ORIGINAL RESEARCH

Synthesis and biological evaluation of (+)-paeoveitol derivatives as novel antidepressants

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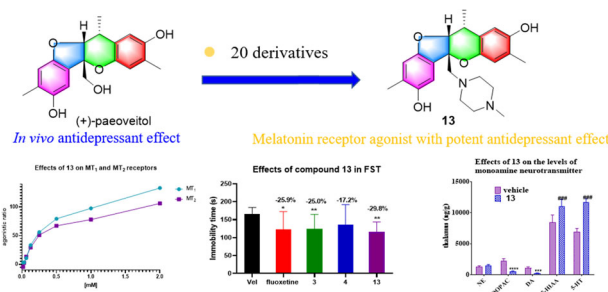
Abstract

The antidepressant activity of (+) and (−)-paeoveitol was first evaluated using the forced swimming test (FST), and (+)-paeoveitol showed potential antidepressant activity by decreasing immobility time of mice (by approximately 26.4%) in the FST at a dose of 20 mg/kg. To explore the structure-activity relationships (SARs) and obtain more potent compounds, twenty derivatives of (+)-paeoveitol were synthesized and evaluated for their agonistic activities on melatonin type I (MT_1) and type II (MT_2) receptors. As a results, compound **13** with an *N*-methylpiperazine fragment exhibited obvious effect on MT_1 and MT_2 receptors with EC_{50} values of 0.20 and 0.24 mM. Moreover, compound **13** dose-dependently decreased the immobility of mice in the FST and showed an inverted U-shaped dose-effect, and the most efficacious dose (at 40 mg/kg) was comparable to fluoxetine (20 mg/kg) with a reduced immobility time of 29.2% and 34.5%, respectively. In vivo neurochemical assays suggested that compound **13** obviously increased 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) and norepinephrine (NE) levels in the mice brain, indicating that its antidepressant effects might be related to the monoaminergic system. In silico ADMET study revealed that **13** has favorable pharmacokinetic properties. These findings suggest that compound **13** could be a potential antidepressant agent.

Graphical abstract

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Introduction

Depression is a common mental disease that seriously endanger human's mental and physical health, affecting approximately 3.8% of the population worldwide [1]. The symptoms of depression include: black mood, loss of interest and pleasure, lack of appetite, difficulty sleeping, fatigue and concentration problems. More than 300 million people worldwide suffer from depression and the number is still increasing. Numerous antidepressants with different mechanisms have been approved and widely used in clinical

Fig. 1 Chemical structures of (+)- and (−)-paeoveitol and paeoveitol D

(-)-paeoveitol   (+)-paeoveitol   paeoveitol D
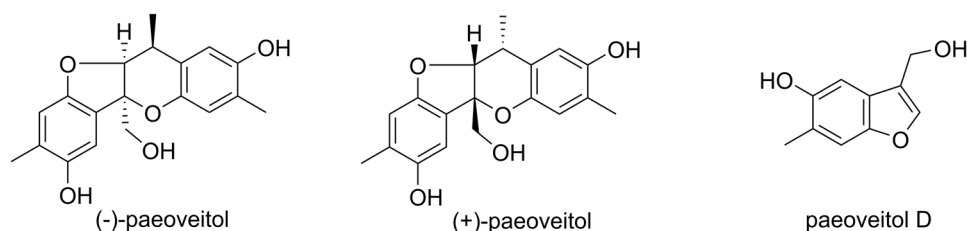
treatment, mainly including selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), norepinephrine and specific serotonergic antidepressants (NaSSAs), and monoamine oxidase inhibitors (MAOIs) [2]. The approval of esketamine [3] and allopregnanolone [4] by the US Food and Drug Administration (FDA) for treatment-resistant depression and postpartum depression represents the most significant achievement in antidepressant drug development in the last three decades. Despite significant developments in antidepressants, they still do not fully meet medical needs since around 30% of patients are resistant to currently available antidepressants. Other disadvantages of the first line antidepressants include slow onset of action and unacceptable side effects. Thus, the search for new antidepressants with novel structures and mechanisms is ever interesting.

A lot of natural products have been reported to have good antidepressant effects [5, 6]. As a part of a drug discovery program, we aimed to discover novel natural antidepressant drugs [7, 8]. Our previous phytochemical investigation of the traditional Chinese medicine *Paeonia veitchii* that is widely used in the formulation of herbal remedies for depression led to the isolation of a pair of structurally novel norditerpene paeoveitol enantiomers (Fig. 1) [9]. Following the discovery of paeoveitol, its structural novelty has attracted great attention from synthetic chemists. So far, four total synthetic routes of paeoveitol have already been reported [10–13]. Our synthetic approach finished the first catalytic asymmetric total synthesis of (+)- and (−)-paeoveitol *via* a phosphoric acid catalyzed hetero-Diels−Alder reaction. By changing the enantiomer of the catalyst, both (+)- and (−)-paeoveitol could be selectively synthesized from the same prochiral substrates in 42% overall yield. However, the biological activity of paeoveitol has not been reported. The efficient and scalable synthesis renders paeoveitol accessible for biological studies.

Paeveitol D, a benzofuran compound that is the biogenetic precursor of paeoveitol, showed activity on melatonin type I (MT_1) and type II receptors with agonistic ratios of 57.5% and 51.6% at a concentration of 1 mM, and our recent structure–activity relationship studies delivered 34 paeoveitol D derivatives with in vivo antidepressant activity [14]. Given the origination of paeoveitol that were produced by plant with antidepressant properties, combined with
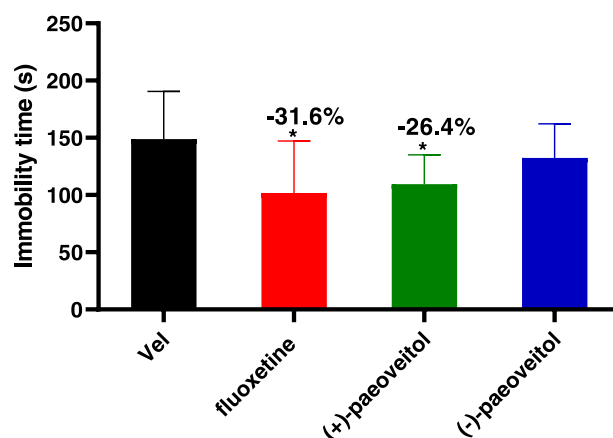

Fig. 2 Effect of treatment of mice with (+) and (−)-paeoveitol on immobility time in the FST

structural correlation between paeoveitol and paeoveitol D, in this study, the antidepressant-like activities of (+) and (−)-paeoveitol were first studied using the forced swimming test (FST). Next, to study the structure-activity relationship of (+)-paeoveitol and find new ones with better antidepressant activities, a series of (+)-paeoveitol derivatives were synthesized and evaluated on MT_1 and MT_2 receptors in vitro. Compound **13** that showed potential agonistic activities was assayed in vivo to evaluate its antidepressant properties using FST and its effects on monoamine neurotransmitters levels in multiple brain regions of mice.

Results and discussion

Chemistry

The stereo configuration of chiral compounds often has a significant effect on their biological activity, with both (+) and (−)-paeoveitol could be selectively synthesized, we first performed a preliminary assessment of their antidepressant activity using the forced swimming method with fluoxetine as a positive control (Fig. 2). It was found that (+)-paeoveitol at a dose of 20 mg/kg significantly shortened the immobility time in the FST, which was comparable to that of fluoxetine. The percent decrease in immobility time of (+)-paeoveitol and fluoxetine was 26.4 and 31.6%, respectively (Table 1). In

Table 1 The effects of (+) and (−)-paeovetiol on immobility time in the FST

Compounds	Doses (mg/kg)	Immobility time (s)	SD	SE	DID (%)	P
Vel	0.1 mL/10 g	148.8	41.8	14.8	—	—
fluoxetine	20	101.7	45.5	16.1	31.65	0.0397*
(+)-paeoveitol	20	109.5	25.5	8.48	26.41	0.0254*
(−)-paeoveitol	20	132.4	29.74	9.91	11.02	0.3544

DID Percentage decrease in immobility duration. Values are significant at $*P < 0.05$ with $n = 12$ in each group

Scheme 1 Synthesis of compounds **2-4**. Reagents and conditions: **a** Acetic anhydride, pyridine, rt, 94%, **b** 1-acetyl-1H-1,2,3-triazolo[4,5-B] pyridine, NaOH, rt, 49% (**3**), 23% (**4**)
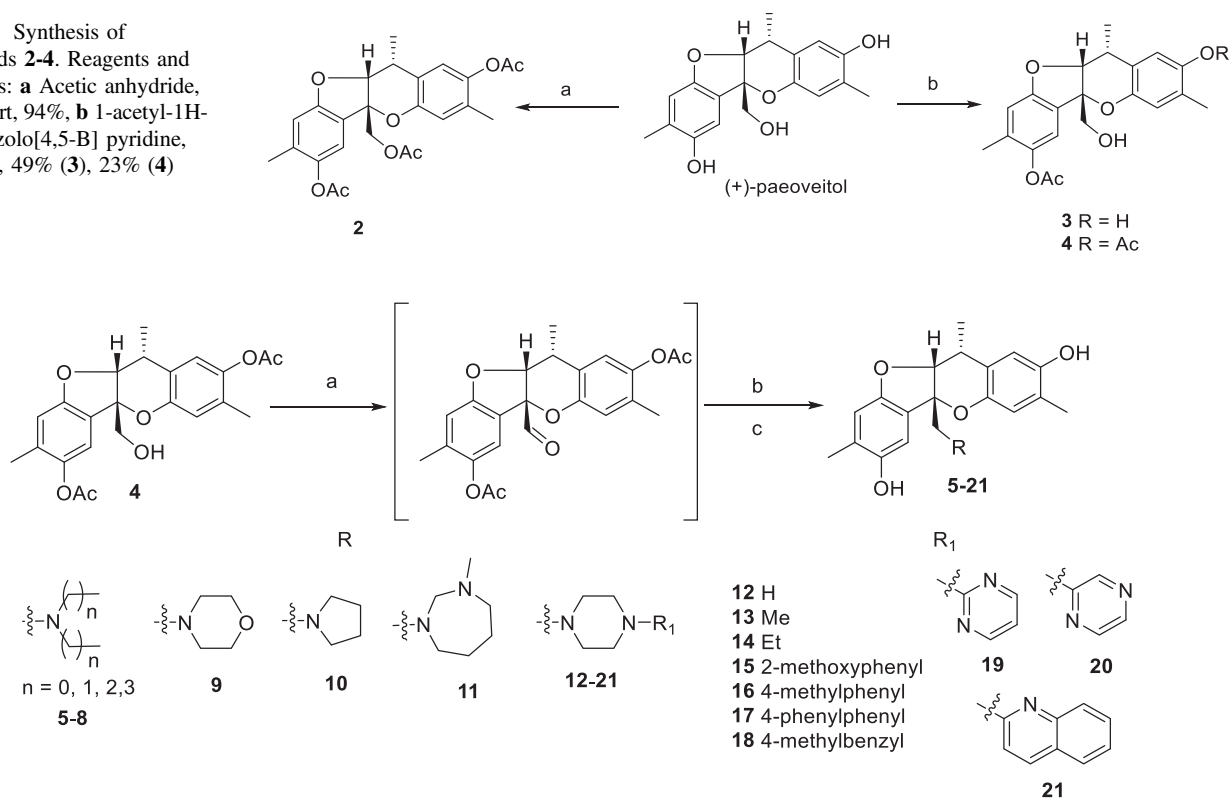
Scheme 2 Synthesis of compounds **5-21**. Reagents and conditions: **a** Dess-Martin periodinane, CH$_2$Cl$_2$, rt, **b** Amine, NaBH(OAc)$_3$, AcOH, CH$_2$Cl$_2$, rt, 8-16 h, **c** NaOH, rt, 52–79% yield over three steps

contrast, (−)-paeoveitol was found to be inactive in FST studies with an immobility time of 132.4 seconds compared to the control group with 148.8 seconds, suggesting that the stereo-configuration of paeoveitol has a significant effect on its antidepressant activity. The structure of paeoveitol is characterized by the unprecedented fusion of [3, 2-b]2, 3-dihydrobenzofuran and chromane in a tetracyclic framework bearing three hydroxyl groups, which was totally different from all available antidepressants that are mainly nitrogen-containing small molecules.

Next, adopting (+)-paeoveitol as a lead compound, we designed and synthesized a series of its derivatives to study the structure-activity relationship with the expectation to improve its efficacy on depression. In order to clarify the influence of hydroxyl groups on the antidepressant activity,

esterification products **2-4** were first synthesized (Scheme 1). The treatment of (+)-paeoveitol with acetic anhydride in pyridine delivered a triacetylated product **2**. The phenolic hydroxyl groups could be selectively acetylated using 1-acetyl-1H-1,2,3-triazolo[4,5-B] pyridine as an acetylation reagent under basic condition to give mono- and diacetylated products **3-4**. Compound **3** was confirmed to be the C-14 monoacetylated product by comparison with the spectral data of the C-2-acetylated paeoveitol in the literature [11].

The tertiary amino moiety is a privileged structural element of a series of antidepressants, and we tried to incorporate diverse tertiary amino-containing fragments into the template of (+)-paeoveitol (Scheme 2). Dess − Martin oxidation of compound **4** led to an aldehyde that was

Table 2 Agonistic activities of (+) and (−)-paeovetiol and their derivatives on melatonin receptors

| Compounds	Agonistic ratio (%)		Compounds	Agonistic ratio (%)	
	MT$_1$	MT$_2$		MT$_1$	MT$_2$
(−)-paeoveitol	6.6	3.2	**11**	58.9	62.2
(+)-paeoveitol	7.3	−0.9	**12**	20.5	30.2
2	1.1	0.6	**13**	95.3	74.5
3	5.5	1.0	**14**	5.8	20.4
4	2.2	2.1	**15**	22.0	32.0
5	0.7	0.5	**16**	7.9	14.6
6	11.9	5.8	**17**	56.4	32.5
7	5.5	5.1	**18**	12.3	7.5
8	−3.6	−4.5	**19**	5.6	19.2
9	−6.4	−5.8	**20**	19.3	7.9
10	1.0	−3.7	**21**	13.5	25.6

[a]Compounds were tested at 1.0 mM

[b]Agomelatine was employed as a positive control with EC$_{50}$ values of 5.3 ± 1.8 nM (MT$_1$) and 15.7 ± 3.6 nM (MT$_2$). The maximum agonistic activity achieved by agomelatine at the highest concentration was set as 100%, the agonistic activities of the tested compounds were determined by comparison with the maximum agonistic activity of agomelatine

directly treated with different secondary amines and sodium triacetoxyborohydride in the presence of a catalytic amount of acetic acid, followed by deacetylation to deliver compounds **5**-**21** in 52–79% yields over three steps. Piperazine structural fragments are an important pharmacophore in drug design and have been reported as key structural moieties of many antidepressants, such as amooxapine, sertraline and vilazodone [15]. Considering that the introduction of the pyrazine fragment may enrich the action targets of paeoveitol and lead to an increase in the activity, derivatives **12**-**21** containing different substituents on the piperazine ring were synthesized.

Biology

The structure and purity (higher than 95%) of the (+)-paeoveitol derivatives were verified by ^{1}H and ^{13}C NMR spectroscopic data, and HRESIMS. Although paeoveitol has no agonistic activity on MT$_1$ and MT$_2$ receptors, but given the reported results that amines could interact with various amino acid residues of the active site of MT$_1$ and MT$_2$ receptors [16] and the introduction of amine groups may lead to a positive effect, the synthesized compounds were initially tested in vitro for their potential agonistic activities on MT$_1$ and MT$_2$ receptors (Table 2). The test was performed at a concentration of 1 mM with agomelatine as a positive control. As a result, compound **11** showed moderate activities on MT$_1$ and MT$_2$ receptors with the agonistic ratios of 58.9%


Effect of 13 on MT$_1$ and MT$_2$ receptors
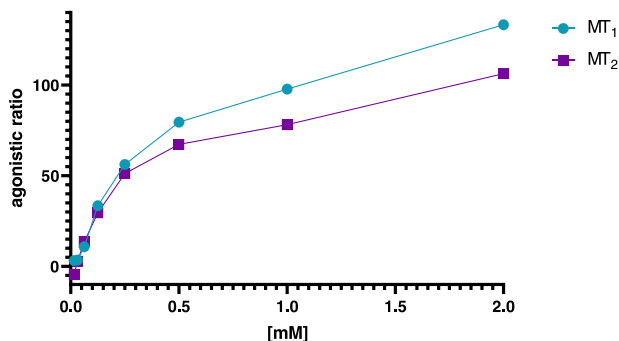
Fig. 3 The dose-dependent effects of derivative **13** on MT$_1$ and MT$_2$ receptors
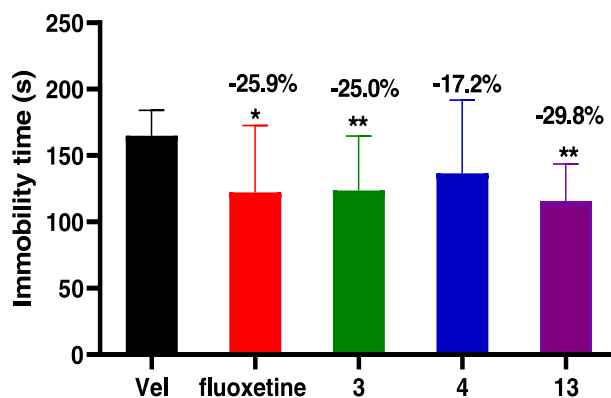
Fig. 4 Effects of derivatives **3**, **4**, and **13** (20 mg/kg) on immobility time in the FST. $*p < 0.05$, $**p < 0.01$

and 62.2%, respectively. Compound **13** displayed favorable agonistic activity on MT$_1$ and MT$_2$ receptors with the values of 95.3% and 74.5%. Other pyrazine containing derivatives, except compound **17**, exhibited no meaningful activity at the tested concentration, indicating substituents on nitrogen had a significant effect on the activity. The dose-dependent effects of the most potent derivative **13** on MT$_1$ and MT$_2$ receptors were studied to reveal EC$_{50}$ values of 0.20 and 0.24 mM, respectively (Fig. 3).

The in vivo antidepressant activity of derivatives **3**, **4**, and **13** was next investigated at a dose of 20 mg/kg using the forced swimming test. The compounds were administered orally twice (1 and 24 hours before testing) and fluoxetine acted as a positive control. As shown in Fig. 4, mono-acetylated derivative **3** also reduced the immobility time of animals in the FST by about 25%, while the antidepressant activity of compound **4** was significantly decreased after both phenolic hydroxyls were acetylated, indicating that the phenolic hydroxyl group at C-2 was important for antidepressant activity. Derivative **13** as the most potent agonists of MT$_1$ and MT$_2$ receptors decreased the immobility time of mice by 29.8%, which was similar to that of fluoxetine (Table 3).

Table 3 The effects of compounds **3**, **4**, and **13** (20 mg/kg) on immobility time in the FST

Compounds	Immobility time (s)	SD	SE	DID (%)	P
Vel	164.9	19.1	6.1	—	—
fluoxetine	122.2	50.3	15.9	25.9	0.0219*
3	123.7	40.9	11.8	25.0	0.0084**
4	136.5	55.3	17.5	17.2	0.1393
13	115.8	27.9	8.8	29.8	0.0046**
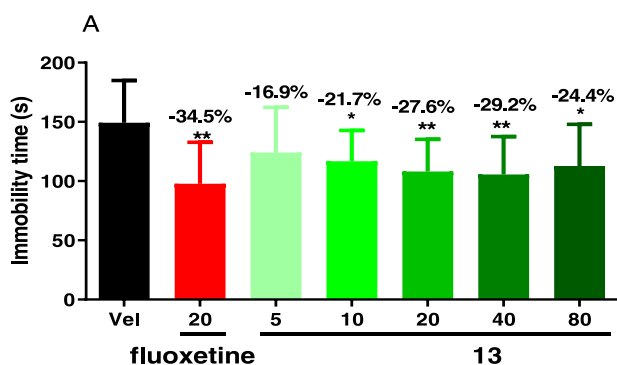
Fig. 5 The dose-dependent effects of derivative **13** on the immobility time in the FST. $*p < 0.05$, $**p < 0.01$

To explore the optimal doses for compound **13**, a wide range of doses were tested in the FST. Interestingly, compound **13** showed an inverted U-shaped dose-effect and decreased immobility of mice at doses of 10, 20, 40, and 80 mg/kg (but not 5 mg/kg) by 21.7%, 27.6%, 29.2% and 24.4%, respectively (Fig. 5). The most effective dose (at 40 mg/kg) of derivative **13** were comparable to fluoxetine (20 mg/kg). When the dosage increased to 80 mg/kg, the antidepressant activity decreased slightly (Table 4).

Clinical studies have demonstrated that depression is relevant to the abnormal expression of monoamine neurotransmitters such as dopamine (DA), 5-hydroxytrylamine (5-HT) and norepinephrine (NE), while antidepressants could ameliorate depression by upregulating the 5-HT and NA levels in the brain [17, 18]. To reveal the role of derivative **13** in the treatment of depression, the contents of neurotransmitters including NE, DA, 5-HT, 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA) in the frontal cortex, hippocampus, striatum, hypothalamus and thalamus after treatment with **13** were assessed using high-performance liquid chromatography (HPLC) with an electrochemical detector (Fig. 6). After consecutive treatment with compound **13** for 14 days at a dose of 20 mg/kg, the levels of 5-HT and 5-HIAA markedly increased ($P < 0.01$) compared with the control group, and exhibited statistically significant differences. The levels of NE in each group were increased to different degrees, with statistic difference in the hippocampus and hypothalamus.

However, the effects of compound **13** on the concentrations of DA and DOPAC were not consistent in different brain regions. The results suggested that the effect of compound **13** may be attributed to its effect on the function of the monoaminergic system by regulating 5-HT, 5-HIAA and NE levels in the brain.

Concerning the results described above, the absorption, distribution, metabolism, excretion, and toxicity (ADMET) and drug-likeness properties of compound **13** and (+)-paeoveitol were predicted using the ADMETlab server [19]. The values of the log S for **13** and (+)-paeoveitol were −3.91 and −4.62, indicating that the compounds have moderate solubility in water (Table 5). In terms of pharmacokinetics, both compounds indicated high Gastro intestinal absorption (GI). (+)-Paeoveitol cannot pass through the blood brain barrier (BBB), while compound **13** showed a positive response to BBB and may be applicable for central nervous system treatment. (+)-Paeoveitol was substrate for P-glycoprotein (P-gp), while compound **13** was non-substrate of P-gp. Both compounds were found to be non-inhibitors of CYP1A2, CYP2C19, CYP2C9 and CYP3A4. In terms of drug-likeness prediction, both compound **13** and (+)-paeoveitol complied with Lipinski, Ghose, Veber, Egan and Muegge rules with bioavailability score of 55%. The above prediction showed that compound **13** had good ADMET profile and drug likeness.

Conclusion

Starting with the antidepressant activity evaluation of (+) and (−)-paeoveitol, (+)-paeoveitol was found to be an orally effective leading to a significant reduction in immobility time in the forced swimming test at a dose of 20 mg/kg. Subsequently, 20 novel derivatives were designed and synthesized to explore the structure-activity relationships and obtain more active compounds. Among these compounds, derivative **13** containing *N*-methyl piperazine moiety showed obvious agonistic activities on MT_1 and MT_2 receptors with EC_{50} values of 0.20 and 0.24 mM. Three selected derivatives (**3**, **4**, **13**) were performed in vivo antidepressant assays using FST and SAR analysis indicated that the phenolic hydroxyl group at C-2 was important for antidepressant activity. Compound **13** was found to be as efficacious as fluoxetine in FST and showed an inverted U-shaped dose-effect with 40 mg/kg as the most potent dose. Furthermore, in vivo neurochemical studies indicated that compound **13** could increase 5-HT, 5-HIAA and NE content in the mice brain. In-silico prediction indicated compound **13** had good AMDET properties and drug likeness. These findings demonstrated compound **13** derived from (+)-paeoveitol had potentiality as a candidate to develop antidepressant agent, the antidepressant target of

Table 4 The dose-dependent effects of derivative **13** on the immobility time in the FST

Compounds	Doses (mg/kg)	Immobility time (s)	SD	SE	DID (%)	P
Vel	0.1 mL/10 g	149.2	35.6	10.3	—	—
fluoxetine	20	97.8	35.0	10.6	34.5	0.0017**
13	5	124.1	38.3	11.0	16.9	0.1094
13	10	116.8	26.0	7.5	21.7	0.0184*
13	20	108.1	27.2	7.8	27.6	0.0043**
13	40	105.6	32.0	9.2	29.2	0.0046**
13	80	112.8	35.1	10.1	24.4	0.0192*
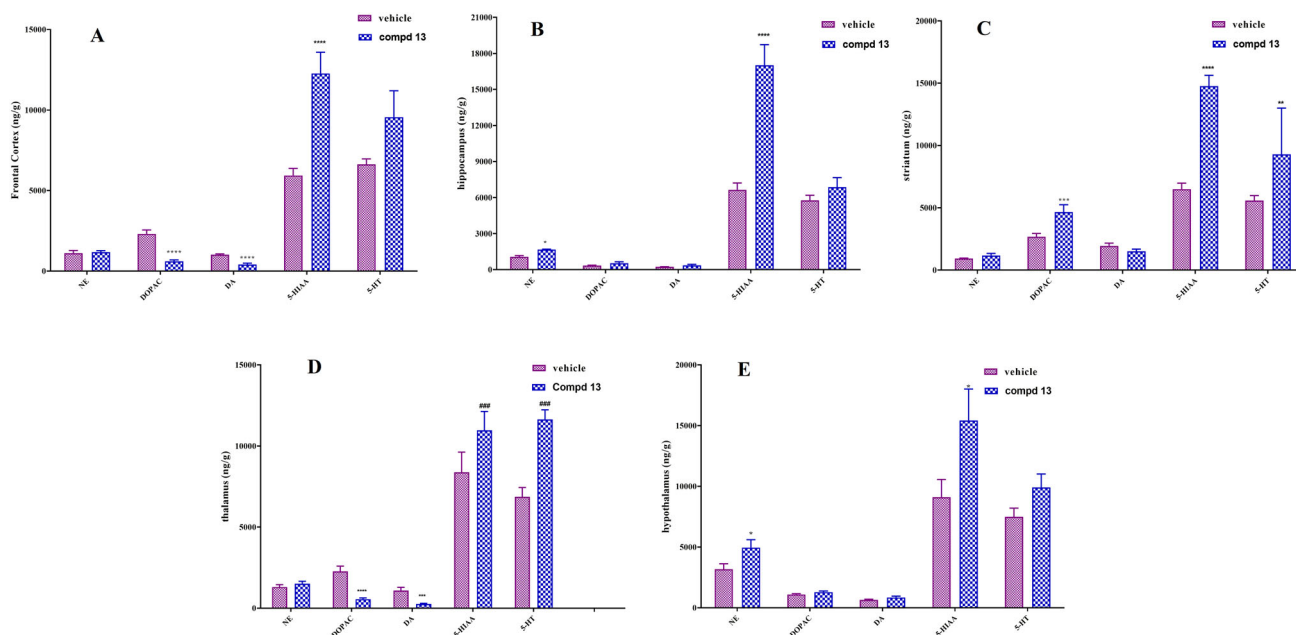
Fig. 6 Effects of compound **13** on the levels of monoamine neurotransmitters in different regions of mice brain. **A** Frontal cortex. **B** Hippocampus. **C** Striatum. **D** Thalamus. **E** Hypothalamus; Data were expressed as means ± SEM with units of ng/g. ($n = 12$) *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$

(+)-paeoveitol and compound **13** is still unclear and further studies are needed to reveal their molecular mechanism.

Experimental section

Chemistry

(+) and (−)-paeoveitol were synthesized according to our previously reported method [12], and other chemical reagents and reaction solvents were purchased from J&K Scientific or Energy Chemical. ^{1}H NMR and ^{13}C NMR spectra were recorded on Avance III HD 400 (Bruker, Germany), Avance III 500 (Bruker, Germany) with TMS as the internal standard. High resolution mass spectra (HRMS) data were obtained from a Shimadzu LC/MS-IT-TOF mass spectrometer (Shimadzu, Kyoto, Japan). Optical rotations were measured on an Autopol VI (Serial #91058) manufactured by Rudolph Research Analytical. All synthetic compounds were purified

by column chromatography on silica gel (200–300 mesh) that was purchased from Qingdao Makall Group Co., Ltd.

(5aS,10aR,11R) - 5a- (acetoxymethyl)- 3,8,11- trimethyl - 5a,10a - dihydro - 11H-benzofuro [3,2-b] chromene-2,7-diyl diacetate (2)

To a solution of (+)-paeoveitol (33 mg, 0.1 mmol) in 1 mL of pyridine was added acetic anhydride (1 mL), the reaction mixture was stirred at 25 °C for 10 h, quenched with 10 mL of water and extracted with ethyl acetate (3 × 10 mL). The combined organic phases were washed with 5% HCl solution, saturated NaHCO$_3$ and NaCl solution in turn, dried with anhydrous Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The residue was subjected to flash column chromatography on silica gel (acetone-petroleum ether, 10:90) to provide compound **2** (43 mg, 94% yield) as a white powder. $[\alpha]_D^{25.0}$ - 17.16 (c 0.076, MeOH); ^{1}H NMR (500 MHz, CDCl$_3$) δ 6.97 (s, 1H, H-15), 6.80 (s, 1H, H-1),

Table 5 In silico ADMET properties and drug-likeness prediction of compound **13** and (+)-paeoveitol

	Molecule	(+)-Paeoveitol	Compound 13
Physicochemical Properties	MW	328.36	410.51
	Rotatable bonds	1	2
	H-bond acceptors	5	6
	H-bond donors	3	2
	MR	89.29	123.67
	TPSA	79.15	65.4
Lipophilicity	Log P	2.66	2.93
	Log S (ESOL)	−3.91	−4.62
	class	Moderately soluble	Moderately soluble
Pharmacokinetics	GI absorption	High	High
	BBB permeant	No	Yes
	Pgp substrate	Yes	No
	CYP1A2 inhibitor	No	No
	CYP2C19 inhibitor	No	No
	CYP2C9 inhibitor	No	No
	CYP2D6 inhibitor	Yes	Yes
	CYP3A4 inhibitor	No	No
	log Kp (cm/s)	−6.35	−6.47
Druglikeness	Lipinski	Yes	Yes
	Ghose	Yes	Yes
	Veber	Yes	Yes
	Egan	Yes	Yes
	Muegge	Yes	Yes
	Bioavailability Score	0.55	0.55
Medicinal Chemistry	PAINS (alert)	0	0
	Brenk(alert)	0	0

6.58 (s, 1H, H-4), 6.49 (s, 1H, H-12), 5.02 (d, $J = 3.0$ Hz, 1H, H-8), 4.75 (d, $J = 14.4$ Hz, 1H, H-18a), 4.48 (d, $J = 13.6$ Hz, 1H, H-18b), 3.11-3.09 (m, 1H, H-7), 2.29 (s, 3H, -OAc), 2.27 (s, 3H, -OAc), 2.09 (s, 3H, -OAc), 2.05 (s, 3H, H-19), 2.00 (s, 3H, H-16), 1.56 (d, $J = 8.4$ Hz, 3H, H-17); ^{13}C NMR (125 MHz, CDCl$_3$) δ 170.6 (C = O), 169.6 (C = O), 169.5 (C = O), 158.0 (C-11), 151.1 (C-2), 144.5 (C-14), 143.1 (C-5), 133.8 (C-13), 129.1 (C-10), 126.5 (C-6), 123.9 (C-2), 120.1 (C-4), 119.3 (C-1), 117.7 (C-12), 112.0 (C-15), 89.2 (C-8), 86.0 (C-9), 67.1 (C-18), 32.2 (C-7), 20.8 (-OAc), 20.8 (-OAc), 20.8 (-OAc), 16.8 (C-19), 15.9 (C-16), 13.2 (C-17); HRMS (ESI, m/z): [M + H]$^+$ calcd for [C$_{25}$H$_{27}$O$_8$]$^+$ 455.1706, found 455.1789.

Synthesis of compounds 2 and 3

To a solution of (+)-paeoveitol (328 mg, 1 mmol) in 4 mL of tetrahydrofuran was added 1 M NaOH solution (1 mL, 1 mmol) and the mixture was stirred for 5 min at room temperature. Then, 1-acetyl-1H-1,2,3-triazolo[4,5-B] pyridine (162 mg, 1 mmol) was added and the reaction mixture was stirred for an additional 30 min. Upon completion, water (5 mL) was added and the mixture was neutralized with 1 M HCl solution. The mixture was extracted with EtOAc (3 × 10 mL), washed with brine, dried with anhydrous Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The residue was subjected to flash column chromatography on silica gel (acetone-petroleum ether, 15:85) to provide compound **3** (181 mg, 49% yield) and compound **4** (95 mg, 23% yield).

(5a*S*,10a*R*,11*R*)-2-hydroxy-5a-(hydroxymethyl)-3,8,11-trimethyl-5a,10a-dihydro-11H-benzofuro [3,2-b] chromen-7-yl acetate (3)

White powder. ^{1}H NMR (500 MHz, CD$_3$OD) δ 7.00 (s, 1H, H-1), 6.63 (s, 1H, H-4), 6.42 (s, 1H, H-15), 6.37 (s, 1H, H-12), 5.10 (d, $J = 3.0$ Hz, 1H, H-8), 4.14 (d, $J = 11.5$ Hz, 1H, H-18a), 3.98 (d, $J = 11.5$ Hz, 1H, H-18b), 3.07-3.02 (m, 1H, H-7), 2.24 (s, 3H, H-19), 2.01 (s, 3H, H-16), 1.95 (s, 3H, -OAc), 1.54 (d, $J = 7.5$ Hz, 3H, H-17); ^{13}C NMR (125 MHz, CD$_3$OD) δ 171.8 (OAc), 159.8 (C-11), 151.6 (C-2), 148.0 (C-5), 144.2 (C-14), 134.2 (C-13), 128.4 (C-6), 126.6 (C-10), 123.9 (C-2), 120.9 (C-4), 118.9 (C-15), 113.2

(C-1), 112.2 (C-12), 91.0 (C-8), 89.5 (C-9), 67.1 (C-18), 33.7 (C-7), 20.7 (OAc), 16.7 (C-16), 16.0 (C-19), 13.6 (C-17); HRMS (ESI, m/z): $[M + H]^+$ calcd for $[C_{21}H_{23}O_6]^+$ 371.1495, found 371.1503.

(5aS,10aR,11R)-5a-(hydroxymethyl)-3,8,11-trimethyl-5a,10a-dihydro-11H-benzofuro [3,2-b] chromene-2,7-diyl diacetate (4)

White powder. $[\alpha]_D^{24.3}$ – 11.0 (c 0.072, MeOH); ^{1}H NMR (400 MHz, CDCl$_3$) δ 6.94 (s, 1H, H-15), 6.81 (s, 1H, H-1), 6.56 (s, 1H, H-4), 6.47 (s, 1H, H-12), 5.10 (d, J = 3.2 Hz, 1H, H-8), 4.20 (d, J = 12.0 Hz, 1H, H-18a), 3.96 (d, J = 13.6 Hz, 1H, H-18a), 3.06-3.03 (m, 1H, H-7), 2.28 (s, 3H, -OAc), 2.26 (s, 3H, -OAc), 2.03 (s, 3H, H-19), 2.00 (s, 3H, H-16), 1.55 (d, J = 6.8 Hz, 3H, H-17); ^{13}C NMR (100 MHz, CDCl$_3$) δ 169.7 (C = O), 169.6 (C = O), 158.3 (C-11), 151.3 (C-5), 144.5 (C-2), 142.9 (C-14), 133.5 (C-13), 129.0 (C-6), 127.0 (C-10), 124.2 (C-3) 120.0 (C-4), 119.3 (C-1), 117.5 (C-15), 112.0 (C-12), 89.4 (C-8), 88.0 (C-9), 67.1 (C-18), 32.6 (C-7), 20.8 (-OAc), 20.7 (-OAc), 16.8 (C-19), 15.9 (C-16), 13.1 (C-17); HRMS (ESI, m/z): $[M + H]^+$ calcd for $[C_{23}H_{25}O_7]^+$ 413.1600, found 413.1604.

General synthetic procedures for compounds 5-21

To a solution of compound **4** (20.6 mg, 0.05 mmol) in CH$_2$Cl$_2$ (10 mL), Dess-Martin periodinane (31.8 mg, 0.075 mmol, 1.5 equiv) was added slowly. After stirring at room temperature for 2 h, the mixture was quenched with saturated Na$_2$S$_2$O$_3$ (10 mL), and the aqueous layer was extracted with CH$_2$Cl$_2$ (3 × 10 mL). The combined organic phases were washed with saturated NaHCO$_3$ (10 mL), and brine (10 mL), dried over anhydrous Na$_2$SO$_4$, filtered and concentrated under reduced pressure to yield crude aldehyde. A mixture of aldehyde, amine (0.075 mmol) and NaBH(OAc)$_3$ (15.9 mg, 0.075 mmol) and AcOH (1 μL) in CH$_2$Cl$_2$ (1 mL) was stirred at room temperature for 12 h. Upon completion, 2 M NaOH solution (1 mL) was added and stirred for another 6 h. The reaction mixture was neutralized with 1 M HCl solution and extracted with CH$_2$Cl$_2$ (3 × 5 mL), washed with brine, dried with anhydrous Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (Et$_3$N-ethyl acetate-petroleum ether, 1:30:70) to afford products **5-21**.

(5aS,10aR,11R)-5a-((dimethylamino)methyl)-3,8,11-trimethyl-5a,10a-dihydro-11H-benzofuro[3,2-b]chromene-2,7-diol (5)

White powder, 12 mg, 69% yield, $[\alpha]_D^{24}$ + 223.53 (c 0.085, MeOH); ^{1}H NMR (400 MHz, pyridine-d_5) δ 7.43 (s, 1H, H-15), 7.11 (s, 1H, H-1), 6.81 (s, 1H, H-4), 6.61 (s, 1H, H-12),

5.36 (d, J = 2.8 Hz, 1H, H-8), 3.33-3.30 (m, 1H, H-7), 3.22 (d, J = 13.6 Hz, 1H, H-18a), 2.94 (d, J = 13.6 Hz, 1H, H-18b), 2.36 (s, 6H, N-Me), 2.25 (s, 3H, H-19), 2.19 (s, 3H, H-16), 1.61 (d, J = 7.2 Hz, 3H, H-17); ^{13}C NMR (100 MHz, pyridine-d_5) δ 153.6 (C-11), 151.7 (C-2), 150.5 (C-14), 147.0 (C-5), 127.8 (C-13), 127.7 (C-6), 126.9 (C-10), 122.9 (C-3), 120.1 (C-4), 112.8 (C-1), 111.6 (C-12), 110. 7 (C-15), 89.7 (C-8), 89.3 (C-9), 65.9 (C-18), 47.8 (N-Me), 32.9 (C-7), 16.9 (C-19), 16.1 (C-16), 13.5 (C-17); HRMS (ESI, m/z): $[M + H]^+$ calcd for $[C_{21}H_{26}NO_4]^+$ 356.1856, found 356.1838.

(5aS,10aR,11R)-5a-((diethylamino)methyl)-3,8,11-trimethyl-5a,10a-dihydro-11H-benzofuro[3,2-b]chromene-2,7-diol (6)

White powder, 14 mg, 71% yield, $[\alpha]_D^{25.0}$ + 163.13 (c 0.055, MeOH); ^{1}H NMR (500 MHz, pyridine-d_5) δ 7.46 (s, 1H, H-15), 7.13 (s, 1H, H-1), 6.82 (s, 1H, H-4), 6.62 (s, 1H, H-12), 5.33 (d, J = 2.5 Hz, 1H, H-8), 3.44-3.35 (m, 2H, H-7, H-18a), 3.06 (d, J = 14.0 Hz, 1H, H-18b), 2.84-2.77 (m, 2H, H-1′), 2.65-2.58 (m, 2H, H-1′), 2.25 (s, 3H, H-19), 2.19 (s, 3H, H-16), 1.65 (d, J = 7.0 Hz, 3H, H-17), 1.00-0.97 (m, 6H, H-2′); ^{13}C NMR (125 MHz, pyridine-d_5) δ 153.8 (C-11), 151.9 (C-2), 150.7 (C-14), 147.3 (C-5), 128.0 (C-13), 127.3 (C-6), 127.3 (C-10), 123.1 (C-2), 120.3 (C-4), 113.0 (C-1), 111.9 (C-11), 111.0 (C-15), 90.1 (C-8), 90.0 (C-9), 60.7 (C-18), 46.6 (C-1′), 33.1 (C-2′), 17.2 (C-19), 16.3 (C-16), 13.8 (C-17); HRMS (ESI, m/z): $[M + H]^+$ calcd for $[C_{23}H_{30}NO_4]^+$ 384.2169, found 384.2146.

(5aS,10aR,11R)-5a-((dipropylamino)methyl)-3,8,11-trimethyl-5a,10a-dihydro-11H-benzofuro[3,2-b]chromene-2,7-diol (7)

White powder, 13 mg, 65% yield, $[\alpha]_D^{24.9}$ + 168.63 (c 0.102, MeOH); ^{1}H NMR (500 MHz, pyridine-d_5) δ 7.45 (s, 1H, H-15), 7.14 (s, 1H, H-1), 6.83 (s, 1H, H-4), 6.62 (s, 1H, H-12), 5.33 (d, J = 3.0 Hz, 1H, H-8), 3.44-3.40 (m, 2H, H-7, H-18a), 3.06 (d, J = 13.0 Hz, 1H, H-18b), 2.73-2.65 (m, 2H, H-1′), 2.52-2.48 (m, 2H, H-1′), 2.25 (s, 3H, H-19), 2.18 (s, 3H, H-16), 1.68 (d, J = 7.0 Hz, 3H, H-17), 1.45-1.39 (m, 4H, H-2′), 0.82 (t, J = 7.0 Hz, 6H, H-3′); ^{13}C NMR (125 MHz, pyridine-d_5) δ 153.9 (C-11), 151.9 (C-2), 150.7 (C-14), 147.4 (C-5), 128.0 (C-13), 127.3 (C-6), 127.3 (C-6), 123.1 (C-3), 120.3 (C-4), 113.0 (C-1), 111.8 (C-12), 111.0 (C-15), 90.2 (C-8), 90.1 (C-9), 62.1 (C-18), 59.2 (C-1′), 33.1 (C-7), 20.7 (C-2′), 17.2 (C-19), 16.4 (C-16), 13.8 (C-17), 11.1(C-3′); HRMS (ESI, m/z): $[M + H]^+$ calcd for $[C_{25}H_{34}NO_4]^+$ 412.2482, found 412.2466.

(5aS,10aR,11R)-5a-((dibutylamino)methyl)-3,8,11-trimethyl-5a,10a-dihydro-11H-benzofuro[3,2-b]chromene-2,7-diol (8)

White powder, 13 mg, 60% yield, $[\alpha]_D^{24.8}$ + 169.43 (c 0.091, MeOH); ^{1}H NMR (500 MHz, pyridine-d_5) δ 7.47 (s, 1H, H-

15), 7.14 (s, 1H, H-1), 6.84 (s, 1H, H-4), 6.62 (s, 1H, H-12), 5.36 (d, $J = 3.0$ Hz, 1H, H-8), 3.45-3.41 (m, 2H, H-7, H-18a), 3.11 (d, $J = 14.5$ Hz, 1H, H-18b), 2.78-2.73 (m, 2H, H-1′), 2.59-2.53 (m, 2H, H-1′), 2.25 (s, 3H, H-19), 2.18 (s, 3H, H-16), 1.68 (d, $J = 7.0$ Hz, 3H, H-17), 1.45-1.40 (m, 4H, H-2′), 1.29-1.24 (m, 4H, H-3′), 0.85 (t, $J = 7.0$ Hz, 6H, H-4′); ^{13}C NMR (125 MHz, pyridine-d_5) δ 153.8 (C-11), 151.9 (C-2), 150.7 (C-14), 147.4 (C-6), 128.0 (C-13), 128.0 (C-6), 127.3 (C-10), 123.1 (C-3), 120.3 (C-4), 113.0 (C-1), 111.9 (C-12), 111.0 (C-15), 90.2 (C-8), 90.1 (C-9), 62.1 (C-18), 55.9 (C-1′), 33.1 (C-7), 29.8 (C-2′), 20.8 (C-3′), 17.2 (C-19), 16.4 (C-16), 14.3 (C-4′), 13.8 (C-17); HRMS (ESI, m/z): $[M + H]^+$ calcd for $[C_{27}H_{28}NO_4]^+$ 440.2795, found 440.2787.

(5aS,10aR,11R)-3,8,11- trimethyl-5a-(morpholinomethyl)-5a,10a-dihydro-11H-benzofuro[3,2-b]chromene-2,7-diol (9)

White powder, 15 mg, 75% yield, $[\alpha]_D^{24.8} + 191.37$ (c 0.035, MeOH); ^{1}H NMR (500 MHz, pyridine-d_5) δ 7.59 (s, 1H, H-15), 7.14 (s, 1H, H-1), 6.82 (s, 1H, H-4), 6.62 (s, 1H, H-12), 5.33 (d, $J = 3.0$ Hz, 1H, H-8), 3.68-3.66 (m, 4H, H-2′), 3.37-3.35 (m, 1H, H-7), 3.31 (d, $J = 14.0$ Hz, 1H, H-18a), 2.95 (d, $J = 14.0$ Hz, 1H, H-18b), 2.84-2.80 (m, 2H, H-1′), 2.64-2.60 (m, 2H, H-1′), 2.25 (s, 3H, H-19), 2.20 (s, 3H, H-16), 1.67 (d, $J = 8.4$ Hz, H-17); ^{1}H NMR (125 MHz, pyridine-d_5) δ 153.8 (C-11), 152.1 (C-2), 150.8 (C-14), 147.1 (C-5), 128.2 (C-13), 128.0 (C-6), 127.1 (C-10), 123.3 (C-3), 120.4 (C-4), 113.1 (C-1), 112.0 (C-12), 110.9 (C-15), 90.1 (C-8), 89.7 (C-9), 67.3 (C-2′), 65.6 (C-18), 56.1 (C-1′), 33.3 (C-3), 17.2 (C-19), 16.4 (C-16), 13.8 (C-17); HRMS (ESI, m/z): $[M + H]^+$ calcd for $[C_{23}H_{28}NO_5]^+$ 398.1962, found 398.1938.

(5aS,10aR,11R)-3,8,11-trimethyl-5a-(pyrrolidin-1-ylmethyl)-5a,10a-dihydro-11H-benzofuro[3,2-b]chromene-2,7-diol (10)

White powder, 13 mg, 71% yield, $[\alpha]_D^{25.0} + 196.59$ (c 0.051, MeOH); ^{1}H NMR (500 MHz, pyridine-d_5) δ 7.44 (s, 1H, H-15), 7.11 (s, 1H, H-1), 6.83 (s, 1H, H-4), 6.61 (s, 1H, H-12), 5.38 (d, $J = 3.0$ Hz, 1H, H-8), 3.39 (d, $J = 13.5$ Hz, 1H, H-18a), 3.31-3.26 (m, 1H, H-7), 3.18 (d, $J = 13.5$ Hz, 1H, H-18b), 2.73-2.62 (m, 4H, H-1′), 2.25 (s, 3H, H-19), 2.20 (s, 3H, H-16), 1.62-1.58 (m, 7H, H-1′, H-17); ^{1}H NMR (125 MHz, pyridine-d_5) δ 153.9 (C-11), 151.9 (C-2), 150.7 (C-14), 147.3 (C-5), 128.1 (C-13), 127.9 (C-6), 127.2 (C-10), 123.2 (C-3), 120.4 (C-4), 113.1 (C-1), 111.9 (C-12), 111.0 (C-15), 90.0 (C-8), 89.2 (C-9), 63.2 (C-18), 56.8 (C-1′), 33.1 (C-7), 23.3 (C-2′), 17.2 (C-19), 16.4 (C-17), 13.8 (C-17); HRMS (ESI, m/z): $[M + H]^+$ calcd for $[C_{23}H_{28}NO_4]^+$ 382.2013, found 382.2007.

(5aS,10aR,11R)-3,8,11-trimethyl-5a-((4-methyl-1,4-diazepan-1-yl)methyl)-5a,10a-dihydro-11H-benzofuro[3,2-b]chromene-2,7-diol (11)

White powder, 13 mg, 62% yield, $[\alpha]_D^{24.9} + 166.09$ (c 0.044, MeOH); ^{1}H NMR (500 MHz, pyridine-d_5) δ 7.44 (s, 1H, H-15), 7.14 (s, 1H, H-1), 6.83 (s, 1H, H-4), 6.62 (s, 1H, H-12), 5.38 (d, $J = 3.0$ Hz, 1H, H-8), 3.54 (d, $J = 14.5$ Hz, 1H, H-18a), 3.44-3.41 (m, 1H, H-7), 3.17 (d, $J = 14.5$ Hz, 1H, H-18b), 3.08-3.05 (m, 2H, H-1′), 2.97-2.93 (m, 2H, H-2′), 2.50-2.52 (m, 2H, H-3′, H-5′), 2.25 (s, 3H, H-19), 2.25 (s, 3H, H-6′), 2.19 (s, 3H, H-16), 1.75-1.71 (m, 2H, H-4′), 1.68 (d, $J = 7.0$ Hz, 3H, H-17); ^{13}C NMR (125 MHz, pyridine-d_5) δ 154.0 (C-11), 151.9 (C-2), 150.7 (C-14), 147.4 (C-5), 128.1 (C-13), 128.0 (C-6), 127.2 (C-10), 123.1 (C-3), 120.4 (C-4), 113.1 (C-1), 111.9 (C-12), 111.0 (C-15), 90.2 (C-9), 90.0 (C-8), 64.9 (C-18), 58.9 (C-2′), 57.6 (C-1′), 57.0 (C-3′), 56.9 (C-5′), 47.1 (C-6′), 33.2 (C-7), 28.3 (C-4′), 17.2 (C-19), 16.4 (C-16), 13.7 (C-17); HRMS (ESI, m/z): $[M + H]^+$ calcd for $[C_{25}H_{33}N_2O_4]^+$ 425.2435, found 425.2387.

(5aS,10aR,11R)-3,8,11-trimethyl-5a-(piperazin-1-ylmethyl)-5a,10a-dihydro-11H-benzofuro[3,2-b]chromene-2,7-diol (12)

White powder, 10 mg, 52% yield, $[\alpha]_D^{24.9} + 158.36$ (c 0.028, MeOH); ^{1}H NMR (500 MHz, pyridine-d_5) δ 7.45 (s, 1H, H-15), 7.14 (s, 1H, H-11), 6.82 (s, 1H, H-4), 6.61 (s, 1H, H-12), 5.35 (d, $J = 3.0$ Hz, 1H, H-8), 3.39-3.38 (m,1H, H-7), 3.33 (d, $J = 14.0$ Hz, 1H, H-18a), 2.96 (d, $J = 14.0$ Hz, 1H, H-18b), 2.89-2.85 (m, 6H, H-2′, H-1′), 2.64-2.62 (m, 2H, H-1′), 2.24 (s, 3H, H-19), 2.19 (s, 3H, H-16), 1.66 (d, $J = 7.0$ Hz, 3H, H-17); ^{13}C NMR (125 MHz, pyridine-d_5) δ 153.8 (C-11), 152.0 (C-2), 150.8 (C-14), 147.2 (C-5), 128.1 (C-13), 128.0 (C-6), 127.2 (C-10), 123.2 (C-3), 120.4 (C-4), 113.1 (C-1), 111.9 (C-12), 110. 9 (C-15), 90.1 (C-8), 89.8 (C-9), 65.8 (C-18), 56.8 (C-1′), 46.6 (C-2′), 33.2 (C-7), 17.2 (C-19), 16.3 (C-16), 13.8 (C-17); HRMS (ESI, m/z): $[M + H]^+$ calcd for $[C_{23}H_{28}N_2O_4]^+$ 397.2122, found 397.2106.

(5aS,10aR,11R)-3,8,11-trimethyl-5a-((4-methylpiperazin-1-yl)methyl)-5a,10a-dihydro-11H-benzofuro[3,2-b]chromene-2,7-diol (13)

White powder, 16 mg, 78% yield, $[\alpha]_{24}^{D} + 174.58$ (c 0.072, MeOH);^{1}H NMR (400 MHz, CD$_3$OD) δ 6.73 (s, 1H, H-15), 6.59 (s, 1H, H-1), 6.33 (s, 1H, H-4), 6.27 (s, 1H, H-12), 5.03 (d, $J = 3.2$ Hz, 1H, H-8), 3.14 (d, $J = 14.0$ Hz, 1H, H-18a), 3.07-3.04 (m, 1H, H-7), 2.87-2.84 (m, 3H, H-18b, H-1′), 2.64-2.49 (m, 6H, H-1′, H-2′), 2.28 (s, 3H, H-3′), 2.06 (s, 3H, H-19), 2.01 (s, 3H, H-16), 1.52 (d, $J = 7.2$ Hz, 3H, H-17); ^{13}C NMR (100 MHz, CD$_3$OD) δ 153.3 (C-11), 149.9 (C-2), 148.7 (C-14), 146.6 (C-5), 127.3 (C-13), 127.2 (C-6), 125.8 (C-10), 122.2 (C-3), 119.3 (C-4), 111.9 (C-1), 110.6

(C-12), 109.5 (C-15), 89.5 (C-8), 89.1 (C-9), 63.9 (C-18), 54.9 (C-1′), 54.2 (C-2′), 44.6 (C-3′), 32.5 (C-7), 15.5 (C-19), 14.6 (C-16), 12.4 (C-17); HRMS (ESI, m/z): $[M + H]^+$ calcd for $[C_{24}H_{30}N_2O_4]^+$ 411.2278, found 411.2248.

(5aS,10aR,11R)-5a-((4-ethylpiperazin-1-yl)methyl)-3,8,11-trimethyl-5a,10a-dihydro-11H-benzofuro[3,2-b]chromene-2,7-diol (14)

White powder, 17 mg, 79% yield, $[\alpha]_D^{24.9}$ + 185.12 (c 0.034, MeOH);^{1}H NMR (500 MHz, pyridine-d_5) δ 7.45 (s, 1H, H-15), 7.14 (s, 1H, H-1), 6.82 (s, 1H, H-4), 6.62 (s, 1H, H-12), 5.34 (d, J = 3.0 Hz, 1H, H-8), 3.41-3.38 (m, 1H, H-7), 3.34 (d, J = 13.5 Hz, 1H, H-18a), 2.99 (d, J = 13.5 Hz, 1H, H-18b), 2.91-2.89 (m, 2H, H-1′), 2.73-2.71 (m, 2H, H-1′), 2.40-2.34 (m, 4H, H-2′), 2.25 (s, 3H, H-19), 2.24 (q, 2H, J = 7.5 Hz, H-3′), 2.19 (s, 3H, H-16), 1.67 (d, J = 6.0 Hz, 3H, H-17), 0.98 (t, 3H, J = 7.5 Hz, H-3′);^{13}C NMR (125 MHz, pyridine-d_5) δ 153.8 (C-11), 152.0 (C-2), 150.8 (C-14), 147.2 (C-5), 128.1 (C-13), 128.0 (C-6), 127.2 (C-10), 123.2 (C-3), 120.4 (C-4), 113.1 (C-1), 111.9 (C-12), 110.9 (C-15), 90.1 (C-8), 89.7 (C-9), 65.9 (C-18), 55.8 (C-1′), 53.5 (C-2′), 52.4 (C-3′), 33.2 (C-7), 17.2 (C-19), 16.3 (C-16), 13.8 (C-17), 12.4 (C-4′); HRMS (ESI): $[M + H]^+$ calcd for $[C_{25}H_{32}N_2O_4]^+$ 425.2435, found 425.2393.

(5aS,10aR,11R)-5a-((4-(2-methoxyphenyl)piperazin-1-yl)methyl)-3,8,11-trimethyl- 5a,10a-dihydro- 11H-benzofuro[3,2-b]chromene-2,7-diol (15)

White powder, 15 mg, 61% yield, $[\alpha]_D^{24.9}$ + 160.77 (c 0.057, MeOH);^{1}H NMR (500 MHz, pyridine-d_5) δ 7.48 (s, 1H, H-15), 7.15 (s, 1H, H-1), 7.07-7.00 (m, 2H, H-5′, H-7′), 6.96-6.93 (m, 2H, H-6′, H-8′), 6.85 (s, 1H, H-4), 6.63 (s, 1H, H-12), 5.37 (d, J = 3.0 Hz, 1H, H-8), 3.75 (s, 3H, OMe), 3.43-3.37 (m, 2H, H-7, H-18a), 3.14-3.04 (m, 7H, H-18b, H-1′, H-2′), 2.88-2.85 (m, 2H, H-1′), 2.26 (s, 3H, H-19), 2.20 (s, 3H, H-16), 1.68 (d, J = 7.0 Hz, 3H, H-17); ^{13}C NMR (125 MHz, pyridine-d_5) δ 153.8 (C-11), 153.0 (C-3′), 152.0 (C-2), 150.8 (C-14), 147.2 (C-5), 142.3 (C-4′), 128.1 (C-13), 128.0 (C-6), 127.2 (C-10), 123.2 (C-8′), 122.9 (C-3), 121.5 (C-6′), 120.4 (C-7′), 118.6 (C-4), 113.1 (C-5′), 112.5 (C-1), 111.9 (C-12), 111.0 (C-15), 90.1 (C-9), 89.7 (C-8), 65.2 (C-18), 55.9 (C-2′), 55.5 (OMe), 51.1 (C-1′), 33.2 (C-7), 17.2 (C-19), 16.4 (C-16), 13.8 (C-17); HRMS (ESI, m/z): $[M + H]^+$ calcd for $[C_{30}H_{35}N_2O_5]^+$ 503.2540, found 503.2506.

(5aS,10aR,11R)-3,8,11-trimethyl-5a-((4-(p-tolyl)piperazin-1-yl)methyl)-5a,10a-dihydro-11H-benzofuro[3,2-b]chromene-2,7-diol (16)

White powder, 14 mg, 59% yield, $[\alpha]_D^{24.9}$ + 132.21 (c 0.037, MeOH); ^{1}H NMR (500 MHz, pyridine-d_5) δ 7.49

(s, 1H, H-15), 7.15-7.13 (m, 3H, H-1, H-5′), 6.93 (d, J = 9.0 Hz, H-4′), 6.85 (s, 1H, H-4), 6.63 (s, 1H, H-15), 5.33 (d, J = 3.0 Hz, 1H, H-8), 3.44-3.34 (m, 2H, H-7, H-18a), 3.11 (d, J = 5.0 Hz, 4H, H-2′), 3.10-2.96 (m, 3H, H-18b, H-1′), 2.82-2.77 (m, 2H, H-1′), 2.26 (s, 1H, H-19), 2.23 (s, 1H, H-7′), 2.20 (s, 1H, H-16), 1.67 (d, J = 7.0 Hz, H-17); ^{13}C NMR (125 MHz, pyridine-d_5) δ 153.8 (C-11), 152.0 (C-2), 150.8 (C-14), 149.9 (C-3′), 147.2 (C-5), 130.0 (C-5′) 128.5 (C-6′), 128.1 (C-13), 128.0 (C-6), 127.1 (C-10), 123.2 (C-2), 120.4 (C-4), 116.4 (C-4′), 113.1 (C-1), 112.0 (C-12), 111.0 (C-15), 90.1 (C-8), 89.7 (C-9), 65.0 (C-18), 55.5 (C-2′), 49.8 (C-1′), 33.2 (C-7), 20.4 (C-7′), 17.2 (C-19), 16.4 (C-16), 13.8 (C-17); HRMS (ESI, m/z): $[M + H]^+$ calcd for $[C_{30}H_{35}N_2O_4]^+$ 487.2591, found 487.2561.

(5aS,10aR,11R) -5a-((4-([1,1′ - biphenyl] - 4- yl) piperazin-1-yl) methyl) - 3,8,11-trimethyl -5a, 10a-dihydro-11H-benzofuro[3,2-b]chromene-2,7-diol (17)

White powder, 17 mg, 63% yield, $[\alpha]_D^{24.9}$ + 119.77 (c 0.026, MeOH);^{1}H NMR (500 MHz, pyridine-d_5) δ 7.74-7.69 (m, 4H, H-8′, H-9′), 7.51 (s, 1H, H-15), 7.49-7.45 (m, 2H, H-5′), 7.34-7.31 (m,1H, H-10′), 7.16 (s, 1H, H-1), 7.08 (d, J = 9.0 Hz, 2H, H-4′), 6.86 (s, 1H, H-2), 6.64 (s, 1H, H-12), 5.34 (d, J = 3.0 Hz, 1H, H-8), 3.42-3.35 (m, 2H, H-7, H-18a), 3.20-3.18 (m, 4H, H-2′), 3.05-2.97 (m, 3H, H-18b, H-1′), 2.83-2.80 (m, 2H, H-1′), 2.27 (s, 3H, H-19), 2.21 (s, 3H, H-16), 1.69 (d, 3H, J = 7.0 Hz, H-17); ^{13}C NMR (125 MHz, pyridine-d_5) δ 153.8 (C-11), 152.1 (C-2), 151.3 (C-3′), 150.8 (C-14),147.2 (C-5), 141.3 (C-7′), 131.7 (C-6′), 129.3 (C-9′), 128.1 (C-13), 128.0 (C-6), 127.9 (C-5′), 127.1 (C-10), 126.8 (C-10′), 126.7 (C-8′), 123.3 (C-2), 120.4 (C-4), 116.2 (C-4′), 113.1 (C-1), 112.0 (C-12), 111.0 (C-15), 90.1 (C-8), 89.7 (C-9), 65.0 (C-18), 55.4 (C-2′), 49.0 (C-1′), 33.2 (C-7), 17.2 (C-19), 16.4 (C-16), 13.8 (C-17); HRMS (ESI, m/z): $[M + H]^+$ calcd for $[C_{35}H_{36}N_2O_4]^+$ 549.2748, found 549.2740.

(5aS,10aR,11R)-3,8,11-trimethyl-5a-((4-(3-methylbenzyl) piperazin-1-yl) methyl) -5a,10a-dihydro-11H-benzofuro[3,2-b]chromene-2,7-diol (18)

White powder, 17 mg, 66% yield, $[\alpha]_D^{24.8}$ + 167.35 (c 0.034, MeOH);^{1}H NMR (400 MHz, CD$_3$OD) δ 7.20-7.06 (m, 4H, H-5′, H-6′, H-7′, H-8′), 6.72 (s, 1H, H-15), 6.58 (s, 1H, H-1), 6.31 (s, 1H, H-4), 6.26 (s, 1H, H-12), 5.01 (s, 1H, H-8), 3.36 (s, 2H, H-3′), 3.34 (d, J = 14.0 Hz, 1H, H-18a), 3.11 (d, J = 14.0 Hz, 1H, H-18b), 3.05-3.03 (m, 1H, H-7), 2.83-2.79 (m, 2H, H-1′),2.59-2.55 (m, 6H, H-1′, H-2′), 2.31 (s, 3H, 10′), 2.04 (s, 3H, H-19), 1.99 (s, 3H, H-16), 1.50 (d, J = 7.2 Hz, 3H, H-17);^{13}C NMR (100 MHz, CD$_3$OD) δ 153.3 (C-11), 149.9 (C-2), 148.7 (C-14), 146.7

(C-5), 137.6 (C-4′), 136.6 (C-6′), 130.2 (C-5′), 127.8 (C-7′), 127.7 (C-8′), 127.3 (C-13), 127.2 (C-6), 126.6 (C-9′), 125.8 (C-10), 122.2 (C-3), 119.3 (C-4), 111.9 (C-1), 110.6 (C-12), 109.5 (C-15), 89.5 (C-8), 89.1 (C-9), 64.0 (C-18), 62.7 (C-3′), 54.3 (C-1′), 53.0 (C-2′), 32.4 (C-7), 20.0 (C-10′), 15.4 (C-19), 14.5 (C-17), 12.3 (C-17); HRMS (ESI, m/z): $[M + H]^+$ calcd for $[C_{31}H_{36}N_2O_4]^+$ 501.2748, found 501.2711.

(5aS,10aR,11R)-3,8,11-trimethyl-5a-((4-(pyrimidin-2-yl) piperazin-1-yl) methyl) -5a,10a-dihydro-11H-benzofuro[3,2-b]chromene-2,7-diol (19)

White powder, 16 mg, 69% yield, $[\alpha]_D^{24.5} + 172.05$ (c 0.083, MeOH); ^1H NMR (400 MHz, CDCl₃) δ 8.31 (d, $J = 4.8$ Hz, 2H, H-4′), 6.72 (s, 1H, H-15), 6.57 (s, 1H, H-1), 6.49 (t, $J = 4.8$ Hz, 1H, H-5′), 6.40 (s, 1H, H-4), 6.36 (s, 1H, H-12), 5.08 (d, $J = 3.2$ Hz, 1H, H-8), 3.80-3.75 (m, 4H, H-2′), 3.20 (d, $J = 14.0$ Hz, 1H, H-18a), 3.13-3.10 (m, 1H, H-7), 2.91-2.85 (m, 2H, H-1′), 2.80 (d, $J = 14.0$ Hz, 1H, H-18b), 2.60-2.55 (m, 2H, H-1′), 2.09 (s, 3H, H-19), 2.04 (s, 3H, H-16), 1.51 (d, $J = 6.8$ Hz, 3H, H-17); ^{13}C NMR (100 MHz, CDCl₃) δ 161.4 (C-3′), 157.8 (C-4′), 153.8 (C-11), 149.1 (C-2), 148.0 (C-14), 147.3 (C-5), 127.5 (C-13), 127.0 (C-6), 126.2 (C-10), 122.1 (C-3), 119.9 (C-4), 112.7 (C-1), 111.5 (C-12), 110.1 (C-15), 109.8 (C-5′), 89.7 (C-8), 89.1 (C-9), 64.9 (C-18), 55.1 (C-1′), 44.2 (C-2′), 32.7 (C-7), 16.5 (C-19), 15.6 (C-16), 13.4 (C-17); HRMS (ESI, m/z): $[M + H]^+$ calcd for $[C_{27}H_{31}N_4O_4]^+$ 475.2340, found 475.2311.

(5aS,10aR,11R)-3,8,11-trimethyl-5a-((4-(pyrazin-2-yl) piperazin-1-yl)methyl)-5a,10a-dihydro-11H-benzofuro[3,2-b]chromene-2,7-diol (20)

White powder, 14 mg, 59% yield, $[\alpha]_D^{24.6} + 173.60$ (c 0.135, MeOH); ^1H NMR (400 MHz, CDCl₃) δ 8.07-8.06 (m, 2H, H-5′, H-6′), 7.82 (d, $J = 2.8$ Hz, 1H, H-4′), 6.76 (s, 1H, H-15), 6.59 (s, 1H, H-1), 6.41 (s, 1H, H-4), 6.35 (s, 1H, H-12), 5.06 (d, $J = 2.8$ Hz, 1H, H-8), 3.52-3.50 (m, 4H, H-2′), 3.18 (d, $J = 14.0$ Hz, 1H, H-18a), 3.11-3.05 (m, 1H, H-7), 2.92-2.87 (m, 2H, H-1′), 2.84 (d, $J = 14.0$ Hz, 1H, H-18b), 2.66-2.62 (m, 2H, H-1′), 2.10 (s, 3H, H-19), 2.04 (s, 3H, H-16), 1.51 (d, $J = 7.2$ Hz, 3H, H-17); ^{13}C NMR (100 MHz, CDCl₃) δ 155.1 (C-3′), 153.7 (C-11), 149.5 (C-14), 148.3 (C-2), 147.1 (C-5), 142.0 (C-6′), 132.4 (C-5′), 130.6 (C-4′), 127.4 (C-13), 127.4 (C-6), 126.0 (C-10), 122.4 (C-3), 119.8 (C-4), 112.7 (C-1), 111.4 (C-12), 110.4 (C-15), 89.7 (C-8), 89.0 (C-9), 64.7 (C-18), 54.6 (C-2′), 44.7 (C-1′), 32.7 (C-7), 16.6 (C-19), 15.7 (C-16), 13.4 (C-17); HRMS (ESI, m/z): $[M + H]^+$ calcd for $[C_{27}H_{30}N_4O_4]^+$ 475.2340, found 475.2341.

(5aS,10aR,11R)-3,8,11-trimethyl-5a-((4-(quinolin-2-yl) piperazin-1-yl)methyl) -5a,10a-dihydro-11H-benzofuro[3,2-b]chromene-2,7-diol (21)

White powder, 12 mg, 56% yield, $[\alpha]_D^{24.6} + 175.09$ (c 0.099, MeOH); ^1H NMR (400 MHz, CDCl₃) δ 7.86 (d, $J = 9.2$ Hz, H-5′), 7.74 (d, $J = 8.4$ Hz, H-7′), 7.59 (d, $J = 8.0$ Hz, H-7′), 7.52 (dd, $J = 7.2, 7.2$ Hz, 1H, H-9′), 7.23 (dd, $J = 7.6, 7.2$ Hz, 1H, H-8′), 6.91 (d, $J = 9.2$ Hz, 1H, H-4′), 6.75 (s, 1H, H-15), 6.53 (s, 1H, H-1), 6.41 (s, 1H, H-4), 6.35 (s, 1H, H-12), 5.08 (d, $J = 2.8$ Hz, 1H, H-8), 3.70-3.61 (m, 4H, H-2′), 3.14 (d, $J = 14.0$ Hz, 1H, H-18a), 3.11-3.08 (m, 1H, H-7), 2.91-2.86 (m, 2H, H-1′), 2.81 (d, $J = 14.0$ Hz, 1H, H-18b), 2.65-2.60 (m, 2H, H-1′), 2.08 (s, 3H, H-19), 2.04 (s, 3H, H-16), 1.47 (d, $J = 7.2$ Hz, 3H, H-17); ^{13}C NMR (100 MHz, CDCl₃) δ 157.7 (C-3′), 153.8 (C-11), 149.1 (C-2), 147.9 (C-14), 147.6 (C-5), 147.3 (C-11′), 137.7 (C-5′), 129.8 (C-9′), 127.5 (C-13), 127.3 (C-7′), 127.2 (C-6), 126.3 (C-10), 126.2 (C-8′), 123.1 (C-3), 122.6 (C-8′), 122.3 (C-6′), 119.8 (C-4), 112.8 (C-4′), 111.5 (C-1), 110.4 (C-12), 109.9 (C-15), 89.7 (C-8), 89.0 (C-9), 64.6 (C-18), 55.0 (C-1′), 45.6 (C-2′), 32.7 (C-7), 16.6 (C-19), 15.6 (C-16), 13.4 (C-17); HRMS (ESI, m/z): $[M + H]^+$ calcd for $[C_{32}H_{34}N_3O_4]^+$ 524.2544, found 524.2516.

Biology

In vitro MT agonistic activity evaluation

Referring to our previous paper [7], HEK293 cell lines that stably express human MT₁ or MT₂ receptor were cultured in Dubecco's modified Eagle's medium supplemented with 10% fetal bovine serum at 37 °C under 95% O₂ /5% CO₂. The cells were incubated in a Matrigel coated 96-well black plate with a plating volume of 100 μL/well and density of 4×10^4/well, and incubated in a CO₂ incubator (Thermo Forma 3310, Gaithersburg, USA.) overnight. The cells were then dyed with an HDB wash free calcium assay kit, and placed in a CO₂ incubator for 1 h. (+) and (−)-paeoveitols and their derivatives were dissolved in 10 μL DMSO and 990 μL HBSS buffer, and a plating volume of 100 μL/well was extracted in a Matrigel coated 96-well clear bottom plate. Placing two 96-well plates into Flexstation 3 Benchtop Multi-Mode Microplate Reader, reading absorption values at room temperature using Flexstation 3 Benchtop Multi-Mode Microplate Reader with wavelength (excitation: 485 nm; emission:525 nm; emission cut-off: 515 nm). EC₅₀ values for derivative **13** were determined from the dose-response curves obtained with seven concentrations from the range of 0.02 to 2 mM using GraphPad Prism 6.0.

Forced swim test (FST)

Male Kunming mice (weight 15−18 g) were purchased from Beijing Hengfu Biotechnology Co., Ltd with license number: SCXK (JING) 2020-0009, Beijing, China). All mice were placed in a group of six animals per cage that could freely access food and water with an ambient temperature of $22 \pm 1\,°C$, and a relative humidity of 55−65% under a normal 12 h light/dark cycle (light turned on at 7 am).

The forced swim test was performed following a literature method [20]. Mice were individually placed into 25 cm high, 15 cm diameter glass cylinders filled with 10 cm high water, and the temperature was maintained at $25 \pm 1\,°C$. Mice were gently placed onto the water and forced to swim for 6 min, and the total time of the last 4 min of immobility was automatically measured by the ANY-maze Video Tracking System (Anymaze, Stoelting Co., Wood Dale, USA).

Neurochemical tests

After continuous administration of vehicle or compound **13** (20 mg/kg) for 14 days, male Kunming mice were executed by cervical dislocation, and brains were collected and rapidly dissected on an ice-cold glass plate to isolate the frontal cortex, hippocampus, striatum, hypothalamus, and thalamus. Weighed tissue samples were homogenized in an ice-cold solution of 0.2 M perchloric acid (10 μ/mg) containing 0.1 mM EDTA, and then centrifuged at 15,000 rpm for 20 min at 4 °C. The supernatant was collected and the amounts of NE, 5-HT, 5-HIAA, DA, and DOPAC were measured by HPLC-ECD equipped with an Agilent 1200 pump (0.6 mL/min) and electrochemical detector (+0.50 V). The mobile phase was 69 mM sodium dihydrogen phosphate, 0.01% (v/v) Et_3N, 0.025 mM EDTA, 1.7 mM sodium octanesulfonate (pH = 3.0) and 12% methanol. The injection volume was 20 μL.

In silico ADMET properties and drug-likeness prediction

The structures of compound **13** and (+)-paeoveitol were drawn using ChemDraw software (ChemDraw® Ultra, version 12), saved separately as an MDL Mol file (*.mol), and upload to the SwissADME server and converted into SMILES format. After that, the "Run" icon was pressed to give the ADMET parameters and related values.

Compliance with Ethical Standards

Conflict of interest The authors declare no competing interests.

Ethical approval "All applicable international, national, and/or institutional guidelines for the care and use of animals were followed."

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