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Research paper

Synthesis and biological evaluation of magnolol derivatives as melatonergic receptor agonists with potential use in depression

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ABSTRACT

Depression is associated with high mortality and morbidity rates worldwide. By our random screening, it was first revealed that 23 magnolol derivatives were synthesized followed by *in vitro* and *in vivo* evaluation of their antidepressive potential. Compound **7c** was found to be the most promising compound, with EC_{50} values of 396.5 and 383.0 μM agitating on MT_1 and MT_2 receptors, respectively. Additionally, we carried out *in vivo* experiments to confirm the efficacy and safety of compound **7c**; the compound was found to be orally bioavailable and highly effective, leading to a significant reduction of immobility time in a mouse model of depression (forced swimming test and tail suspension test); the acting mechanism was explored by determining its effect on the levels of monoamine neurotransmitters and their metabolites in different mice brain regions; the acute toxicity study showed that the 50% lethal dose (LD50) of **7c** was higher than 2000 mg/kg, p. o. A total of 25 metabolites of **7c** were identified, including 5 metabolites in phase I and 20 metabolites in phase II. Altogether, these results indicate that magnolol derivative **7c** is a promising lead compound for the development of a new chemical class of antidepressant drugs.

1. Introduction

Depression is the leading cause of disability and a major contributor to the overall global burden of disease, with a lifetime prevalence of 17% in the USA, and it is estimated that 300 million people affecting worldwide [1,2]. According to the World Health Organization (WHO), almost 800,000 lives are lost yearly due to suicide, and by the year 2020 is projected to be second only to ischemic heart disease in the amount of disability experienced by sufferers. Current pharmacotherapy is based on monoamine deficiency as the underlying etiology and pathogenesis of depression. There are several drugs on the market which have anti-depressant properties such as inhibitors of the enzyme monoamine oxidase (MAO), selective monoamine reuptake inhibitors, and, more recently, triple anti-depressants [3,4]. Although first-line antidepressants offer therapeutic benefit, the response to antidepressants is not immediate and usually occurs between the second and fourth weeks as well as causing side effects, as sedation and weight gain [5–7]. Furthermore, about 35% of depressed patients are not adequately treated, creating a large unmet medical need [8]. Evidence from preclinical and clinical studies implicates melatonin hypofunction in the pathophysiology of depression, and the potent MT_1 and MT_2 melatonergic agonist agomelatine displayed effective in the treatment of depressed patients [9,10]. Thus, during the past decade, a great number of structurally different MT receptor ligands which range from simple indole derivatives and their bioisosteres to phenylalkyl amides and constrained melatoninergic agents, have been reported in the literature [11]. The above reports clearly confirm that melatonergic agonists are emerging as an important target for the treatment of depression. In an attempt to develop a set of melatonergic agonists inspired by natural products, and magnolol displayed moderate activity on $MT_{1/2}$ receptors (agonistic rates: 40.24% and 20.04%, respectively at a concentration of 1.0 mM) in our earlier study, thus, we have selected magnolol as the parent scaffold. Although simple chemical modifications of the magnolol via the acetylation and methylation reported, their bioactivities only determined with anti-inflammatory activity or activation of cannabinoid receptors [12,13].

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In this report, we designed and synthesized a series of magnolol derivatives via suitable chemical transformation, then these derivatives evaluated for agitating activity on $MT_{1/2}$ receptors. The most potent derivative **7c** was further studied to confirm the efficacy *in vivo* by the behavioral test. Then the safety of compound **7c** was estimated with acute toxicity and its metabolites from urine, plasma and faeces were also detected and identified.

2. Results and discussion

2.1. Chemistry

The synthetic routes for the target compounds outline in Scheme 1. Magnolol treated with corresponding acids in the presence of *N*, *N*-dicyclohexylcarbodiimide and 4-dimethylaminopyridine to afford 1-substituted magnolol derivatives **1a**-**1g** and 1, 2-substituted derivatives **2a**-**2e**. To obtain the epoxides compounds **3**–**4**, magnolol was reacted with meta-chloroperbenzoic acid (*m*-CPBA) in dichloromethane at room temperature. Compounds **5**–**6** prepared by the α-D-glucopyranosyl bromide tetraacetate and magnolol with tetrabutylammonium bromide (TBAB). Stirring compounds **5** or **6** in sodium methoxide-methanol solution for 4–8 h furnished the corresponding derivatives **7a**-**7d**. A mixture of compound **7c** and appropriate carboxylic acid in a solution of DCC and DMAP to get target compounds **8a**-**8c**.

2.2. In vitro agonistic activities on $MT_{1/2}$ receptors

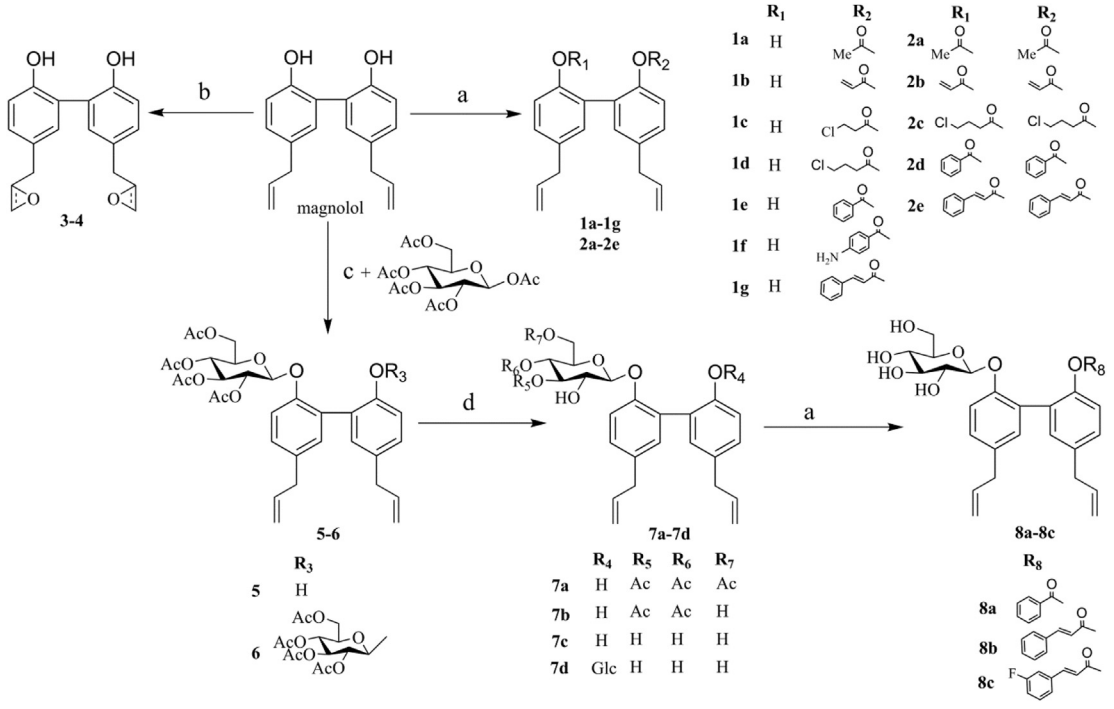
MT_1 and MT_2 melatonin receptors are important targets for the development of novel antidepressants. Thus, all synthesized compounds for their agonistic activities on $MT_{1/2}$ receptors evaluated. The results were summarized, and the details of the bioassay procedures described in the Experimental Section. As shown in Table 1, the mono-acetylated derivative **1a** exhibited stronger agonistic activities on $MT_{1/2}$ receptors compared with magnolol. Interestingly, derivatives **1d** and **7b** can only improve agitating activity on MT_2 (nearly 3.5–4.5 folds compared with magnolol). Compounds **1e**-**1g** showed no activities indicating that different aromatic groups at the position of R_2 were not beneficial because of steric hindrance. Compared mono-substituted (**1a** and **1d**) with bis-substituted (**2a** and **2c**), the agonistic activities decreased dramatically suggesting hydroxyl at the position of R_1 was very much beneficial to the activity. To confirm the role of double bonds, we synthesized compounds **3**–**4**. Unfortunately, the introduction of epoxide groups could not improve their biological activities, indicating the double bond may be the binding site to the receptors.

To develop more potent derivatives, another kind of compound with a glucosyl group was synthesized. Notably, compound **7c** exhibited excellent agonistic activity on $MT_{1/2}$ receptors with the values of 107.95% and 150.67%, respectively at a concentration of 1.0 mM. As shown in Table 1, the result suggested decrement in the

Table 1
Agonistic activities of magnolol and its derivatives on melatonin receptors.

Comp.	Agonistic activities (%)[b]		Comp.	Agonistic activities (%)[b]	
	MT_1	MT_2		MT_1	MT_2
Ago.[a]	100.00 ± 1.98	100.00 ± 3.21	**2e**	28.34 ± 1.80	3.34 ± 0.36
Mag.	40.24 ± 2.17	20.04 ± 0.85	**3**	53.77 ± 3.38	−3.08 ± 0.22
1a	91.43 ± 2.74	77.60 ± 3.06	**4**	2.18 ± 0.35	−3.39 ± 0.41
1b	1.31 ± 0.22	2.93 ± 1.09	**5**	−0.58 ± 0.02	−2.09 ± 0.08
1c	21.01 ± 2.21	30.15 ± 3.18	**6**	7.09 ± 1.33	0.04 ± 1.09
1d	32.05 ± 2.04	69.68 ± 3.41	**7a**	10.98 ± 1.20	19.06 ± 2.16
1e	−6.50 ± 1.35	−0.04 ± 0.42	**7b**	31.03 ± 1.83	89.00 ± 3.52
1f	−1.55 ± 0.33	5.17 ± 0.53	**7c**	107.95 ± 3.12	150.67 ± 2.75
1g	56.78 ± 2.57	25.56 ± 1.79	**7d**	2.97 ± 0.08	10.85 ± 0.96
2a	−1.86 ± 0.30	−2.52 ± 0.59	**8a**	13.24 ± 2.37	−5.71 ± 0.53
2b	0.04 ± 0.14	4.57 ± 0.23	**8b**	50.31 ± 5.36	−10.12 ± 1.67
2c	8.14 ± 0.77	2.11 ± 0.10	**8c**	5.30 ± 1.11	−10.50 ± 1.28
2d	−5.93 ± 0.51	−1.72 ± 3.11			

[a] Agomelatine was as a positive control and tested at the concentration of 3.33 μM and other compounds were tested at the concentration of 1.00 mM.
[b] The agonistic activities expressed as $\overline{X}$ ±SD (n = 3).



Scheme 1. (A) DMAP, DCC, CH_2Cl_2, rt, 4–6 h; (b) *m*-CPBA, CH_2Cl_2, rt, 4 h; (c) HBr, CH_2Cl_2, ice-bath, 6 h; Bu_4NBr, NaOH, $CHCl_3$, H_2O, rt, 2–4 h; (d) NaOMe, MeOH, rt, 4–8 h.

activity observed in the case of two acetyls of glucosyl analog (**7b**, 31.0% and 89.0% agitation on $MT_{1/2}$ receptors at 1.0 mM, respectively) and complete loss of activity observed in three and four acetyls of glucosyl analogs **7a** (10.98% and 19.06% agitation on $MT_{1/2}$ receptors at 1.0 mM, respectively) and **5** (−0.58% and −2.09% agitation on $MT_{1/2}$ receptors at 1.0 mM, respectively). By comparing compound **7c** and **7d**, it also suggested that the hydroxyl was essential to induce agitating effects.

According to these results, the dose-response curves for the most potent derivative **7c** was investigated to provide EC_{50} values of 396.5 and 383.0 μM on MT_1 and MT_2 receptors, respectively (Fig. 1). Dose-response of calcium activity was performed in triplicate and monitored with FlexStation plate reader. Based on the result, compound **7c** was also selected to study its antidepressant-like activity *in vivo*.

2.3. Effects of **7c** on behavioral evaluations

The forced swimming test (FST) and tail suspension test (TST) are the primary behavioral screen for detecting potential antidepressant drugs. Due to 7c was a potent agonist on MT1/2 receptors, and its antidepressant-like effect was further studied. Accordingly, FST and TST were employed in animals treating with different dosages of **7c** (10, 20 and 40 mg/kg, p. o.).

2.3.1. Effect of **7c** on locomotor activity

In order to eliminate the excitatory or inhibitory effects of **7c** on behavior test, the locomotion counts determined in the open-field test before depression behavior test (Fig. 2). The data showed that **7c** did not affect the locomotor activity compared to the vehicle group on the number of line crosses ($p > 0.05$, Fig. 2A) and on the locomotor distance ($p > 0.05$, Fig. 2B). These results indicated that the anti-immobility effect observed in the FST and TST couldn't be attributable to a psychostimulant activity.

2.3.2. Effect of **7c** in the FST

As shown in Fig. 3A, fluoxetine and agomelatine as positive standards, significantly reduced the immobility time in FST when compared with vehicle control ($p < 0.001$ and $p < 0.005$, respectively). Oral administration of **7c** at doses of 10, 20 and 40 mg/kg produced a dose-dependent decrease in the immobility duration, and the reduction in immobility time was significant when compared with vehicle control ($p < 0.001$ or $p < 0.05$). Additionally, **7c** at a dose of 40 mg/kg o. p. significantly ($p < 0.001$) decreased the immobility time of mice similar to fluoxetine and agomelatine during FST and percent decrease in immobility time was 37.66%.

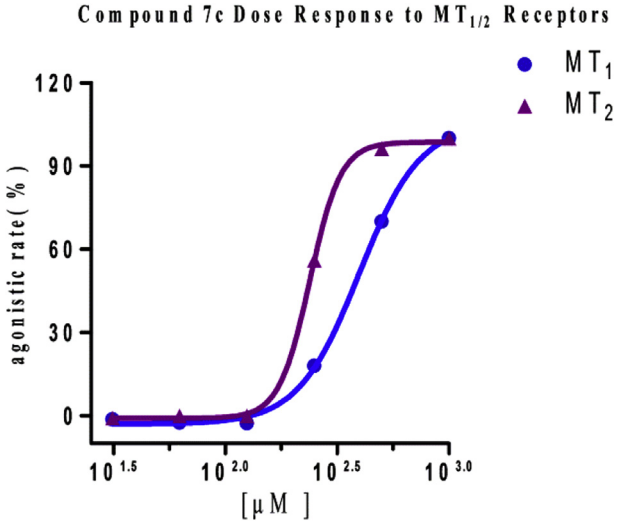


Fig. 1. The dose-dependent effects of derivative **7c** on MT_1 and MT_2 receptors.

2.3.3. Effect of **7c** in the TST

The effects of **7c**, fluoxetine and agomelatine on immobility time in the mouse TST were shown in Fig. 3B. The results obtained shown that **7c** ($p < 0.005$ or $p < 0.05$), fluoxetine ($p < 0.001$) and agomelatine ($p < 0.005$) induced a significantly decrease in immobility time in the TST when compared to normal control mice. **7c** produced a decrease in immobility time, the minimum effective dose value being 10 mg/kg ($p < 0.05$). Therefore, the treatment with the compound appeared to induce an equivalent antidepressive response to fluoxetine and agomelatine under the premise that this treatment may have antidepressant potential based on the efficacy presented.

2.4. Neurochemical tests

According to results of behavioral tests, derivative **7c** at 40 mg/kg was the most effective dose, so we used that to analyze monoamine neurotransmitter levels with an HPLC-ECD method (Fig. 4). Effects of derivative **7c** on levels of norepinephrine (NE), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindole-3-acetic acid (5-HIAA), dopamine (DA), 4-hydroxy-3-methoxyphenylacetic acid (HVA) and 5-hydroxytryptamine (5-HT), together with the ratios of 5-HIAA/5-HT and (DOPAC + HVA)/DA in frontal cortex, hippocampus, striatum, hypothalamus and thalamus were shown in Fig. 5. The mice treatment of derivative **7c** (40 mg/kg) significantly elevated the contents of NE ($p < 0.001$) in the frontal cortex, hippocampus, striatum, hypothalamus and thalamus of mice, respectively, as compared with the vehicle control group. Evidence for the involvement of NE in depression is abundant and therapeutic agents which specifically increase NE activity are effective antidepressants [14,15]. Further, NE also was a neurotransmitter intimately involved in the synthesis of melatonin, and it might be anticipated that melatonin concentration would be diminished in depression [16]. Moreover, no obvious difference in 5-HT level observed in the frontal cortex, hippocampus and hypothalamus, whereas 5-HIAA and the 5-HIAA/5-HT ratio significantly increased ($p < 0.001$ or $p < 0.01$), which suggested derivative **7c** could increase 5-HT indirectly.

In the hippocampus, thalamus and hypothalamus, compound **7c** induced obviously decrease in DA level ($p < 0.001$, $p < 0.005$ or $p < 0.05$, respectively), and significantly increased the contents of DA metabolites of HVA in the thalamus and hypothalamus ($p < 0.001$ or $p < 0.005$) and DOPAC in the hypothalamus ($p < 0.05$). Furthermore, the ratios of the (DOPAC + HVA)/DA markedly increased in these brain regions ($p < 0.001$) as well as in the frontal cortex ($p < 0.05$). In addition, there were no alterations in monoamine neurotransmitter levels except noradrenaline in the striatum.

2.5. Acute toxicity

Based on the excellent antidepressant activity of compound **7c**, the safety evaluation of **7c** was undertaken to determine the acute toxicity. Oral administration of compound **7c** (2000 mg/kg) did not produce obvious changes in behavior, breathing, cutaneous effects (irritation, ulceration, caustic injuries and skin rashes), sensory nervous system responses, and gastrointestinal effects in the animals during the first 4 h. No deaths occurred in any of the groups during the entire period of treatment. Therefore, the **7c** seemed to be safe at a dose level of 2000 mg/kg body weight, and the LD50

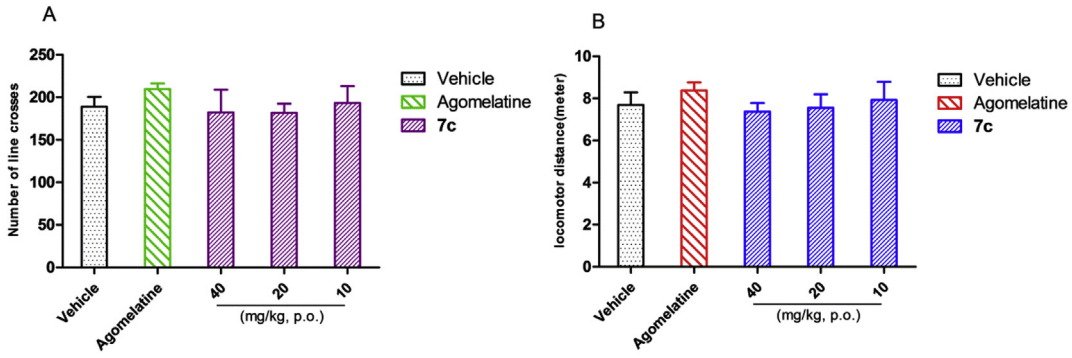


Fig. 2. Effects of **7c** on locomotor activity in mice, (A) The number of line crosses, (B) The locomotor distance. The data are reported as means ± SEM (n = 10). Agomelatine as positive control, 20 mg/kg, p. o.

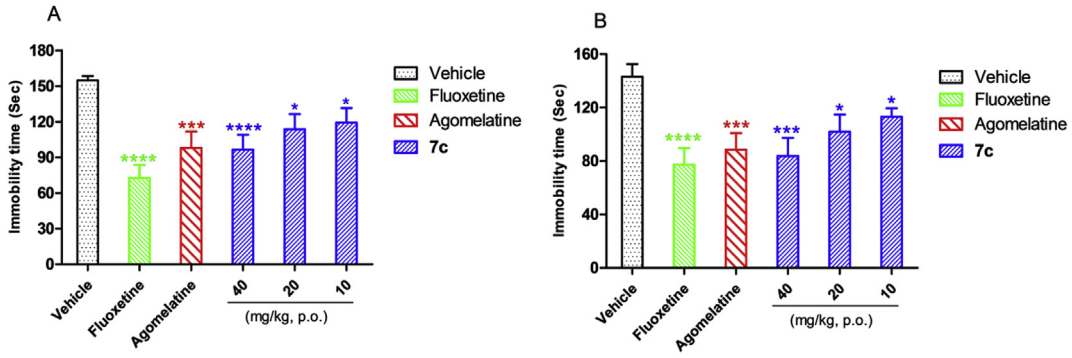


Fig. 3. Effects of different doses of **7c** (10, 20 and 40 mg/kg, O.P.) in the FST (A) and TST (B) in mice. Fluoxetine and agomelatine (20 mg/kg, o. p.) were used as a positive control group. Values are expressed as the mean ± S.E.M (n = 10). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$ and $^{****}p < 0.001$ compared with the vehicle-treated group.

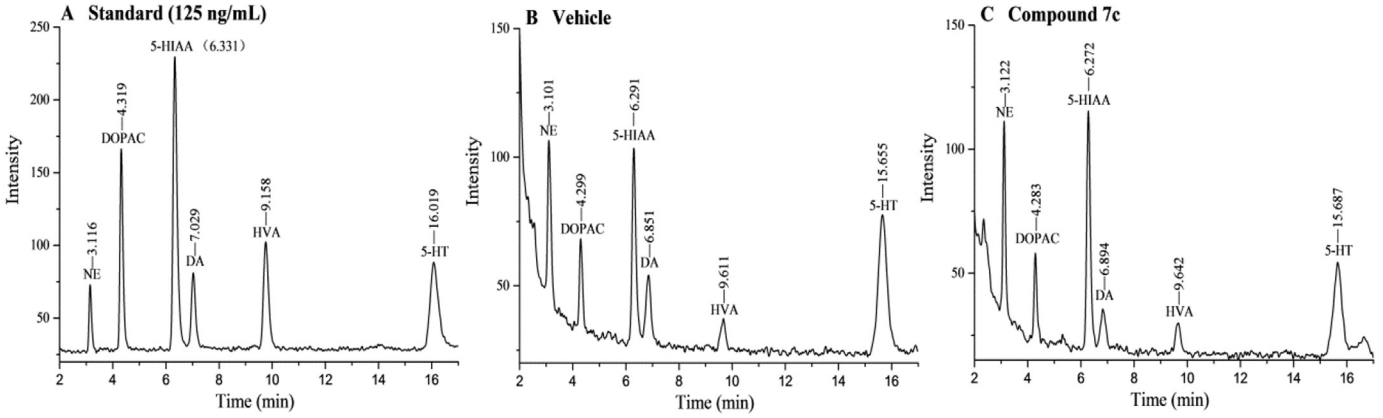


Fig. 4. A–C Chromatograms of standard catecholamine, serotonin and their metabolite agents (A), as well as the hypothalamus homogenate sample by HPLC-ECD in the mice pretreatment with vehicle (B) or compound **7c** (C) at a dose of 40 mg/kg.

considered be > 2000 mg/kg. These results also suggested that the **7c** had no influence on the behavioral and physical appearance of the animals. The absence of diarrhea indicated that the **7c** does not stimulate intestinal peristalsis.

2.6. *Identification and characterization of* ***7c*** *metabolites*

Drug metabolism plays a key role in drug discovery and development, and drug metabolism research can also offer assistance to the explanation for the compound mechanism of action. Therefore, the metabolic profile of **7c** *in vivo* was investigated using the ultra-high performance liquid chromatography coupled with ion trap and time-of-flight mass spectrometry (UPLC-IT-TOF-MS). A total of 25 metabolites of **7c** were identified, including 5 metabolites in phase I and 20 metabolites in phase II. The results showed that the phase I metabolites were a reduction, hydrolyzation and hydroxylation metabolites and the phase II metabolites mainly included glucuronidation, sulfation and methylation metabolites (Scheme 2 and Table 2).

2.6.1. *Phase I metabolites*

The metabolite **M13** was found in the chromatogram at a retention time of 9.15 min and had an $[M-H]^-$ ion at *m/z* 443.1708 (elemental composition $C_{24}H_{28}O_8$) suggesting the addition of an

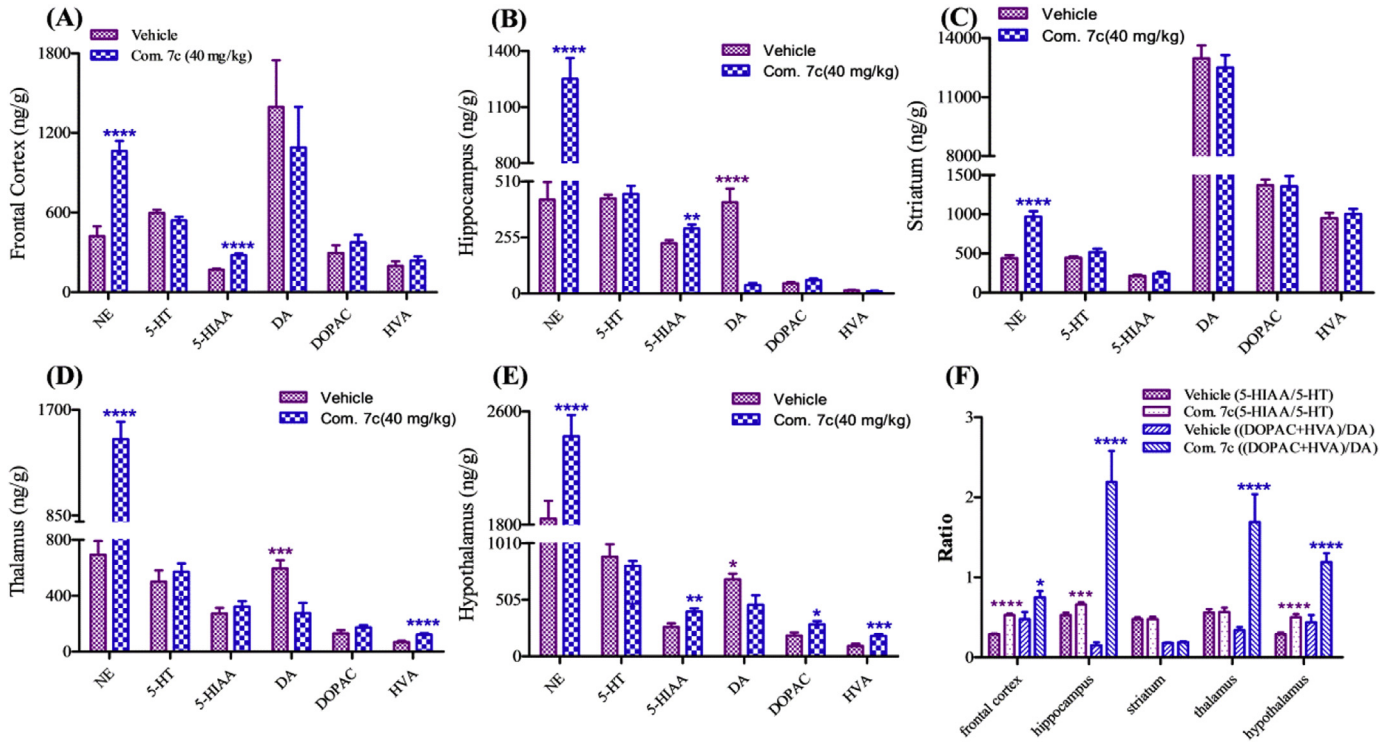


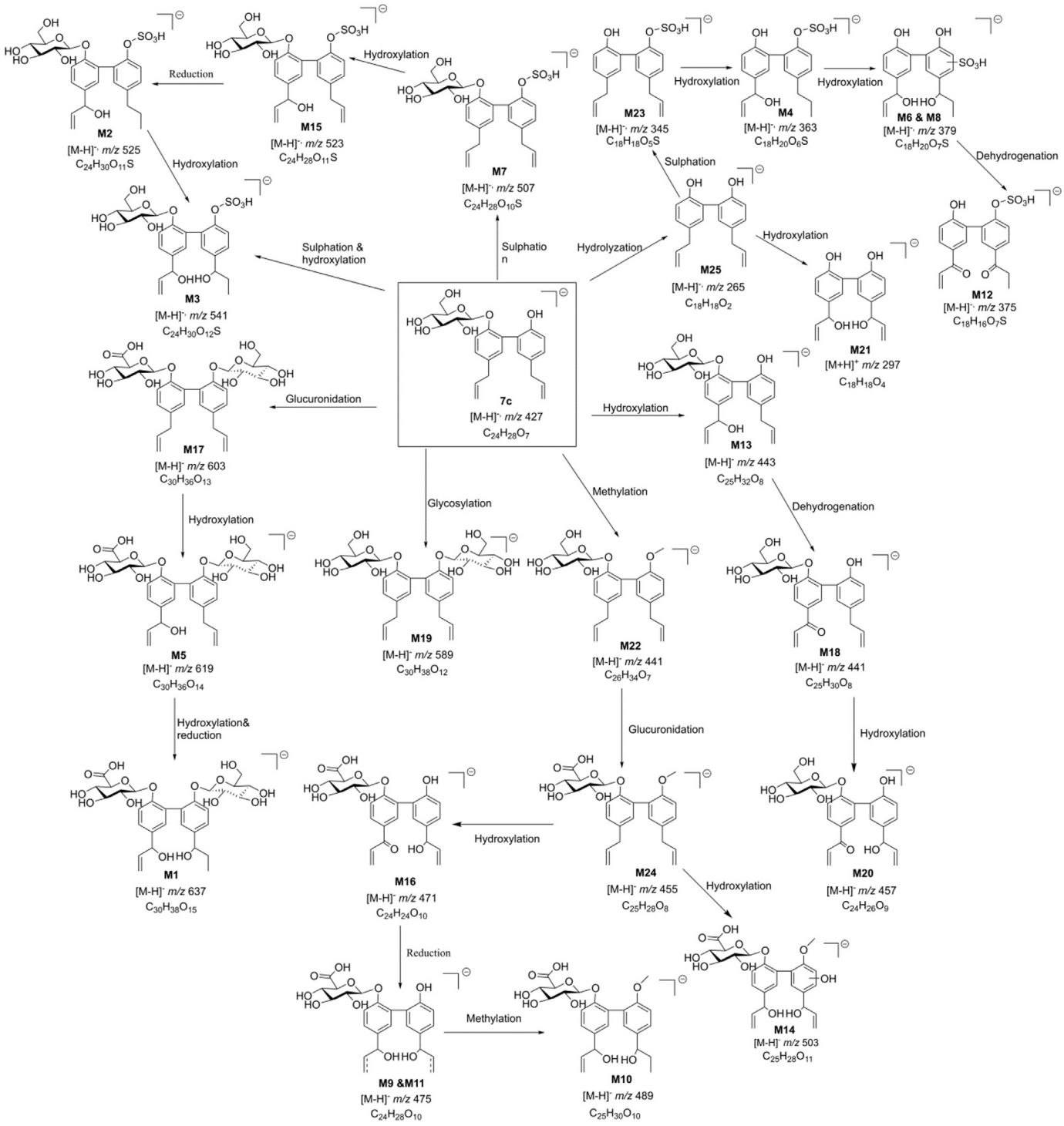
Fig. 5. Effects of compound **7c** on monoamine neurotransmitter and its metabolites levels in different regions of mice brain. (A) Frontal Cortex; (B) Hippocampus; (C) Striatum; (D) Thalamus; (E) Hypothalamus; (F) Ratios of 5-HIAA/5-HT and (DOPAC + HVA)/DA. Data were expressed as means ± SEM with units of ng/g. (n = 10) $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$, $^{****}P < 0.001$ vs. vehicle.

oxygen atom. In its MS/MS spectrum, the $[M-H]^-$ ion showed fragment ions at *m/z* 281.1141 ($C_{18}H_{18}O_3$, loss of $C_6H_{10}O_5$, a glucosyl) which was also 16 Da than that of magnolol. Therefore, **M13** was determined to be hydroxylation of **7c**. **M18** (Rt = 10.59 min) exhibited a molecular formula of $C_{24}H_{26}O_8$ ($[M-H]^-$ *m/z* 441.1893). In the MS/MS spectra, the $[M-H]^-$ ion showed a fragment ion at *m/z* 279.1129 ($C_{18}H_{16}O_3$). Therefore, M18 was determined as the dehydrogenation of **M13**. At a retention time of 10.88 min in the chromatogram, the MS spectrum showed that the molecular formula could be identified as $C_{24}H_{26}O_9$ (*m/z* 457.1555) indicating the addition of one oxygen atom than that of **M18**. In addition, it had a fragment ion at *m/z* 295.0790 ($C_{18}H_{16}O_4$, [M-glucosyl]) which showed one more hydroxyl. Thus, **M20** was presumed to be the hydroxylation of **M18**. **M25** (Rt = 14.33 min), had an $[M-H]^-$ ion at *m/z* 265.1237 ($C_{18}H_{18}O_2$) and was identified as magnolol by comparison with a standard. So **M25** was the hydrolyzation metabolite of **7c**. A chromatogram peak at a retention time of 11.06 min was detected with a molecular weight at *m/z* 297.1138 ($C_{18}H_{18}O_4$) displaying the addition of two oxygen atoms than that of magnolol. Therefore, **M21** was identified as hydrolyzation and dihydroxylated metabolites of **7c**.

2.6.2. Phase II metabolites

In the sulfate-related metabolites group, nine target peaks were detected and identified. The metabolite **M7** (Rt = 8.17 min) were detected in faeces and showed $[M-H]^-$ ion at *m/z* 507.1325 ($C_{24}H_{28}O_{10}S$). The addition of SO_3 to the **7c** clearly suggested the metabolite **M7** to be a sulfate metabolite. The formation of characteristic product ions at *m/z* 429.1968 confirmed the sulphation. The metabolite **M15** at *m/z* 523.1274 ($[M-H]^-$, $C_{24}H_{28}O_{11}S$) eluted at 9.24 min indicating the addition of one oxygen atom than that of **M7** which suggested **M15** was hydroxylation of **M7**. The MS/MS spectrum displayed product ions at *m/z* 361.0779 ($C_{18}H_{18}O_6S$, loss of $C_6H_{10}O_5$ from 523) and at *m/z* 281.1084 ($C_{18}H_{18}O_3$, loss of SO_3 from 361). The metabolite **M2** (Rt = 6.91 min) showed its $[M-H]^-$ ion peak at *m/z* 525.1443 with an elemental composition $C_{24}H_{30}O_{11}S$ suggesting that it was a reduction of **M15**. In its MS/MS spectrum, the $[M-H]^-$ ion showed fragment ions at *m/z* 445.1881 ($C_{24}H_{30}O_8$, loss of SO_3), 363.0892 ($C_{18}H_{20}O_6S$, loss of Glu) and 269.1151 ($C_{17}H_{18}O_3$, loss of SO_3, CH_3 and Glu). Therefore, **M2** was identified as the reduction of **M15**. The metabolite **M3** (Rt = 7.39 min) was found in urine and the ESI mass spectrum exhibited the $[M-H]^-$ ion at *m/z* 541.1397 ($C_{24}H_{30}O_{12}S$), which was 16 Da more than that of **M2**, indicating that the metabolite **M3** was formed by the hydroxylation of **M2**. **M23** was detected at a retention time of 11.49 min. The MS spectrum showed that the molecular formula was $C_{18}H_{18}O_5S$ (*m/z* 345.0794). In its MS/MS spectrum, the $[M-H]^-$ ion showed fragment ions at *m/z* 265.1226 ($C_{18}H_{18}O_2$, loss of SO_3). Thus, **M23** was identified as the sulphation of magnolol. **M4** (Rt = 7.55 min) had the molecular formula of $C_{18}H_{20}O_6S$, suggesting the addition of two hydrogen atoms and one oxygen atom than that of **M23**. The MS/MS spectra displayed product ions at *m/z* 327.0669 ($C_{18}H_{16}O_4S$, loss of 2 H_2O from *m/z* 363) and *m/z* 281.1198 ($C_{18}H_{18}O_3$, loss of SO_3 and H_2 from *m/z* 363). At retention times of 8.01 and 8.21 min in the chromatogram, the MS spectrum showed that the molecular formula was $C_{18}H_{20}O_7S$ (*m/z* 379.0856) suggesting the addition of one oxygen atom than that of **M4**. In its MS/MS spectrum, the $[M-H]^-$ ion showed fragment ions at *m/z* 299.1267 ($C_{18}H_{20}O_4$, loss of SO_3). Therefore, **M6** and **M8** were determined as isomers of hydroxylation of **M4**. The metabolite **M12** at *m/z* 375.0557 ($[M-H]^-$, $C_{18}H_{16}O_7S$) eluted at 9.05 min indicating the reduction of four hydrogen atoms than that of **M6** which suggested **M12** was dehydrogenation of **M6**. Its MS/MS spectrum showed the product ion at *m/z* 295.1002 ($C_{18}H_{16}O_4$, loss of SO_3 from *m/z* 375) which suggested that the metabolite was the dehydrogenation of **M6**.

In the glucuronide-related metabolites group, nine target peaks



Scheme 2. Proposed biotransformation pathway of **7c** in rat based on its metabolites observed in urine, plasma and faeces.

were detected and identified. **M17** (Rt = 9.94 min) were detected and showed $[M-H]^-$ ion at *m/z* 603.2073 ($C_{30}H_{36}O_{13}$). The addition of $C_6H_8O_6$ to the **7c** clearly suggested the metabolite **M17** to be a glucuronide metabolite. In its MS/MS spectrum, the $[M-H]^-$ ion showed fragment ions at *m/z* 427.1779 ($C_{24}H_{28}O_7$, loss of $C_6H_8O_6$ from 603) and 265.1189 ($C_{18}H_{18}O_2$, loss of $C_6H_{10}O_5$ from 427). **M5** was eluted at 7.79 min and showed the molecular formula of $C_{30}H_{36}O_{14}$ ($[M-H]^-$ *m/z* 619.2048), which was 16 Da (the addition of oxygen) higher than the **M17**. Therefore, **M5** was tentatively determined as the hydroxylation conjugate of **M17**. **M1** (Rt = 6.75 min) exhibited a molecular formula of $C_{30}H_{38}O_{15}$ ($[M-H]^-$ *m/z* 637.2130). In the MS/MS spectra, the $[M-H]^-$ ion showed fragment ion at *m/z* 475.1584 [M-glc] $^-$ and 299.1286 [M-glc-gluA]$^-$.Therefore, **M1** was determined as the hydroxylation and reduction of **M5**. **M24** (Rt = 12.40 min) exhibited a molecular formula of $C_{25}H_{28}O_8$ ($[M-H]^-$ *m/z* 455.1709). In the MS/MS spectra, the $[M-H]^-$ ion showed fragment ion at *m/z* 265.1219 [M-glcUA-CH_3] $^-$. Therefore, **M24** was determined as the glucuronide and

Table 2
UPLC-IT-TOF-MS retention times and fragment ions of the metabolites of **7c** in rats.

NO.	Retention time (min)	**[M-H]⁻** Observed	**[M-H]⁻** Calculated	Error in mDa	Formula	Wavelength λ_{max}: **nm**	MS/MS fragments ions	Plasma	Urine	Feces
M1	6.75	637.2130	637.2138	−0.8	$C_{30}H_{38}O_{15}$	218, 280	637.2130 ($C_{30}H_{38}O_{15}$), 475.1584 ($C_{24}H_{28}O_{10}$), 299.1286($C_{18}H_{20}O_4$)	−	+	+
M2	6.91	525.1443	525.1436	0.7	$C_{24}H_{30}O_{11}S$	210, 273	525.1443 ($C_{24}H_{30}O_{11}S$), 445.1881 ($C_{24}H_{30}O_8$), 363.0892 ($C_{18}H_{20}O_6S$), 269.1151 ($C_{17}H_{18}O_3$)	−	−	+
M3	7.39	541.1397	541.1385	1.2	$C_{24}H_{30}O_{12}S$	240, 292	541.1397 ($C_{24}H_{30}O_{12}S$), 461.1768 ($C_{24}H_{30}O_9$)	−	+	−
M4	7.55	363.0888	363.0908	−2.0	$C_{18}H_{20}O_6S$	216, 288	363.0888 ($C_{18}H_{20}O_6S$), 327.0669 ($C_{18}H_{16}O_4S$), 281.1198 ($C_{18}H_{18}O_3$)	−	−	+
M5	7.79	619.2048	619.2032	1.6	$C_{30}H_{36}O_{14}$	219, 282	619.2048 ($C_{30}H_{36}O_{14}$), 281.1190 ($C_{18}H_{18}O_3$)	−	+	+
M6	8.01	379.0856	379.0857	−0.1	$C_{18}H_{20}O_7S$	219, 273	379.0856 ($C_{18}H_{20}O_7S$), 299.1267 ($C_{18}H_{20}O_4$)	−	+	−
M7	8.17	507.1325	507.1330	−0.5	$C_{24}H_{28}O_{10}S$	217, 276	507.1325 ($C_{24}H_{28}O_{10}S$), 429.1968 ($C_{24}H_{30}O_7$)	−	−	+
M8	8.21	379.0856	379.0857	−0.1	$C_{18}H_{20}O_7S$	280, 219	379.0856 ($C_{18}H_{20}O_7S$), 299.1287 ($C_{18}H_{20}O_4$)	−	+	−
M9	8.37	475.1617	475.1610	0.7	$C_{24}H_{28}O_{10}$	220, 283	475.1617 ($C_{24}H_{28}O_{10}$), 299.1284 ($C_{18}H_{20}O_4$)	−	+	−
M10	8.70	489.1764	489.1766	−0.2	$C_{25}H_{30}O_{10}$	213, 288	489.1764 ($C_{25}H_{30}O_{10}$), 475.1602 ($C_{24}H_{28}O_{10}$), 299.1300 ($C_{18}H_{20}O_4$)	−	+	−
M11	8.74	475.1614	475.1610	0.4	$C_{24}H_{28}O_{10}$	220, 282	475.1614 ($C_{24}H_{28}O_{10}$), 299.1284 ($C_{18}H_{20}O_4$)	−	+	−
M12	9.05	375.0557	375.0544	1.3	$C_{18}H_{16}O_7S$	220, 283	375.0557 ($C_{18}H_{16}O_7S$), 295.1002 ($C_{18}H_{16}O_4$)	−	+	−
M13	9.15	443.1708	443.1711	−0.3	$C_{24}H_{28}O_8$	219, 283	443.1711 ($C_{24}H_{28}O_8$), 281.1141 ($C_{18}H_{18}O_3$)	−	+	−
M14	9.22	503.1563	503.1559	0.4	$C_{25}H_{28}O_{11}$	217, 283	503.1563 ($C_{25}H_{28}O_{11}$), 473.1458 ($C_{24}H_{26}O_{10}$) 297.1111 ($C_{18}H_{18}O_4$)	−	+	
M15	9.24	523.1274	523.1280	−0.6	$C_{24}H_{28}O_{11}S$	212, 273	523.1274 ($C_{24}H_{28}O_{11}S$), 361.0779 ($C_{18}H_{18}O_6$ S), 281.1084 ($C_{18}H_{18}O_3$)	−	−	+
M16	9.47	471.1294	471.1297	−0.3	$C_{24}H_{24}O_{10}$	217, 292	471.1294 ($C_{24}H_{24}O_{10}$), 295.0976 ($C_{18}H_{16}O_4$)	−	+	−
M17	9.94	603.2073	603.2083	−1.0	$C_{30}H_{36}O_{13}$	213, 260	603.2073 ($C_{30}H_{36}O_{13}$), 427.1779 ($C_{24}H_{28}O_7$) 265.1189 ($C_{18}H_{18}O_2$)	−	+	+
M18	10.59	441.1893	441.1919	−2.6	$C_{24}H_{26}O_8$	220, 287	441.1893 ($C_{24}H_{26}O_8$), 279.1129 ($C_{18}H_{16}O_3$)	−	+	−
M19	10.63	589.2269	589.2291	−2.2	$C_{30}H_{38}O_{12}$	217, 285	589.2291 ($C_{30}H_{38}O_{12}$), 427.1769 ($C_{24}H_{28}O_7$) 265.1219 ($C_{18}H_{18}O_2$)	−	+	+
M20	10.88	457.1555	457.1504	5.1	$C_{24}H_{26}O_9$	220, 291	457.1555 ($C_{24}H_{26}O_9$), 295.0970 ($C_{18}H_{16}O_4$)	−	+	−
M21	11.06	297.1138	297.1132	0.6	$C_{18}H_{18}O_4$	216, 288	297.1138 ($C_{18}H_{18}O_4$), 281.1148 ($C_{18}H_{18}O_3$)	−	+	+
M0 (7c)	11.23	427.1764	427.1762	0.2	$C_{24}H_{28}O_7$	206, 284	427.1764 ($C_{24}H_{28}O_7$), 265.1246 ($C_{18}H_{18}O_2$) 247.1105 ($C_{18}H_{16}O$), 199.0927 ($C_{13}H_{12}O_2$)	+	−	−
M22	11.32	441.1559	441.1555	0.4	$C_{25}H_{30}O_7$	216, 282	441.1559 ($C_{25}H_{30}O_7$), 427.1774 ($C_{24}H_{28}O_7$) 265.1230 ($C_{18}H_{18}O_2$)	+	+	+
M23	11.49	345.0794	345.0802	−0.8	$C_{18}H_{18}O_5S$	216, 279	345.0794 ($C_{18}H_{18}O_5S$), 265.1226 ($C_{18}H_{18}O_2$)	−	−	+
M24	12.40	455.1709	455.1711	−0.2	$C_{25}H_{28}O_8$	220, 283	455.1709 ($C_{25}H_{28}O_8$), 265.1219 ($C_{18}H_{18}O_2$)	−	+	−
M25	14.33	265.1237	265.1234	0.3	$C_{18}H_{18}O_2$	219, 290	265.1237 ($C_{18}H_{18}O_2$), 247.1045 ($C_{18}H_{16}O$)	−	+	+

methyl conjugate of magnolol. One target peak was found at retention times of 9.22 min. The MS spectrum showed the $[M-H]^-$ ion at *m/z* 503.1563 ($C_{25}H_{28}O_{11}$), which yielded the characteristic fragment ions at *m/z* 473.1458 ($[M-H]^-$, loss of OCH_3) and 297.1111 ($[M-H]^-$, loss of gluA). Therefore, **M14** was determined as trihydroxylation of **M24**. **M16** (Rt = 9.47 min) exhibited the molecular formula of $C_{25}H_{28}O_{11}$ ($[M-H]^-$, *m/z* 471.1294). In its MS/MS spectra, the $[M-H]^-$ ions showed product ion at *m/z* 295.0976 [M-gluA]$^-$. Therefore, **M16** was determined as glucuronide, hydroxylation and carbonyl conjugates of magnolol. At a retention time of 8.37 and 8.74 min, **M9** and **M11** were found in the chromatogram. The MS spectrum showed that the molecular formula was $C_{24}H_{28}O_{10}$ (*m/z* 475.1614). In their MS/MS spectra, the $[M-H]^-$ ion showed fragment ions at *m/z* 299.1284 ($C_{18}H_{20}O_4$, loss of gluA). Therefore, **M9** and **M11** were determined as isomers of reduction of **M16**. **M10** (Rt = 8.70 min) had the molecular formula of $C_{25}H_{30}O_{10}$ ($[M-H]^-$ *m/z* 489.1764), 14 Da (the addition of CH_2) larger than that of **M9**. The MS/MS spectra of $[M-H]^-$ ions of **M10** showed product ions at *m/z* 475.1602 (loss of CH_2 from *m/z* 489) and *m/z* 299.1300 (loss of gluA from *m/z* 475).

Two other phase II metabolites were also detected and identified. **M22** (Rt = 11.32 min) had the molecular formula of $C_{25}H_{30}O_7$ (*m/z* 441.1559), 14 Da (the addition of CH_2) higher than the **7c**, which had the product ion at *m/z* 427.1769 [M − CH_3], similar to **7c** (**M0**). Therefore, **M24** was determined as the methyl of **7c**. **M19** (Rt = 10.63 min) had the molecular formula of $C_{30}H_{38}O_{12}$ (*m/z* 589.2269), 162 Da (the addition of $C_6H_{10}O_5$) higher than the **7c**, which had the product ion at *m/z* 427.1769 [M-$C_6H_{10}O_5$], similar to **7c** (**M0**). Therefore, **M24** was determined as the glucosyl of **7c**.

3. Conclusions

In this work, we have designed and synthesized a series of new magnolol derivatives. The SAR was discussed and the preliminary results revealed the essential structural elements for the activity, namely, the glucosyl moiety attached to one hydroxyl group was beneficial for improving the activity, while the other free hydroxyl was essential to induce agitating effects. Among these compounds, derivative **1a** showed improved activity agitating on $MT_{1/2}$ receptors compared with magnolol. Compounds **1d** and **7b** can only improve agitating activity on the MT_2 receptor (nearly 3.5–4.5 folds higher than magnolol). Compound **7c** emerged as the most potent agonistic activity on both MT_1 and MT_2 receptors, with EC_{50} values of 396.5 and 383.0 μM, respectively. Specifically, compound **7c** was found to be orally effective to a significant reduction of immobility time in a mouse model of depression (FST and TST) without affecting the mouse locomotor activity. It was also found that compound **7c** significantly increased the concentrations of the main neurotransmitters NE, 5-HIAA along with the metabolites of DOPAC and HVA and decreases of DA in the mice brain. Furthermore, the safety evaluation of **7c** was undertaken and the LD50 was considered be > 2000 mg/kg. The pharmaco-metabonomics of **7c** on rats was studied, and 25 metabolites of **7c** were identified, including 5 metabolites in phase I and 20 metabolites in phase II. These results indicate that derivative **7c** is a promising lead compound for the development of a new chemical class of antidepressant drugs.

4. Experimental section

4.1. Chemistry

All commercial chemicals were used as obtained and all solvents were purified by distillation prior to use applying the standard procedures. Melting points (m.p.) were measured by an SGW® X-4B melting point apparatus. Reactions were monitored by TLC with silica gel plates under UV light (λ = 254 nm) and colorization with 10% H_2SO_4 in ethanol. ^1H NMR and ^{13}C NMR spectra were measured against the peak of tetramethylsilane using a Bruker AVANCE III-400 or AVANCE III-600 spectrometers. High-resolution mass spectra (HRMS) were determined in ESI ionization on a Shimadzu UFLC/MS-IT-TOF.

4.2. General synthetic procedures for **1a-1g**, **2a-2e**

To a solution of the magnolol (0.3 mM) in anhydrous CH_2Cl_2 (5 mL) were added appropriate carboxylic acid (1.1 or 2.3 equiv.) and 4-dimethylaminopyridine (DMAP, 0.6 equiv.). Subsequently, the *N*, *N*-dicyclohexylcarbodiimide (DCC, 1.2 equiv.) dissolved in dry CH_2Cl_2 (5 mL) was slowly dropped into the above solution and stirred for 4–6 h at room temperature monitored by TLC. The reaction mixture was diluted with 20 mL $CHCl_3$ and further washed by 5% HCl (3 × 50 mL), saturated $NaHCO_3$ (3 × 50 mL) and saturated NaCl (3 × 50 mL) respectively. The organic layer was dried with anhydrous Na_2SO_4 and concentrated to dryness *in vacuo* [17]. Purification by column chromatography yielded target compounds (**1a**-**1g**, **2a-2e**).

4.2.1. 4′-O-acetyl-magnolol (**1a**)

White solid, m. p. 85.1–86.3 °C, yield 68.79%, ^1H NMR (400 MHz, $CDCl_3$) δ_H: 7.26 (d, 1H, J = 1.6 Hz, H-2′), 7.20 (dd, 1H, J = 8.4, 1.6 Hz, H-6′), 7.16 (d, 1H, J = 1.8 Hz, H-2), 7.05 (d, 1H, J = 8.4 Hz, H-5′), 7.03 (dd, 1H, J = 8.4, 1.8 Hz, H-6), 6.97 (d, 1H, J = 8.4 Hz, H-5), 5.94 (m, 1H, H-8′), 5.90 (m, 1H, H-8), 5.09 (dd, 1H, J = 17.2, 1.4 Hz, H-9′a), 5.05 (dd, 1H, J = 16.8, 1.6 Hz, H-9a), 5.03 (dd, 1H, J = 9.2, 1.4 Hz, H-9′b), 5.00 (dd, 1H, J = 9.6, 1.6 Hz, H-9b), 3.38 (d, 2H, J = 6.8 Hz, H-7′), 3.39 (d, 2H, J = 6.6 Hz, H-7), 2.00 (s, 3H, Me). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 137.7 (s, C-1), 131.6 (d, C-2), 130.2 (s, C-3), 151.4 (s, C-4), 122.7 (d, C-5), 129.3 (d, C-6), 39.2 (t, C-7), 136.5 (d, C-8), 116.0 (t, C-9), 138.4 (s, C-1′), 131.7 (d, C-2′), 130.6 (s, C-3′), 147.3 (s, C-4′), 122.8 (d, C-5′), 129.5 (d, C-6′), 39.5 (t, C-7′), 136.7 (d, C-8′), 116.3 (t, C-9′), 169.7 (s, COO), 20.6 (q, Me). HRESIMS *m/z*: $[M + H]^+$ 309.1480 ($C_{20}H_{20}O_3$), cal. 309.1485.

4.2.2. 4′-O-acryl-magnolol (**1b**)

White oil, yield 76.34%, ^1H NMR (400 MHz, $CDCl_3$) δ_H: 7.29 (d, 1H, J = 1.7 Hz, H-2′), 7.26 (d, 1H, J = 1.8 Hz, H-2), 7.22 (dd, 1H, J = 8.0, 1.7 Hz, H-6′), 7.13 (dd, 1H, J = 6.8, 1.8 Hz, H-6), 7.05 (d, 1H, J = 8.0 Hz, H-5′), 6.98 (d, 1H, J = 6.8 Hz, H-5), 6.37 (dd, 1H, J = 17.2, 2.1 Hz, H-3″b), 6.09 (dd, 1H, J = 17.2, 10.8 Hz, H-2″), 6.01 (m, 1H, H-8′), 5.97 (m, 1H, H-8), 5.92 (dd, 1H, J = 10.8, 2.1 Hz, H-3″a), 5.12 (dd, 1H, J = 17.2, 1.7 Hz, H-9′b), 5.10 (dd, 1H, J = 17.6, 1.6 Hz, H-9b), 5.07 (dd, 1H, J = 10.0, 1.7 Hz, H-9′a), 5.04 (dd, 1H, J = 11.2, 1.6 Hz, H-9a), 3.41 (d, 2H, J = 6.8 Hz, H-7′), 3.39 (d, 2H, J = 6.4 Hz, H-7). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 137.7 (s, C-1), 130.0 (d, C-2), 131.1 (s, C-3), 154.4 (s, C-4), 122.3 (d, C-5), 129.0 (d, C-6), 39.2 (t, C-7), 136.8 (d, C-8), 116.1 (t, C-9), 137.9 (s, C-1′), 130.5 (d, C-2′), 131.2 (s, C-3′), 146.0 (s, C-4′), 122.8 (d, C-5′), 129.6 (d, C-6′), 39.4 (t, C-7′), 136.9 (d, C-8′), 116.2 (t, C-9′), 168.9 (s, C-1″), 127.6 (d, C-2″), 132.3 (t, C-3″). HRESIMS *m/z*: $[M + H]^+$ 321.1490 ($C_{21}H_{20}O_3$), cal. 321.1485.

4.2.3. 4′-O-(3-chloropropionyl)-magnolol (**1c**)

Yellow oil, yield 71.2%, ^1H NMR (600 MHz, $CDCl_3$) δ_H: 7.27 (dd, 1H, J = 2.0, 8.2 Hz, H-6′), 7.20 (d, 1H, J = 2.0 Hz, H-2′), 7.12 (d, 1H, J = 8.2 Hz, H-5′), 7.08 (dd, 1H, J = 8.4, 1.9 Hz, H-6), 6.92 (d, 1H, J = 1.9 Hz, H-2), 6.90 (d, 1H, J = 8.4 Hz, H-5), 5.97 (m, 1H, H-8′), 5.934 (m, 1H, H-8), 5.14 (dd, 1H, J = 16.8, 1.5 Hz, H-9′a), 5.10 (dd, 1H, J = 16.0, 1.2 Hz, H-9a), 5.09 (dd, 1H, J = 9.6, 1.5 Hz, H-9′b), 5.05 (dd, 1H, J = 9.2, 1.2 Hz, H-9b), 3.58 (t, 2H, J = 6.9 Hz, H-3″), 3.43 (d, 2H, J = 6.6 Hz, H-7′), 3.33 (d, 2H, J = 6.6 Hz, H-7), 2.76 (t, 2H, J = 6.9 Hz, H-2″). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 132.0 (s, C-1), 130.5 (d, C-2), 123.6 (s, C-3), 151.2 (s, C-4), 116.2 (d, C-5), 129.8 (d, C-6), 39.3 (t, C-7), 136.6 (d, C-8), 115.6 (t, C-9), 139.0 (s, C-1′), 131.8 (d, C-2′), 129.8 (s, C-3′), 146.6 (s, C-4′), 122.8 (d, C-5′), 129.7 (d, C-6′), 39.5 (t, C-7′), 137.7 (d, C-8′), 116.6 (t, C-9′), 169.1 (s, C-1″), 37.3 (t, C-2″), 38.4 (t, C-3″). HRESIMS *m/z*: $[M + H]^+$ 357.1252 ($C_{21}H_{21}O_3Cl$), cal. 357.1252.

4.2.4. 4′-O-(4-chlorobutyl)-magnolol (**1d**)

Yellow oil, yield 81.2%, ^1H NMR (600 MHz, $CDCl_3$) δ_H: 7.27 (dd, 1H, J = 1.8, 8.4 Hz, H-6′), 7.20 (d, 1H, J = 1.8 Hz, H-2′), 7.10 (d, 1H, J = 8.4 Hz, H-5′), 7.09 (dd, 1H, J = 8.4, 2.0 Hz, H-6), 6.93 (d, 1H, J = 2.0 Hz, H-2), 6.91 (d, 1H, J = 8.4 Hz, H-5), 5.99 (m, 1H, H-8′), 5.94 (m, 1H, H-8), 5.13 (dd, 1H, J = 17.0, 1.5 Hz, H-9′a), 5.11 (dd, 1H, J = 16.2, 1.3 Hz, H-9a), 5.10 (dd, 1H, J = 10.2, 1.5 Hz, H-9′b), 5.07 (dd, 1H, J = 9.5, 1.3 Hz, H-9b), 3.43 (t, 2H, J = 6.8 Hz, H-4″), 3.34 (d, 2H, J = 6.2 Hz, H-7′), 3.33 (d, 2H, J = 6.0 Hz, H-7), 2.49 (t, 2H, J = 7.2 Hz, H-2″), 1.89 (m, 2H, H-3″). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 137.0 (s, C-1), 130.5 (d, C-2), 123.8 (s, C-3), 151.2 (s, C-4), 116.2 (d, C-5), 129.7 (d, C-6), 39.3 (t, C-7), 136.6 (d, C-8), 115.6 (t, C-9), 138.8 (s, C-1′), 131.1 (d, C-2′), 129.9 (s, C-3′), 146.8 (s, C-4′), 122.7 (d, C-5′), 129.8 (d, C-6′), 39.5 (t, C-7′), 137.6 (d, C-8′), 116.5 (t, C-9′), 171.5 (s, C-1″), 30.9 (t, C-2″), 27.4 (t, C-3″), 43.6 (t, C-4″). HRESIMS *m/z*: $[M + H]^+$ 371.1406 ($C_{22}H_{23}O_3Cl$), cal. 371.1408.

4.2.5. 4′-O-benzoyl-magnolol (**1e**)

White solid, m. p. 69.6–70.1 °C, yield 86.74%, ^1H NMR (400 MHz, $CDCl_3$) δ_H: 8.14 (dd, 2H, J = 7.6, 2.4 Hz, H-3″, 7″), 7.98 (d, 1H, J = 1.8 Hz, H-2′), 7.61 (d, 1H, J = 1.4 Hz, H-2), 7.56–7.49 (m, 3H, H-4″, 5″, 6″), 7.41 (dd, 1H, J = 8.2, 1.8 Hz, H-6′), 7.29 (d, 1H, J = 8.2 Hz, H-5′), 7.00 (dd, 1H, J = 8.0, 1.4 Hz, H-6), 6.86 (d, 1H, J = 8.0 Hz, H-5), 6.05–5.95 (m, 1H, H-8′), 5.90–5.80 (m, 1H, H-8), 5.14 (dd, 1H, J = 15.6, 1.8 Hz, H-9′b), 5.13 (dd, 1H, J = 16.4, 1.6 Hz, H-9b), 4.97 (dd, 1H, J = 10.0, 1.8 Hz, H-9′a), 4.96 (dd, 1H, J = 10.1, 1.6 Hz, H-9a), 3.46 (d, 2H, J = 6.6 Hz, H-7′), 3.27 (d, 2H, J = 6.4 Hz, H-7). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 133.4 (s, C-1), 131.8 (d, C-2), 129.5 (s, C-3), 151.2 (s, C-4), 116.1 (d, C-5), 129.3 (d, C-6), 39.1 (t, C-7), 136.7 (d, C-8), 115.3 (t, C-9), 138.5 (s, C-1′), 128.3 (d, C-2′), 131.6 (s, C-3′), 147.0 (s, C-4′), 116.4 (d, C-5′), 130.6 (d, C-6′), 39.5 (t, C-7′), 137.6 (d, C-8′), 115.4 (t, C-9′), 165.5 (s, C-1″), 130.0 (s, C-2″), 130.1 (d, C-3″, 7″), 128.4 (d, C-4″, 6″), 133.7 (d, C-5″). HRESIMS *m/z*: $[M - H]^-$ 369.1492($C_{25}H_{22}O_3$), cal. 369.1496.

4.2.6. 4′-O-(4-aminobenzoyl)-magnolol (**1f**)

White solid, m. p. 71.3–72.8 °C, yield 86.4%, ^1H NMR (500 MHz, $(CD_3)_2CO$) δ_H: 7.68 (d, 2H, J = 7.0 Hz, H-3″, 7″), 7.66 (d, 2H, J = 7.0 Hz, H-4″, 6″), 7.21 (dd, 1H, J = 7.8, 1.6 Hz, H-6′), 6.98 (d, 1H, J = 1.6 Hz, H-2′), 6.93 (d, 1H, J = 7.8 Hz, H-5′), 6.80 (dd, 1H, J = 8.0, 2.0 Hz, H-6), 6.63 (d, 1H, J = 2.0 Hz, H-2), 6.61 (d, 1H, J = 8.0 Hz, H-5), 6.01 (m, 1H, H-8′), 5.82 (m, 1H, H-8), 5.15 (dd, 1H, J = 17.6, 2.0 Hz, H-9′a), 5.12 (dd, 1H, J = 16.0, 2.0 Hz, H-9a), 5.05 (dd, 1H, J = 8.6, 2.0 Hz, H-9′b), 5.03 (dd, 1H, J = 8.4, 2.0 Hz, H-9b), 3.43 (t, 2H, J = 6.5 Hz, H-7′), 3.22 (d, 2H, J = 6.5 Hz, H-7). ^{13}C NMR (125 MHz, $(CD_3)_2CO$) δ: 137.6 (s, C-1), 132.0 (d, C-2), 125.4 (s, C-3), 154.3 (s, C-4), 117.8 (d, C-5), 128.8 (d, C-6), 39.8 (t, C-7), 138.5 (d, C-8), 116.0 (t, C-9), 138.5 (s, C-1′), 132.3 (d, C-2′), 131.3 (s, C-3′), 153.6 (s, C-4′), 123.9 (d, C-5′), 129.6 (d, C-6′), 40.2 (t, C-7′), 139.0 (d, C-8′), 116.5 (t, C-9′), 165.4 (s, C-1″), 148.3 (s, C-2″), 132.7 (d, C-3″, 7″), 132.5 (d, C-4″, 6″), 137.6 (s, C-5″). HRESIMS *m/z*: $[M + H]^+$ 386.1732 ($C_{25}H_{23}NO_3$), cal. 386.1751.

*4.2.7. 4′-O-cinnamoyl-magnolol (**1g**)*

White solid, m. p. 90.6–91.8 °C, yield 80.51%, ^{1}H NMR (500 MHz, $CDCl_3$) δ_H:7.68 (d, 1H, J = 16.0 Hz, H-3″), 7.29 (dd, 1H, J = 8.3, 1.7 Hz, H-6′), 7.25 (d, 1H, J = 1.7 Hz, H-2′), 7.66–7.38 (m, 5H, H-5″, 6″, 7″, 8″, 9″), 7.20 (dd, 1H, J = 8.3, 1.2 Hz, H-6), 7.04 (d, 1H, J = 8.3 Hz, H-5′), 7.00 (d, 1H, J = 1.2 Hz, H-2), 6.90 (d, 1H, J = 8.3 Hz, H-5), 6.42 (d, 1H, J = 16.0 Hz, H-2″), 5.98 (m, 2H, H-8, 8′), 5.13 (dd, 2H, J = 17.3, 2.0 Hz, H-9b, 9′b),5.00 (dd, 2H, J = 11.0, 2.0 Hz, H-9a, 9′a), 3.41 (d, 2H, J = 6.6 Hz, H-7′), 3.31 (d, 2H, J = 6.5 Hz, H-7), ^{13}C NMR (125 MHz, $CDCl_3$) δ: 131.7 (s, C-1), 131.8 (d, C-2), 123.9 (s, C-3), 151.3 (s, C-4), 116.4 (d, C-5), 130.6 (d, C-6), 39.2 (t, C-7), 136.7 (d, C-8), 115.4 (t, C-9), 138.5 (s, C-1′), 130.6 (d, C-2′), 130.1 (s, C-3′), 146.8 (s, C-4′), 122.8 (d, C-5′), 129.6 (d, C-6′), 39.5 (t, C-7′), 137.7 (d, C-8′), 116.2 (t, C-9′), 165.6 (s, C-1″), 116.5 (d, C-2″), 146.7 (d, C-3″), 133.9 (s, C-4″), 128.8 (d, C-5″, 9″), 129.4 (d, C-6″, 8″), 128.2 (d, C-7″). HRESIMS *m/z*: [M-H]−395.1651 ($C_{27}H_{24}O_3$), cal. 395.1653.

*4.2.8. 4, 4′-di-O-acetyl-magnolol (**2a**)*

White solid, m. p. 101.6–103.0 °C, yield 60.01%, H NMR (400 MHz, $CDCl_3$) δ_H:7.21 (dd, 2H, J = 8.2, 1.8 Hz, H-6, 6′), 7.12 (d, 2H, J = 1.8 Hz, H-2, 2′), 7.08 (d, 2H, J = 8.2 Hz, H-5, 5′), 5.98 (m, 2H, H-8, 8′), 5.11 (dd, 2H, J = 17.2, 1.8 Hz, H-9b, 9′b),5.09 (dd, 2H, J = 9.6, 1.8 Hz, H-9a, 9′a), 3.42 (d, 4H, J = 6.4 Hz, H-7, 7′),2.05 (s, 6H, Me). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 137.6 (s, C-1, 1′), 130. 2 (d, C-2, 2′), 131.2 (s, C-3, 3′), 146.2 (s, C-4, 4′), 122.3 (d, C-5, 5′), 128.9 (d, C-6, 6′), 39.4 (t, C-7, 7′), 136.9 (d, C-8, 8′), 116.2 (t, C-9, 9′), 169.4 (s, COO), 20.7 (q, Me). HRESIMS *m/z*: [M + H] $^+$ 351.1589 ($C_{22}H_{22}O_4$), cal. 351.1591.

*4.2.9. 4, 4′-di-O-acryl-magnolol (**2b**)*

Yellow oil, yield 68.14%, ^{1}H NMR (400 MHz, $CDCl_3$) δ_H:7.26 (d, 2H, J = 1.8 Hz, H-2, 2′), 7.20 (dd, 2H, J = 8.4, 1.8 Hz, H-6, 6′), 7.12 (d, 2H, J = 8.4 Hz, H-5, 5′), 6.37 (dd, 2H, J = 17.2, 10.4 Hz, H-2″, 2‴), 6.10 (dd, 2H, J = 17.2, 1.8 Hz, H-3″b, 3‴b), 5.94 (m, 2H, H-8, 8′), 5.84 (dd, 2H, J = 10.4, 1.8 Hz, H-3″a, 3‴a), 5.09 (dd, 2H, J = 16.8, 1.6 Hz, H-9b, 9′b),5.06 (dd, 2H, J = 10.6, 1.6 Hz, H-9a, 9′a), 3.39 (d, 4H, J = 6.6 Hz, H-7, 7′). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 137.5 (s, C-1, 1′), 130. 0 (d, C-2, 2′), 131.1 (s, C-3, 3′), 146.1(s, C-4, 4′), 122.2 (d, C-5, 5′), 128.9 (d, C-6, 6′), 39.4 (t, C-7, 7′), 136.8 (d, C-8, 8′), 116.1 (t, C-9, 9′), 164.3 (s, C-1″, 1‴), 127.6 (d, C-2″, 2‴), 132.1 (t, C-3″, 3‴). HRESIMS *m/z*: [M + H] $^+$ 375.1589 ($C_{24}H_{22}O_4$), cal. 375.1591.

*4.2.10. 4, 4′-di-(4-chlorobutyl)-magnolol (**2c**)*

Yellow oil, yield 80.5%, ^{1}H NMR (600 MHz, $CDCl_3$) δ_H: 7.21 (dd, 2H, J = 1.8, 8.4 Hz, H-6, 6′), 7.11 (d, 2H, J = 1.8 Hz, H-2, 2′), 7.06 (d, 2H, J = 8.4 Hz, H-5, 5′), 5.96 (m, 2H, H-8, 8′), 5.09 (m, 4H, H-9, 9′), 3.41 (t, 4H, J = 6.6 Hz, H-4″, 4‴), 3.38 (d, 4H, J = 6.4 Hz, H-7, 7′), 2.47 (t, 4H, J = 7.2 Hz, H-2″, 2‴), 1.92 (m, 4H, H-3″, 3‴). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 137.8 (s, C-1, 1′), 131.1 (d, C-2, 2′), 130.2 (s, C-3, 3′), 146.2 (s, C-4, 4′), 122.3 (d, C-5, 5′), 129.1 (d, C-6, 6′), 39.5 (t, C-7, 7′), 136.8 (d, C-8, 8′), 116.3 (t, C-9, 9′), 171.0 (s, C-1″, 1‴), 31.0 (t, C-2″, 2‴), 27.4 (t, C-3″, 3‴), 43.6 (t, C-4″, 4‴). HRESIMS *m/z*: [M + H] $^+$ 475.1439 ($C_{26}H_{28}O_4Cl_2$), cal. 475.1437.

*4.2.11. 4, 4′-di-O-benzoyl-magnolol (**2d**)*

White solid, m. p. 64.3–65.4 °C, yield 88.15%, ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 8.00 (dd, 4H, J = 8.0, 1.2 Hz, H-3″, 3‴, 7″, 7‴), 7.62 (d, 2H, J = 1.9 Hz, H-2, 2′), 7.58–7.40 (m, 6H, H-4″,4‴, 5″, 5‴, 6″, 6‴), 7.22 (d, 2H, J = 8.4, Hz, H-5, 5′), 7.19 (dd, 2H, J = 8.4, 1.9 Hz, H-6, 6′), 5.82 (m, 2H, H-8, 8′), 5.01 (dd, 2H, J = 12.4, 1.8 Hz, H-9b, 9′b), 4.98 (dd, 2H, J = 8.8, 1.8 Hz, H-9a, 9′a), 3.33 (d, 4H, J = 6.4 Hz, H-7, 7′). ^{13}C NMR (100 MHz, $CDCl_3$) δ:137.5 (s, C-1, 1′), 133.3 (d, C-2, 2′), 131.2 (s, C-3, 3′), 146.6 (s, C-4, 4′), 122.5 (d, C-5, 5′), 128.4(d, C-6, 6′), 39.4 (t, C-7, 7′), 137.5 (d, C-8, 8′), 116.0 (t, C-9, 9′), 165.1 (s, C-1″, 1‴), 130.1 (s, C-2″, 2‴), 130.2 (d, C-3″, 3‴, 7″, 7‴), 129.0 (d, C-4″, 4‴, 6″, 6‴), 133.7 (d, C-5″, 5‴). HRESIMS *m/z*: [M + H] $^+$ 475.1904 ($C_{32}H_{26}O_4$), cal. 475.1904.

*4.2.12. 4, 4′-di-O-cinnamoyl-magnolol (**2e**)*

White solid, m. p. 116.7–117.4 °C, yield 70.01%, ^{1}H NMR (400 MHz, $CDCl_3$) δ_H:7.67 (d, 2H, J = 16.0 Hz, H-3″, 3‴), 7.51 (d, 2H, J = 1.7 Hz, H-2, 2′), 7.50–7.39 (m, 10H, H-5″, 5‴, 6″, 6‴, 7″, 7‴, 8″, 8‴,9″, 9‴), 7.23 (dd, 2H, J = 8.4, 1.7 Hz, H-6, 6′), 7.21 (d, 2H, J = 8.4 Hz, H-5, 5′), 6.49 (d, 2H, J = 16.0 Hz, H-2″, 2‴)5.95 (m, 2H, H-8, 8′), 5.08 (dd, 2H, J = 17.6, 2.0 Hz, H-9b, 9′b),5.04 (dd, 2H, J = 11.2, 2.0 Hz, H-9a, 9′a), 3.41 (d, 4H, J = 6.4 Hz, H-7, 7′). ^{13}C NMR (100 MHz, $CDCl_3$) δ:137.4 (s, C-1, 1′), 130.4 (d, C-2, 2′), 131.2 (s, C-3, 3′), 146.4 (s, C-4, 4′), 122.4 (d, C-5, 5′), 137.4 (d, C-6, 6′), 39.5 (t, C-7, 7′), 136.9 (d, C-8, 8′), 116.1 (t, C-9, 9′), 165.3 (s, C-1″, 1‴), 117.2 (d, C-2″, 2‴), 146.1 (d, C-3″, 3‴), 134.2 (s, C-4″, 4‴), 128.9 (d, C-5″, 5‴, 9″, 9‴), 130.2 (d, C-6″, 6‴, 8″, 8‴), 128.2 (d, C-7″, 7‴). HRESIMS *m/z*: [M + H] $^+$ 527.2223 ($C_{36}H_{30}O_4$), cal. 527.2217.

*4.3. General synthetic procedures for **3**–**4***

To a solution of magnolol in CH_2Cl_2 (5 mL), 3-Chloroperbenzoic acid (*m*-CPBA, 1.0 or 2.1 equiv.) and potassium carbonate (1.0 or 2.1 equiv.) were added. The reaction mixture was stirred at room temperature for 4 h monitored by TLC [17]. The reaction mixture was diluted with chloroform (50 mL), and concentrated to dryness under reduced pressure. The obtained residue was purified by column chromatography over silica gel to afford compounds **3**–**4**.

*4.3.1. 8′, 9′-epoxide-magnolol (**3**)*

White solid, m. p. 79.8–80.5 °C, yield 79.11%, ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 8.09 (d, 1H, J = 2.0 Hz, H-2′), 7.99 (d, 1H, J = 1.9 Hz, H-2), 7.16 (dd, 1H, J = 7.2, 1.9 Hz, H-6′), 7.13 (d, 1H, J = 7.2, Hz, H-5′), 7.09 (dd, 1H, J = 8.4, 2.0 Hz, H- 6), 6.94 (d, 1H, J = 8.4 Hz, H-5), 5.97 (m, 1H, H-8), 5.09 (dd, 1H, J = 16.4, 1.6 Hz, H-9a), 5.05 (dd, 1H, J = 9.6, 1.6 Hz, H-9b), 3.36 (d, 2H, J = 6.5 Hz,H-7), 3.20 (m, 1H, H-8′), 2.94 (dd, 1H, J = 4.8, 4.2 Hz, H-9b), 2.60 (dd, 1H, J = 4.8, 2.7 Hz, H-9a). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 133.8 (s, C-1), 131.7 (d, C-2), 124.4 (s, C-3), 151.0 (s, C-4), 116.8 (d, C-5), 129.8 (d, C-6), 39.3 (t, C-7), 137.5 (d, C-8), 115.8 (t, C-9), 133.2 (s, C-1′), 131.3 (d, C-2′), 128.3 (d, C-3′), 151.6 (s, C-4′), 116.7 (d, C-5′), 130.2 (d, C-6′), 37.8 (t, C-7′), 52.9 (d, C-8′), 47.2 (t, C-9′). HRESIMS *m/z*: [M + H]$^+$ 283.1325 ($C_{18}H_{18}O_3$), cal. 283.1329.

*4.3.2. 8, 8′, 9, 9′-di-epoxide-magnolol (**4**)*

White solid, m. p. 128.1–129.4 °C, yield 70.11%, ^{1}H NMR (400 MHz, $CDCl_3$) δ_H:7.58 (d, 2H, J = 2.4 Hz, H-2, 2′), 7.13 (dd, 2H, J = 8.4, 2.4 Hz, H-6, 6′), 6.90 (d, 2H, J = 8.4 Hz, H-5, 5′), 3.17 (m, 2H, H-8, 8′), 2.83 (dd, 2H, J = 4.8, 4.4 Hz, H-9b, 9′b),2.79 (d, 4H, J = 6.0 Hz, H-7, 7′), 2.59 (dd, 2H, J = 4.8, 2.8 Hz, H-9a, 9′a). ^{13}C NMR (100 MHz, $CDCl_3$) δ:131.9 (s, C-1,1′), 133.7 (d, C-2, 2′), 124.9 (s, C-3, 3′), 151.6 (s, C-4, 4′), 116.9 (d, C-5, 5′), 129.8 (d, C-6, 6′), 37.7 (t, C-7, 7′), 53.0 (d, C-8, 8′), 47.2 (t, C-9, 9′). HRESIMS *m/z*: [M − H]$^-$ 297.1127 ($C_{18}H_{18}O_4$), cal. 297.1132.

*4.4. General synthetic procedures for **5**–**6** and **7a**-**7d***

The 30% HBr (8 mL) was added to the solution of pentalacetylated glucose (3.8 mmol) in anhydrous CH_2Cl_2 (10 mL) and stirred at 0 °C for 6 h until the starting material was not observed by TLC check. The crude solution was filtered and washed with CH_2Cl_2 (2 × 20 mL), and the CH_2Cl_2 solution was further washed with distilled water (3 × 30 mL). Then the organic layer was dried over anhydrous Na_2SO_4 and concentrated to dryness under reduced pressure to yield the α-D-glucopyranosyl bromide tetra-acetate. Magnolol was dissolved in 10 mL NaOH solution (0.8 N) and stirred for 30 min and then added to the solution of α-D-

glucopyranosyl bromide tetra-acetate (1 or 2 equiv.) and tetrabutyl ammonium bromide (TBAB, 1 or 2 equiv.) in 10 mL $CHCl_3$. After stirring for 3 h, the reaction mixture was filtered and washed with $CHCl_3$ (3 × 20 mL). Subsequently, the $CHCl_3$ solution was washed by 5% HCl (3 × 50 mL), saturated $NaHCO_3$ (3 × 50 mL) and saturated NaCl (3 × 50 mL) respectively and further concentrated to dryness *in vacuo* [18]. Purification by column chromatography on silica gel to get the target compounds **5** and **6**. The obtained compounds **5** or **6** was further deacetylated in sodium methoxide-methanol solution (0.8 equiv.) for different hours (4–8 h), and then purified by column chromatography over silica gel to afford **7a**-**7d**.

4.4.1. Magnolol-4′-O-(2″, 3″, 4″, 6″-4 acetyl)-β-D-glucopyranoside (**5**)

White solid, m. p. 67.7–68.5 °C, yield 68.7%, ^{1}H NMR (400 MHz, CD_3OD) δ_H: 7.22 (d, 1H, J = 8.4 Hz, H-5′), 7.14 (dd, 1H, J = 2.0, 8.4 Hz, H-6′), 7.06 (d, 1H, J = 2.0 Hz, H-2′), 7.00 (dd, 1H, J = 8.2, 2.0 Hz, H-6), 6.91 (d, 1H, J = 2.0 Hz, H-2), 6.78 (d, 1H, J = 8.2 Hz, H-5), 6.00 (m, 1H, H-8′), 5.98 (m, 1H, H-8), 5.11 (m, 2H, H-9′), 5.08 (m, 2H, H-9), 5.01 (d, 1H, J = 8.1, H-1″), 4.32 (dd, 1H, J = 5.2, 12.4 Hz, H-6″a), 4.18 (dd, 1H, J = 2.4, 12.4 Hz, H-6″b), 4.00 (m, 1H, H-5″), 3.38 (d, 2H, J = 6.8 Hz, H-7′), 3.36 (d, 2H, J = 6.2 Hz, H-7), 2.09 (s, 3H), 2.03 (s, 3H), 1.94 (s, 3H), 1.71 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ: 134.7 (s, C-1), 131.2 (d, C-2), 129.2 (s, C-3), 151.6 (s, C-4), 116.6 (d, C-5), 128.3 (d, C-6), 38.9 (t, C-7), 137.6 (d, C-8), 114.1 (t, C-9), 136.8 (s, C-1′), 131.7 (d, C-2′), 130.6 (s, C-3′), 152.7 (s, C-4′), 116.6 (d, C-5′), 128.1 (d, C-6′), 39.0 (t, C-7′), 138.1 (d, C-8′), 114.5 (t, C-9′), 170.9 (s), 170.1 (s), 169.8 (s), 169.4 (s), 99.2 (d, C-1″), 70.9 (d, C-2″), 72.9 (d, C-3″), 68.4 (d, C-4″), 71.5 (d, C-5″), 61.8 (t, C-6″), 19.3 (q), 19.3 (q), 19.1 (q), 19.1 (q). HRESIMS m/z: $[M-H]^-$ 595.2185 ($C_{32}H_{36}O_{11}$), cal. 595.2185.

4.4.2. Magnolol-4, 4′-di-O-(2″, 3″, 4″, 6″-4 acetyl)-β-D-glucopyranoside (**6**)

White solid, m. p. 66.3–67.2 °C, yield 78.6%, ^{1}H NMR (400 MHz, CD_3OD) δ_H: 7.18 (dd, 2H, J = 8.4, 1.8 Hz, H-6, 6′), 7.14 (d, 2H, J = 1.8 Hz, H-2, 2′), 7.03 (d, 2H, J = 8.4 Hz, H-5, 5′), 6.00 (m, 2H, H-8, 8′), 5.11 (dd, 2H, J = 1.5, 16.8 Hz, H-9, 9′a), 5.06 (dd, 2H, J = 1.5, 10.0 Hz, H-9, 9′b), 4.96 (d, 2H, J = 8.0 Hz, H-1″, 1‴), 4.30 (dd, 2H, J = 5.2, 12.3 Hz, H-6″a, 6‴a), 4.16 (dd, 2H, J = 2.1, 12.3 Hz, H-6″b, 6‴b), 3.95 (m, 2H, H-5″, 5‴), 3.37 (d, 4H, J = 6.8 Hz, H-7, 7′), 2.09 (s, 3H), 2.03 (s, 3H), 1.96 (s, 3H), 1.75 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ: 134.8 (s, C-1, 1′), 131.7 (d, C-2, 2′), 128.9 (s, C-3, 3′), 152.4 (s, C-4, 4′), 117.3 (d, C-5, 5′), 128.4 (d, C-6, 6′), 38.9 (t, C-7, 7′), 137.5 (d, C-8, 8′), 114.8 (t, C-9, 9′), 171.2 (s), 170.4 (s), 170.1 (s), 169.5 (s), 99.5 (d, C-1″), 71.2 (d, C-2″), 72.9 (d, C-3″), 68.4 (d, C-4″), 71.5 (d, C-5″), 61.8 (t, C-6″), 19.4 (q), 19.3 (q), 19.2 (q), 19.1 (q). HRESIMS m/z: $[M+Na]^+$ 949.3099 ($C_{46}H_{54}O_{20}$), cal. 949.3101.

4.4.3. Magnolol-4′-O-(3″, 4″, 6″-3 acetyl)-β-D-glucopyranoside (**7a**)

White solid, m. p. 60.1–61.4 °C, yield 30.8%, ^{1}H NMR (400 MHz, CD_3OD) δ_H: 7.20 (dd, 1H, J = 8.2, 1.8 Hz, H-6′), 7.13 (d, 1H, J = 1.8 Hz, H-6′), 7.06 (d, 1H, J = 8.2 Hz, H-2′), 6.91 (dd, 1H, J = 8.4, 1.8 Hz, H-6), 6.88 (d, 1H, J = 2.0 Hz, H-2), 6.78 (d, 1H, J = 8.2 Hz, H-5), 5.89 (m, 1H, H-8′), 5.87 (m, 1H, H-8), 5.04 (m, 2H, H-9′), 5.01 (m, 2H, H-9), 4.99 (d, 1H, J = 6.8, H-1″), 4.16 (dd, 1H, J = 5.2, 12.4 Hz, H-6″a), 4.01 (dd, 1H, J = 2.4, 12.4 Hz, H-6″b), 3.87 (m, 1H, H-5″), 3.27 (d, 2H, J = 6.4 Hz, H-7′), 3.22 (d, 2H, J = 6.8 Hz, H-7), 1.92 (s, 3H), 1.91 (s, 3H), 1.90 (s, 3H). ^{13}C NMR (150 MHz, CD_3OD) δ: 133.0 (s, C-1), 132.9 (d, C-2), 127.7 (s, C-3), 153.1 (s, C-4), 116.4 (d, C-5), 129.7 (d, C-6), 40.4 (t, C-7), 139.0 (d, C-8), 115.6 (t, C-9), 135.6 (s, C-1′), 133.0 (d, C-2′), 130.2 (s, C-3′), 154.1 (s, C-4′), 117.5 (d, C-5′), 129.6 (d, C-6′), 40.4 (t, C-7′), 139.5 (d, C-8′), 115.9 (t, C-9′), 172.3 (s), 172.0 (s), 171.4 (s), 102.1 (d, C-1″), 72.9 (d, C-2″), 76.1 (d, C-3″), 69.9 (d, C-4″), 72.9 (d, C-5″), 63.2 (t, C-6″), 20.8 (q), 20.7 (q), 20.6 (q). HRESIMS m/z: $[M-H]^-$ 553.2086 ($C_{30}H_{34}O_{10}$), cal. 553.2079.

4.4.4. Magnolol-4′-O-(3″, 4″-2acetyl)-β-D-glucopyranoside (**7b**)

White solid, m. p. 64.8–65.6 °C, yield 23.1%, ^{1}H NMR (600 MHz, CD_3OD) δ_H: 7.23 (d, 1H, J = 7.8 Hz, H-5′), 7.17 (d, 1H, J = 1.8 Hz, H-6′), 7.10 (d, 1H, J = 7.8 Hz, H-2′), 7.22 (d, 1H, J = 7.2 Hz, H-6), 7.14 (d, 1H, J = 7.2 Hz, H-5), 7.01 (d, 1H, J = 1.6 Hz, H-2), 6.04 (m, 1H, H-8′), 5.99 (m, 1H, H-8), 5.13 (m, 2H, H-9′), 5.06 (m, 2H, H-9), 4.81 (d, 1H, J = 6.1, H-1″), 4.18–3.27 (m, 6H), 3.34 (m, 4H, H-7, 7′), 2.07 (s, 3H), 2.06 (s, 3H). ^{13}C NMR (150 MHz, CD_3OD) δ: 132.5 (s, C-1), 132.4 (d, C-2), 128.9 (s, C-3), 147.8 (s, C-4), 116.5 (d, C-5), 130.1 (d, C-6), 40.2 (t, C-7), 138.9 (d, C-8), 115.9 (t, C-9), 135.4 (s, C-1′), 132.8 (d, C-2′), 132.2 (s, C-3′), 154.0 (s, C-4′), 117.8 (d, C-5′), 129.4 (d, C-6′), 40.5 (t, C-7′), 139.0 (d, C-8′), 116.3 (t, C-9′), 172.5 (s), 171.8 (s), 102.1 (d, C-1″), 75.2 (d, C-2″), 77.6 (d, C-3″), 74.2 (d, C-4″), 71.4 (d, C-5″), 64.5 (t, C-6″), 21.1 (q), 20.7 (q). HRESIMS m/z: $[M+Na]^+$ 535.1940 ($C_{28}H_{32}O_9$), cal. 535.1939.

4.4.5. Magnolol-4′-O-β-D-glucopyranoside (**7c**)

White solid, m. p. 68.9–70.1 °C, yield 79.8%, ^{1}H NMR (400 MHz, CD_3OD) δ_H: 7.19 (dd, 1H, J = 8.4, 1.8 Hz, H-6′), 7.16 (d, 1H, J = 1.8 Hz, H-2′), 7.14 (d, 1H, J = 8.4 Hz, H-5′), 7.03 (dd, 1H, J = 8.0, 2.0 Hz, H-6), 6.98 (d, 1H, J = 2.0 Hz, H-2), 6.85 (d, 1H, J = 8.0 Hz, H-5), 5.96 (m, 1H, H-8′), 5.95 (m, 1H, H-8), 5.11 (m, 2H, H-9′), 5.09 (m, 2H, H-9), 4.99 (d, 1H, J = 7.7, H-1″), 3.96 (dd, 1H, J = 1.8, 12.4 Hz, H-6″a), 3.69 (dd, 1H, J = 5.6, 12.4 Hz, H-6″b), 3.38 (d, 2H, J = 6.1 Hz, H-7′), 3.33 (d, 2H, J = 7.8 Hz, H-7), 1.92 (s, 3H), 1.91 (s, 3H), 1.90 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ: 131.8 (s, C-1), 131.3 (d, C-2), 126.8 (s, C-3), 151.7 (s, C-4), 116.2 (d, C-5), 128.3 (d, C-6), 39.0 (t, C-7), 137.7 (d, C-8), 114.2 (t, C-9), 133.8 (s, C-1′), 131.4 (d, C-2′), 128.5(s, C-3′), 153.0(s, C-4′), 116.3(d, C-5′), 128.4(d, C-6′), 39.0(t, C-7′), 138.0(d, C-8′), 114.5(t, C-9′), 101.0(d, C-1″), 73.4(d, C-2″), 76.8 (d, C-3″), 69.9 (d, C-4″), 76.4 (d, C-5″), 61.2 (t, C-6″). HRESIMS m/z: $[M+Na]^+$ 451.1724 ($C_{24}H_{28}O_7$), cal. 451.1727.

4.4.6. Magnolol-4, 4′-di-β-D-glucopyranoside (**7d**)

White solid, m. p. 89.9–91.4 °C, yield 80.6%, ^{1}H NMR (400 MHz, CD_3OD) δ_H: 7.18 (dd, 2H, J = 8.4, 1.8 Hz, H-6, 6′), 7.14 (d, 2H, J = 1.8 Hz, H-2, 2′), 7.06 (d, 2H, J = 8.4 Hz, H-5, 5′), 6.00 (m, 2H, H-8, 8′), 5.11 (dd, 2H, J = 1.5, 16.8 Hz, H-9a, 9′a), 5.07 (dd, 2H, J = 1.5, 10.0 Hz, H-9b, 9′b), 4.92 (d, 2H, J = 8.0 Hz, H-1″, 1‴), 3.85 (d, 2H, J = 11.3 Hz, H-6″a, 6‴a), 3.69 (dd, 2H, J = 4.5, 11.3 Hz, H-6″b, 6‴b), 3.42–3.34 (m, 8H, H-2″, 2‴, 3″, 3‴, 4″, 4‴, 5″, 5‴), 3.37 (m, 4H, H-7, 7′). ^{13}C NMR (100 MHz, CD_3OD) δ: 133.9 (s, C-1, 1′), 131.4 (d, C-2, 2′), 129.0 (s, C-3, 3′), 152.6 (s, C-4, 4′), 115.8 (d, C-5, 5′), 128.3 (d, C-6, 6′), 39.0 (t, C-7, 7′), 137.7 (d, C-8, 8′), 114.6 (t, C-9, 9′), 100.6 (d, C-1″, 1‴), 73.4 (d, C-2″, 2‴), 76.6 (d, C-3″, 3‴), 70.0 (d, C-4″, 4‴), 76.6 (d, C-5″, 5‴), 61.2 (t, C-6″, 6‴). HRESIMS m/z: $[M-H]^-$ 589.2286 ($C_{30}H_{38}O_{12}$), cal. 589.2291.

4.5. General synthetic procedures for **8a**-**8c**

These derivatives were synthesized by the Steglich esterification reaction of **7c** (0.5 mmol) with the corresponding acid (1.2 equiv.) and DCC (1.2 equiv.) in the presence of DMAP (0.6 equiv.), which had the similar treatment process to preparation of compounds **1a**-**1g**.

4.5.1. Magnolol-4-O-benzoyl-4′-O- β-D-glucopyranoside (**8a**)

White solid, m. p. 76.8–77.5 °C, yield 87.2%, ^{1}H NMR (600 MHz, CD_3OD) δ_H: 7.92 (d, 2H, J = 7.2 Hz, H-3‴, 7‴), 7.42 (t, 1H, J = 7.8 Hz, H-5‴), 7.31 (dd, 2H, J = 7.2, 7.8 Hz, H-4‴, 6‴), 7.25 (dd, 1H, J = 7.8, 1.8 Hz, H-6′), 7.19 (d, 1H, J = 1.8 Hz, H-2′), 7.12 (d, 1H, J = 7.8 Hz, H-5′), 7.11 (dd, 1H, J = 8.0, 2.2 Hz, H-6), 7.09 (d, 1H, J = 2.2 Hz, H-2), 7.05 (d, 1H, J = 8.0 Hz, H-5), 6.04 (m, 1H, H-8′), 5.78 (m, 1H, H-8), 5.14 (m, 2H, H-9′), 5.06 (m, 2H, H-9), 5.01 (d, 1H, J = 7.2, H-1″), 3.76

(dd, 1H, J = 1.6, 12.6 Hz, H-6″a), 3.45 (dd, 1H, J = 5.2, 12.6 Hz, H-6″b), 3.32 (d, 2H, J = 6.2 Hz, H-7′), 3.24 (d, 2H, J = 6.6 Hz, H-7). ^{13}C NMR (150 MHz, CD_3OD) δ: 135.7 (s, C-1), 130.2 (d, C-2), 128.6 (s, C-3), 147.9 (s, C-4), 117.6 (d, C-5), 129.6 (d, C-6), 40.2 (t, C-7), 138.8 (d, C-8), 116.9 (t, C-9), 138.9 (s, C-1′), 131.1 (d, C-2′), 130.8(s, C-3′), 154.1(s, C-4′), 123.6(d, C-5′), 129.6(d, C-6′), 40.5(t, C-7′), 139.1(d, C-8′), 116.3(t, C-9′), 102.5(d, C-1″), 74.6(d, C-2″), 78.0 (d, C-3″), 71.4 (d, C-4″), 74.6 (d, C-5″), 62.6 (t, C-6″), 167.2 (s, C-1‴), 135.2 (s, C-2‴), 132.2 (s, C-3‴, C-7‴), 128.6 (s, C-4‴, C-6‴), 134.7 (s, C-5‴). HRESIMS m/z: $[M + Na]^+$ 555.1979 ($C_{31}H_{32}O_8$), cal. 555.1989.

*4.5.2. Magnolol-4-O-cinnamyl-4′-O- β-D-glucopyranoside (**8b**)*

White solid, m. p. 78.8–80.2 °C, yield 87.2%, ^{1}H NMR (600 MHz, CD_3OD) δ_H: 7.68 (d, 1H, J = 16.0 Hz, H-3‴), 7.56 (d, 2H, J = 7.0 Hz, H-5‴, 9‴), 7.52 (t, 1H, J = 7.5 Hz, H-7‴), 7.37 (dd, 2H, J = 7.0, 7.5 Hz, H-6‴, 8‴), 7.25 (dd, 1H, J = 8.2, 2.0 Hz, H-6′), 7.21 (d, 1H, J = 2.0 Hz, H-2′), 7.12 (d, 1H, J = 8.2 Hz, H-5′), 7.10 (dd, 1H, J = 7.8 2.2 Hz, H-6), 7.08 (d, 1H, J = 2.2 Hz, H-2), 7.01 (d, 1H, J = 7.8 Hz, H-5), 6.48 (d, 1H, J = 16.0 Hz, H-2‴), 6.00 (m, 1H, H-8′), 5.89 (m, 1H, H-8), 5.13 (m, 2H, H-9′), 5.05 (m, 2H, H-9), 5.00 (d, 1H, J = 7.8, H-1″), 3.75 (dd, 1H, J = 2.0, 12.0 Hz, H-6″a), 3.41 (dd, 1H, J = 6.5, 12.0 Hz, H-6″b), 3.32 (d, 2H, J = 7.5 Hz, H-7′), 3.26 (d, 2H, J = 6.5 Hz, H-7). ^{13}C NMR (125 MHz, CD_3OD) δ: 138.9 (s, C-1), 130.1 (d, C-2), 128.7 (s, C-3), 147.8 (s, C-4), 118.2 (d, C-5), 129.5 (d, C-6), 40.3 (t, C-7), 138.7 (d, C-8), 116.0 (t, C-9), 139.0 (s, C-1′), 131.8 (d, C-2′), 132.7(s, C-3′), 154.2(s, C-4′), 123.6(d, C-5′), 129.6(d, C-6′), 40.6(t, C-7′), 138.9(d, C-8′), 116.4(t, C-9′), 102.5(d, C-1″), 74.6(d, C-2″), 78.1 (d, C-3″), 71.5 (d, C-4″), 77.8 (d, C-5″), 62.6 (t, C-6″), 167.6 (s, C-1‴), 117.5 (s, C-2‴), 147.8 (s, C-3‴), 138.7 (s, C-4‴), 135.3 (d, C-5‴, C-9‴), 133.2 (d, C-6‴, C-8‴), 130.4 (d, C-7‴). HRESIMS m/z: $[M + Na]^+$ 581.2148 ($C_{33}H_{34}O_8$), cal. 581.2146.

*4.5.3. Magnolol-4-O- (3-fluoro-cinnamyl)-4′-O- β-D-glucopyranoside (**8c**)*

White solid, m. p. 83.6–84.7 °C, yield 87.2%, ^{1}H NMR (500 MHz, CD_3OD) δ_H: 7.63 (d, 1H, J = 16.0 Hz, H-3‴), 7.39 (d, 1H, J = 7.6 Hz, H-9‴), 7.37 (dd, 1H, J = 7.6, 8.0 Hz, H-8‴), 7.25 (dd, 1H, J = 8.5, 2.0 Hz, H-6′), 7.21 (d, 1H, J = 2.0 Hz, H-2′), 7.14 (d, 1H, J = 8.5 Hz, H-5′), 7.10 (dd, 1H, J = 8.2 2.0 Hz, H-6), 7.08 (d, 1H, J = 2.0 Hz, H-2), 7.01 (d, 1H, J = 8.2 Hz, H-5), 6.52 (d, 1H, J = 16.0 Hz, H-2‴), 6.01 (m, 1H, H-8′), 5.87 (m, 1H, H-8), 5.10 (m, 2H, H-9′), 5.06 (m, 2H, H-9), 5.00 (d, 1H, J = 7.5, H-1″), 3.76 (dd, 1H, J = 2.0, 11.6 Hz, H-6″a), 3.42 (dd, 1H, J = 6.5, 11.6 Hz, H-6″b), 3.33 (d, 2H, J = 6.0 Hz, H-7′), 3.29 (d, 2H, J = 6.5 Hz, H-7). ^{13}C NMR (125 MHz, CD_3OD) δ: 138.9 (s, C-1), 130.3 (d, C-2), 128.7 (s, C-3), 147.8 (s, C-4), 118.5 (d, C-5), 129.6 (d, C-6), 40.3 (t, C-7), 138.0 (d, C-8), 115.9 (t, C-9), 139.1 (s, C-1′), 131.8 (d, C-2′), 132.6(s, C-3′), 154.2(s, C-4′), 123.6(d, C-5′), 130.4(d, C-6′), 40.5(t, C-7′), 138.7(d, C-8′), 116.4(t, C-9′), 102.5(d, C-1″), 74.6(d, C-2″), 78.1 (d, C-3″), 71.5 (d, C-4″), 71.8 (d, C-5″), 62.6 (t, C-6″), 167.2 (s, C-1‴), 117.4 (d, C-2‴), 147.8 (d, C-3‴), 137.9 (s, C-4‴), 135.3 (d, C-5‴), 133.2 (d, C-6‴), 130.4 (d, C-7‴), 146.2 (s, C-8‴), 125.7 (d, C-7‴). HRESIMS m/z: $[M + Na]^+$ 599.2061 ($C_{33}H_{33}FO_8$), cal. 599.2052.

4.6. Biological evaluation

All *in vivo* experiments were performed in strict accordance with the guidelines established in the guide for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee of the Kunming Institute of Botany, Chinese Academy of Sciences.

4.6.1. In vitro MT agonistic activity assay

The effect of the compounds at different concentrations was determined using the Fluo-8 calcium assay on the HEK293 cell lines stably expressing the human melatonin MT_1 or MT_2 receptors. According to the procedure reported by X.J. Yin [19], the cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and cultured at 37 °C (95% O_2/5% CO_2), then seeded into a 96-well plate (4×10^4/well) and incubated for 24 h. Tested compounds or positive drug were dissolved in 10 μL DMSO and 990 μL HBSS Buffer, respectively, and determined by FlexStation 3 Benchtop Multi-Mode Microplate Reader at room temperature with wavelength (excitation: 485 nm; emission: 525 nm; emission cut-off: 515 nm). Each test was prepared in triplicate, and the experiments were carried out three times. EC_{50} values were obtained by nonlinear regression (GraphPad Prism. 5.0).

4.6.2. Behavioral tests

Male Kunming mice, weighing 18–20 g, were bought from Beijing HFK Bioscience CO. Ltd. (License: SCXK (JING) 2014–0004, Beijing, China). The quality of the experimental animals review is the Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences (Beijing, China). All mice were housed in a group of five animals per cage at an ambient temperature of 22 ± 2 °C and relative humidity of 55–65% under a normal 12 h light/dark cycle (lights on at 7:00 a.m.), with free access to food and water.

4.6.2.1. Open field test. The mice spontaneous locomotor activity was measured using the open field. The apparatus (24 × 24 × 40 cm) had black floor and walls [20,21]. The animals were individually placed in the center of the box for 30 s for adaptation and then allowed to freely explore the area for 6 min. The animal's locomotor activity was registered as the total number of times (counts) and the distance travelled (in meters) that the animal crossed a square during the test. After each trial, the apparatus was cleaned with 70% ethanol to remove the odour clues left by the previous mice.

4.6.2.2. Forced swim test (FST). This test was performed according to Porsolt et al. [22,23]. Mice were individually placed into glass cylinders (height: 25 cm, diameter: 15 cm) filling with water to a height of 10 cm and the temperature was maintained at 25 ± 1 °C. Mice were gently placed onto the water and forced to swim for 6 min, and the total duration of immobility during the last 4 min was automatically measured by the ANY-maze Video Tracking System (Anymaze, Stoelting Co., Wood Dale, USA).

4.6.2.3. Tail suspension test (TST). The tail suspension test (TST) was based on the method of Steru [24]. The animals were acoustically and visually isolated and individually suspended 50 cm above the surface of a wooden box using adhesive tape placed approximately 1 cm from the tip of the tail for 6 min. The duration of immobility (seconds) was automatically recorded during the last 4 min of the test by the ANY-maze Video Tracking System (Anymaze, Stoelting Co., Wood Dale, USA).

4.6.3. Neurochemical tests

Male Kunming Mice were sacrificed by the method of cervical dislocation and following decapitated, and brains were rapidly removed and dissected on an ice-chilled glass plate, including the frontal cortex, hippocampus, striatum, hypothalamus and thalamus, after administration of vehicle or compound **7c** (40 mg/kg) for 7 days. The tissue samples were weighed and homogenized in an ice-cold solution of 0.2 M perchloric acid (10 μL/mg) containing 0.1 mM EDTA, and then centrifuged at 15,000 rpm for 20 min at 4 °C. The supernatant was reserved and the contents of NE, 5-HT, 5-HIAA, DA, DOPAC and HVA were measured by HPLC-ECD as described previously. An Agilent 1200 pump (Agilent, California, U.S.A) was set at 0.6 mL/min. The detector (Coulochem III-ECD, Thermo Fisher Scientific) was set at +0.50 V. The mobile phase

consisted of 69 mM sodium dihydrogen phosphate, 0.01% (v/v) triethylamine, 0.025 mM EDTA, 1.7 mM sodium octanesulfonate (pH = 3.0) and 12% methanol. The volume of injection was 20 μL [25].

4.6.4. Acute oral toxicity

Adult Sprague Dawley (SD) rats used for the study, weighing 180–220 g, were obtained from the Beijing HFK Bioscience CO. Ltd. An acute oral toxicity study was conducted according to the guidelines of the Organization for Economic Co-operation and Development (OECD, 425) [26]. The animals were randomly allocated into three groups of six animals each. Group I as a blank control: animals were administered orally with vehicle normal 0.5% CMC-Na. Group II (male rats) and III (female rats) administered orally with 2000 mg/kg body weight of **7c**. The animals were observed continuously for the first 4 h and then every hour for the next 24 h and at 6 hourly intervals for the following 48 h after administering the drug [27].

4.6.5. Statistical analysis

Results were presented as the mean ± S.E.M. Data were analyzed using GraphPad 5.0 (GraphPad Software Inc., San Diego, USA). All data were carried out by one-way analysis of variance (ANOVA), followed by Tukey test. Differences were considered statistically significant when $p < 0.05$.

4.6.6. Metabolic profiling of **7c**

The rats (n = 6) were maintained in metabolic cages separately, and a reference standard of **7c** was orally administered to rats at a dose of 100.0 mg/kg. A 0.5% solution of CMC-Na was orally administered to rats in the control group according to the same protocol. The method for collecting and preparing samples (plasma, urine and faeces) was performed according to Chavan and Wu [28,29].

Metabolic profiling was carried out by employing LC-MS technique. Liquid chromatography was performed with a Shimadzu LCMS-IT-TOF system. Chromatographic separation was achieved on an Agilent Eclipse Plus C_{18} column (100 × 2.1 mm i. d., 1.8 μm, Agilent Technologies) at 30 °C. The mobile phases for LC-MS analysis were A: formic acid/water (0.05/100, v/v) and B: formic acid/acetonitrile (0.05/100, v/v) at a flow rate of 0.2 mL/min. Gradient elution was performed as follows: linear gradient 5–100% B from 0 to 18.0 min. The injection volume was 2 or 5 μL for each LC-MS analysis. MS and MS^n experiments were achieved in an automatic pattern in both positive and negative ion modes. The analytical conditions were as follows: spray voltage, 4.50 kV or −3.50 kV; detector voltage, 1.58 kV; pressure of the TOF region, 1.6×10^{-4} Pa; pressure of the IT, 1.5×10^{-2} Pa; rotary pump (RP) area vacuum, 70.0 Pa; drying gas pressure, 110.0 kPa; nebulizing gas (N_2) flow, 1.5 L/min.

Conflicts of interest

The authors have no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejmech.2018.07.027.

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