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SYNTHESIS AND SEDATIVE ACTIVITY OF 5-(4′-β-D-ALLOPYRANOSYLOXYPHENYL)-3-ARYL-4,5-DIHYDROPYRAZOLE-1-CARBOTHIOAMIDES

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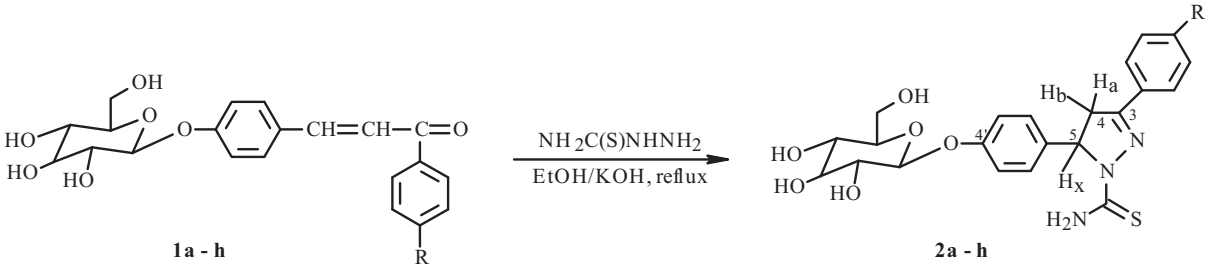
*5-(4′-β-D-Allopyranosyloxyphenyl)-3-aryl-4,5-dihydropyrazole-1-carbothioamides (**2a–2h**) were synthesized by the reaction of (**1a–1h**) with thiosemicarbazide and KOH in ethanol. The structures of all new compounds were characterized by ^{1}HNMR, IR, and MS (HR-MS) spectra. A preliminary bioassay test of **1f–1h** and **2a–2h** suggested that most of these helicid analogues showed mild to strong activity. Compounds **1g**, **2a**, **2c**, and **2f** at a dose of 200 mg·kg^{-1} were better than that of the parent helicid.*

Keywords: helicid, Claisen-Schmidt reaction, pyrazoline, carbothioamide.

Natural helicid (4-formylphenyl-β-D-allopyranoside, $C_{13}H_{16}O_7$), a major active ingredient in Chinese herbal medicine, is a pure compound extracted from the fruit of *Helicia nilagirica* Beed [1], which is distributed widely in Yunnan Province of China. It has been reported that helicid possesses a variety of biological activities, including sedative, hypnotic, analgesic, anticonvulsant, and so on [2]. But helicid also has side effects, such as the large dose required, slow action, and low biological utilization. Therefore, multiple approaches are being sought to overcome these limitations. Our laboratory′s attention was focused on modification at the aldehyde group of helicid [3–6], which gave good results. In recent years, increasing evidence suggests that pyrazoline derivatives possess a broad spectrum of biological activities [7–13], such as antibacterial, antifungal, antimicrobial, antidepressant, and anticonvulsant. Herein, in order to obtain helicid analogues with excellent therapeutic effect and low side effect, we successfully used pyrazoline in the structure modification of helicid. In this paper, we synthesized eleven helicid derivatives through Schmidt-Claisen condensation and addition reaction. The structures of all new compounds were characterized by ^{1}H NMR, IR, and MS spectra, and the sedative-hypnotic activity produced by the target derivatives was investigated by the spontaneous activity test. Compounds **1g**, **2a**, **2c**, and **2f** at a dose of 200 mg·kg^{-1} was better than that of the parent helicid.



1a - h **2a - h**

1a,2a: R = H; **1b,2b:** R = CH_3; **1c,2c:** R = OCH_3; **1d,2d:** R = Cl
1e,2e: R = Br; **1f,2f:** R = F; **1g,2g:** R = C_2H_5; **1h,2h:** R = NO_2

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TABLE 1. Spontaneous Motion Results of Target Compounds in Mice

Compound[a]	Pre-administration, $\bar{x} \pm s$	Post-administration, $\bar{x} \pm s$		
		30 min	60 min	90 min
0.05% CMC	161.00±21.72	141.00±20.55	121.33±77.81	103.33±56.10
Diazepam[b]	163.17±40.91	34.83±23.27**	18.00±26.65*	8.00±19.60**
Helicid	163.83±76.08	162.5±50.23**	130.67±69.04	70.67±44.27**
1f	151.33±36.01	144.00±102.73	112.83±35.75	91.17±92.29
1g	162.17±49.47	116.17±77.22	114.50±40.89	60.17±34.65**
1h	169.47±59.67	159.51±64.12	168.27±68.34	115.85±45.46*
2a	161.00±47.62	92.17±49.79	71.50±16.90	42.00±53.35**
2b	181.67±28.76	115.83±68.05	90.33±54.13	96.00±65.78
2c	167.67±51.84	86.67±43.36*	103.00±51.79	34.33±24.38**
2d	163.50±58.75	107.60±74.39	78.67±73.05	73.50±49.29
2e	166.83±55.64	182.83±123.71	119.50±37.77	83.17±61.69
2f	171.83±55.87	105.33±53.40	99.00±48.15	39.83±35.27**
2g	162.00±38.62	157.67±53.40	122.17±28.29	91.17±49.96
2h	152.33±42.53	119.50±54.12	88.17±78.94	99.00±72.96

*P<0.05; **P<0.01 compared with 0.05% CMC. [a]Dose: 200 mg/kg. [b]Diazepam: 20 mg/kg. Number of animals: 6.

In the present study, the title compounds were synthesized by a two-step reaction as shown in Scheme 1. All of the new compounds were identified using IR, ^{1}H NMR, and MS (HR-MS). The IR spectra of compounds **2a–2h** showed NH_2 stretching bands at 3435–3427 cm^{-1} and 3376–3343 cm^{-1} and C=N stretching bands at 1595–1577 cm^{-1} due to ring closure, and C=S stretching bands at 1407–1344 cm^{-1}. In ^{1}H NMR spectra, protons CH_2-CH of the pyrazoline fragment of the synthesized compounds **2a–2h** show characteristic patterns of an ABX system. The chemical shifts of H_A, H_B, and H_X have been assigned to about δ 3.00–3.22, δ 3.35–3.45, and δ 5.76–5.93, respectively, with corresponding coupling constants of J_{AB} = 17.8–18.4 Hz, J_{BX} = 7–11.4 Hz, and J_{AX} = 4.2–7.4 Hz. According to the currently accepted mechanism, formation of the cyclized pyrazoline analogues proceeds via the thiosemicarbazone, which undergoes cyclization under basic conditions to form the desired pyrazoline ring in all the compounds [14].

To summarize, a concise and effective procedure has been successfully developed for the synthesis of helicid derivatives containing pyrazoline. The results of the present investigation should be of value in the synthesis of structural analogs.

EXPERIMENTAL

Helicid was purchased from Yunnan Chemical Company of China. Mice (Kunming strain) weighing 18–22 g were obtained from West China School of Pharmacy, Sichuan University (Chengdu China). Flash column chromatography was performed on silica gel (300–400 mesh, Qingdao, China). Thin-layer chromatography (TLC) was performed on percolated Merck silica gel 60F_{254} plates. Diazepam injects was purchased from Tianjin Jiaozuo Pharmaceutical Co. Ltd. (China) (Batch number 030708-1). All the other reagents and solvent were commercially available and used without further purification. Compounds **1a–1e** were prepared according to the method in the literature [5].

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were taken on a Bruker AV-400 MHz spectrometer with TMS as an internal standard. IR spectra were recorded on a Perkin–Elmer FT-IR spectrometer (KBr disk). Mass spectra were obtained on a Finnigan-LCQDECA mass spectrometer (ESI-LR-MS) and a Bruker Daltoics Bio TOF-Q mass spectrometer (ESI-HR-MS). An electronic balance (Sartorius BS210S) and multi-function mice locomotor activity recorder (YLS-1A) were used.

Procedure for the Preparation of Compounds 1f–1h [15].

***E*-(4-*β*-D-Allopyranosyloxyphenyl)-1-(4-nitrophenyl)-propenone (1h)**. This compound was prepared from 4-nitroacetophenone. Yield: 310 mg, 72%, pale yellow solid, mp 85–87°C.

IR (KBr, cm^{-1}): 3391, 2918, 1662, 1574, 1519, 1452, 1343, 1247, 1108, 1083, 1030, 986, 830. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.40–3.95 (6H, m), 5.20 (1H, d, J = 8.0, H-1), 7.82 (1H, d, J = 16.0, CH=CH), 7.09–8.39 (8H, m,

PhH), 8.34 (1H, d, J = 16.0, CH=CHCO), 4.51–5.11 (4H, br, 4-OH). ESI-LR-MS *m/z*: 466.0887 $[M + Cl]^-$; calcd for $C_{21}H_{21}Cl_1O_9N_1$, 466.0899.

General Procedure for the Preparation of Compounds 2a–2h. In a round bottomed flask, a mixture of **1a–1h** (1 mmol), thiosemicarbazide (1.2 mmol), and potassium hydroxide (0.11 g) in ethanol (15 mL) was introduced. The mixture was heated to reflux, and the progress of the reaction was monitored by TLC. After the reaction ended, the mixture was acidified with diluted hydrochloric acid (1 mol/L) to give a yellow solid, which was filtered. The solution was concentrated under reduced pressure, and the residue was subjected to column chromatography. It was first eluted with ethyl acetate to remove impurities, and then with chloroform, hexane, and methanol (7:5:2, volume ratio) as solvents to give products **2a–2h**.

5-(4′-β-D-Allopyranosyloxyphenyl)-3-phenyl-4,5-dihydropyrazole-1-carbothioamide (2a). This compound was prepared from **1a**. Yield: 266 mg, 58%, yellow solid, mp 156–158°C.

IR (KBr, cm^{-1}): 3434, 3376, 2920, 1606, 1587, 1509, 1478, 1344, 1079, 1033, 976, 837, 692.

^1H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.13 (1H, dd, J_{ax} = 6.4, J_{ab} = 18.0, Ha-4), 3.44 (1H, dd, J_{bx} = 11.2, J_{ab} = 18.0, Hb-4), 3.37–3.90 (6H, m), 5.08 (1H, d, J = 8.0, 1-H), 5.87 (1H, dd, J_{bx} = 11.2, J_{ax} = 6.4, Hx-5), 6.93–7.88 (9H, m, PhH), 4.46–4.93 (4H, br, 4OH), 7.89–8.02 (2H, br, NH_2).

ESI-LR-MS *m/z*: 482.1363 $[M + Na]^+$; calcd for $C_{22}H_{25}O_6N_3SNa$, 482.1367.

5-(4′-β-D-Allopyranosyloxyphenyl)-3-(4-methylphenyl)-4,5-dihydropyrazole-1-carbothioamide (2b). This compound was prepared from **1b**. Yield: 255 mg, 54%, yellow solid, mp 143–145°C.

IR (KBr, cm^{-1}): 3431, 3376, 2921, 1602, 1588, 1509, 1478, 1446, 1361, 1346, 1233, 1080, 1033, 917, 692. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.35 (3H, s, CH_3) 3.10 (1H, dd, J_{ax} = 4.2, J_{ab} = 18.0, Ha-4), 3.44 (1H, dd, J_{bx} = 11.2, J_{ab} = 18.0, Hb-4), 3.35–3.90 (6H, m), 5.08 (1H, d, J = 8.0, H-1), 5.85 (1H, dd, J_{bx} = 11.2, J_{ax} = 4.2, Hx-5), 6.93–7.84 (8H, m, PhH), 4.49–5.05 (4H, br, 4OH), 7.98–8.58 (2H, br, NH_2). ESI-LR-MS *m/z*: 496.1524 $[M + Na]^+$; calcd for $C_{23}H_{27}O_6N_3SNa$, 496.1524.

5-(4′-β-D-Allopyranosyloxyphenyl)-3-(4-methoxyphenyl)-4,5-dihydropyrazole-1-carbothioamide (2c). This compound was prepared from **1c**. Yield: 254 mg, 52%, yellow solid, mp 141–143°C.

IR (KBr, cm^{-1}): 3427, 3351, 2921, 1607, 1580, 1509, 1360, 1252, 1081, 1036, 973, 793, 699. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.10 (1H, dd, J_{ax} = 4.4, J_{ab} = 17.8, Ha-4), 3.44 (1H, dd, J_{bx} = 7.6, J_{ab} = 17.8, Hb-4), 3.36–3.91 (9H, m), 5.08 (1H, d, J = 8.0, H-1), 5.84 (1H, dd, J_{bx} = 7.6, J_{ax} = 4.4, Hx-5), 6.93–7.83 (8H, m, PhH), 4.42–5.00 (4H, br, 4OH), 7.75–7.88 (2H, br, NH_2). ESI-LR-MS *m/z*: 512.1464 $[M + Na]^+$; calcd for $C_{23}H_{27}O_7N_3SNa$, 512.1473.

5-(4′-β-D-Allopyranosyloxyphenyl)-3-(4-chlorophenyl)-4,5-dihydropyrazole-1-carbothioamide (2d). This compound was prepared from **1d**. Yield: 306 mg, 62%, yellow solid, mp 170–172°C.

IR (KBr, cm^{-1}): 3435, 3369, 2913, 1595, 1509, 1471, 1360, 1233, 1082, 1035, 977, 828, 695. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.13 (1H, dd, J_{ax} = 6.0, J_{ab} = 18.0, Ha-4), 3.44 (1H, dd, J_{bx} = 11.4, J_{ab} = 18.0, Hb-4), 3.37–3.90 (6H, m), 5.08 (1H, d, J = 8.0, H-1), 5.87 (1H, dd, J_{bx} = 11.4, J_{ax} = 6.0, Hx-5), 6.93–7.92 (8H, m, PhH), 4.49–5.05 (4H, br, 4OH), 7.95–8.05 (2H, br, NH_2). ESI-LR-MS *m/z*: 516.0953 $[M + Na]^+$; calcd for $C_{22}H_{24}Cl_1O_6N_3SNa$, 516.0967.

5-(4′-β-D-Allopyranosyloxyphenyl)-3-(4-bromophenyl)-4,5-dihydropyrazole-1-carbothioamide (2e). This compound was prepared from **1e**. Yield: 301 mg, 56%, yellow solid, mp 157–159°C.

IR (KBr, cm^{-1}): 3434, 3369, 2920, 1606, 1589, 1509, 1359, 1233, 1072, 1008, 912, 825, 713. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.13 (1H, dd, J_{ax} = 4.2, J_{ab} = 17.8, Ha-4), 3.44 (1H, dd, J_{bx} = 9.2, J_{ab} = 17.8, Hb-4), 3.37–3.90 (6H, m), 5.08 (1H, d, J = 8.0, H-1), 5.87 (1H, dd, J_{bx} = 9.2, J_{ax} = 4.2, Hx-5), 6.93–7.84 (8H, m, PhH), 4.49–5.05 (4H, br, 4OH), 7.95–8.05 (2H, br, NH_2). ESI-LR-MS *m/z*: 560.0450 $[M + Na]^+$; calcd for $C_{22}H_{24}BrO_6N_3$ SNa, 560.0461.

5-(4′-β-D-Allopyranosyloxyphenyl)-3-(4-fluorophenyl)-4,5-dihydropyrazole-1-carbothioamide (2f). This compound was prepared from **1f**. Yield: 253 mg, 53%, yellow solid, mp 148–150°C.

IR (KBr, cm^{-1}): 3427, 3353, 2913, 1601, 1505, 1479, 1411, 1361, 1231, 1036, 977, 838, 713. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.13 (1H, dd, J_{ax} = 7.4, J_{ab} = 18.0, Ha-4), 3.44 (1H, dd, J_{bx} = 11.2, J_{ab} = 18.0, Hb-4), 3.33–3.90 (6H, m), 5.08 (1H, d, J = 8.0, H-1), 5.86 (1H, dd, J_{bx} = 11.2, J_{ax} = 7.4, Hx-5), 6.93–7.88 (8H, m, PhH), 4.47–5.05 (4H, br, 4OH), 7.96–8.02 (2H, br, NH_2). ESI-LR-MS *m/z*: 500.1245 $[M + Na]^+$; calcd for $C_{22}H_{24}FO_6N_3SNa$, 500.1262.

5-(4′-β-D-Allopyranosyloxyphenyl)-3-(4-ethylphenyl)-4,5-dihydropyrazole-1-carbothioamide (2g). This compound was prepared from **1g**. Yield: 297 mg, 61%, yellow solid, mp 144–146°C.

IR (KBr, cm^{-1}): 3435, 3376, 2920, 1608, 1577, 1467, 1407, 1338, 1234, 1083, 977, 850, 691. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.15–1.19 (3H, t, CH_3), 2.62 (2H, q, J = 15.2, CH_2), 3.02 (1H, dd, J_{ax} = 6.8, J_{ab} = 18.0, Ha-4), 3.36 (1H, dd, J_{bx} = 11.0, J_{ab} = 18.4, Hb-4), 3.30–3.83 (6H, m), 5.01 (1H, d, J = 8.0, H-1), 5.78 (1H, dd, J_{bx} = 11.0, J_{ax} = 6.8, Hx-5),

6.84–7.22 (8H, m, PhH), 4.40–4.98 (4H, br, 4OH), 7.70–7.72 (2H, br, NH_2). ESI-LR-MS *m/z*: 510.1669 $[M + Na]^+$; calcd for $C_{22}H_{29}O_6N_3SNa$, 510.1669.

5-(4′-β-D-Allopyranosyloxyphenyl)-3-(4-nitrophenyl)-4,5-dihydropyrazole-1-carbothioamide (2h). This compound was prepared from **1h**. Yield: 217 mg, 43%, yellow solid, mp 160–162°C.

IR (KBr, cm^{-1}): 3505, 3376, 2920, 1608, 1577, 1511, 1407, 1338, 1234, 1180, 1083, 1036, 977, 850, 691. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.20 (1H, dd, J_{ax} = 6.0, J_{ab} = 18.0, Ha-4), 3.43 (1H, dd, J_{bx} = 11.4, J_{ab} = 18.0, Hb-4), 3.34–3.94 (6H, m), 5.08 (1H, d, J = 8.0, H-1), 5.91 (1H, dd, J_{bx} = 11.4, J_{ax} = 6.0, Hx-5), 6.93–8.30 (8H, m, PhH), 4.49–5.06 (4H, br, 4OH), 8.21–8.27 (2H, br, NH_2). ESI-LR-MS *m/z*: 527.1189 $[M + Na]^+$; calcd for $C_{22}H_{24}O_8N_4SNa$, 527.1207.

The sedative-hypnotic activity of all the compounds was investigated by recording the number of spontaneous activities in mice using a recorder; all samples were dissolved in 0.05%CMC (carboxymethyl cellulose) to form a solution (200 mg/kg) for use later. Diazepam injects were dissolved in saline to a form solution (20 mg/kg) for use later. Eighty-four mice were randomized in the experiment and divided into 14 groups of 6 mice each (3 male and 3 female). The mice were placed in the recorder for 5 min before the experiments and maintained in suitable environmental conditions throughout the experiments. When testing, the prepared solutions were injected into the mouse stomach with a syringe in a volume of 0.2 mL·10 g^{-1} body weight, and then the number of spontaneous activities was recorded every 5 min at 30 min intervals. The data were recorded as number of movements per minute and are listed in Table 1. Compounds **1g**, **2a**, **2c**, and **2f** at a dose of 200 mg·kg^{-1} were better than that of the parent compound helicid. Thus, further modification of helicid should be worthwhile.

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