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Design and synthesis of novel 3-substituted-indole derivatives as selective H_3 receptor antagonists and potent free radical scavengers

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ABSTRACT

A series of novel 3-substituted-indole derivatives with a benzyl tertiary amino moiety were designed, synthesized and evaluated as H_3 receptor antagonists and free radical scavengers for Alzheimer's disease therapy. Most of these synthesized compounds exhibited moderate to potent antagonistic activities in CREs driven luciferase assay. In particular, compound **2d** demonstrated the most favorable H_3 receptor antagonistic activity with the IC_{50} value of 0.049 μM. Besides, it also displayed high binding affinity to H_3 receptor (K_i = 4.26 ± 2.55 nM) and high selectivity over other three histamine receptors. Moreover, **2d** and other two 3-substituted indole derivatives **1d** and **3d** exerted potent ABTS radical cation scavenging capacities similar to melatonin. Above results illustrate that **2d** is an interesting lead for extensive optimization to explore new drug candidate for AD therapy.

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1. Introduction

As one of four members of the G-protein coupled receptor (GPCR) binding to histamine, H_3 receptor was pharmacologically identified by Arrang in 1983 and successfully cloned in 1999.[1,2] It is predominantly expressed in the central nervous system (CNS) and functions as an autoreceptor that negatively regulates the release of histamine. More importantly, as a heteroreceptor, H_3 receptor participates in modulating the release of key neurotransmitters including acetylcholine, serotonin, noradrenaline, and dopamine that are involved in attention, vigilance and cognition.[3,4] Therefore, H_3 receptor has been recognized as an effective therapeutic target for the treatment of CNS disorders, exemplified by narcolepsy, Alzheimer's disease (AD), attention deficit hyperactivity (ADHD), obesity, and schizophrenia.[5]

There are numerous of new H_3 receptor antagonists with different chemical scaffolds have been developed in the past few years, most of these newly developed compounds are characterized by a tertiary amine, a central core and a flexible part chosen from either a polar group, a second basic amine or a lipophilic residue.[6–13] Recently, several of them being investigated in clinical trials for the potential treatment of above mentioned CNS disorders, for example, Pitolisant (BF2.649, phase III, Fig. 1), PF-03654746 (phase II, Fig. 1) and GSK-189254 (phase II, Fig. 1) act as wake-promoting agents for narcoleptic patients. Clinical trials with JNJ-31001074 (phase I, Fig. 1) are being carried out in adult and pediatric ADHD groups. For Alzheimer's disease (AD), MK-0249 (phase II, Fig. 1), PF-03654746 (phase I, Fig. 1) and Irdabisant (CEP-26401, phase I, Fig. 1) are used to alleviate the symptoms and slow the progression of mild to moderate AD patients.[14,15]

Although the etiology of AD is not yet fully understood, there are numerous studies prove that the imbalance between the generation of free radicals and reactive oxygen species (ROS) may be involved in the pathogenesis of AD. Many free radical scavengers have produced promising results for the treatment of AD in preclinical stage and clinical trials. For example, melatonin protects neuronal cells from Aβ-induced oxidative damage in vitro and increases survival in a transgenic model of AD.[16] Moreover, in a controlled clinical trial, vitamin E (a-tocopherol) delay clinically important functional deterioration in patients with Alzheimer's disease.[17]

Based on above research results, we hypothesized that compounds with dual activities of H_3 receptor antagonism and free radical scavenging may achieve better efficacy by a complementary manner.[18] Our strategy to design such molecules was to combine pharmacophores of H_3 receptor antagonist and free radical scavenger into one molecule.

The benzyl tertiary amine moiety was chosen as the pharmcophore of H_3 receptor antagonists because it was frequently employed into H_3 receptor antagonists, such as PF-03654746 and JNJ-31001074. Furthermore, the molecular docking analysis of

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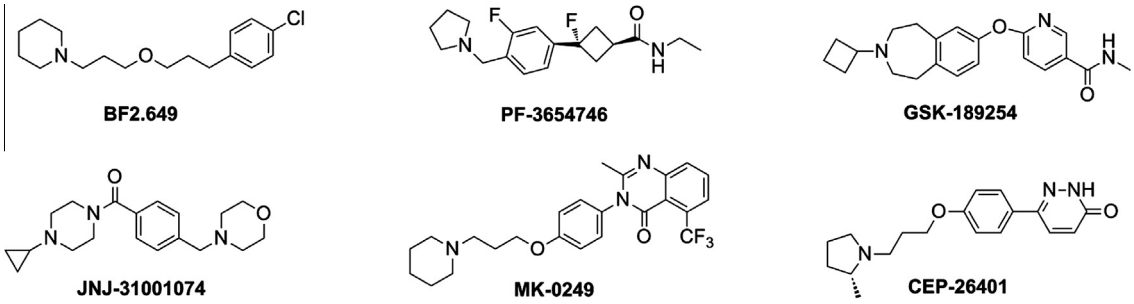


Figure 1. Structures of non-imidazole H_3 receptor antagonists in clinical trials.

PF-03654746 bound to H_3 receptor suggested that the protonated nitrogen of benzyl piperidine interact with crucial residue Asp114 in transmembrane helix 3 (TM3), which is highly conserved in G-protein-coupled receptors (GPCRs).[19,20]

Melatonin was a potent free radical scavenger and a broad spectrum antioxidant, in which the indole moiety was validated as the reactive center of interaction with cations and radicals due to its high resonance stability and very low activation energy barrier towards the free radical reactions.[21] Moreover, this moiety was widely present in various CNS-active drugs, such as anti-migraine drug Rizatriptan, psychotherapeutic drug Oxypertine (Fig. 2).

Therefore, a new series of 3-substituted-indole derivatives are designed, synthesized and evaluated as H_3 receptor antagonists and free radical scavengers through tethering of benzyl tertiary amine to the 3-position of indole. In these designed target compounds, the indole moiety not only serves as a free radical scavenger pharmacophore, but also acts as the flexible part in H_3 receptor antagonists, and the benzyl tertiary amine is suppose to form critical hydrogen bond with the amino acid residue in H_3 receptor. Meanwhile, different kinds of linkers (alkyl amide, alkyl amino and alkyl ester, Fig. 2) are employed to connect the two moieties to explore the optimum one.

2. Results and discussion

2.1. Chemistry

The synthetic route for these new 3-substituted-indole derivatives is outlined in Scheme 1. Reaction of 4-nitro-benzylbromide **4** with different secondary amines in refluxing acetonitrile gave corresponding 4-nitro-benzyl amines **5a**–**d**, which was followed by catalytic hydrogenation with 5% Pd/C to furnish 4-amino-benzyl tertiary amines **6a**–**d**.[22] Subsequent condensation of **6a**–**d** with indole-3-carboxylic acid, indole-3-acetic acid or indole-3-propionic acid in the presence of HOBt/EDC resulted in amides **1a**–**i**. Further reduction of amide group of compounds **1a**–**1e** and **1h** with $LiAlH_4$ afforded compounds **2a**–**f**. Finally, condensation of phenol derivatives **7a**–**d** with indole-3-carboxylic acid, indole-3-acetic acid or indole-3-propionic acid in the presence of DCC/DMAP provided compounds **3a**–**i**.

2.2. Biological activities and SAR

2.2.1. H_3 receptor antagonistic activity and binding affinity evaluation

cAMP-response elements (CREs) driven luciferase assay has been widely utilized to measure the function of GPCR agonists and antagonists. The H_3 receptor antagonistic activities of these newly synthesized 3-substituted indole derivatives have been evaluated in HEK-293 cell lines expressing the human H_3 receptor and a reporter gene consisting of the firefly luciferase coding region that is under the control of minimal promoter containing CREs.[18,23] Thioperamide is employed as the positive control and results are summarized in Table 1.

As shown in Table 1, most of the synthetic compounds demonstrated moderate to potent H_3 receptor antagonistic activities and seven compounds displayed submicromolar IC_{50} values. Compounds **2b** (IC_{50} = 0.080 μM) and **2d** (IC_{50} = 0.049 μM) exhibited the most potent H_3 receptor antagonistic activities, which were more potent than that of Thioperamide (IC_{50} = 0.12 μM) in CRE-driven luciferase assay.

The variation of linker between indole and benzene ring of benzylamine moieties significantly influenced the H_3 receptor antagonistic activities. Indole-3-alkylamino derivatives (**2a**–**f**) were more potent than corresponding indole-3-amide derivatives (**1a**–**e**, **1h**) and indole-3-carboxylate derivatives (**3a**–**e**, **3h**). For example, compound **2b** (IC_{50} = 0.080 μM) displayed more potent H_3 receptor antagonistic activity than that of compounds **1b** (IC_{50} = 2.84 μM)

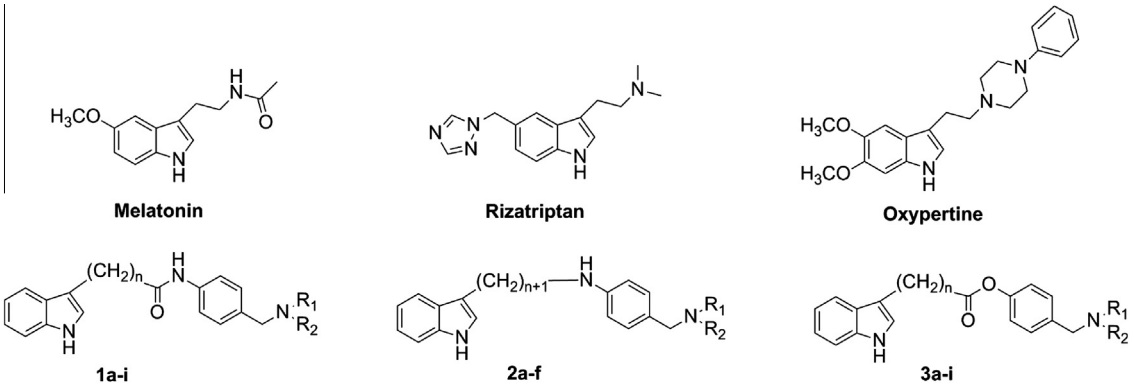
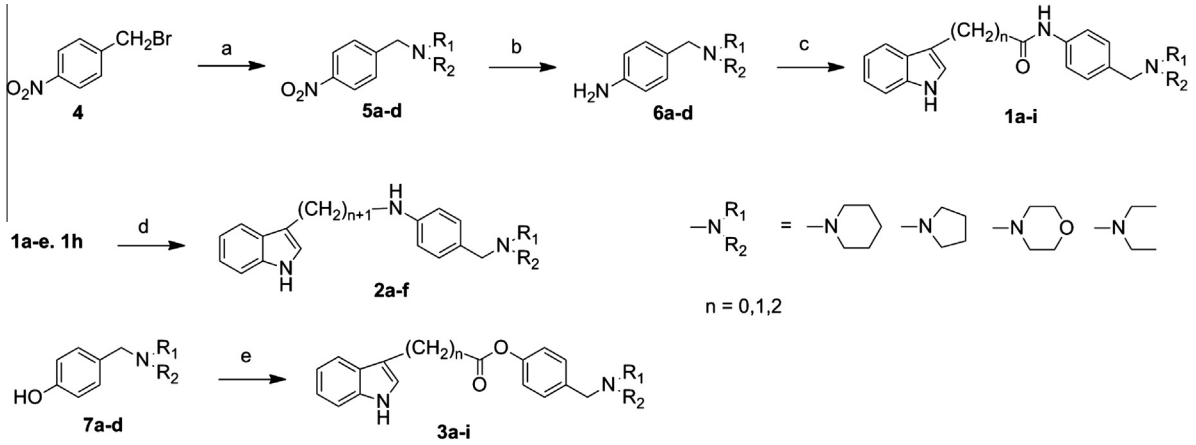
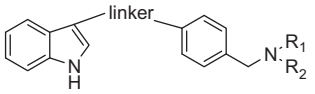


Figure 2. CNS drugs containing indole moiety and our target 3-substituted-indole derivatives.



Scheme 1. Reagents and conditions: (a) secondary amine, acetonitrile, reflux 2–4 h; (b) 5% Pd/C, methanol, rt 6–10 h; (c) indole-3-carboxylic acid or indole-3-acetic acid or indole-3-propionic acid, HOBt/EDC, CH_2Cl_2/THF, rt overnight; (d) $LiAlH_4$, THF, reflux 4–6 h; (e) indole-3-carboxylic acid or indole-3-acetic acid or indole-3-propionic acid, DCC/DMAP, CH_2Cl_2/THF, rt over night.

Table 1
The H_3 receptor antagonist activity and H_3 receptor binding affinity of 3-substituted indole derivatives



Compounds	Linker	*n*	NR_1R_2	H_3R antagonistic activity[a] (IC_{50}, μM)
Thioperamide				0.12
1a	$-(CH_2)_nCONH-$	0	Piperidine	1.21
1b		1	Piperidine	2.84
1c			Pyrrolidine	1.37
1d			Morpholine	2.19
1e			Diethylamine	3.50
1f		2	Piperidine	>10
1g			Pyrrolidine	>10
1h			Morpholine	>10
1i			Diethylamine	>10
2a	$-(CH_2)_{n+1}NH-$	0	Piperidine	0.54
2b		1	Piperidine	0.080
2c			Pyrrolidine	0.18
2d			Morpholine	0.049[b]
2e			Diethylamine	0.29
2f		2	Morpholine	0.49
3a	$-(CH_2)_nCOO-$	0	Piperidine	2.03
3b		1	Piperidine	1.04
3c			Pyrrolidine	2.46
3d			Morpholine	0.79
3e			Diethylamine	4.61
3f		2	Piperidine	>10
3g			Pyrrolidine	>10
3h			Morpholine	>10
3i			Diethylamine	>10

[a] Results are presented as the mean of at least three independent experiments.
[b] H_3 receptor binding affinity, K_i = 4.26 ± 2.55 nM.

and **3b** (IC_{50} = 1.04 μM). The indole-3-amide derivatives (**1a**–**e**) exerted H_3 receptor antagonistic activities similar to indole-3-carboxylate derivatives (**3a**–**e**), with IC_{50} values ranging from 1.21 to 3.50 μM and 0.79 to 4.61 μM, respectively. The compromised activities of **1f**–**i** and **3f**–**i** (IC_{50} >10 μM) suggested that the introduction of longer linker with rigidity (propionamide, propionate) was detrimental for H_3 receptor antagonistic activities.

The most potent 3-indole ethylamino derivative **2d** was selected to perform H_3 receptor binding affinity evaluation which was measured in terms of displacement of [^{3}H]-(*R*)-a-methyl-histamine binding to membranes of HEK-293 cells expressing the human H_3 receptor in the presence of the compounds.[23] As a result, compound **2d** showed high binding affinity to H_3 receptor with K_i value of 4.26 ± 1.55 nM.

2.2.2. Homology model and molecular docking

To gain insight into the molecular determinants that modulate the antagonistic activities of these compounds, molecular docking study of PF-03654746, **2d**, **1d** and **1h** with H_3 receptor homology model were performed, which was generated based on the crystal structure of H_1 receptor (PDB ID: 3RZE).[24] The results are reported in Figure 3.

As expected, in Figure 3A, PF-3654746 binds to H_3 receptor through three key hydrogen-bond interactions with Asp114, Glu206 and Tyr374, which are fully consistent with the reported results.[20] Figure 3B reveals that compound **2d** has a favorable fit into hydrophobic cavity in TMs 3-5-6 region of H_3 receptor. The benzyl morpholine and indole moiety of **2d** endows two common hydrogen bonds with crucial residues Asp114 and Glu206, respectively. In addition, the nitrogen of ethyl amino linker forms the third hydrogen bond with Tyr374. Although there is only minor structural difference between compounds **1d** (–CH_2CONH– as linker) and **2d** (–CH_2CH_2NH– as linker), Figure 3C reveals that the direction of indole moiety in **1d** is slightly different from **2d** and thereby result in the lost of hydrogen bond between indole and Glu206, which may account for the 40-fold decrease of potency for compound **1d**. Figure 3D shows that compound **1h** inserted deeper than **2d** into the hydrophobic cavity of TMs 3-5-6 region in H_3 receptor, which may be responsible for the loss of three hydrogen bonds with crucial residues of H_3 receptor.

2.2.3. Histamine receptor selectivity

Provided the distinct biological function of the four subtypes of histamine GPCRs, the selectivity is an important issue in designing H_3 receptor antagonist. Indole-3-ethylamino derivative **2d** was selected to evaluate for its biological activities against other three subtypes of histamine receptors (H_1R, H_2R and H_4R) using the same CRE-driven luciferase assay as H_3 receptor. As shown in Figure 4, compound **2d** did not influence the responses induced by 5 μM histamine in the luciferase activity in cells expressing H_1R, H_2R or H_4R. These results demonstrated that compound **2d** is an H_3 receptor antagonist with preferable selectivity versus H_1, H_2 and H_4 receptors.

2.2.4. ABTS radical cation scavenging capacity assay

ABTS [2,29-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation ($ABTS^{\cdot+}$) was a blue-colored cation with absorption maxima at wavelengths 415 nm, 645 nm, 734 nm and 815 nm. Addition of antioxidants to the solution of pre-formed radical cation will reduce it, and the scavenging activities of the antioxidants is determined by the degree of blanching of $ABTS^{\cdot+}$.[25] Three 3-substituted indole derivatives with different linkers (**1d**, **2d** and **3d**) were selected to evaluate their free radical scavenging capacities with Melatonin as positive control. The results are shown in Figure 5.

The data of Figure 5 indicated that melatonin, **1d**, **2d** and **3d** scavenge $ABTS^{\cdot+}$ in a concentration dependent style, **1d**, **2d** and **3d** exhibited potent ABTS radical cation scavenging capacities with IC_{50} value of 4.71 μM, 3.05 μM and 5.32 μM, respectively, similar to that of Melatonin (IC_{50} = 1.92 μM), which confirmed our hypothesis that compounds containing indole moiety was able to confer potent ABTS radical cation scavenging capacities.

2.2.5. hERG patch-clamp assay

Many of reported H_3 antagonists have shown hERG inhibitory activities which may result in a potential safety liability. Three most potent compounds (**2b**, **2c**, **2d**) and indole-3-amide derivative **1d**, indole-3-ester derivative **3d** were chosen to evaluate hERG ion channel inhibitory activities. The results are shown in Table 2.

The results demonstrated that compound **1d** and **3d** almost did not exhibit any hERG inhibition at 3 μM as measured by patch clamp assay, while all three 3-indole ethylamino derivatives **2b**, **2c** and **2d** showed moderate hERG inhibition at the same condition, these results implies that more work need to be done to find a promising new drug candidate with satisfactory hERG profile.

3. Conclusion

With the goal of identifying dual-acting compounds with potent H_3 receptor antagonistic activities and free radical scavenging properties, a series of novel 3-substituted-indole derivatives were designed through incorporating benzyl tertiary amine into 3-position of indole skeleton with different linkers. Most of these synthesized compounds exhibited moderate to potent H_3 receptor

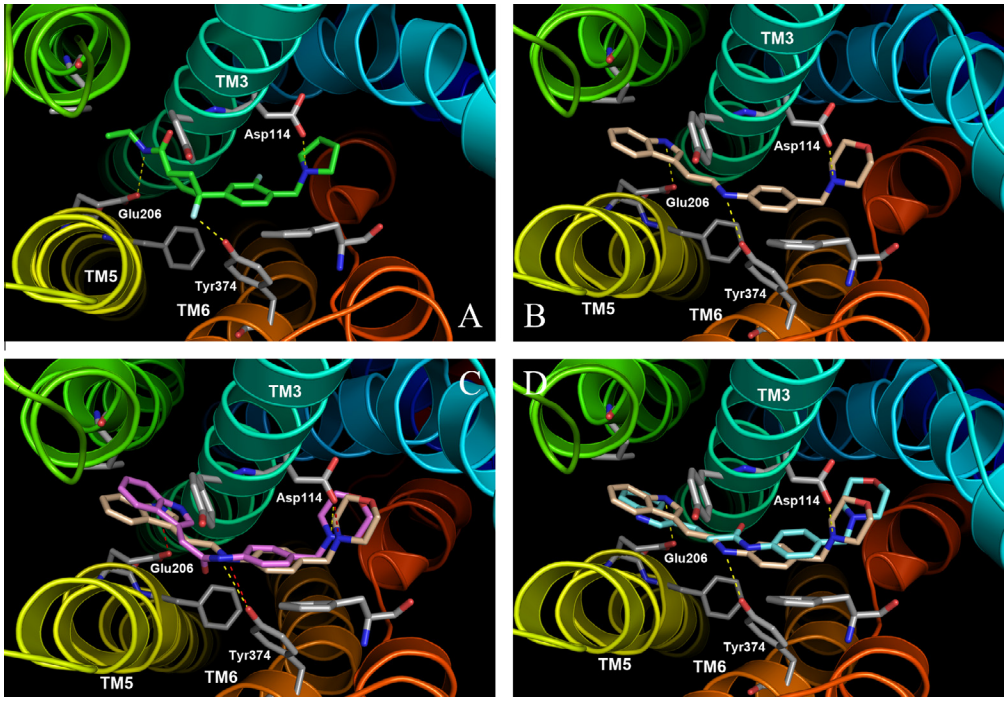


Figure 3. The predicted best configuration of PF-03654746, **2d**, **1d** and **1h** bound to H_3 receptor. PF-03654746 (A) and **2d** (B) bound to H_3 receptor with similar mode, while **1d** (purple in C) and **1h** (blue in D) exhibited different binding mode with H_3 receptor in comparison with **2d** (gold in C and D).

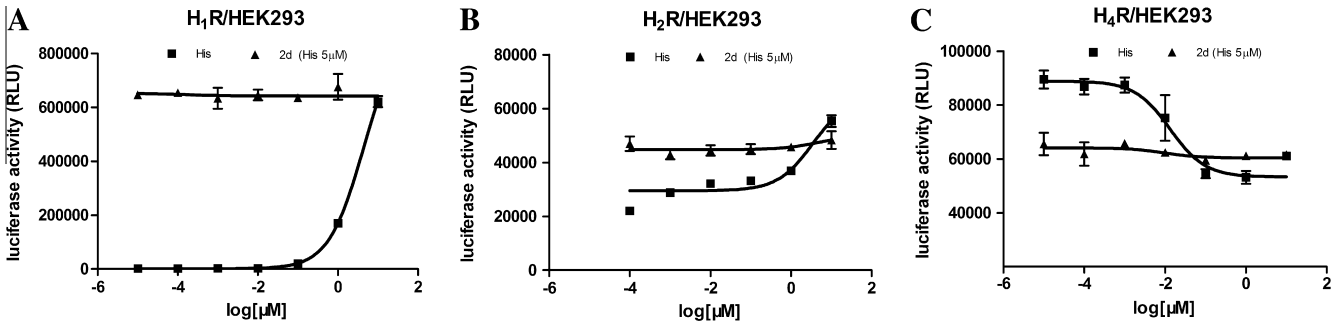


Figure 4. The selectivity of compound **2d** for human histamine receptors by CRE-luciferase transcription assay. HEK-293 cells stably expressing human H_1 receptor (A), human H_2 receptor (B), or human H_4 receptor (C) were treated with different concentrations of compound **2d** in the presence of 5 μM histamine, single histamine response curve used as the control separately. The presented data points are the mean ± SE of triplicate values from a single experiment and are representative of three separate experiments.

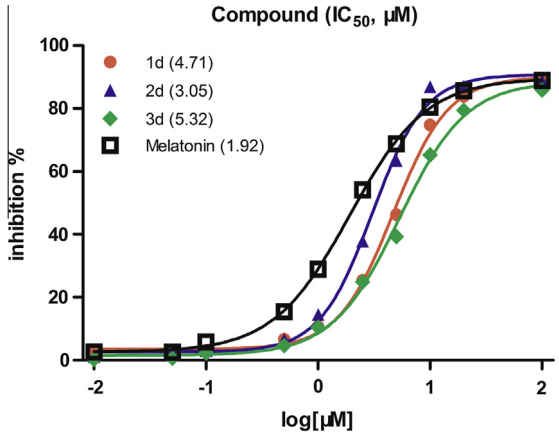


Figure 5. Concentration dependence in scavenging of $ABTS^{\cdot+}$ by **1d**, **2d**, **3d** and Melatonin with the results being obtained at the end of a 6-min incubation.

Table 2
The hERG inhibitory activities of 3-substituted indole derivatives

Compounds	1d	2b	2c	2d	3d	Cisepride (100 nM)
hERG (%Inh.,3 μM)	6	87	73	54	12	89

antagonistic activities. The preliminary SAR reveals that alkyl-amino linker is favorable for H_3 receptor antagonistic activity. The most potent H_3 antagonist **2d** (IC_{50} = 0.049 μM) also shows high binding affinity to H_3 receptor with K_i value of 4.26 ± 2.55 nM, and good selectivity over other three histamine receptors. Molecular docking analysis demonstrates that **2d** bind to H_3 receptor via three key hydrogen-bond interactions with Asp114, Glu206, and Tyr374, respectively. Moreover, three 3-substituted indole derivatives with different linkers (**1d**, **2d** and **3d**) all exerted potent ABTS radical cation scavenging activities similar to melatonin. All these results suggested that **2d** can be explored as an interesting dual-functional lead for the development of AD therapeutic drugs.

4. Experimental section

4.1. Synthesis

General, all reagents and solvents used were purchased from common commercial of analytical grade. Melting points were recorded on a B-540 Buchi melting-point apparatus and uncorrected, ^{1}H NMR were recorded on a BRUKER AVIII 500 M spectrometer with TMS as the internal standard. Proton Chemical shifts are expressed in parts per million (ppm) and coupling constants in Hz. Mass spectra (ESI-MS) were performed on a *Finnigan LCQ DecaXP* ion trap mass spectrometry.

4.1.1. General procedure for preparation of compounds 6a–d

To a solution of 1-(bromomethyl)-4-nitrobenzene **4** (1.0 g, 4.6 mmol) in 15 mL acetonitrile, was added a solution of piperidine in 5 mL acetonitrile at room temperature. The mixture was refluxed for 2 h and the solvent was removed under vacuum to nearly dryness. The residue was acidified with 20 mL 2 mol/L hydrochloric acid and extracted with ethyl acetate (10 mL × 3). Concentrated ammonium hydroxide was added to aqueous solution up to clearly basic, and the product was extracted with ethyl acetate (20 mL × 3). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and removed solvent under reduced pressure to give **5a** which was used in the following reaction without further purification. A mixture of **5a** (1.0 mmol) in methanol (20 mL) and 5% palladium on carbon (20 mg) was stirred under hydrogen (balloon) for 6–10 h. The mixture was then filtered through celite and then concentrated to obtain crude product, which was purified by silica gel column chromatography eluting with petroleum, ethyl acetate and triethylamine (20:20:1) to afford **6a**.

4.1.1.1. 4-(Piperidin-1-ylmethyl)-aniline (6a). Yellow solid (yield: 59.0%); mp: 115–117 °C (lit. 120–123 °C).[26]

4.1.1.2. 4-(Pyrrolidin-1-ylmethyl)-aniline (6b). Yellow oil (yield: 63.0%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.11 (d, J = 7.5 Hz, 2H), 6.64 (d, J = 7.5 Hz, 2H), 3.62 (s, 2H), 3.51 (s, 2H), 2.47–2.48 (m, 4H), 1.78 (m, 4H).

4.1.1.3. 4-(Morpholinomethyl)-aniline (6c). Yellow solid (yield 56.0%); mp: 99–100 °C (lit. 100.8–102.2 °C).[22]

4.1.1.4. 4-(Diethylaminomethyl)-aniline (6d). Yellow solid (yield 53.0%); mp: 40–42 °C (lit. 46–47 °C).[27]

4.1.2. General procedure for preparation of compounds 1a–1i

To a mixture of 4-amino benzyl amine **6a** (0.42 mmol) and indole-3-carboxylic acid (0.50 mmol) in 8 mL CH_2Cl_2 and 2 mL THF, was added HOBt (1.0 mmol), EDC (1.0 mmol) and triethyl amine (1.0 mmol). The mixture was stirred over night at room temperature and stopped by addition of 10 mL 5% $NaHCO_3$ solution, then, the mixture was extracted with CH_2Cl_2 (15 × 3 mL) and organic layers were combined, washed with brine, dried over Na_2SO_4. The solvent was evaporated under vacuum to give crude

product, which was purified by silica gel column chromatography eluting with petroleum, ethyl acetate and triethylamine (20:20:1) to afford **1a**.

4.1.2.1. *N*-(4-(Piperidin-1-ylmethyl)phenyl)-1*H*-indole-3-carboxamide (1a). Yellow solid (yield 19.8%); mp: 108–110 °C; IR (KBr) 3434, 3369, 3109, 2947, 1632, 1598, 1531, 1411, 748 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 9.54 (br s, 1H, H-1), 8.09–8.07 (m, 1H, H-4), 7.89 (s, 1H, H-2), 7.78 (s, 1H, H-7), 7.61 (d, J = 8.5 Hz, 2H, H-3′ and H-5′), 7.45–7.43 (m, 1H, H-5), 7.30–7.26 (m, 3H, H-2′, H-6′, H-6), 3.49 (s, 2H, benzylic-CH_2), 2.43–2.41 (m, 4H, piperidine-CH_2, H-2, H-6), 1.62–1.57 (m, 4H, piperidine-CH_2, H-3, H-5), 1.44–1.42 (m, 2H, piperidine-CH_2, H-4); MS (ESI) m/z = 334.4 M+H]$^+$.

4.1.2.2. 2-(1*H*-Indol-3-yl)-*N*-(4-(piperidin-1-ylmethyl)phenyl) acetamide (1b). White solid (yield: 24.4%). Mp: 185–187 °C; IR (KBr) 3298, 3261, 3067, 2937, 1661, 1607, 1536, 1413, 741 cm^{-1}; ^1H NMR (500 MHz,$CDCl_3$): δ 8.43 (br s, 1H, H-1), 7.65 (d, J = 8.0 Hz, 1H, H-4), 7.46 (d, J = 8.0 Hz, 1H, H-7), 7.40 (s, 1H, H-2), 7.29–7.26 (m, 2H, H-3′, H-5′), 7.24–7.18 (m, 4H, H-2′, H-6′, H-5, H-6), 3.91 (s, 2H, CH_2CO), 3.42 (s, 2H, benzylic-CH_2), 2.35–2.30 (m, 4H, piperidine-CH_2, H-2, H-6), 1.58–1.53 (m, 4H, piperidine-CH_2, H-3, H-5), 1.42–1.40 (m, 2H, piperidine-CH_2, H-4); MS (ESI) m/z = 348.3 M+H]$^+$.

4.1.2.3. 2-(1*H*-Indol-3-yl)-*N*-(4-(pyrrolidin-1-ylmethyl)phenyl) acetamide (1c). White solid (yield 28.1%); mp: 177–178 °C; IR (KBr) 3292, 3255, 3052, 2968, 1655, 1605, 1536, 1412, 738 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.40 (br s, 1H, H-1), 7.65 (d, J = 8.0 Hz, 1H, H-4), 7.46 (d, J = 8.5 Hz, 1H, H-7), 7.40 (s, 1H, H-2), 7.30–7.27 (m, 2H, H-3′, H-5′), 7.24–7.18 (m, 4H, H-2′, H-6′, H-5, H-6), 3.91 (s, 2H, CH_2CO), 3.57 (s, 2H, benzylic-CH_2), 2.49–2.45 (m, 4H, pyrrolidine-CH_2, H-2, H-5), 1.78–1.76 (m, 4H, pyrrolidine-CH_2, H-3, H-4); MS (ESI) m/z = 334.3 [M+H]$^+$.

4.1.2.4. 2-(1*H*-Indol-3-yl)-*N*-(4-(morpholinomethyl)phenyl) acetamide (1d). White solid (yield 23.2%); mp: 128–130 °C; IR (KBr) 3290, 3256, 3064, 2962, 1658, 1607, 1535, 1415, 1116, 743 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.34 (br s, 1H, H-1), 7.63 (d, J = 7.5 Hz, 1H, H-4), 7.45 (d, J = 8.5 Hz, 1H, H-7), 7.39 (s, 1H, CONH), 7.30–7.26 (m, 3H, H-2, H-3′, H-5′), 7.23–7.16 (m, 4H, H-2′, H-6′, H-5, H-6), 3.90 (s, 2H, CH_2CO), 3.68 (m, 4H, morpholine-CH_2, H-2, H-6), 3.41 (s, 2H, benzylic-CH_2), 2.40 (m, 4H, morpholine-CH_2, H-3, H-5); MS (ESI) m/z = 350.3 [M+H]$^+$.

4.1.2.5. *N*-(4-((Diethylamino)methyl)phenyl)-2-(1*H*-indol-3-yl) acetamide (1e). Slightly yellow solid (yield 23.9%); mp: 133–135 °C; IR (KBr) 3291, 3256, 3051, 2967, 1657, 1605, 1535, 1412, 740 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.30 (br s, 1H, H-1), 7.64 (d, J = 8.0 Hz, 1H, H-4), 7.44 (d, J = 8.0 Hz, 1H, H-7), 7.36 (s, 1H, CONH), 7.28–7.25 (m, 3H, H-2, H-3′, H-5′), 7.23–7.10 (m, 4H, H-2′, H-6′, H-5, H-6), 3.90 (s, 2H, CH_2CO), 3.49 (s, 2H, benzylic-CH_2), 2.52 (t, J = 7.5 Hz, 4H, $CH_2 \times 2$), 1.01 (t, J = 7.5 Hz, 6H, $CH_3 \times 2$); MS (ESI) m/z = 336.3 [M+H]$^+$.

4.1.2.6. 3-(1*H*-Indol-3-yl)-*N*-(4-(piperidin-1-ylmethyl)phenyl) propanamide (1f). White solid (yield 43.5%); mp: 152–154 °C; IR (KBr) 3437, 3295, 3053, 2933, 1657, 1600, 1542, 1410, 744 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.24 (br s, 1H, H-1), 7.65 (d, J = 8.0 Hz, 1H, H-4), 7.38 (d, J = 8.0 Hz, 1H, H-7), 7.31 (d, J = 8.5 Hz, 2H, H-3′, H-5′), 7.22–7.20 (m, 3H, CONH, H-2′, H-6′), 7.15–7.13 (m, 2H, H-5, H-6), 7.00 (s, H-2), 3.43 (s, 2H, benzylic-CH_2), 3.22 (t, J = 7.5 Hz, 2H, CH_2), 2.76 (t, J = 7.5 Hz, 2H, CH_2), 2.37–2.32 (m, 4H, piperidine-CH_2, H-2, H-6), 1.58–1.55 (m, 4H, piperidine-CH_2, H-3, H-5), 1.43–1.41 (m, 2H, piperidine-CH_2, H-4); MS (ESI) m/z = 362.3 [M+H]$^+$.

4.1.2.7. 3-(1*H*-Indol-3-yl)-*N*-(4-(pyrrolidin-1-ylmethyl)phenyl) propanamide (1g). Slightly yellow solid (yield: 36.5%); mp: 135–137 °C; IR (KBr) 3434, 3284, 3054, 2959, 1656, 1600, 1544, 1411, 734 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.24 (br s, 1H, H-1), 7.65 (d, J = 8.0 Hz, 1H, H-4), 7.39 (d, J = 8.0 Hz, 1H, H-7), 7.34 (d, 2H, H-3′, H-5′), 7.25–7.20 (m, 4H, CONH, H-2′, H-6′, H-5), 7.16–7.13 (m, 1H, H-6), 7.00 (s, 1H, H-2), 3.62 (s, 2H, benzylic-CH_2), 3.23 (t, J = 7.5 Hz, 2H, CH_2), 2.78 (t, J = 7.5 Hz, 2H, CH_2), 2.57–2.54 (m, 4H, pyrrolidine-CH_2, H-2, H-5), 1.81–1.79 (m, 4H, pyrrolidine-CH_2, H-3, H-4); MS (ESI) m/z = 348.4 [M+H]$^+$.

4.1.2.8. 3-(1*H*-Indol-3-yl)-*N*-(4-(morpholinomethyl)phenyl)propanamide (1h). White solid (yield 41.2%); mp: 114–116 °C; IR (KBr) 3435, 3288, 3054, 2958, 1656, 1600, 1543, 1411, 1114, 740 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.05 (br s, 1H, H-1), 7.64 (d, J = 7.5 Hz, 1H, H-4), 7.38 (d, J = 8.0 Hz, 1H, H-7), 7.32 (d, J = 8.5 Hz, 2H, H-3′, H-5′), 7.23–7.20 (m, 3H, CONH, H-2, H-5), 7.15 (m, 1H, H-6), 7.07–7.04 (m, 2H, H-2′, H-6′), 3.73–3.69 (m, 4H, morpholine-CH_2, H-2, H-6), 3.46 (s, 2H, benzylic-CH_2), 3.22 (t, J = 7.0 Hz, 2H, CH_2), 2.76 (t, J = 7.0 Hz, 2H, CH_2), 2.42–2.40 (m, 4H, morpholine-CH_2, H-3, H-5); MS (ESI) m/z = 364.4 [M+H]$^+$.

4.1.2.9. *N*-(4-((Diethylamino)methyl)phenyl)-3-(1*H*-indol-3-yl) propanamide (1i). Slightly yellow solid (yield 39.5%); mp: 73–75 °C; IR (KBr) 3454, 3300, 3054, 2970, 1656, 1600, 1540, 1410, 742 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.50 (br s, 1H, H-1), 8.33 (br s, 1H, CONH), 7.60 (d, J = 7.5 Hz, 1H, H-4), 7.32–7.30 (m, 3H, H-7, H-3′, H-5′), 7.22–7.17 (m, 3H, H-2, H-2′, H-6′), 7.12–7.10 (m, 1H, H-5), 6.90 (m, 1H, H-6), 3.53 (s, 2H, benzylic-CH_2), 3.17 (t, J = 7.0 Hz, 2H, CH_2), 2.71 (t, J = 7.0 Hz, 2H, CH_2), 2.53 (q, J = 7.0 Hz, 4H, $CH_2 \times 2$), 1.08 (t, J = 7.5 Hz, 6H, $CH_3 \times 2$); MS (ESI) m/z = 350.4 [M+H]$^+$.

4.1.3. General procedure for preparation of compounds 2a–f

To a solution of compound **1a** (0.50 mmol) in 15 mL anhydrous THF, $LiAlH_4$ (1.0 mmol) was added in one portion. The resulting mixture was refluxed for 4 h and carefully quenched with H_2O (5.0 mL). The organic layer was separated and the aqueous solution was extracted with EtOAc (20 mL × 3). The combined organic layer was dried (Na_2SO_4) and evaporated to dryness to obtain crude product, which was purified by silica gel column chromatography eluting with petroleum, ethyl acetate and triethylamine (20:20:1) to afford **2a**.

4.1.3.1. *N*-((1*H*-Indol-3-yl)methyl)-4-(piperidin-1-ylmethyl)aniline (2a). Yellow oil (yield 65.4%); IR (KBr) 3410, 3055, 2934, 1616, 1522, 1411, 742 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.22 (br s, 1H, H-1), 7.69 (d, J = 8.0 Hz, 1H, H-4), 7.40 (d, J = 8.0 Hz, 1H, H-7), 7.24 (m, 1H, H-5), 7.19–7.10 (m, 3H, H-2, H-3′, H-5′), 6.68 (d, J = 8.0 Hz, 2H, H-2′, H-6′), 4.48 (s, 2H, CH_2NH), 3.46 (s, 2H, benzylic-CH_2), 2.44–2.40 (m, 4H, piperidine-CH_2, H-2, H-6), 1.64–1.60 (m, 4H, piperidine-CH_2, H-3, H-5), 1.44–1.42 (m, 2H, piperidine-CH_2, H-4); MS (ESI) m/z = 320.2 [M+H]$^+$.

4.1.3.2. *N*-(2-(1*H*-Indol-3-yl)ethyl)-4-(piperidin-1-ylmethyl)aniline (2b). Yellow solid (yield 69.3%); mp: 126–128 °C; IR (KBr) 3404, 3204, 2961, 1617, 1526, 1413, 820, 744 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.56 (br s, 1H, H-1), 8.47 (br s, 1H, CONH), 7.69 (d, J = 8.0 Hz, 1H, H-4), 7.42 (d, J = 8.0 Hz, 1H, H-7), 7.28 (m, 1H, H-5), 7.20–7.15 (m, 3H, H-6, H-3′, H-5′), 7.07 (s, 1H, H-2), 6.62 (d, J = 8.0 Hz, 2H, H-2′, H-6′), 3.79 (br s, 1H, NH) 3.54 (t, J = 6.5 Hz, 2H, NCH_2), 3.45 (s, 2H, benzylic-CH_2), 3.16 (t, J = 6.5 Hz, 2H, CH_2), 2.45–2.42 (m, 4H, piperidine-CH_2, H-2, H-6), 1.65–

1.61 (m, 4H, piperidine-CH_2, H-3, H-5), 1.49–1.47 (m, 2H, piperidine-CH_2, H-4); MS (ESI) m/z = 334.5 $[M+H]^+$.

4.1.3.3. *N*-(2-(1*H*-Indol-3-yl)ethyl)-4-(pyrrolidin-1-ylmethyl) aniline (2c). Yellow solid (yield 63.2%); mp: 78–80 °C; IR (KBr) 3394, 3136, 2966, 1614, 1528, 1453, 820, 742 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.13 (br s, 1H, H-1), 7.64 (d, J = 10.0 Hz, 1H, H-4), 7.40 (d, J = 10.0 Hz, 1H, H-7), 7.23 (m, 1H, H-5), 7.15–7.11 (m, 3H, H-3′, H-5′, H-6), 7.06 (d, J = 2.5 Hz, 1H, H-2), 6.58 (d, J = 10.5 Hz, 2H, H-2′, H-6′), 3.56 (s, 2H, benzylic-CH_2), 3.49 (t, J = 8.5 Hz, 2H, NCH_2), 3.11 (t, J = 8.5 Hz, 2H, CH_2), 2.56–2.54 (m, 4H, pyrrolidine-CH_2, H-2, H-5), 1.80–1.78 (m, 4H, pyrrolidine-CH_2, H-3, H-4); MS (ESI) m/z = 320.3 M+H]$^+$.

4.1.3.4. *N*-(2-(1*H*-Indol-3-yl)ethyl)-4-(morpholinomethyl)aniline (2d). Yellow oil (yield 59.8%); IR (KBr) 3407, 3106, 2955, 1614, 1521, 1113, 742 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.26 (br s, 1H, H-1), 7.65 (d, J = 8.0 Hz, 1H, H-4), 7.37 (d, J = 8.0 Hz, 1H, H-7), 7.23 (t, J = 7.5 Hz, 1H, H-5), 7.17–7.13 (m, 3H, H-3′, H-5′, H-6), 7.03 (s, 1H, H-2), 6.60 (d, J = 8.5 Hz, 2H, H-2′, H-6′), 3.74 (m, 4H, morpholine-CH_2, H-2, H-6), 3.50 (t, J = 7.0 Hz, 2H, NCH_2), 3.43 (s, 2H, benzylic-CH_2), 3.12 (t, J = 7.0 Hz, 2H, CH_2), 2.46–2.44 (m, 4H, morpholine-CH_2, H-3, H-5); MS (ESI) m/z = 336.4 $[M+H]^+$.

4.1.3.5. *N*-(2-(1*H*-Indol-3-yl)ethyl)-4-((diethylamino)methyl) aniline (2e). Yellow oil (yield 70.8%); IR (KBr) 3410, 3102, 2968, 1614, 1519, 1455, 741 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.14 (br s, 1H, H-1), 7.68 (d, J = 8.0 Hz, 1H, H-4), 7.42 (d, J = 8.0 Hz, 1H, H-7), 7.28 (m, 1H, H-5), 7.19–7.15 (m, 3H, H-6, H-3′, H-5′), 7.10 (d, J = 2.0 Hz, 1H, H-2), 6.63 (d, J = 8.0 Hz, 2H, H-2′, H-6′), 3.53 (m, 4H, NCH_2, benzylic-CH_2), 3.15 (t, J = 6.5 Hz, 2H, CH_2), 2.57–2.55 (q, 4H, $CH_2 \times 2$), 1.11 (t, J = 7.0 Hz, 6H, $CH_3 \times 2$); MS (ESI) m/z = 322.4 $[M+H]^+$.

4.1.3.6. *N*-(3-(1*H*-Indol-3-yl)propyl)-4-(morpholinomethyl)aniline (2f). Slightly yellow oil (yield 70.8%); IR (KBr) 3410, 3105, 2956, 1614, 1521, 1113, 863, 742 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.05 (br s, 1H, H-1), 7.62 (d, J = 8.0 Hz, 1H, H-4), 7.42 (d, J = 8.0 Hz, 1H, H-7), 7.22 (t, J = 7.5 Hz, 1H, H-5), 7.14–7.10 (m, 3H, H-6, H-3′, H-5′), 6.98 (d, J = 2.0 Hz, 1H, H-2), 6.56 (d, J = 8.0 Hz, 2H, H-2′, H-6′), 3.72 (m, 4H, morpholine-CH_2, H-2, H-6), 3.40 (s, 2H, benzylic-CH_2), 3.22 (t, J = 7.0 Hz, 2H, NCH_2), 2.91 (t, J = 7.5 Hz, 2H, CH_2), 2.44–2.42 (m, 4H, morpholine-CH_2, H-3, H-5), 2.08–2.02 (m, 2H, CH_2); MS (ESI) m/z = 350.4 $[M+H]^+$.

4.1.4. General procedure for preparation of compounds 3a–i

To a solution of indole-3-carboxylic acid (1.55 mmol) in 10 mL dichloromethane and 2 mL THF, was added DCC (1.71 mmol), DMAP (1.55 mmol) and 4-piperidin-1-yl-methyl-phenol **7a** (1.55 mmol). The mixture was stirred at room temperature over night and stopped by addition of 4.0 mL water, followed by extracting with dichloromethane (20 mL × 3), the combined organic layer was washed with $NaHCO_3$, saline, dried over anhydrous Na_2SO_4 and evaporated to dryness to get crude product, which was purified by silica gel column chromatography eluting with petroleum, ethyl acetate and triethylamine (20:20:1) to afford **3a**.

4.1.4.1. 4-(Piperidin-1-ylmethyl)phenyl-1*H*-indole-3-carboxylate (3a). Yellow oil (yield 27.1%); IR (KBr) 3287, 3052, 2928, 2852, 1677, 1611, 1440, 1170, 1010, 749 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 9.46 (br s, 1H, H-1), 8.01 (m, 1H, H-4), 7.80 (s, 1H, H-2), 7.70 (s, 1H, H-7), 7.53 (d, J = 8.0 Hz, 2H, H-3′, H-5′), 7.36–7.34 (m, 1H, H-5), 7.22–7.19 (m, 3H, H-2′, H-6′), 3.40 (s, 2H, benzylic-CH_2), 2.35–2.33 (m, 4H, piperidine-CH_2, H-2, H-6), 1.53–1.49 (m, 4H, piperidine-CH_2, H-3, H-5), 1.35–1.32 (m, 2H, piperidine-CH_2, H-4); MS (ESI) m/z = 335.3 $[M+H]^+$.

4.1.4.2. 4-(Piperidin-1-ylmethyl)phenyl-2-(1*H*-indol-3-yl)acetate (3b). Yellow oil (yield 28.1%); IR (KBr) 3407, 3016, 2932, 2852, 1751, 1625, 1592, 1505, 1197, 1133, 741 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.43 (br s, 1H, H-1), 8.37 (br s, 1H, CONH), 7.72 (d, J = 8.0 Hz, 1H, H-4), 7.36 (d, J = 8.0 Hz, 1H, H-7), 7.30 (d, J = 8.5 Hz, 2H, H-3′, H-5′), 7.24–7.21 (m, 2H, H-2, H-5), 7.18 (m, 1H, H-6), 7.02 (d, J = 8.5 Hz, 2H, H-2′, H-6′), 4.02 (s, 2H, CH_2CO), 3.46 (s, 2H, benzylic-CH_2), 2.38–2.36 (m, 4H, piperidine-CH_2, H-2, H-6), 1.60–1.55 (m, 4H, piperidine-CH_2, H-3, H-5), 1.44–1.42 (m, 2H, piperidine-CH_2, H-4); MS (ESI) m/z = 349.3 $[M+H]^+$.

4.1.4.3. 4-(Pyrrolidin-1-ylmethyl)phenyl-2-(1*H*-indol-3-yl)acetate (3c). Slightly yellow oil, yield 24.7%; IR (KBr) 3408, 3022, 2929, 1751, 1619, 1596, 1505, 1193, 1112, 742 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.32 (br s, 1H, H-1), 7.71 (d, J = 8.0 Hz, 1H, H-4), 7.37 (d, J = 8.0 Hz, 1H, H-7), 7.31 (d, J = 8.5 Hz, 2H, H-3′, H-5′), 7.23–7.20 (m, 2H, H-2, H-5), 7.17 (m, 1H, H-6), 7.01 (d, J = 8.5 Hz, 2H, H-2′, H-6′), 4.01 (s, 2H, CH_2CO), 3.62 (s, 2H, benzylic-CH_2), 2.55–2.52 (m, 4H, pyrrolidine-CH_2, H-2, H-5), 1.82–1.80 (m, 4H, pyrrolidine-CH_2, H-3, H-4); MS (ESI) m/z = 335.4 $[M+H]^+$.

4.1.4.4. 4-(Morpholinomethyl)phenyl-2-(1*H*-indol-3-yl)acetate (3d). Slightly yellow oil, yield 30.5%; IR (KBr) 3405, 3057, 2956, 1751, 1608, 1596, 1505, 1196, 1116, 865, 743 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.18 (br s, 1H, H-1), 7.71 (d, J = 9.5 Hz, 1H, H-4), 7.38 (d, J = 10.0 Hz, 1H, H-7), 7.31 (d, J = 10.0 Hz, 2H, H-3′, H-5′), 7.24–7.20 (m, 2H, H-2, H-5), 7.18–7.14 (m, 1H, H-6), 7.02 (d, J = 10.0 Hz, 2H, H-2′, H-6′), 4.01 (s, 2H, CH_2CO), 3.70 (m, 4H, morpholine-CH_2, H-2, H-6), 3.46 (s, 2H, benzylic-CH_2), 2.43 (m, 4H, morpholine-CH_2, H-3, H-5); MS (ESI) m/z = 351.5 $[M+H]^+$.

4.1.4.5. 4-((Diethylamino)methyl)phenyl)-2-(1*H*-indol-3-yl)acetate (3e). Slightly yellow oil (yield 40.8%); IR (KBr) 3407, 3147, 2925, 1751, 1618, 1504, 1457, 1198, 743 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.30 (br s, 1H, H-1), 7.73 (d, J = 8.0 Hz, 1H, H-4), 7.35–7.31 (m, 3H, H-7, H-3′, H-5′), 7.22–7.16 (m, 3H, H-5, H-6, H-2), 7.01 (d, 2H, J = 8.0 Hz, H-2′, H-6′), 4.02 (s, 2H, CH_2CO), 3.55 (s, 2H, benzylic-CH_2), 2.54 (q, J = 7.0 Hz, 4H, $CH_2 \times 2$), 1.05 (t, J = 7.5 Hz, 6H, $CH_3 \times 2$); MS (ESI) m/z = 337.5 $[M+H]^+$.

4.1.4.6. 4-(Piperidin-1-ylmethyl)phenyl-3-(1*H*-indol-3-yl)propanoate (3f). White solid (yield 48.3%); mp: 89–90 °C; IR (KBr) 3403, 3137, 2925, 1751, 1629, 1543, 1280, 1157, 736 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.01 (br s, 1H, H-1), 7.66 (d, J = 7.5 Hz, 1H, H-4), 7.38 (d, J = 8.0 Hz, 1H, H-7), 7.30 (d, J = 8.5 Hz, 2H, H-3′, H-5′), 7.23 (t, J = 7.0 Hz, 1H, H-5), 7.16 (t, J = 7.5 Hz, 1H, H-6), 7.08 (d, J = 2.0 Hz, 1H, H-2), 6.96 (d, J = 8.5 Hz, 2H, H-2′, H-6′), 3.44 (s, 2H, benzylic-CH_2), 3.25 (t, J = 7.5 Hz, 2H, CH_2CO), 2.98 (t, J = 7.5 Hz, 2H, CH_2), 2.36–2.34 (m, 4H, piperidine-CH_2, H-2, H-6), 1.60–1.54 (m, 4H, piperidine-CH_2, H-3, H-5), 1.44–1.42 (m, 2H, piperidine-CH_2, H-4); MS (ESI) m/z = 363.5 $[M+H]^+$.

4.1.4.7. 4-(Pyrrolidin-1-ylmethyl)phenyl-3-(1*H*-indol-3-yl)propanoate (3g). White solid (yield 50.6%); mp: 98–100 °C; IR (KBr) 3403, 3137, 2925, 1751, 1629, 1543, 1280, 1157, 736 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.05 (s, 1H, H-1), 7.70 (d, J = 8.0 Hz, 1H, H-4), 7.42 (d, J = 8.0 Hz, 1H, H-7), 7.36 (d, J = 8.5 Hz, 2H, H-3′, H-5′), 7.23 (t, J = 7.5 Hz, 1H, H-5), 7.17 (t, J = 7.5 Hz, 1H, H-6), 7.09 (d, J = 2.0 Hz, 1H, H-2), 7.00 (d, J = 8.5 Hz, 2H, H-2′, H-6′), 3.63 (s, 2H, benzylic-CH_2), 3.28 (t, J = 7.5 Hz, 2H, CH_2CO)), 3.02 (t, J = 7.5 Hz, 2H, CH_2), 2.54–2.52 (m, 4H, pyrolidine-CH_2, H-2, H-5),

1.83–1.81 (m, 4H, pyrolidine-CH_2, H-3, H-4); MS (ESI) m/z = 349.3 $[M+H]^+$.

4.1.4.8. 4-(Morpholinomethyl)phenyl-3-(1*H*-indol-3-yl)propanoate (3h). Slightly yellow oil, yield 45.7%; IR (KBr) 3406, 3058, 2956, 1750, 1609, 1543, 1196, 1116, 863, 738 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.11 (br s, 1H, H-1), 7.66 (d, J = 7.5 Hz, 1H, H-4), 7.38 (d, J = 8.0 Hz, 1H, H-7), 7.31 (d, J = 8.5 Hz, 2H, H-3′, H-5′), 7.22 (t, J = 7.0 Hz, 1H, H-5), 7.15 (t, J = 7.0 Hz, 1H, H-6), 7.06 (d, J = 2.5 Hz, 1H, H-2), 6.97 (d, J = 8.5 Hz, 2H, H-2′, H-6′), 3.74 (m, 4H, morpholine-CH_2, H-2, H-6), 3.48 (s, 2H, benzylic-CH_2), 3.24 (t, J = 7.5 Hz, 2H, CH_2CO), 2.98 (t, J = 7.5 Hz, 2H, CH_2), 2.44–2.42 (m, 4H, morpholine-CH_2, H-3, H-5); MS (ESI) m/z = 365.4 $[M+H]^+$.

4.1.4.9. 4-((Diethylamino)methyl)phenyl-3-(1*H*-indol-3-yl)propanoate (3i). Slightly yellow oil (yield 51.2%); IR (KBr) 3406, 3145, 2928, 1750, 1619, 1505, 1456, 1196, 742 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.04 (s, 1H, H-1), 7.66 (d, J = 8.0 Hz, 1H, H-4), 7.38 (d, J = 8.0 Hz, 1H, H-7), 7.32 (d, J = 8.5 Hz, 2H, H-3′, H-5′), 7.23 (m, 1H, H-5), 7.16 (t, J = 7.5 Hz, 1H, H-6), 7.08 (d, J = 1.5 Hz, 1H, H-2), 6.96 (d, J = 8.5 Hz, 2H, H-2′, H-6′), 3.54 (s, 2H, benzylic-CH_2), 3.25 (t, J = 7.5 Hz, 2H, CH_2CO), 2.98 (t, J = 7.5 Hz, 2H, CH_2), 2.55 (q, J = 7.0 Hz, 4H, $CH_2 \times 2$), 1.05 (t, J = 7.0 Hz, 6H, $CH_3 \times 2$); MS (ESI) m/z = 351.2 $[M+H]^+$.

4.2. Pharmacology

4.2.1. Constructs, cell culture and generation of stable cell lines[23]

The human H_1 receptor and human H_2 receptor genes were cloned from HEK-293 genomic DNA by PCR. The human H_3 gene was cloned using human thalamus poly-A RNA (Clontech, Palo Alto, CA, USA) with RT-PCR methods. The human H_4 receptor gene was cloned from human bone marrow Marathon-Ready cDNAs (Clontech, Basingstoke, UK) by PCR. Primers were designed according to the published human histamine receptor gene sequences (GenBank accession nos. X76786, M64799, AF140538, and AB044934).

HEK-293 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μL streptomycin in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. For transfection, plasmid constructs were transfected or co-transfected into HEK-293 cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Stable transfectants were selected in the presence of 800 μg/mL G418.

4.2.2. CRE-driven reporter gene assay[18,23]

Stable HEK-293 cells co-transfected with H_3 receptor (or H_1, H_2, H_4 receptor) and pCRE-Luc were seeded in a 48-well plate overnight and were grown to 90–95% confluence. Afterwards, the cells were stimulated with 10 μM forskolin or 10 μM forskolin plus different concentrations of histamine or compound in serum-free DMEM, and the cells were incubated for 5 h at 37 °C. Luciferase activity was detected using a firefly luciferase kit (Promega, Madison, WI, USA).

4.2.3. Human histamine H_3 receptor binding assay[23]

HEK-293 cells stably expressing human H_3 receptor were harvested and membrane was gained by centrifugal classification. The membranes (50 μg of protein) were incubated with [^{3}H] *N*-α-methylhistamine at various concentrations (from 0.03 nM to 50 nM) for saturation binding assays. For competitive binding assays, the membranes (50 μg of protein) were incubated with 2 nM [^{3}H] *N*-α-methylhistamine for recombinant hH_3 receptor in the presence of various compound concentrations in the buffer (50 mM Tris–HCl, pH 7.4). The reaction mixtures were incubated for 90–120 min at room temperature to achieve binding equilibrium. After washing, the membrane-bound radioactivity was measured on a Topcount (PerkinElmer) at 20 °C. Non-specific binding was determined in the presence of excessive cold histamine (final concd, 300 μM). IC_{50} values were converted to K_i values using the Cheng–Prusoff equation. Each experiment was performed in duplicate and repeated for three to six times.

4.2.4. ABTS radical cation scavenging activity assay[25]

2,2′-Azino-bis-2-ethybenz-thiazoline-6-sulfonic acid (ABTS) was dissolved in purified water to a 7 mM concentration. ABTS radical cation ($ABTS^{\cdot+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for at least 18 h before use. The stock solution of $ABTS^{\cdot+}$ was serially diluted with sodium phosphate buffer (50 mM, pH 7.4) to 100 μM. Melatonin and **1d**, **2d** and **3d**, at different concentrations (total volume of 50 μL) were added to 150 μL of 100 μM $ABTS^{\cdot+}$ solution, respectively. After the addition of either melatonin or another antioxidant to the $ABTS^{\cdot+}$ solution, complete mixing of reactants was achieved by bubbling three to four times using plastic pipettes. The optical absorbance of $ABTS^{\cdot+}$ at 415 nm was measured at 6 min after addition and equilibrated at 30 °C. Each individual treatment was repeated for three times and the results of the experiments were compared.

4.2.5. hERG patch-clamp assay

HEK 293 cells stably expressing the hERG ion channel were used. hERG current was recorded and analysed using the suite of pCLAMP programs (version 10, Molecular Devices, Union City, CA, USA). Onset and steady-state block of hERG tail current due to the test compounds were measured by a depolarizing test pulse depolarization at −40 mV for 2 s after a prepulse to 40 mV for 4 s from a holding potential of −80 mV. This pulse pattern was repeated at 20 s intervals. Peak tail currents were measured from the test pulse and monitored during repetitive pulsing to reach a steady state. Patch pipettes were made from glass capillaries using a PC-10 micropipette puller (Narishige, Japan). The pipette solution contained (in mM): K Aspartate, 130; $MgCl_2$, 5; EGTA 5; HEPES, 10; Tris-ATP 4; pH 7.2 (adjusted with KOH).

4.3. Computational chemistry

4.3.1. Homology modeling

The H_3 receptor homology model was constructed starting from the preliminary transmembrane models based on the recently reported 3.1 Å crystal structure of the histamine H_1 receptor (PDB ID: 3RZE)[24] H_1 receptor has a sequence identity of 22.09% in overall and 32.12% in transmembrane regions to H_3 receptor. The primary sequence of human histamine H_3 receptor was obtained from the Universal Protein Resource (UniProt ID: Q9Y5N1). To align the sequence of H_3 receptor with that of H_1 receptor, the ClustalW program was employed and the same alignment was got as Soo-Kyung Kim reported.[20] In this study, COMPOSER program in SYBYL6.9 software (Tripos Inc., St. Louis, MO, USA) was applied to build a three-dimensional model of the histamine H_3 receptor structure.

4.3.2. Molecular docking

The docking study was performed by using the FlexiDock program in SYBYL6.9 software. The binding pocket of H_3 receptor was defined to cover all residues within 5 Å of the ligand in the homology modeling. Ligand with the most basic moiety was manually placed in the active site to interact with Glu206 in TM5 and/or Asp114 in TM3 and then, the ligand–H_3 receptor complex was

further optimized. All single bonds of residue side chains inside the binding pocket were regarded as rotatable, and the docked ligand was allowed to rotate on all the single bonds and move flexibly within the tentative binding pocket. Kollman all-atom charges were loaded to the protein, and Gasteiger–Huckel charges were assigned to the ligand atoms. The structural optimization was performed for 100,000 generations using a TRIPOS forcefield and genetic algorithm. As a result, 20 best scoring ligand–protein complexes were kept for further analysis.

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