SYNTHESIS AND CALMING ACTIVITY OF 6*H*-2-AMINO-4-ARYL-6-(4-β-D-ALLOPYRANOSYLOXYPHENYL)-1,3-THIAZINE

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*E-(4-β-D-Allopyranosyloxyphenyl)-1-(4-substituted phenyl)propenone derivatives (**1a–g**) have been synthesized by the Claisen-Schmidt condensation of helicid with 4-substituted acetophenone using 10% NaOH aqueous solution as a catalyst. 6H-2-Amino-4-aryl-6-(4-β-D-allopyranosyloxyphenyl)-1,3-thiazine (**2a–g**) were synthesized by the 1,4-Michael reaction of **1a–g** with thiourea. The structures of all the new products were established by ^{1}H NMR, IR, and MS spectroscopy. Compound **2b** (200 mg·kg^{-1}) showed better sedative-hypnotic activity, so further modification of helicid should be worthwhile.*

Keywords: helicid, Claisen-Schmidt reaction, 1,3-thiazine, synthesis.

Helicid [4-formylphenyl-β-D-allopyranoside, $C_{13}H_{16}O_7$] is a pure compound extracted from the fruit of *Helicia nilagirica* Beed [1], which is distributed widely in Yunnan Province of China, and has been successfully used in the treatment of patients with insomnia in China. Its structure and pharmacological mechanism are similar to gastrodin, but it has special properties because it is a rare form of allopyranoside. Helicid is a safe sedative-hypnotic and anticonvulsant [2]. However, it possesses some disadvantages, such as the large dose, required slow action, and low biologic utilization. It has been reported that heterocyclic pharmaceutical agents containing sulfur have various biological activities [3–5]. Up to now, 1,3-thiazine has not been introduced to structure of helicid. Therefore, in order to obtain helicid analogs with better therapeutic effect and low side effect [6], we synthesized nine helicid derivatives through the Claisen-Schmidt condensation and 1,4-Michael reaction. 1,3-Thiazine has been successfully used in its structure modification. The structures of all the new compounds were characterized by ^{1}H NMR, IR, and MS spectroscopy. We also performed preliminary bioassay tests. Compound **2b** (200 mg·kg^{-1}) showed better sedative-hypnotic activity.

In the present study, a series of helicid derivatives was synthesized (Scheme 1). *E*-(4-β-D-Allopyranosyloxyphenyl)-1-(4-substituted phenyl) propenone derivatives (**1a–g**) have been synthesized by the Claisen-Schmidt condensation of helicid with the 4-substituted acetophenone using 10% NaOH aqueous solution as a catalyst. 6*H*-2-Amino-4-aryl-6-(4-β-D-allopyranosyloxyphenyl)-1,3-thiazine (**2a–g**) was synthesized by the 1,4-Michael reaction of **1a–g** with thiourea. Thiourea exhibited tautomerism in alkaline solution, and 1,3-thiazine was synthesized by 1,4-Michael addition reaction and intramolecular cyclization. Some comparative experiments were carried out. Potassium hydroxide is a better catalyst than other alkalis. The alkalinity of other alkalis is either too strong or too weak, which is inappropriate for this reaction. In the ^{1}H NMR spectra, compounds **2a–g**, due to H_5, H_6 protons of the thiazine ring, exibited a double peak at 5.25–5.40 and 5.03–5.08 ppm (J_5 = 4.4–4.8, J_6 = 4.4–6.8).

To summarize, a concise and effective procedure has been successfully developed for the synthesis of helicid derivatives containing 1,3-thiazine. The results of the present investigation should be of value in the synthesis of structural analogs.

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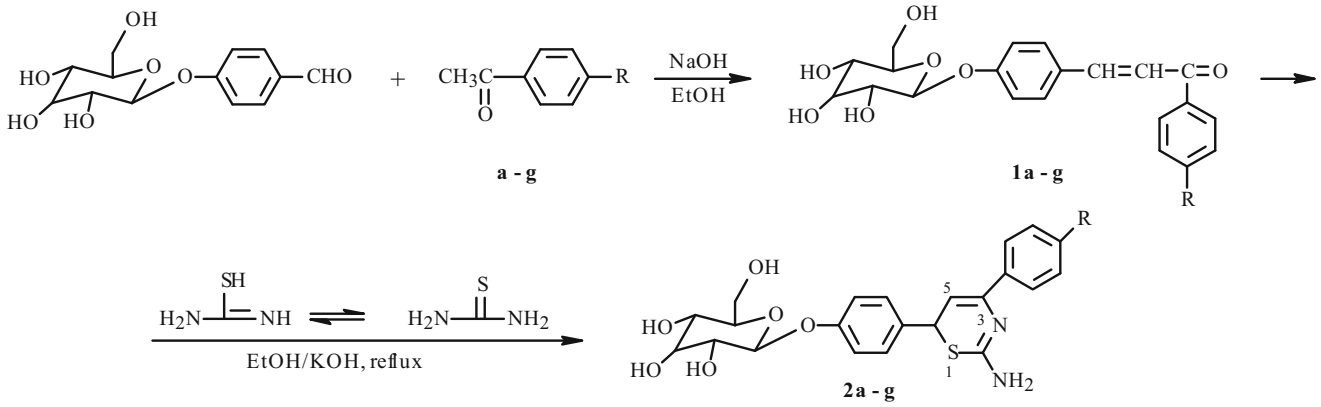
TABLE 1. Spontaneous Motion Results of Target Compounds Administered to Mice, min

Entry	Pre-administration, $\bar{x} \pm s$	Post-administration, $\bar{x} \pm s$		
		30 min	60 min	90 min
0.05% CMC	179.32±49.17	161.00±32.55	176.33±77.11	185.33±76.18
Diazepam[a]	170.17±61.80	37.92±34.45**	13.33±17.88**	11.33±16.59***
Helicid	187.73±76.08	165.55±51.57*	162.67±44.47	110.45±69.74
2a	167.50±38.03	149.17±59.79	153.50±46.15	164.45±53.35
2b	183.67±67.67	119.73±38.35*	84.33±59.53**	71.99±45.98**
2c	189.75±81.98	166.67±53.21	171.57±61.82	166.45±55.68
2d	183.50±60.75	147.60±54.39	159.67±67.45	171.89±69.10
2e	174.83±55.64	160.83±99.71	182.67±46.78	183.17±61.69
2f	187.33±75.87	155.43±43.49	99.15±38.97*	119.53±54.67*
2g	172.41±68.57	158.67±43.46	165.37±58.29	171.17±59.34

*P < 0.05; **P < 0.01, ***P < 0.001 compared with 0.05% CMC. Dose, mg/kg – 200. [a]Diazepam – 20 mg/kg.



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

Scheme 1

EXPERIMENTAL

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 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). Flash column chromatography was performed on silica gel (300–400 mesh, Qingdao, China). Thin-layer chromatography (TLC) was performed on precoated Merck silica gel $60F_{254}$ plates. An electronic balance (Satorius BS210S) and multi-function mouse locomotor activity recorder (YLS-1A) were used. All the other reagents and solvent were commercially available and used without further purification. According to the literature [6], compounds **1a–e** were obtained.

General Procedure for the Preparation of Compounds 1f–g. To a solution of helicid (1.420 g, 5 mmoL) in anhydrous ethanol (30 mL), 10% sodium hydroxide aqueous solution was added until helicid dissolved in the ice bath, and then 4-substituted acetophenone (5.5 mmol) was added. The progress of the reaction was monitored by TLC. The mixture was neutralized with diluted hydrochloric acid (3 mol/L) when the reaction ended, the solvent was removed under reduced pressure, and water (30 mL) was added. The solution was extracted three times with ethyl acetate (25 mL), and the combined organic layers were dried over anhydrous sodium sulfate after being concentrated by evaporation in *vacuo*. The residue thus separated was subjected to column chromatography. It was first eluted with dichloromethane to remove impurities and then with chloroform–methanol (8:1) to give a pale yellow solid.

***E*-(4-β-D-Allopyranosyloxyphenyl)-1-(4-flourophenyl)-propenone (1f)**. This compound was prepared from 4-fluoroacetophenone. Yield: 263 mg (65%), pale yellow solid, mp 108–110°C.

^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.45–3.95 (6H, m), 5.20 (1H, d, J = 8.0, H-1), 7.73 (1H, d, J = 16.0, CH=CH), 7.07–7.88 (6H, d, PhH), 7.83 (1H, d, J = 16.0, CH=CHCO), 8.23–8.26 (2H, d, PhH), 4.51–5.11 (4H, br, 4OH).

IR (KBr, ν_{max}, cm^{-1}): 3400, 3072, 2900, 1655, 1598, 1509, 1426, 1333, 1180, 1084, 1030, 870, 824.

ESI-LR-MS *m/z* 405.1 $[M + H]^+$, ESI-LR-MS *m/z* calcd for $C_{21}H_{22}O_7F$ $[M + H]^+$ 405.1344, found 405.1362.

***E*-(4-β-D-Allopyranosyloxyphenyl)-1-(4-ethylphenyl)-propenone (1g)**. This compound was prepared from 4-ethylacetophenone. Yield: 290 mg (70%), pale yellow solid, mp 85–87°C.

^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.12–1.16 (3H, t, CH_3), 2.62 (2H, q, J = 15.2, CH_2), 3.31–3.87 (6H, m), 5.12 (1H, d, J = 8.0, H-1), 7.63 (1H, d, J = 16.0, CH=CH), 7.00–7.79 (6H, d, PhH), 7.75 (1H, d, J = 16.0, CH=CHCO), 8.01 (2H, d, PhH), 4.45–5.05 (4H, br, 4OH).

IR (KBr, ν_{max}, cm^{-1}): 3437, 2935, 2891, 1651, 1606, 1509, 1334, 1175, 1078, 1030, 978, 845, 821. ESI-LR-MS *m/z* 437.1 $[M + Na]^+$; ESI-LR-MS *m/z* calcd for $C_{23}H_{26}O_7Na$ $[M + Na]^+$ 437.1571, found 437.1584.

General Procedure for the Preparation of Compounds 2a–g. In a round bottomed flask, a mixture of **2a–g** (1 mmol), thiourea (1.2 mmol), and potassium hydroxide (110 mg) in 95° ethanol (15 mL) was introduced. The mixture was heated to reflux, and the progress of the reaction was monitored by TLC. It was acidified with dilute hydrochloric acid (1 mol/L) to give a yellow solid when the reaction ended, then filtered. The solution was concentrated, and the residue thus separated was subjected to column chromatography. It was first eluted with ethyl acetate to remove impurities, then with chloroform–hexane–methanol (3:1:1) to give a solid.

6*H*-2-Amino-4-phenyl-6-(4-β-D-allopyranosyloxyphenyl)-1,3-thiazine (2a). This compound was prepared from **1a**. Yield: 231 mg (52%), yellow solid, mp 138–140°C.

^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.13–3.92 (6H, m), 5.07 (1H, d, J = 6.4, H-6), 5.11 (1H, d, J = 8.0, H-1), 5.36 (1H, d, J = 4.8, H-5), 7.02–7.52 (9H, d, PhH), 4.47–5.06 (4H, br, 4OH), 9.07–9.82 (2H, br, NH_2).

IR (KBr, ν_{max}, cm^{-1}): 3368, 2925, 1608, 1559, 1508, 1232, 1178, 1080, 1035, 832, 760, 696. ESI-LR-MS *m/z* 467.1 $[M + Na]^+$; ESI-LR-MS *m/z* calcd for $C_{22}H_{24}O_6N_2SNa$ $[M + Na]^+$ 467.1247, found 467.1254.

6*H*-2-Amino-4-(4-methylphenyl)-6-(4-β-D-allopyranosyloxyphenyl)-1,3-thiazine (2b). This compound was prepared from **1b**. Yield: 254 mg (55%), yellow solid, mp 160–162°C.

^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.31 (3H, s, CH_3) 3.12–3.92 (6H, m), 5.04 (1H, d, J = 4.4, H-6), 5.11 (1H, d, J = 8.0, H-1), 5.31 (1H, d, J = 4.8, H-5), 7.02–7.41 (8H, d, PhH), 4.41–5.04 (4H, br, 4OH), 9.05–9.80 (2H, br, NH_2).

IR (KBr, ν_{max}, cm^{-1}): 3370, 2925, 1608, 1559, 1508, 1233, 1178, 1081, 1031, 812, 636. ESI-LR-MS *m/z* 481.1 $[M + Na]^+$; ESI-LR-MS *m/z* calcd for $C_{23}H_{26}O_6N_2SNa$ $[M + Na]^+$ 484.1404, found 481.1421.

6*H*-2-Amino-4-(4-methoxyphenyl)-6-(4-β-D-allopyranosyloxyphenyl)-1,3-thiazine (2c). This compound was prepared from **1c**. Yield: 237 mg (50%), yellow solid, mp 150–152°C.

^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.37 (3H, s, OCH_3) 3.33–3.91 (6H, m), 5.07 (1H, d, J = 6.8, H-6), 5.11 (1H, d, J = 8.0, H-1), 5.26 (1H, d, J = 4.8, H-5), 6.92–7.46 (8H, d, PhH), 4.48–5.04 (4H, br, 4OH), 9.04–9.73 (2H, br, NH_2).

IR (KBr, ν_{max}, cm^{-1}): 3381, 2924, 1608, 1567, 1510, 1510, 1464, 1235, 1178, 1081, 1035, 834, 631. ESI-LR-MS *m/z* 497.1 $[M + Na]^+$; ESI-LR-MS *m/z* calcd for $C_{23}H_{26}O_7N_2SNa$ $[M + Na]^+$ 497.1353, found 497.1357.

6*H*-2-Amino-4-(4-chlorophenyl)-6-(4-β-D-allopyranosyloxyphenyl)-1,3-thiazine (2d). This compound was prepared from **1d**. Yield: 296 mg (62%), yellow solid, mp 155–157°C.

^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.17–3.92 (6H, m), 5.06 (1H, d, J = 4.8, H-6), 5.11 (1H, d, J = 8.0, H-1), 5.39 (1H, d, J = 4.8, H-5), 7.02–7.57 (8H, d, PhH), 3.62–5.05 (4H, br, 4OH), 9.01–9.91 (2H, br, NH_2).

IR (KBr, ν_{max}, cm^{-1}): 3384, 2925, 1602, 1566, 1508, 1233, 1178, 1084, 1036, 832, 630. ESI-LR-MS *m/z* 501.0 $[M + Na]^+$; ESI-LR-MS *m/z* calcd for $C_{22}H_{23}Cl_1O_6N_2SNa$ $[M + Na]^+$ 501.0858, found 501.0836.

6*H*-2-Amino-4-(4-bromophenyl)-6-(4-β-D-allopyranosyloxyphenyl)-1,3-thiazine (2e). This compound was prepared from **1e**. Yield: 298 mg (57%), yellow solid, mp 170–172°C.

^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.33–3.92 (6H, m), 5.04 (1H, d, J = 4.8, H-6), 5.11 (1H, d, J = 8.0, H-1), 5.40 (1H, d, J = 4.4, H-5), 7.02–7.68 (8H, d, PhH), 4.47–5.05 (4H, br, 4OH), 9.09–9.91 (2H, br, NH_2).

IR (KBr, ν_{max}, cm^{-1}): 3376, 2925, 1609, 1559, 1508, 1483, 1233, 1178, 1078, 1037, 830, 630. ESI-LR-MS *m/z* 545.0 $[M + Na]^+$; ESI-LR-MS *m/z* calcd for $C_{22}H_{23}BrO_6N_2SNa$ $[M + Na]^+$ 545.0352, found 545.0353.

6*H*-2-Amino-4-(4-fluorophenyl)-6-(4-β-D-allopyranosyloxyphenyl)-1,3-thiazine (2f). This compound was prepared from **1f**. Yield: 245 mg (53%), yellow solid, mp 173–175°C.

^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.32–3.92 (6H, m), 5.06 (1H, d, J = 4.8, H-6), 5.11 (1H, d, J = 8.0, H-1), 5.31 (1H, d, J = 4.8, H-5), 7.01–7.34 (8H, d, PhH), 4.49–5.05 (4H, br, 4OH), 9.02–9.92 (2H, br, NH_2).

IR (KBr, ν_{max}, cm^{-1}): 3391, 2925, 1602, 1573, 1505, 1409, 1233, 1178, 1079, 1038, 836, 574. ESI-LR-MS *m/z* 485.1 $[M + Na]^+$; ESI-LR-MS *m/z* calcd for $C_{22}H_{23}FO_6N_2SNa$ $[M + Na]^+$ 485.1153, found 485.1126.

6*H*-2-Amino-4-(4-ethylphenyl)-6-(4-*β*-D-allopyranosyloxyphenyl)-1,3-thiazine (2g). This compound was prepared from **1g**. Yield: 255 mg (54%), yellow solid, mp 154–156°C.

^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.18 (3H, t, CH_3), 2.61 (2H, q, J = 15.2, CH_2), 3.33–3.92 (6H, m), 5.07 (1H, d, J = 6.8, H-6), 5.11 (1H, d, J = 8.0, H-1), 5.32 (1H, d, J = 4.8, H-5), 7.02–7.42 (8H, d, PhH), 4.47–5.05 (4H, br, 4OH), 9.05–9.75 (2H, br, NH_2).

IR (KBr, ν_{max}, cm^{-1}): 3369, 2926, 1608, 1557, 1508, 1470, 1233, 1177, 1081, 1035, 835, 637. ESI-LR-MS *m/z* 495.1 $[M + Na]^+$; ESI-LR-MS *m/z* calcd for $C_{24}H_{28}O_6N_2SNa$ $[M + Na]^+$ 495.1560, found 495.1564.

Mice (Kunming strain) weighing 18–22 g were obtained from West China School of Pharmacy of Sichuan University (Chengdu China). Diazepam injects were purchased from Huayin Jinqiancheng Pharmaceutical Co. (China) Ltd. The sedative-hypnotic activity of samples was evaluated as inhibition of spontaneous motion resulting from administration of the target compounds to mice. All samples were dissolved in 0.05% CMC (carboxymethylcellulose) to form solutions of concentration 200 mg/kg for use later. Diazepam injects were dissolved in saline to form solutions of concentration 200 mg/kg for use later. Sixty mice were randomized in the experiment and divided into 10 groups of 6 mice each (3 male and 3 female). The mice were placed in a recorder for 5 min before the experiment, and the same conditions were maintained throughout the experiment. In the tests, the prepared solutions were injected into the mouse stomach with a syringe at a volume of 0.2 mL·10 g^{-1}, and the spontaneous activity was recorded after the first 5 min, then after 30, 60, and 90 min. The data were recorded in the form of the number of movements per minute. Compound **2b** (200 $mg \cdot kg^{-1}$) showed better activity than the parent helicid. So further modification of helicid should be worthwhile.

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