proteins, as well as a β-lactamase reporter gene under the control of a UAS response element. The first fusion protein was human H3R linked to a Gal4-VP16 transcription factor through the TEV protease site, and the other was the β-arrestin/TEV protease fusion protein. H3-bla U2OS cells were cultured in McCoy’s 5A Medium supplemented with 10% foetal bovine serum (FBS; Gibco, Shanghai, China) at 37°C in a humidified atmosphere with 5% CO2. To each well in a 384-well plate was seeded exponentially growing cells in a density of 6.5 × 10^3 cells/mL in 32 μL of media. The plate was incubated at 37°C, 18–24 h, 5% CO2 for cell adherence.

Fluorescent H3R assay

Stock solutions of test compounds (10 mM) were prepared in DMSO and then diluted 100 times in media. Cells were exposed to 4 μL of test compounds and the control compound thioperaamide (Sigma-Aldrich, St. Louis, Missouri) for 30 min and then stimulated with 4 μL of methylhistamine at 400 nM (Sigma-Aldrich) for 5 h. Then, 8 μL of LiveBLAzer-FRET B/G Substrate (CCF4-AM; Invitrogen) was added and incubation continued for 2 h. Plates were subjected to the fluorescence reading with a Spectra Max M5 microplate reader (BioTek, Winooski, Vermont); equipped with 410 nm excitation and 460 nm and 530 nm emission filters. The inhibition percentage was calculated based on the fluorescence according to the following equation: % inhibition = (ModelResponse ratio - CompoundResponse ratio)/ModelResponse ratio. And IC50 values were determined from log concentration – inhibition curves. At least three separate tests were carried out.

Molecular docking

We chose the most active compounds for molecular docking studies to predict how molecules and proteins work. A homology modelling of H3R was built as our previous report. The 3D structure of compound 2h was built using DS MODELER (Discovery Studio 2016, BIOVIA Inc, San Diego, CA) and evaluated the model
according to the PDF Total Energy and the Profile-3D procedure. Flexible Docking was used for the docking procedure. The 3D model of H3R with the lowest PDF Total Energy was chosen for docking. Water and the cognate ligand (doxepin) were removed from the model, and hydrogen atoms were added to amino acid residues. The binding mode was shown by DS visualizer.

Results and discussion

Structure–activity relationship

The compounds were initially evaluated for inhibition rate on H3R at a fixed concentration of 10 \( \mu \text{M} \) (Tables 1 and 2). Of the 27 compounds evaluated, four compounds (1c, 2c, 2h, 2o) performed satisfactory inhibitory effect (Figure 2). According to reports in the literature, H3R inhibitory activities were increased by the introduction of pyrrolidine and piperidine to the iso-flavone scaffold. Thus, we introduced various pyrrolidine, piperidine, piperazine and morpholine moieties onto 6- or 8-position of iso-flavone. The results for series 1 are shown in Table 1. The advantage of piperidine groups outweighed pyrrolidine moieties. As for substituted piperazine and morpholine moieties, the subsequent data did not give satisfactory results. Then, we modified daidzein and formononetin with substituted piperidine and pyrrolidine fragments. It should be noted that further steric modification on piperidine was detrimental for the inhibitory activities. For example, 4-hydroxy-methyl, 3-hydroxy piperidine (compound 2b, 2e) attached to the structure of daidzein led the inhibitory activity to decrease. However, the 2-methyl piperidine group (compound 2h) showed very strong inhibition. Interestingly, for formononetin, 3-methyl piperidine (compound 2o) and pyrrolidine (compound 2m) fragments showed unexpected inhibitory effect. Structurally, substituted piperidine (such as methyl- and hydroxyl-) or pyrrolidine groups could improve bioactivity but bulky substitutions may hinder binding H3 pockets, namely, binding affinity would loss. Comparing different iso-flavone structures, even though 4-flavone scaffold showed significant fluctuation in bioactivity level according to the data shown in Table 2, in most cases, daidzein derivatives have advantages over formononetin as H3R antagonists, for example, compound 2c vs 2l, 2h vs 2t.

Binding modes of compound 2h

The results showed that compound 2h bound with H3R through multiple sites (Figure 3). The protonated amine of the pyridine group interacted with Glu206 through a salt bridge. The Tyr-115 and Phe-198 bound to the aromatic ring structural on one side of compound 2h by \( \pi-\pi \) T-shape interactions. In addition to this, compound 2h also formed hydrophobic interaction, \( \pi-\sigma \) and \( \pi-\text{alkyl} \) interaction with the protein.

Conclusions

In this work, two series of iso-flavone derivatives were synthesised and evaluated for their H3R inhibitory activity. Ultimately, we identified compound 1c, 2c, 2h, 2o which possessed favourable H3R inhibitory activity. The structure–activity relationship (SAR) study identified the piperazine group in the 8-position of iso-flavone was essential for the H3R inhibitory activity (compound 2h). Molecular docking showed 2'-methyl piperidine substituent of 2h formed a salt bridge and hydrophobic interactions with the protein. In this paper, we creatively modified the iso-flavone derivatives and determined this scaffold possessing the potential H3R
inhibitory activity. Moreover, these results also provided clues for the development of novel H3R antagonists.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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