Synthesis of 2,4-dihydroxychalcone Derivatives as Potential Antidepressant Effect

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Abstract
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In this study, twelve 2,4-dihydroxychalcone derivatives were synthesized and evaluated for antidepressant activities using the forced swimming test (FST). The pharmacological test showed that 6 compounds significantly reduced the immobility times in the FST at a dose of 10 mg/kg, indicative of antidepressant activity. Among the derivatives, compounds designated 3d and 3h exhibited the best antidepressant activity, with reduced immobility time by 32.05% and 34.33%, respectively. In the 5-hydroxytryptophan-induced head-twitch test and yohimbine-induced mortality test, compounds 3d and 3h increased head-twitch and increased the mortality rate. The mechanisms of the antidepressant effects of compounds 3d and 3h may be related with the 5-HTP and NE nervous system.

Introduction
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Depression is one of the most prevalent psychopathologies. Symptoms of depression include lowered mood, and reduced interest and pleasure. It is predicted by the World Health Organization to become the second leading cause of disease-related disability by the year 2020 [1,2]. Despite the introduction of tricyclic and monoamine oxidase inhibitors (MAOIs) and selective serotonin reuptake inhibitors (SSRIs) to treat depression, the therapeutic results have not been satisfactory. Only one-third of afflicted individuals have remission and the subjects always suffer from the side effects, such as anxiety, sleep disturbance, weight gain, and sexual dysfunction [3–5]. Therefore, there is an unmet need for new antidepressants. Chalcones (*trans*-1,3-diphenyl-2-propen-1-ones) are biogenetic precursors of flavonoids [6]. Their chemical structure consists of open-chain flavonoids in which 2 aromatic rings are joined by a 3-carbon α,β-unsaturated carbonyl system. They present a broad spectrum of biological activities, such as anti-tumor, anti-oxidation, anti-inflammatory, antiviral, antilipidemic, and antidepressant activities [7–12].
Previously, our research team found that isoliquiritigenin and butein (○ **Fig. 1**) showed antidepressant-like effects in the forced swimming test (FST; $p < 0.05$ and $p < 0.01$, respectively) in mice,

but had weaker activity than the reference drug fluoxetine ($p < 0.001$) [13]. To obtain new compounds with better antidepressant effects, in this paper, we tried to chemically modify chalcone to improve its bioavailability, therefore a series of 5,7-dihydroxychalcone derivatives were designed and synthesized. The antidepressant activity of the synthesized compounds were also determined by using Porsolt's behavioral despair (Forced Swimming Test: FST) and Steru's behavioral despair (Tail Suspension Test: TST) [14,15]. Because the monoaminergic system is one of the most important targets in the pathophysiology and therapies for depression [16,17], in this study we used 2 behavioural models to investigate the possible monoaminergic antidepressant effects of 2 compounds, designated 3d and 3h.

Materials and Methods
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Chemistry
Melting points were determined in open capillary tubes and were uncorrected. IR spectra were recorded (in KBr) on a FT-IR1730 (Bruker, Switzerland), ^{1}H-NMR and ^{13}C-NMR spectras were measured on an AV-300 (Bruker, Switzerland), and all chemical shifts were given in ppm relative to tetramethysilane. Mass spectra were measured on an HP1100LC (Agilent Technologies,
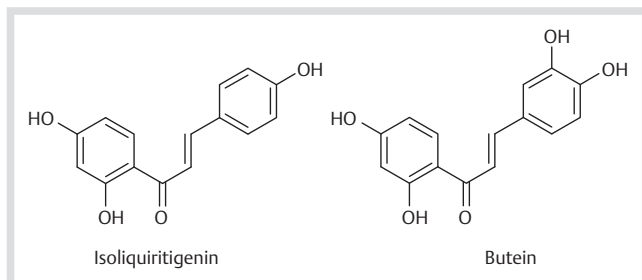
Fig. 1 Structure of Isoliquiritigenin and Butein.

USA). Elemental analysis(CHN) were performed on a Perkin Elmer 204Q CHN or a Heraeus CHN Rapid Analyzer. The major chemicals were purchased from Aldrich Chemical Corporation. All other chemicals were the analytical grade.

Synthesis of 2′-hydroxy-3-bromo-4′-methoxymethoxychalcone(2d)

To a stirred solution of 15% KOH cooled to 0 °C in an ice bath was added dropwise a solution of 2-hydroxy-4-methoxymethoxy-acetophenone (2.0 mmol) and 3-bromobenzaldehyde (4.0 mmol) in ethanol under nitrogen. The reaction mixture was kept at room for 8 h. The mixture was poured into the ice-water, adjusted to pH 2–3 with 1 M HCl, and the extracted with ethyl acetate. The organic layer was washed with water and saturated brine, dried over anhydrous Na_2SO_4[18]. After removing solvents, the product was recrystallized from ethanol. The yellow solid was obtained. Yield 83%; ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 3.46 (s, 3H, OCH_3), 5.34 (s, 2H, CH_2), 6.37–7.54 (m, 6H, Ar-H). 7.69 (d, 1H, J=15.6 Hz, H_α), 7.88 (d, 1H, J=15.6 Hz, H_β), 12.78 (s, H, -OH); IR (KBr) cm^{-1}: 3364, 1658; MS m/z: 363(M+1).

General procedure for the preparation of compounds(3a–l)

In a round-bottomed flask, to a stirred solution of chalcones 2a-l (0.25 mmol) in methanol was added dropwise 3 M HCl, the mixture was refluxed for 30 min. The solvents were removed under the reduced pressure and diluted with water, and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4. After concentration under reduced pressure, the resultant was recrystallized from ethanol[19]. The yield, melting point and spectral data of part compounds are given below.

2′,4′-dihydroxychalcone 3a: Yield 82.5%; m.p. 80.2–80.4 °C; ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 6.40–7.47(m, 3H, -C_6H_3), 7.68(d, 1H, J=15 Hz, =CH), 7.21–7.39(m, 5H, -C_6H_5), 7.78(d, 1H, J=15 Hz, =CH), 10.54 (s, H, -OH), 12.57 (s, H, -OH); ^{13}C-NMR (DMSO-d_6, 300 MHz):103.4, 106.4, 114.8, 117.1, 124.8, 127.7, 128.6, 131.3, 132.6, 134.8, 136.2, 141.2, 154.3, 166.5, 183.4; IR (KBr) cm^{-1}: 3318, 1643; Anal. Calcd. for $C_{15}H_{12}O_3$: C, 74.99; H, 5.03; O, 19.98. Found: C, 74.86; H, 4.92; O, 19.87. MS: m/z [M+1] 241.

3-Fluoro-2′,4′-dihydroxychalcone 3b: Yield 65%; m.p. 176 °C; ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 6.42–7.52(m, 3H, -C_6H_3), 7.50(d, 1H, J=15 Hz, =CH), 7.03–7.32(m, 4H, -C_6H_4), 7.92(d, 1H, J=15 Hz, =CH), 10.78 (s, H, -OH), 12.54 (s, H, -OH); ^{13}C-NMR (DMSO-d_6, 300 MHz): 103.7, 108.4, 115.5, 116.6, 123.7, 127.7, 128.6, 130.9, 133.5, 133.8, 135.5, 142.8, 162.3, 164.7, 185.7; IR (KBr) cm^{-1}: 3324, 1628; Anal. Calcd. for $C_{15}H_{11}FO_3$: C, 69.76; H,

4.29; O, 18.59. Found: C, 69.68; H, 4.20; O, 18.48. MS: m/z [M+1] 259.

3-Chloro-2′,4′-dihydroxychalcone 3c: Yield 80.6%; m.p. 166.5–166.7 °C; ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 6.36–7.57(m, 3H, -C_6H_3), 7.59(d, 1H, J=15 Hz, =CH), 7.21–7.35(m, 4H, -C_6H_4), 7.88(d, 1H, J=15 Hz, =CH), 11.12 (s, H, -OH), 13.09 (s, H, -OH); ^{13}C-NMR (DMSO-d_6, 300 MHz): 104.6, 108.9, 117.6, 122.6, 124.7, 127.6, 128.8, 130.5, 132.6, 133.6, 134.5, 142.8, 157.3, 166.7, 187.7; IR (KBr) cm^{-1}: 3314, 1630; Anal. Calcd. for $C_{15}H_{11}ClO_3$: C, 65.58; H, 4.04; O, 17.47. Found: C, 65.46; H, 4.11; O, 17.34. MS: m/z [M+1] 275.

3-Bromo-2′,4′-dihydroxychalcone 3d: Yield 73%; m.p. 127–129 °C; ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 6.45–7.54(m, 3H, -C_6H_3), 7.53(d, 1H, J=15 Hz, =CH), 7.18–7.43(m, 4H, -C_6H_4), 7.95(d, 1H, J=15 Hz, =CH), 9.78 (s, H, -OH), 11.78 (s, H, -OH); ^{13}C-NMR (DMSO-d_6, 300 MHz): 102.7, 108.6, 114.6, 117.6, 123.3, 127.5, 128.1, 130.4, 133.2, 133.6, 135.7, 141.8, 161.4, 165.7, 186.7; IR (KBr) cm^{-1}: 3329, 1629; Anal. Calcd. for $C_{15}H_{11}BrO_3$: C, 56.45; H, 3.47; O, 15.04. Found: C, 56.32; H, 3.31; O, 15.11. MS: m/z [M+1] 319.

4-Fluoro-2′,4′-dihydroxychalcone 3e: Yield 64%; m.p. 76.3–77 °C; ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 6.39–7.54(m, 3H, -C_6H_3), 7.49(d, 1H, J=15 Hz, =CH), 6.98–7.29(m, 4H, -C_6H_4), 7.96(d, 1H, J=15 Hz, =CH), 10.23 (s, H, -OH), 12.76 (s, H, -OH); ^{13}C-NMR (DMSO-d_6, 300 MHz): 101.7, 108.8, 114.5, 118.6, 122.7, 126.9, 128.3, 129.9, 131.5, 132.9, 134.8, 141.8, 158.3, 163.8, 187.7; IR (KBr) cm^{-1}: 3324, 1628; Anal. Calcd. for $C_{15}H_{11}FO_3$: C, 69.76; H, 4.29; O, 18.59. Found: C, 69.60; H, 4.18; O, 18.45. MS: m/z [M+1] 259.

4-Chloro-2′,4′-dihydroxychalcone 3f: Yield 81%; m.p. 186.1–186.9 °C (see in literature[20])

4-Bromo-2′,4′-dihydroxychalcone 3g: Yield 74%; m.p. 187–188 °C; ^{1}H NMR(300 MHz, DMSO-d_6, TMS): δ 6.42–7.58(m, 3H, -C_6H_3), 7.55(d, 1H, J=15 Hz, =CH), 7.13–7.35(m, 4H, -C_6H_4), 7.97(d, 1H, J=15 Hz, =CH), 9.98 (s, H, -OH), 12.65 (s, H, -OH); ^{13}C-NMR (DMSO-d_6, 300 MHz): 102.8, 108.9, 115.8, 117.6, 124.7, 128.7, 129.5, 132.7, 133.4, 134.7, 136.5, 141.8, 158.2, 165.6, 188.1; IR (KBr) cm^{-1}: 3330, 1631; Anal. Calcd. for $C_{15}H_{11}BrO_3$: C, 56.45; H, 3.47; O, 15.04. Found: C, 56.35; H, 3.34; O, 15.14. MS: m/z [M+1] 319.

2,6-Dichloro-2′,4′-′-dihydroxychalcone 3h: Yield 68.7%; m.p. 99.2–99.9 °C; ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 6.38–7.48(m, 3H, -C_6H_3), 7.45(d, 1H, J=15 Hz, =CH), 7.11–7.28(m, 3H, -C_6H_3), 8.13(d, 1H, J=15 Hz, =CH), 10.82 (s, H, -OH), 13.13 (s, H, -OH); ^{13}C-NMR (DMSO-d_6, 300 MHz): 102.9, 109.4, 117.3, 124.1, 127.2, 129.3, 132.4, 134.1, 135.2, 135.8, 141.4, 143.6, 158.5, 165.3, 187.8; IR (KBr) cm^{-1}: 3329, 1628; Anal. Calcd. for $C_{15}H_{10}Cl_2O_3$: C, 58.28; H, 3.26; O, 15.53. Found: C, 58.17; H, 3.20; O, 15.46. MS: m/z [M+1] 309.

4-Methyl-2′,4′-dihydroxychalcone 3i: Yield 84%; m.p. 103–104 °C; ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 2.36(s, 3H, CH_3), 6.40–7.51(m, 3H, -C_6H_3), 7.46(d, 1H, J=15 Hz, =CH), 7.01–7.22(m, 4H, -C_6H_4), 7.96(d, 1H, J=15 Hz, =CH), 10.65 (s, H, -OH), 12.72 (s, H, -OH); ^{13}C-NMR (DMSO-d_6, 300 MHz): 20.4, 102.6, 108.6, 116.6, 120.4, 123.0, 126.5, 127.2, 129.4, 131.3, 132.5,

133.3, 134.2, 140.8, 157.3, 166.7, 187.9; IR (KBr) cm^{-1}: 3326, 1629; Anal. Calcd. for $C_{16}H_{14}O_3$: C, 75.57; H, 5.55; O, 18.88. Found: C, 75.45; H, 5.41; O, 18.76. MS: m/z [M+1]255.

4-dimethylamino-2′,4′-dihydroxychalcone 3j: Yield 79%; m.p. 192℃(see in literature[20])

1-(2,4-dihydroxyphenyl)-3-(furan-2-yl)prop-2-en-1-one 3k: Yield 68.6%; m.p. 117–119 °C; ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 6.40–7.56(m, 3H, -C$_6$H$_3$), 7.55(d, 1H, J = 15 Hz, =CH), 6.75–7.84(m, 3H, -furan), 7.95(d, 1H, J = 15 Hz, =CH), 10.12 (s, H, -OH), 12.76 (s, H, -OH); ^{13}C-NMR (DMSO-d_6, 300 MHz): 103.3, 108.9, 111.5, 112.6, 117.3, 128.9, 142.7, 145.8, 155.6, 159.6, 166.2, 187.7; IR (KBr) cm^{-1}: 3328, 1623; Anal. Calcd. for $C_{13}H_{10}O_4$: C, 67.82; H, 4.38; O, 27.80. Found: C, 67.76; H, 4.29; O, 27.75. MS: m/z [M+1] 231.

1-(2,4-dihydroxyphenyl)-3-(naphthalen-1-yl)prop-2-en-1-one 3l: Yield 84.7%; m.p. 191–192 °C; ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 6.42–7.59(m, 3H, -C$_6$H$_3$), 7.48(d, 1H, J = 15 Hz, =CH), 7.23–7.73(m, 7H, -naphthalen), 7.97(d, 1H, J = 15 Hz, =CH), 10.32 (s, H, -OH), 12.78 (s, H, -OH); ^{13}C-NMR (DMSO-d_6, 300 MHz): 103.2, 108.5, 116.8, 120.6, 123.4, 125.4, 126.8, 127.4, 128.8, 131.5, 132.6, 133.5, 135.7, 136.8, 142.7, 161.3, 166.8, 188.6; IR (KBr) cm^{-1}: 3331, 1629; Anal. Calcd. for $C_{19}H_{14}O_3$: C, 78.61; H, 4.86; O, 16.53. Found: C, 78.57; H, 4.78; O, 16.43. MS: m/z [M+1] 291.

Pharmacology
Animals
Male Kunming mice weighing 20–24 g (Laboratory of Animal Research, College of Pharmacy, Yanbian University, Yanji, Jilin, China) were used in this study. Mice were housed collectively in groups of 10 in polycarbonate cages. They were maintained on a 12 h light/dark cycle in a temperature controlled (22–25 °C) laboratory. Food and water were available ad libitum. All procedures used in the present study were in accordance with the Guide for the Care and Use of Laboratory Animals as adapted by the NIH (Bethesda, MD, USA). All efforts were made to minimize animal suffering and to reduce the number of animals used.

Forced swimming test (FST)
The FST used was the same as described in detail elsewhere Porsolt [14, 15]. The synthesized compounds were screened for their antidepressant activity. Local breed, male Kunming mice (20–24 g) were used in the forced swimming test under standard conditions with free access to food and water. They were housed in groups of 6, In the experiment, mice were assigned into different groups (n = 10 for each group). The synthesized compounds (10 mg/kg) and fluoxetine as a reference antidepressant drug (10 mg/kg) were dissolved in DMSO through injected intraperitoneally (*ip*) in a standard volume of 0.05 mL/20 g body weight, 30 min prior to the test. Briefly, mice were individually placed in a glass cylinder (25 cm in height, 10 cm in diameter filled with 10-cm high of water 22–25 °C). Each mouse was given a 6-min swimming test, and the duration of immobility was observed and measured during the final 4-min interval of the test. All test swim sessions were recorded by a video camera positioned directly above the cylinder. 2 competent observers, who were unaware of the treatment each mouse had received, scored the videotapes. Immobility period was regarded as the time spent by the mouse floating in the water without struggling and mak-

ing only those movements necessary to keep its head above the water. Following swimming sessions, they were then towel dried and returned to their housing condition. The animals were used only once in this test. All FSTs were performed between 12:00 a.m. and 19:00 p.m.

Tail suspension test (TST)
The TST was conducted as previously described [21]. Briefly, the synthesized compounds (10 mg/kg) and fluoxetine as a reference antidepressant drug (10 mg/kg) were dissolved in DMSO through injected intraperitoneally (*ip*) in a standard volume of 0.05 mL/20 g body weight, 30 min prior to the test. Then, mice were individually suspended by tail with clamp (2 cm from the tip of the end) in a box (25 × 25 × 30 cm) with the head 5 cm to the bottom. Testing was carried out in a darkened room with minimal background noise. All animals were suspended for total 6 min, and the duration of immobility was observed and measured during the final 4-min interval of the test. All test sessions were recorded by a video camera positioned directly above the box. 2 competent observers blind to treatment scored the videotapes. Mice consider immobile only when they hung passively and completely motionless. The animals were used only once in this test. All TSTs were performed between 12:00 a.m. and 16:00 p.m.

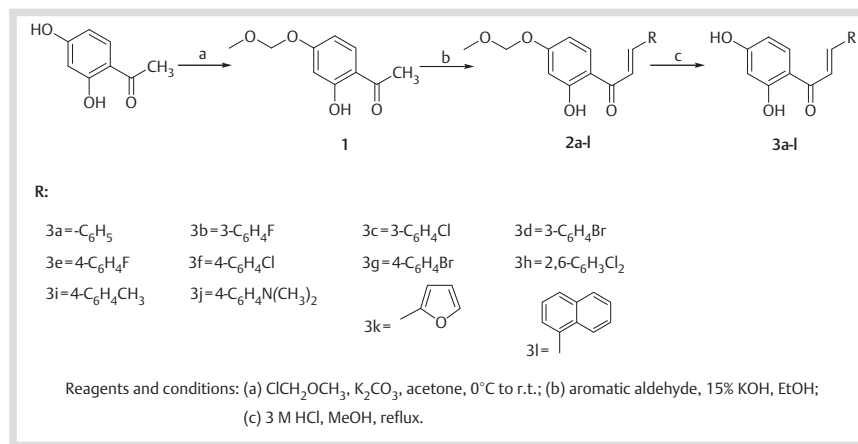### Hydroxytryptophan(5-HTP) induced mouse head-twitch test
To investigate whether the serotonergic system was involved in the antidepressant-like effect of compounds 3d and 3 h, we performed a 5-HTP induced head-twitch test [22,23]. Totally 24 male Kunming (20–25 g) mice were randomly chosen and divided into 3 groups normal control group, group of compounds 3d and 3 h, and positive control (fluoxetine). Mice were administered a i.p. injection with compounds 3d and 3 h (30 mg/kg), fluoxetine (30 mg/kg) 60 min before 5-HTP (100 mg/kg, i.p.). Immediately after the second injection, mice were placed into plastic cages. 10 min later, the cumulative number of head twitches (rapid movements of the head with little or no involvement of the trunk) was recorded for 6 min. All test sessions were recorded by a video camera. The animals were used only once in this test, the head-twitch tests were performed between 10:00 a.m. and 12:00.
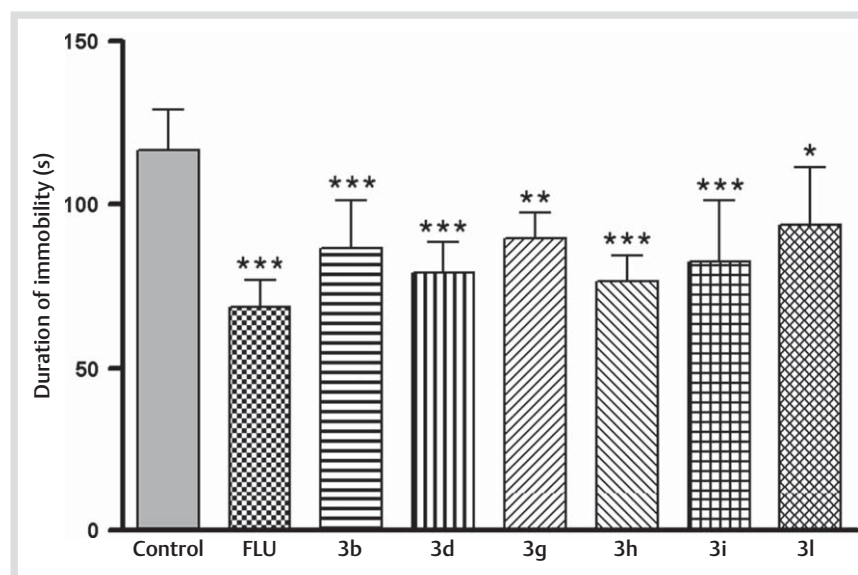
Yohimbine toxicity potentiation test
To trveal whether the noradrenergic system is involved in the antidepressant-like effect of the compounds 3d and 3 h, the yohimbine toxicity potentiation test was performed [22].Totally 24 male Kunming (20–25 g) mice were randomly chosen and divided into 3 groups: normal control group, group of the compounds 3d and 3 h, and positive control group (clomipramine), clomipramine (30 mg/kg)(i.p.), 1 h prior to yohimbine administration (20 mg/kg, s.c.). The number of dead mice was calculated during a 20 h period after the injection of yohimbine.

Statistical analysis
Results are expressed as mean ± S.E.M.; n represents the number of animals. Data obtained from pharmacological experiments were analyzed with 1-way analysis of variance (ANOVA) followed by Dunnet's post hoc test, using Pharmacologic Calculation System Version 4.1.(Microcomputer Specialists). A *p*-value of less than 0.05 was considered statistically significant.

Fig. 2 The synthesis route of target compounds 3a–l.

R:

3a = -C₆H₅   3b = 3-C₆H₄F   3c = 3-C₆H₄Cl   3d = 3-C₆H₄Br
3e = 4-C₆H₄F   3f = 4-C₆H₄Cl   3g = 4-C₆H₄Br   3h = 2,6-C₆H₃Cl₂
3i = 4-C₆H₄CH₃   3j = 4-C₆H₄N(CH₃)₂

3k =

3l =

Reagents and conditions: (a) ClCH₂OCH₃, K₂CO₃, acetone, 0°C to r.t.; (b) aromatic aldehyde, 15% KOH, EtOH; (c) 3 M HCl, MeOH, reflux.

Fig. 3 Effects of the acute treatments with the compounds (10 mg/kg) and fluoxetine (FLU, 10 mg/kg i.p.) on the immobility time in FTS. Each column represents the mean ± S.E.M. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ as compared with control (all comparisons were made by ANOVA followed by Dunnett's test).

Results and Discussion

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Chemistry

The target compounds 3a–l were synthesized as outlined ● **Fig. 2**. Compound 1 was prepared as reported previously in the literature [13]. The intermediates 2a–l were prepared by the Claisen-Schmidt condensation reaction from compound 1, with appropriate aromatic aldehydes, protected as chloromethyl methyl ether in EtOH at room temperature in high yields [19, 24]. Then 2,4-dihydroxychalcone derivatives (3a–l) were obtained with 3 M HCl in methanol in good yield [25] (● **Fig. 2**). The structures of these compounds were confirmed by IR, ¹H-NMR, or ¹³C-NMR, mass spectrometry, and elementary analysis.

Pharmacological evaluations

The forced swimming test (FST) and the tail suspension test (TST) were designed by Porsolt et al. and Steru L et al. as a primary screening test for antidepressants. FST and TST are behavioral tests used to predict the efficacy of antidepressant treatments [14, 15]. They are two of the best models for this purpose for several reasons. They are used effectively in predicting the activity of a wide variety of antidepressants, and are low-cost, fast and reliable models to test potential antidepressant treatments with strong predictive validity. The immobility time observed in the test reflects a state of lowered mood or hopelessness in the animal. Thus, these animal models are the most widely used tools for preclinical screening of putative antidepressant agents, and have good predictive value for antidepressant potency in humans [26, 27].

In the present study, 12 compounds were described for their antidepressant activities in mice using the FST. The pharmacological tests revealed that 6 compounds showed good antidepressant activities. Acute treatment with compounds 3b, 3d, 3 g, 3 h, 3i, and 3 l significantly promoted a decrease in the immobility time in the FST at 10 mg/kg, as depicted in ● **Fig. 3** (control = 116.7 ± 12.3; 3b = 86.7 ± 14.5; 3d = 79.3 ± 9.2; 3g = 89.8 ± 7.8; 3h = 76.7 ± 7.9; 3i = 82.5 ± 18.7; 3l = 93.8 ± 17.7; fluoxetine = 68.6 ± 8.3). The immobility time of mice treated with compounds 3a, 3c, 3e, 3f, 3j, and 3k did not statistically differ from control values, as shown in ● **Fig. 4** (control = 116.7 ± 12.3; 3a = 96.3 ± 31.2; 3c = 95.2 ± 12.6; 3e = 111.7 ± 10.5; 3f = 105.8 ± 11.6; 3j = 97.5 ± 15.6; 3k = 98.5 ± 6.5; fluoxetine = 68.6 ± 8.3). Among, 2 compounds, 3d (3-bromo-2′,4′-dihydrox-ychalcone) and 3h (2,6-dichloro-2′,4′-dihydroxychalcone) were found to be the most potent, and significantly reduced the duration of immobility times at the 10 mg/kg dose level when compared with the control ($p < 0.001$).

Generally, the activity of an organic compound is markedly increased after the introduction of a halogen atom. Therefore, in this paper some halogen-substituted derivatives were designed and synthesized. Analyzing the antidepressant activity of the synthesized compounds 3a–l, the following SAR was derived.
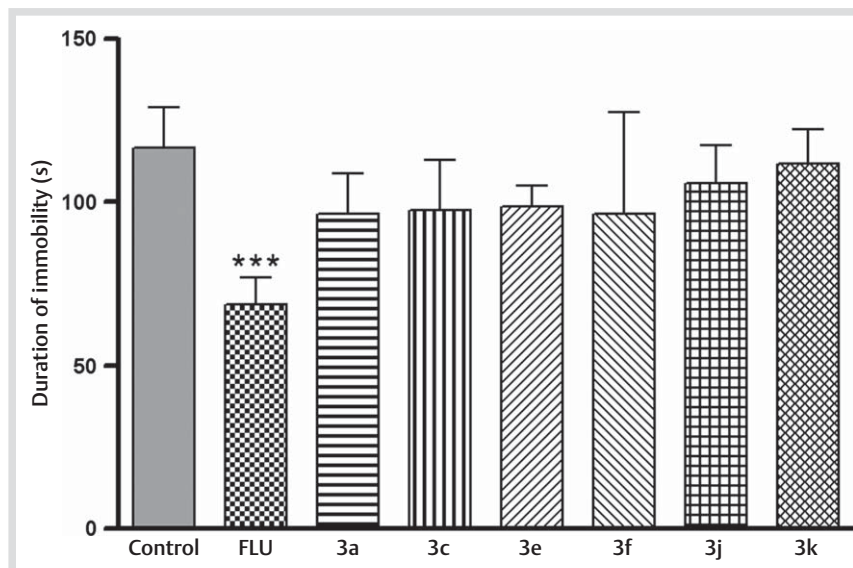
Fig. 4 Effects of the acute treatments with the compounds(10 mg/kg) and fluoxetine (FLU, 10 mg/kg i.p.) on the immobility time in FST. Each column represents the mean ± S.E.M. *p < 0.05 as compared with control (all comparisons were made by ANOVA followed by Dunnett's test).
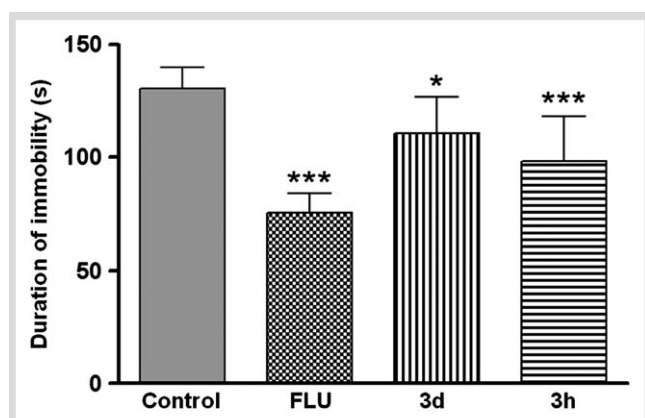
Fig. 5 Immobility time of the compounds 3d and 3h in mouse TST after intraperitoneal administration in mice (i.p.) at 10 mg/kg. Data are expressed as mean ± S.E.M. (n = 10). Statistical analysis of the data was carried out by one-way analysis of variance followed by the t-test. *p < 0.05, ***p < 0.001 vs. control.

Table 1 Effects of compound 3d, 3h and fluoxetine on the number of 5-HTP induced head twitches in mice.

Compounds	Dose (mg/kg)	5-HTP (mg/kg)	Number of head twitchs
3d	30	100	36.5 ± 12.4*
3h	30	100	28.7 ± 10.2*
Fluoxetine	30	100	42.7 ± 11.3**
Control	–	100	15.7 ± 5.2

Values represent the mean ± S.E.M. (n = 10)

*Statistical significance compared with control (Dunnet's test: *p < 0.05, **p < 0.01)

The halogen-substituted derivatives (3b–h), compounds except 3c, 3e, and 3f displayed antidepressant activity in the FST. The Br atom contributed more to antidepressant activity than the F and Cl atoms. The rank order of activity of the halogen-substituted compounds was Br > F > Cl. Among the compounds, 3d and 3h showed the most antidepressant activity, with reduced the immobility time by 32.05% and 34.33%, respectively, at 10 mg/kg. Next, the position of the halogen substituted on the phenyl ring greatly influenced the antidepressant activity, compared with compounds with different Cl-substituted positions on the

phenyl ring. Their rank order of activity was 2,6-Cl$_2$ > 3-Cl > 4-Cl. Regarding the electron-donor group contributing to the antidepressant activity (Fig. 3), their contribution order was 4-CH$_3$ > 4-N(CH$_3$)$_2$ > -H. In addition, 2 aromatic heterocyclic compounds (3k, 3l) were also designed and synthesized. The pharmacological test revealed that compound 3l possessed good antidepressant activity at the 10 mg/kg dose level when compared with the control (p < 0.01) (Fig. 3, 4).

As shown in Fig. 5, immobility in the FST was significantly reduced after treatment with compounds 3d, 3h with better activities, similar to fluoxetine, indicating a significant antidepressant-like effect. The decrease in immobility time in the TST was similar to that seen in the FST. Compounds 3d and 3h showed good antidepressant activity and promoted a significant decrease in the immobility time at 10 mg/kg (control = 130.5 ± 9.6; 3d = 112.8 ± 16.0; 3h = 98.3 ± 19.8; fluoxetine = 75.6 ± 8.3). Both the FST and the TST are the accepted stress models of depression. The immobility has been shown to reflect a state of 'behavioral despair and variants' or 'failure to adapt to stress' [26]. The immobility displayed in both of these behavioural despair models has been hypothesized to reflect depressive disorders in human. There was a significant correlation between clinical potency and the potency of antidepressants in both models. Compounds 3d and 3h significantly produced antidepressant-like activity in both the FST and TST in mice, which indicates that compounds 3d and 3 h possess some antidepressant effects.

Finally, 2 behavioural models were used to investigate the possible monoaminergic participation in the antidepressant activity. Compounds 3d and 3h, as the most active compounds, were chosen for the behavioural tests. Compounds 3d and 3h significantly increased the cumulative number of head twitches (p < 0.05 vs. control) in the 5-HTP-induced mouse head-twitch test, whereas it enhanced the mouse lethality (p < 0.05 vs. control) induced by yohimbine. The results indicate that the serotonergic, but not the noradrenergic system, was involved in the antidepressant-like effect of compounds 3d and 3h (Table 1, 2).

Several lines of evidence indicate that serotonergic, dopaminergic, and noradrenergic neurotransmissions are involved in the expression of an antidepressant-like effect in the behavioural despair models of depression [27]. Many antidepressant drugs exert their effects by modulating these neurotransmission systems [28,29]. The 5-HTP-induced mouse head-twitch test is an

Table 2 Effects of compound 3d, 3h and clomipramine on yohimbine induced lethality in mice.

Compounds	Dose (mg/kg)	yohimbine (mg/kg)	Lethality	
			Total	Died
3d	30	20	10	4
3h	30	20	10	3
Clomi-pramine	30	20	10	8
Control	–	20	10	2

Values represent the mean ± S.E.M. ($n = 10$)

*Statistical significance compared with control(Fisher's exact: *$p < 0.05$)

effective method to evaluate serotonergic effects of drugs in vivo [30]. It has been generally accepted that the number of head twitches represent the level of 5-hydroxytryptamine (5-HT) in the synapses. Yohimbine is an a_2-adrenergic released by its antagonistic action on the presynaptic a_2-adrenoceptor [31]. The yohimbine toxicity potentiation test is usually used for the evaluation of noradrenergic effect of antidepressants [32].

Conclusions
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In conclusion, twelve 2,4-dihydroxychalcone compounds were synthesized and evaluated for their antidepressant activity using the FST and TST tests. 6 compounds showed antidepressant activity at a dose of 10 mg/kg i.p. Among the compounds, 3d and 3h had the most antidepressant activity, and significantly reduced the duration of immobility times when compared with the control ($p < 0.001$) in the FST. Further studies are needed to reveal the mechanism of the antidepressant-like effect of compounds 3d and 3h.

Acknowledgments
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Conflict of Interest
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We declare that we have no conflict of interest with respect to this study.

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