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Research Article

# Design, Synthesis, and Potential Antidepressant-like Activity of 7-prenyloxy-2,3-dihydroflavanone Derivatives

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**A series of 7-prenyloxy-2, 3-dihydroflavanone derivatives were synthesized and screened for their antidepressant-like activity. Among them, it was observed that compounds 5j and 5k were found to be the most antidepressant-like activity. In addition, it was found that compounds 5j and 5k significantly increased the concentrations of the main neurotransmitters 5-HT and NE in the hippocampus, hypothalamus, and cortex. Compounds 5j and 5k also significantly increased the contents of 5-HIAA in the hippocampus and cortex, shut down 5-HT metabolism compared with mice treated with stress vehicle. These results suggested that compounds 5j and 5k displayed potent antidepressant-like properties that were mediated via neurochemical systems.**

**Key words:** 7-Prenyloxy-2,3-dihydroflavanon, antidepressant activity, FST, neurotransmitters, TST

**Abbreviations:** 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; CNS, central nervous system; DA, dopamine; FLU, fluoxetine; FST, forced swimming test; NE, noradrenaline; TST, tail suspension test.

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Depression is one of the most prevalent psychopathologies. Symptoms of depression include lowered mood and reduced pleasure and interest. It is expected to become the second leading cause of disease-related disability by 2020 (1,2). Although there are many effective antidepressants available today, the current remission of therapy is often inadequate with unsatisfactory results in about one-third of all subjects treated (3,4). Therefore, the discovery of new antidepressant drugs with fewer side-effects and better efficacy is necessary.

It reports that gamma-amino butyric acid and brain-derived neurotrophic factor are associated with depressive disorders, but a metabolic disorder of monoamine neurotransmitters in the CNS is believed to be the main biochemical cause of depression. The monoamine hypothesis supposed that depression was a result of the depletion of NE, 5-HT, and DA in addition to activation of monoamine oxidase in the CNS. Depression can be alleviated by increasing the levels of monoamine neurotransmitters in the CNS. NE, 5-HT, and DA play important roles in mediating behavioral effects of antidepressant drugs (5,6).

In recent years, it has been reported that flavonoids possess antidepressant-like activities (7–12). Grosso *et al.* (13) reported that flavonoids displayed the antioxidant activities thus preventing oxidative stress, which is believe to be one of the causes of disorders affecting the central nervous system. Moreover, the benefits of flavonoids have been found in the diet in the treatment of depression, Alzheimer's disease, Parkinson's disease, and epilepsy. Pathak *et al.* (14) also reported that the natural polyphenols modulate monoaminergic neurotransmission in the brain and thus possess antidepressant-like activity in animal models of depression, such as amentoflavone and rutin. Flavanones are a group of flavonoids, a previous study from our laboratory found that 2′-hydroxy- 4′-prenyloxychalcone derivatives(I) and 5,7-dihydroxyflavanone derivatives(II) demonstrated the antidepressant effect in the FST and TST in mice (15,16) (Figure 1). The prenyl fragment is featured widely in many drugs and natural products (17,18). The underlying hypothesis was that introduction of a substituted prenyl group would increase the lipophilic property of compounds and increase their permeability across the blood–brain barrier, which would probably enhance their antidepressant-like activity. So, in this study, we introduce the prenyl group to the 7 position on compounds II, and a series of 7-prenyloxy-2,3-dihydroflavanone



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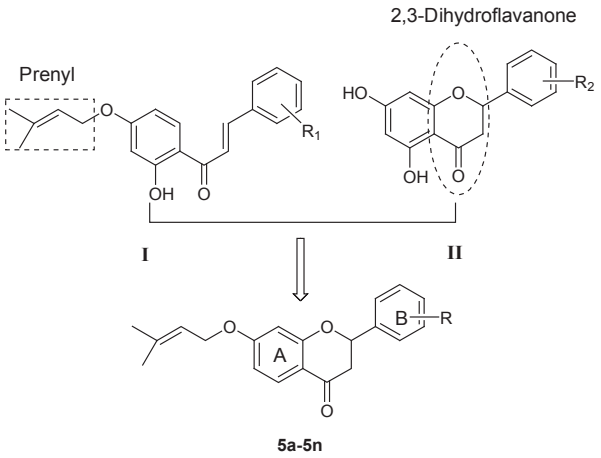



**Figure 1:** Design of compounds **5a**–**5n**.

derivatives were obtained and investigated to determine their antidepressant-like activities. In addition, the probable mechanism of antidepressant-like activity was explored by analyzing monoamine neurotransmitters in the mice brain.

## Methods and Materials

### *Chemistry*

Melting points were determined in open capillary tubes and were uncorrected. IR spectra were recorded (in KBr) on a FT-IR1730 (Bruker, Karlsruhe, Germany), $^{1}$H-NMR and $^{13}$C-NMR spectra were measured on an AV-300 (Bruker, Rheinstetten, Germany), and all chemical shifts were given in ppm relative to tetramethysilane. High-resolution mass spectra were measured on an MALDI-TOF/TOF mass spectrometer (Bruker Daltonik, Bremen, Germany). The major chemicals were purchased from Aldrich Chemical Corporation (Shanghai, China). All other chemicals were of the analytical grade.

For synthesis of compound **1**, compounds **2a**–**2n**, **3a**–**3n**, and **4a**–**4n**, see in references [Xie *et al.* (15); Guan *et al.* (19; Guan *et al.* (20)].

### *Synthesis of 7-prenyloxy-2,3-dihydroflavanone derivatives (5a–5n)*

A stirred solution of **4a**–**4n** (0.35 mmol) and sodium acetate in 30 mL methanol, anhydrous $K_2CO_3$ (1.40 mmol) was added to the above solution, the mixture was stirred at 50 °C for 1 h, and then, prenyl bromide (0.50 mmol and 4 mL anhydrous acetone) was added slowly to the above mixture. The mixture was refluxed for 5–8 h, after the completion of the reaction (monitored by TLC). Filtered $K_2CO_3$ was washed with acetone. After concentration under reduced pressure, the resultant was recrystallized from ethanol. The yield, melting point, and spectral data of each compound are given as below.

### 7-prenyloxy-2,3-dihydroflavanone (5a)

Yield: 66.7%. $^{1}$H NMR($CDCl_3$, 300 MHz) $\delta$: 1.69(s, 3H, -$CH_3$), 1.74(s, 3H, -$CH_3$), 2.73(d, 2H, $CH_2$), 2.97(t, 1H, -CH), 4.51 (d, 2H, -$CH_2$), 5.46(t, 1H, =CH), 6.48-6.56(m, 3H, -$C_6H_3$), 7.34-7.94(m, 5H, -$C_6H_5$). $^{13}$C NMR($CDCl_3$, 75 MHz) $\delta$: 18.37, 25.86, 44.11, 65.30, 79.67, 101.76, 110.65, 114.69, 119.12, 126.48, 128.38, 128.68, 128.78, 138.42, 139.10, 163.39, 165.34, 189.95. IR (KBr) cm$^{-1}$: 2928, 2913, 1676, 1446, 1261, 960. ESI-HRMS calcd for $C_{20}H_{20}O_3^+$([M + H]$^+$): 309.1412; found: 309.1426.

### 2′-Fluoro-7-prenyloxy-2,3-dihydroflavanone (5b)

Yield: 66.9%. $^{1}$H NMR ($CDCl_3$, 300 MHz) $\delta$: 1.68(s, 3H, -$CH_3$), 1.73(s, 3H, -$CH_3$), 2.86(d, 2H, -$CH_2$), 3.01(t, 1H, -CH), 4.51(d, 2H, -$CH_2$), 5.39(t, 1H, =CH), 6.48–6.58 (m, 3H, -$C_6H_3$), 7.07–7.91(m, 4H, -$C_6H_4$). $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$: 18.36, 25.85, 42.99, 65.33, 74.09, 101.73, 110.85, 114.57, 115.63, 115.91, 119.06, 124.79, 128.00, 128.47, 130.48, 130.59, 138.48, 163.31, 165.36, 189.56. IR (KBr) cm$^{-1}$: 2927, 2915, 1675, 1444, 1260, 961. ESI-HRMS calcd for $C_{20}H_{19}FO_3^+$([M + H]$^+$):327.1318; found: 327.1322.

### 3′-Fluoro-7-prenyloxy-2,3-dihydroflavanone (5c)

Yield: 65.7%. $^{1}$H NMR($CDCl_3$, 300 MHz) $\delta$: 1.68(s, 3H, -$CH_3$), 1.73(s, 3H, -$CH_3$), 2.75(d, 2H, -$CH_2$), 2.92(t, 1H, -CH), 4.50(d, 2H, -$CH_2$), 5.39(t, 1H, =CH), 6.47–6.56 (m, 3H, -$C_6H_3$), 6.99–7.80(m, 4H, -$C_6H_4$). $^{13}$C NMR ($CDCl_3$, 75 MHz)$\delta$: 18.34, 25.85, 44.10, 65.31, 101.72, 110.80, 113.13, 113.42, 114.63, 115.25, 115.54, 118.93, 122.02, 128.41, 130.62, 138.60, 141.77, 163.12, 165.41, 189.61. IR (KBr) cm$^{-1}$: 2930, 2917, 1677, 1445, 1265, 960. ESI-HRMS calcd for $C_{20}H_{19}FO_3^+$([M + H]$^+$): 327.1318; found: 327.1324.

### 4′-Fluoro-7-prenyloxy-2,3-dihydroflavanone (5d)

Yield: 76.5%. $^{1}$H NMR($CDCl_3$, 300 MHz)$\delta$: 1.69(s, 3H, -$CH_3$), 1.74(s, 3H, -$CH_3$), 2.75(d, 2H, -$CH_2$), 3.00(t, 1H, -CH), 4.53(d, 2H, -$CH_2$), 5.40(t, 1H, =CH), 6.50–6.54(m, 3H, -$C_6H_3$), 7.09–7.71(m, 4H, -$C_6H_4$). $^{13}$CNMR($CDCl_3$, 75 MHz)$\delta$: 18.40, 25.87, 44.03, 101.82, 110.74, 114.68, 115.47, 115.76, 119.25, 129.37, 128.77, 135.32, 138.34, 149.99, 163.32, 164.10, 165.33, 189.85. IR (KBr) cm$^{-1}$: 2925, 2920, 1674, 1446, 1263, 962. ESI-HRMS calcd for $C_{20}H_{19}FO_3^+$([M +H]$^+$):327.1318; found: 327.1314.

### 2′-Chloro-7-prenyloxy-2,3-dihydroflavanone (5e)

Yield: 65.0%. $^{1}$H NMR($CDCl_3$, 300 MHz)$\delta$: 1.69(s, 3H, -$CH_3$), 1.74(s, 3H, -$CH_3$), 2.78(d, 2H, -$CH_2$), 2.81(t, 1H, -CH), 4.53(d, 2H, -$CH_2$), 5.40(t, 1H, =CH), 6.51–6.60 (m, 3H, -$C_6H_3$), 7.30–7.99(m, 4H, -$C_6H_4$). $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$: 18.39, 25.87, 42.87, 65.37, 76.64, 98.04, 101.82, 110.93, 114.58, 119.14, 127.74, 128.48, 129.75, 130.03, 131.55, 136.63, 138.44, 163.33, 165.38,

189.45. IR (KBr) $cm^{-1}$: 2930, 2917, 1673, 1446, 1261, 960. ESI-HRMS calcd for $C_{20}H_{19}ClO_3^+$([M + H]$^+$):343.1023; found: 343.1029.

### 3′-Chloro-7-prenyloxy-2,3-dihydroflavanone (5f)

Yield: 75.0%. $^1$H NMR($CDCl_3$, 300 MHz): $\delta$ 1.75(s, 3H, -$CH_3$), 1.80(s, 3H, -$CH_3$), 2.84(d, 2H, -$CH_2$), 2.93(t, 1H, -CH), 4.56 (d, 2H, -$CH_2$), 5.47(t, 1H, =CH), 6.51–6.63(m, 3H, -$C_6H_3$), 7.37–7.82(m, 4H, -$C_6H_4$). $^{13}$C NMR($CDCl_3$, 75 MHz) $\delta$: 23.02, 30.56, 48.92, 70.03, 83.65, 106.35, 115.64, 119.30, 123.40, 129.10, 131.01, 133.25, 134.95, 139.23, 143.69, 145.74, 165.01, 167.84, 170.23, 194.47. IR (KBr) $cm^{-1}$: 2929, 2919, 1676, 1445, 1263, 962. ESI-HRMS calcd for $C_{20}H_{19}ClO_3^+$([M + H]$^+$): 343.1023; found: 343.1025.

### 4′-Chloro-7-prenyloxy-2,3-dihydroflavanone (5g)

Yield: 75.3%. $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$: 1.69(s, 3H, -$CH_3$), 1.73(s, 3H, -$CH_3$), 2.76(d, 2H, -$CH_2$), 2.92(t, 1H, -CH), 4.51 (d, 2H, -$CH_2$), 5.45(t, 1H, =CH), 6.45–6.56(m, 3H, -$C_6H_3$), 7.33–7.83(m, 4H, -$C_6H_4$). $^{13}$C NMR($CDCl_3$, 75 MHz) $\delta$: 18.35, 25.85, 44.06, 65.31, 78.10, 101.74, 110.22, 110.76, 114.63, 118.97, 123.10, 127.98, 128.41, 128.84, 133.98, 137.81, 138.57, 163.20, 165.40, 189.69. IR (KBr) $cm^{-1}$: 2930, 2915, 1675, 1443, 1269, 960. ESI-HRMS calcd for $C_{20}H_{19}ClO_3^+$([M + H]$^+$):343.1023; found: 343.1028.

### 2′-Bromo-7-prenyloxy-2,3-dihydroflavanone (5h)

Yield: 73.2%. $^1$H NMR($CDCl_3$, 300 MHz) $\delta$: 1.57(s, 3H, -$CH_3$), 1.63(s, 3H, -$CH_3$), 2.73(d, 2H, -$CH_2$), 2.97(t, 1H, -CH), 4.39(d, 2H, -$CH_2$), 5.30(t, 1H, =CH), 6.35–7.08 (m, 3H, -$C_6H_3$), 7.11–7.69(m, 4H, -$C_6H_4$). $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$: 18.14, 25.70, 43.05, 65.17, 77.81, 101.50, 110.80, 114.48, 118.52, 121.29, 127.43, 127.98, 128.52, 129.85, 132.82, 138.16, 138.91, 163.25, 165.36, 189.77. IR (KBr) $cm^{-1}$: 2928, 2919, 1676, 1444, 1263, 961. ESI-HRMS calcd for $C_{20}H_{19}BrO_3^+$([M + H]$^+$): 386. 0518; found: 387.0511.

### 3′-Bromo-7-prenyloxy-2,3-dihydroflavanone (5i)

Yield: 85.7%, mp: $^1$H NMR($CDCl_3$, 300 MHz) $\delta$: 1.76(s, 3H, -$CH_3$), 1.83(s, 3H, -$CH_3$), 2.87(d, 2H, -$CH_2$), 2.99(t, 1H, -CH), 4.57(d, 2H, -$CH_2$), 5.48(t, 1H, =CH), 6.52–6.67 (m, 3H, -$C_6H_3$), 7.28–7.89(m, 4H, -$C_6H_4$). $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$: 18.27, 25.84, 44.34, 65.32, 79.05, 101.55, 111.05, 114.62, 118.61, 122.93, 124.65, 128.76, 129.26, 130.41, 131.77, 129.24, 141.16, 163.15, 165.61, 189.95. IR (KBr) $cm^{-1}$: 2927, 2912, 1675, 1445, 1255, 961. ESI-HRMS calcd for $C_{20}H_{19}BrO_3^+$([M + H]$^+$): 386.0518; found: 387.0521.

### 4′-Bromo-7-prenyloxy-2,3-dihydroflavanone (5j)

Yield: 77.1%. $^1$H NMR($CDCl_3$, 300 MHz) $\delta$: 1.67(s, 3H, -$CH_3$), 1.73(s, 3H, -$CH_3$), 2.75(d, 2H, -$CH_2$), 2.90(t, 1H, -CH), 4.49(d, 2H, -$CH_2$), 5.41(t, 1H, =CH), 6.43–6.55 (m, 3H, -$C_6H_3$), 7.33–7.75(m, 4H, -$C_6H_4$). $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$: 18.33, 25.85, 44.03, 65.30, 78.93, 101.70, 110.77, 114.60, 118.90, 122.28, 128.20, 128.42, 131.79, 138.23, 138.63, 159.76, 163.16, 165.40, 189.66. IR (KBr) $cm^{-1}$: 2928, 2913, 1676, 1444, 1261, 960. ESI-HRMS calcd for $C_{20}H_{19}BrO_3^+$([M + H]$^+$): 386.0518; found: 387.0522.

### 2′,4′-Dichloro-7-prenyloxy-2,3-dihydroflavanone (5k)

Yield: 66.7%. $^1$H NMR($CDCl_3$, 300 MHz) $\delta$: 1.67(s, 3H, -$CH_3$), 1.72(s, 3H, -$CH_3$), 2.77(d, 2H, -$CH_2$), 2.79(t, 1H, -CH), 4.49(d, 2H, -$CH_2$), 5.38(t, 1H, =CH), 6.44-6.57(m, 3H, -$C_6H_3$), 7.31–7.74(m, 3H, -$C_6H_3$). $^{13}$C NMR($CDCl_3$, 75 MHz) $\delta$: 18.30, 25.82, 42.85, 65.32, 76.23, 101.69, 110.99, 114.50, 118.76, 127.86, 128.60, 129.34, 132.21, 134.57, 135.49, 138.79, 160.95, 163.14, 165.45, 189.34. IR (KBr) $cm^{-1}$: 2927, 2915, 1675, 1446, 1259, 962. ESI-HRMS calcd for $C_{20}H_{18}Cl_2O_3^+$([M + H]$^+$): 377.0633; found: 377.0637.

### 2′,6′-Dichloro-7-prenyloxy-2,3-dihydroflavanone (5l)

Yield: 76.3%. $^1$H NMR($CDCl_3$, 300 MHz) $\delta$: 1.69(s, 3H, -$CH_3$), 1.74(s, 3H, -$CH_3$), 2.54(d, 2H, -$CH_2$), 3.59(t, 1H, -CH), 4.55(d, 2H, -$CH_2$), 5.40(t, 1H, =CH), 6.60–6.63 (m, 3H, -$C_6H_3$), 7.36–7.74(m, 3H, -$C_6H_3$). $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$: 18.26, 26.07, 43.50, 65.19, 76.65, 103.81, 110.93, 113.72, 114.31, 115.32, 119.33, 128.60, 130.11, 131.21, 132.19, 134.89, 138.38, 163.09, 165.44, 188.95. IR (KBr) $cm^{-1}$: 2928, 2916, 1674, 1444, 1265, 960. ESI-HRMS calcd for $C_{20}H_{18}Cl_2O_3^+$([M + H]$^+$): 377. 0633; found: 377.0630.

### 4′-Methyl-7-prenyloxy-2,3-dihydroflavanone (5m)

Yield: 82.6%. $^1$H NMR($CDCl_3$, 300 MHz) $\delta$: 1.67(s, 3H, -$CH_3$), 1.73(s, 3H, -$CH_3$), 2.30(s, 3H, -$CH_3$), 2.72(d, 2H, -$CH_2$), 2.89(t, 1H, -CH), 4.47(d, 2H, -$CH_2$), 5.40(t, 1H, =CH), 6.41–6.45(m, 3H, -$C_6H_3$), 7.14-7.72(m, 4H, -$C_6H_4$). $^{13}$C NMR($CDCl_3$, 75M Hz) $\delta$: 18.29, 20.92, 21.22, 25.82, 44.10, 45.15, 65.23, 79.67, 101.64, 110.58, 114.63, 118.86, 126.30, 128.41, 129.39, 135.91, 138.38, 138.67, 163.45, 165.37, 190.27. IR (KBr) $cm^{-1}$: 2930, 2917, 1677, 1445, 1263, 961. ESI-HRMS calcd for $C_{21}H_{22}O_3^+$ ([M + H]$^+$): 323.1569; found: 323.1572.

### 4′-Methoxy-7-prenyloxy-2,3-dihydroflavanone (5n)

Yield: 74.0%. $^1$H NMR($CDCl_3$, 300 MHz) $\delta$: 1.69(s, 3H, -$CH_3$), 1.74(s, 3H, -$CH_3$), 2.70(d, 2H, -$CH_2$), 3.04(t, 1H, -CH), 4.52(d, 2H, -$CH_2$), 5.43(t, 1H, =CH), 6.49–6.92 (m, 3H, -$C_6H_3$), 7.38–7.70(m, 4H, -$C_6H_4$). $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$: 18.40, 25.86, 40.93, 55.39, 65.33, 99.97, 101.82, 110.58, 114.11, 114.70, 115.89, 119.31,

127.15, 128.21, 128.34, 131.14, 138.28, 159.80, 163.50, 165.40, 190.21. IR (KBr) $cm^{-1}$: 2929, 2915, 1671, 1446, 1265, 960. ESI-HRMS calcd for $C_{21}H_{22}O_4^+$([M + H]$^+$):339.1518; found: 339.1522.

### Pharmacology

The target compounds synthesized in this study were screened for their antidepressant-like activities using Porsolt's behavioral despair (forced swimming test) (21). Compounds **5j** and **5k** were also evaluated using the tail suspension test (22,23) and the open-field test (24,25) to further confirm its antidepressant activity. The tested compounds in the pharmacology experiments were dissolved in DMSO, and administrated intraperitoneally (*i.p.*) to Balb/e mice (20 ± 2 g). The mice were housed collectively in polycarbonate cages in groups of ten, where they were maintained on a 12-h light/dark cycle in a temperature controlled (25 ± 2 °C) laboratory with free access to food and water. Each animal was used only once. All efforts were made to minimize both the suffering of the animals and the number of animals used in the experiments. All procedures used in this study were in accordance with the guide for the Care and Use of Laboratory Animals as adapted by the NIH, and the laboratory got ethical approval from National Science and Technology Commission of China.

### The forced swimming test (FST)

Local breed, male Balb/e mice (20 ± 2 g) were used in the FST under standard conditions with free access to food and water. They were housed in groups of six. On the test day, mice were dropped one at a time into a plexiglass cylinder (height 25 cm, diameter 10 cm) containing 10 cm of water at 22 ± 3 °C. On this day, mice were assigned into different groups ($n$ = 10 for each group). Then, the mice were dropped individually into the plexiglass cylinder and left in the water for 6 min. After the first 2 min of the initial vigorous struggling, the animals were immobile. The duration of immobility was recorded during the last 4 min of the 6 min test. All test swim sessions were recorded by a video camera positioned directly above the cylinder. Two 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 making 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 11:00 a.m. and 17:00 p.m.

### The tail suspension test (TST)

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. Two competent observers blind to treatment scored the videotapes. Mice were considered to be immobile only when they hung passively and completely motionless. The animals were used only once in this test. All TSTs were performed between 11:00 a.m. and 17:00 p.m.

### The open-field test

Open-field tests were used to evaluate the exploratory activity of the animal (26). The investigated compounds were administered 60 min before the experiment. The study was carried out on mice according to Archer's method (24), with slight modifications. Each mouse was placed individually in the center of the open-field apparatus, and the locomotor activity was assessed. The open-field apparatus was a nontransparent plastic container (80 × 60 × 30 cm), with the underside divided into 48 units of size 10 × 10 cm, without walls. The animals were gently placed in the center of the platform and were allowed to explore their surroundings. Hand-operated counters were used to score locomotion (ambulation, numbers of crossing lines with all four paws) and rearing frequencies (number of times an animal stood on its hind legs) for 3 min. The researchers, who did not know which groups had been treated, scored the behaviors in the open field. The experiments were performed in a dark room, and the apparatus was illuminated by a 60-W bulb giving a yellowish light, positioned 1 m above the center of the apparatus.

### The sample preparation

The doses of 10 mg/kg compounds **5j**, **5k**, and FLU were employed for testing the effect of these on monoamine neurotransmitter concentrations in the mouse brain. A vehicle control group (vehicle was DMSO) without any stress was added as a control group for normal condition comparison. The vehicle group in animal test was marked as vehicle + stress group. When the activity tests were completed, mice were killed by decapitation, the brains were rapidly removed and the hippocampus, hypothalamus and cortex were dissected on an ice-cold plate, and stored at −80 °C until assay. All brain regions were weighted and homogenized by ultrasonication in ice-cold 0.02 M perchloric acid. An internal standard (DHBA) at a concentration of 1.0 $\mu$m was also included. The homogenates were centrifuged at 11 000 × $g$ for 15 min at 4 °C, and the supernatant was separated and filtered through a membrane filter pore size 0.45 $\mu$m.

### HPLC condition and test

Following decapitation, the brains were rapidly removed, dissected on an ice-chilled glass plate, and subsequently, prefrontal hypothalamus, hippocampus, and cortex were

isolated. The tissues were weighed, sonicated in 0.1 M $NaH_2PO_4$ aqueous solution including 0.85 mM OSA, 0.5 mM EDTA·$Na_2$ and centrifuged (13 000 × *g* for 30 min). Then, 5-HT, 5-HIAA, NE, DA, and DHBA were assayed by HPLC-ECD. The HPLC system consisted of a microbore reverse-phase column (Shimadzu LC-10ATVP HPLC system, Tokyo, Japan, Shimadzu L-ECD-6A electrochemical detector, N2000 HPLC workstation software, Hypersil ODS C18 Column 4.6 × 150 mm 5 μm, Thermo, Waltham, MA, USA). The mobile phase consisted of 0.1M $NaH_2PO_4$ aqueous solution including 0.85 mM OSA, 0.5 mM EDTA·$Na_2$ and 11% methanol adjusted to pH 3.4 with phosphate acid and filtered through 0.45 μm pore size filter. External standard curves were used to quantify the amounts of 5-HT, 5-HIAA, NE and DA in each sample calculated by area under curve (AUC). The volume of injection was 20 μL. The detection limit of the assay was 20 pg/g sample. The filtrate sample was used for quantification of NE ($y$ = 0.03731 × +0.003125, $r$ = 0.9992), 5-HT ($y$ = 0.04382 × +0.002763, $r$ = 0.9983), DA ($y$ = 0.07152 × +0.00563, $r$ = 0.9985), and 5-HIAA ($y$ = 0.05482 × +0.003442, $r$ = 0.9989) by HPLC coupled with electro-chemical detection in all brain regions.

### Statistical analysis

Results are expressed as mean ± SEM *n* represents the number of animals. Data obtained from pharmacological experiments were analyzed with the Turkey's multiple comparison tests, using GRAPHPAD PRISM program (GraphPad Software, Inc., San Diego, CA, USA). A p-value of $< 0.05$ was considered statistically significant.

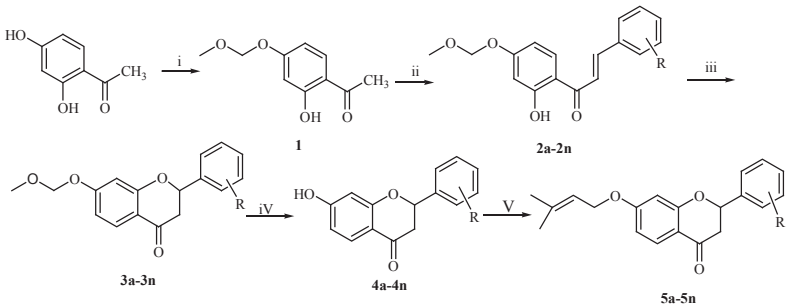
## Results and Discussion

### Chemistry

Target compounds were prepared as outlined in Scheme 1. Compound **1** was prepared as reported previously (21). The intermediates **2a**–**2n** were synthesized by the Claisen–Schmidt condensation of compound **1** protected as methoxymethyl ether with substituted aromatic aldehydes (15,27). Then, the intermediates **2a**–**2n** were treated with 3M HCl in methanol to yield the hydroxychalcones **3a**–**3n** (22). The latter compounds subsequently underwent a substitution reaction with prenyl bromide in acetone under reflux to give compounds **4a**–**4n**. The derivatives **5a**–**5n** were obtained with NaOAc in ethanol in good yield. The chemical structures of target compounds were characterized by IR, $^1$H NMR, $^{13}$C NMR, and high-resolution mass spectroscopy. The IR spectra of the compounds afforded C=O stretching (1761–1767/cm) and C-O-C stretching (1441–1446/cm). In the $^1$H-NMR spectra of the compounds, =CH protons of prenyloxy group were observed at 5.30–5.48 ppm, and $^{13}$C-NMR spectra of the compounds C=O were seen at 188.95–194.47 ppm.

### Effects on immobility periods in FST and TST through i.p. injection

Both the FST and TST are the accepted stress models of depression. Immobility has been shown to reflect a state of 'behavioral despair and variants' or 'failure to adapt to stress' (28). Immobility displayed in both of these behavioral despair models has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in human (29). There was a significant correlation between clinical potency and the potency of antidepressants in both models. Thus, two models are usually used to screen or evaluate antidepressant activity (30). In the present study, a series of 7-prenyloxy-2, 3-dihydroflavanone derivatives have been designed, synthesized, and evaluated for their antidepressant-like activities. The dose-course effects of compounds **5a**–**5n** in the FST test after the *i.p.* injection. The pharmacological experiments showed that the percentage of duration of immobility was the maximum value at the dose of 10 mg/kg. Moreover, the percentage of duration of immobility was steady from




R:

| | | | | |
|---|---|---|---|---|
| 5a = H | 5b = 2-F | 5c = 3-F | 5d = 4-F | 5e = 2-Cl |
| 5f = 3-Cl | 5g = 4-Cl | 5h = 2-Br | 5i = 3-Br | 5j = 4-Br |
| 5k = 2,4-$Cl_2$ | 5l = 2,6-$Cl_2$ | 5m = 4-$CH_3$ | 5n = 4-$OCH_3$ | |

Reagents and conditions:

(i) $ClCH_2OCH_3$, $K_2CO_3$; (ii) aromatic aldehyde, KOH; (iii) NaOAc; (iV) 3 M HCl; (V) prenyl bromide, anhydrous $K_2CO_3$

**Scheme 1:** Synthesis routes of target compounds **5a**–**5n**.

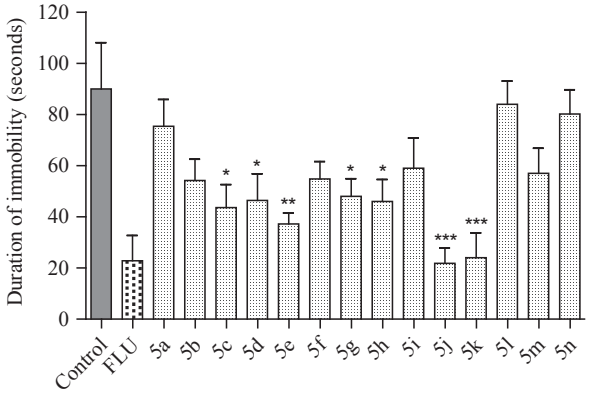



**Figure 2:** Effect of **5a**–**5n** and FLU on immobility time in the forced swimming test in mice. Data are expressed as the mean ± SEM ($n$ = 10). Compounds and FLU were administered intraperitoneally at 10 mg/kg. Symbol (*, ***, or ***) indicates statistically significance in comparison with vehicle at $p < 0.05$, $p < 0.01$, $p < 0.001$.

an oral dose of 10–40 mg/kg. The dose of 10 mg/kg was chosen to evaluate the antidepressant-like activity in the FST. Compounds **5a**–**5n** and the reference drug fluoxetine on immobility time at a dose of 10 mg/kg are shown in Figure 2. The immobility times were significantly reduced after treatment with seven compounds (**5c**–**5e**, **5g**, **5h**, **5j**, and **5k**) in the FST, indicating to possess antidepressant-like effects. Compounds **5j** and **5k** in particular led to significant reductions in the duration of the immobility time compared with the control group with $p < 0.001$, and this result is similar to that following administration of the positive control fluoxetine. For a more detailed and comprehensive understanding of the antidepressant-like effects of the tested compounds **5a**–**5n** and fluoxetine, their values for the percentage decrease in the immobility duration (% DID) were calculated using the following formula: % DID = $[(Y\text{-}X)/Y] \times 100$, where $Y$ is the duration of immobility (s) in the control group and $X$ is the duration of immobility (s) in the test group.

As shown in Table 1, seven compounds examined reduced the duration of immobility and gave high % DID values. Among them, the % DID for compounds **5j** and **5k** was similar to that for fluoxetine in the FST, and exhibited higher % DID values of 75.78% and 73.33%, respectively, similar to fluoxetine (74.67%), and appeared to provide higher levels of antidepressant-like effects.

Consideration of the biological activities of the synthesized compounds revealed the following structure activity relationships (SARs). In general, the activity of an organic compound may be changed after the introduction of a halogen atom. For compounds **5b**–**5l**, the electron-withdrawing groups with F, Cl, and Br groups, were substituted on the B ring of flavanone fragment, which variations in activity were observed. Pleasingly, the introduction of an

**Table 1:** Evaluation of the antidepressant-like activity of compounds **5a**–**5n** in the FST

| | | Antidepressant activity[a] | |
|---|---|---|---|
| Compounds | R | Duration of immobility | DID (%)[b] |
| **5a** | H | 75.40 ± 10.50 | 16.22 |
| **5b** | 2-F | 54.20 ± 8.30 | 39.78 |
| **5c** | 3-F | 43.60 ± 9.00* | 51.56 |
| **5d** | 4-F | 46.40 ± 10.40* | 48.44 |
| **5e** | 2-Cl | 37.20 ± 4.30** | 58.67 |
| **5f** | 3-Cl | 54.80 ± 6.80 | 39.11 |
| **5g** | 4-Cl | 48.00 ± 6.90* | 46.67 |
| **5h** | 2-Br | 46.00 ± 8.60* | 48.89 |
| **5i** | 3-Br | 59.00 ± 11.80 | 34.44 |
| **5j** | 4-Br | 21.80 ± 6.00*** | 75.78 |
| **5k** | 2,4-$Cl_2$ | 24.00 ± 9.70*** | 73.33 |
| **5l** | 2,6-$Cl_2$ | 84.00 ± 9.03 | 6.67 |
| **5m** | 4-$CH_3$ | 57.00 ± 9.80 | 36.67 |
| **5n** | 4-$OCH_3$ | 80.20 ± 9.50 | 10.89 |
| Fluoxetine | — | 22.80 ± 9.96*** | 74.67 |
| Control | — | 90.00 ± 18.01 | — |

Values are the mean ± SEM ($n$ = 10).
Significantly different compared with control (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).
[a]Compounds and fluoxetine were administered intraperitoneally at 10 mg/kg.
[b]%DID: percentage decrease in immobility duration.

F atom (**5c** and **5d**), a Cl atom (**5g**, **5h** and **5k**), or Br atom (**5h** and **5j**) led to decrease significantly the immobility times compared with **5a**. And it was found that the position of the halogen atom substituted on the B ring of flavanone greatly influenced the antidepressant-like activity. Compared with compounds with different Br-substituted positions on the phenyl ring, the order of activity was 4-Br > 2-Br > 3-Br, the order of activity was observed for F-substituted positions with 3-F > 4-F > 2-F, and 2-Cl > 4-Cl > 3-Cl for F-substituted positions. The order of activity for with different Cl-substituted positions on the phenyl ring was 2, 4-$Cl_2$ > 2-Cl > 4-Cl > 3-Cl > 6-$Cl_2$. Among, the introduction of a 4-Br atom and two Cl atoms to give compounds **5j** and **5k** showed the most antidepressant-like effect, similar to the positive control drug fluoxetine. In contrast, two electron-donor groups with the introduction of -$CH_3$ and –$OCH_3$ to give compounds **5m** and **5n** resulted in no antidepressant-like activity in the FST.

Compounds **5j** and **5k** were exhibited the most potent antidepressant-like activity in the FST and were further evaluated in the TST to effectively confirm its antidepressant-like activity. Next, the antidepressant-like effect of **5j**, and **5k** in the TST test after the *i.p.* injection was shown in Figure 3. The pharmacological experiments showed that the percentage of duration of immobility was the maximum value at the dose of 10 mg/kg. Moreover, the percentage of duration of immobility was steady from an oral dose of 10–30 mg/kg. The dose of 10 mg/kg was chosen to evaluate the antidepressant-like activity in the TST. Compounds

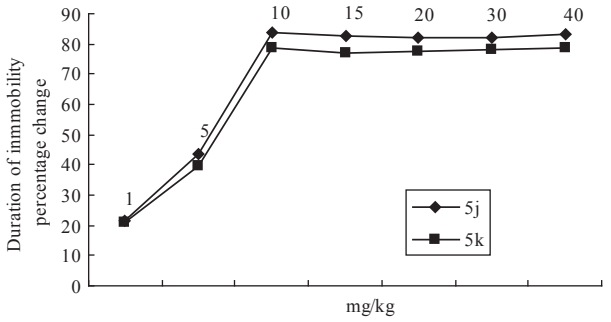



**Figure 3:** The dose course of **5j** and **5k** in the TST test (the number of animals at each point was 10).

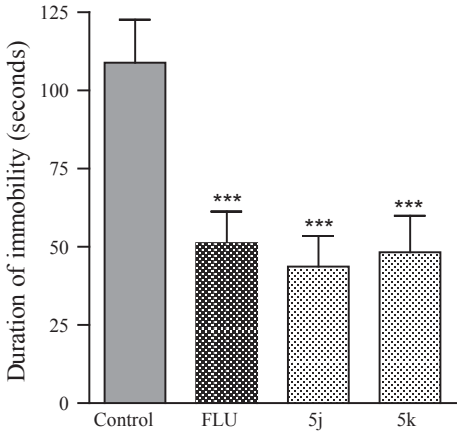



**Figure 4:** Effect of **5j**, **5k** on immobility time in the TST in mice. Data are expressed as the mean ± SEM ($n$ = 12). **5j**, **5k**, and FLU were administered intraperitoneally at 10 mg/kg. Symbol (***) indicates statistically significant in comparison with vehicle at $p < 0.001$.

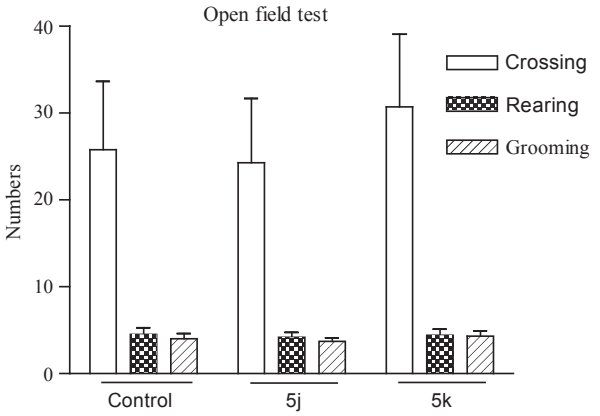



**Figure 5:** Exploratory activity (counts) in the open-field test. The behavioral parameters were recorded for 3 min. Locomotion: number of line crossings; rearing: number of times seen standing on hind legs; grooming: number of modifications; **5j** and **5k** was administered 60 min before the test. **5j** and **5k** were administered intraperitoneally at 10 mg/kg. The values represent the mean ± SEM ($n$ = 10).

**5j** and **5k** produced a marked reduction in immobility time at a dose of 10 mg/kg, and the clinically effective antidepressant fluoxetine significantly reduced immobility time at the same dose points. The results are shown in Figure 4. The immobility displayed in both rodent behavioral test of behavioral despair has been hypothesized to reflect depressive disorders in humans. These findings suggested that compounds **5j** and **5k** displayed the antidepressant-like effects.

### *Effects of the acute treatment in the open-field test*

As some compounds that alter motor activity may give false-positive/negative effects in the FST, in particular

**Table 2:** Effects of FST exposure and **5j**, **5k** treatment on monoamine neurotransmitter concentrations in mouse brain

| Region | Group | 5-HT | 5-HIAA | NE | DA |
|---|---|---|---|---|---|
| Hippocampus | Stress vehicle | 302.5 ± 18.7$^{y}$ | 327.7 ± 49.3$^{y}$ | 93.4 ± 9.3$^{z}$ | 32.7 ± 8.4 |
| | **5j** | 495.2 ± 15.6$^{b,y}$ | 876.8 ± 25.4$^{a,z}$ | 143.9 ± 10.4$^{b}$ | 29.6 ± 5.6$^{z}$ |
| | **5k** | 476.8 ± 18.7$^{b,z}$ | 946.8 ± 38.7$^{a}$ | 156.8 ± 11.7$^{a}$ | 28.8 ± 8.1$^{z}$ |
| | Fluoxetine | 507.8 ± 13.4$^{a,y}$ | 396.8 ± 55.4$^{a,x}$ | 129.7 ± 10.2$^{a}$ | 30.3 ± 5.3$^{z}$ |
| | Vehicle control | 701.2 ± 65.9$^{b}$ | 1001.6 ± 67.4$^{a}$ | 181.6 ± 9.6$^{c}$ | 52.3 ± 7.7 |
| Hypothalamus | Stress vehicle | 356.8 ± 10.8$^{z}$ | 1787.5 ± 68.3 | 137.5 ± 10.5$^{z}$ | 149.3 ± 10.3 |
| | **5j** | 602.2 ± 60.6$^{a}$ | 3843.6 ± 121$^{a,y}$ | 389.6 ± 35.4$^{a,y}$ | 145.9 ± 14.3 |
| | **5k** | 571.4 ± 58.3$^{b}$ | 3676.3 ± 78.9$^{a,z}$ | 275.7 ± 28.8$^{a}$ | 136.9 ± 13.6 |
| | Fluoxetine | 617.6 ± 60.2.6$^{a}$ | 3808.6 ± 41.6$^{a,y}$ | 437.3 ± 37.0$^{a,y}$ | 148.6 ± 9.9 |
| | Vehicle control | 703.2 ± 99.8$^{c}$ | 1845.4 ± 167 | 473.7 ± 70.7$^{c}$ | 176.3 ± 33.8 |
| Cortex | Stress vehicle | 203.9 ± 20.3$^{x}$ | 264.6 ± 18.1$^{y}$ | 179.5 ± 11.8$^{z}$ | 2068.0 ± 77.9$^{z}$ |
| | **5j** | 544.7 ± 35.7$^{a,z}$ | 289.4 ± 28.2$^{z}$ | 217.7 ± 9.7$^{a}$ | 2463.4 ± 93.8$^{y}$ |
| | **5k** | 516.1 ± 28.7$^{a}$ | 276.5 ± 31.7$^{z}$ | 200.8 ± 8.7$^{b}$ | 2376.7 ± 58.7$^{z}$ |
| | Fluoxetine | 549.0 ± 38.7$^{a}$ | 220.6 ± 19.4$^{y}$ | 228.7 ± 6.4$^{a}$ | 2553.7 ± 86.5$^{z}$ |
| | Vehicle control | 696.4 ± 60.8$^{a}$ | 393.4 ± 13.8$^{b}$ | 199.0 ± 21.8$^{c}$ | 3429 ± 160$^{c}$ |

The dose of **5j**, **5k**, and fluoxetine was 10 mg/kg. Neurotransmitter concentrations were expressed as ng/g per brain region wet weight. Data expressed as mean ± SEM ($n$ = 10). Statistical analysis of data was carried out by one-way analysis of variance followed by Turkey's test. $^{a}p < 0.001$, $^{b}p < 0.01$, $^{c}p < 0.05$ versus Stress vehicle, $^{x}p < 0.001$, $^{y}p < 0.01$, $^{z}p < 0.05$ versus vehicle control.

psychomotor stimulants and drugs enhancing motor activity, which decrease immobility time by stimulating locomotor activity (25), an additional measurement was carried out with the specific aim of observing motor activity. To determine whether the observed reductions in immobility were associated with alterations in motor activity, in this study, the effects of compounds **5j** and **5k** on spontaneous locomotor activity were evaluated in the open-field test, a classical animal test used to evaluate the autonomic effects of drugs and general activity of animals. This study demonstrated that compounds **5j** and **5k** did not significantly change the motor activity (crossing, rearing, or grooming) in mice (Figure 5), indicating that the immobility reductions were not caused by possible CNS-stimulating effects in the FST and TST. The results suggest that compounds **5j** and **5k** possess the antidepressant-like effect in mice.

### *Effects on monoamine neurotransmitter concentrations in the mouse brain*

Monoamine neurotransmitters are believed to be involved in mental depression and play important roles in mediating behavioral effects of antidepressant drugs. The monoamine hypothesis supposed that depression was a result of the depletion of NE, 5-HT, and DA in addition to activation of monoamine oxidase in the CNS (31). Depression can be alleviated by increasing the levels of monoamine neurotransmitters in the CNS (32,33). The effects of compounds **5j** and **5k** and fluoxetine after on whole brain monoamine neurotransmitter levels in stressed and normal mice were summarized in Table 2. We found that compounds **5j** and **5k** significantly increased 5-HT and NE levels in mouse hippocampus, hypothalamus, and cortex, similar to the positive control fluoxetine in the FST. However, no significant change in brain DA level was observed in all measured brain regions. Compared with the stress vehicle group, compounds **5j** and **5k** administration are significantly changes in 5-HIAA levels in some brain parts, especially in hypothalamus, compounds **5j** and **5k** exhibited to increase 5-HIAA contents in all measured brain regions. As the ratio of neurotransmitter compared to its metabolites can be used as an index of neurotransmitter metabolism (34), the reduction in metabolites/neurotransmitters suggests a shut down in the metabolism of neurotransmitters (35). Therefore, 5-HIAA was increased in the present study, it is likely that the metabolism of 5-HT was shut down. No significant changes in DA content were observed following the FST. Synthesis of DA occurs in the striatum and remains relatively stable in the brain regions investigated in this study (36). So, the probable mechanism of action of compounds **5j** and **5k** is thought to be related to the increase in 5-HT and NE in the CNS.

## Conclusion

In the present study, a series of 7-prenyloxy-2, 3-dihydroflavanone derivatives were synthesized and evaluated for their antidepressant activities. The results showed that compounds **5j** and **5k** significantly decreased the immobility times and showed the most antidepressant-like activities in the FST and TST. And it suggested that the mechanisms of action of compounds **5j** and **5k** are likely related to the increase in 5-HT and NE in the CNS, although other neurochemical systems may also be involved. These results suggest that compounds **5j** and **5k** are a potential candidate for the treatment of depression.

## Acknowledgments

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