Chemistry of Natural Compounds, Vol. 46, No. 5, 2010

SYNTHESIS AND CALMING ACTIVITY OF 2-AMINO-4-(4-β-D-ALLO-PYRANOSIDE-PHENYL)-6-3(4)-SUBSTITUTED PHENYLPYRIMIDINES

XiuJuan Yin, Lei Zheng, Ying Li, and ShuFan Yin*

UDC 547.495.9

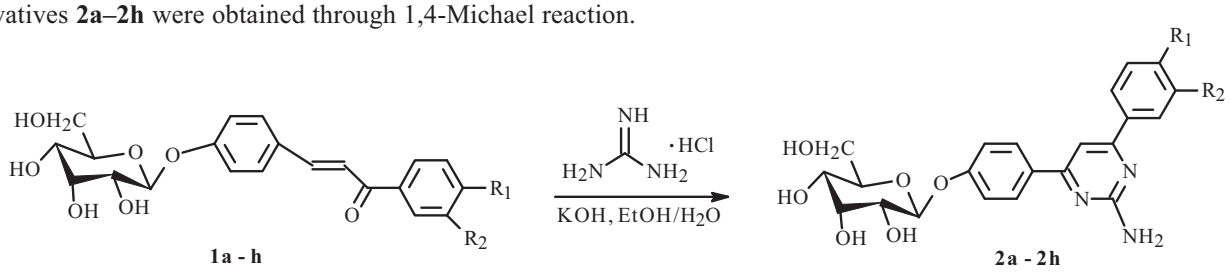
Using helicid as starting material, E-*4*-β-*D-allopyranoside-cinnamic-4-substituted phenylketones (**1a–1h**) containing the structure of chalcones were synthesized; then these chalcones were reacted with guanidine hydrochloride through a 1,4-Michael reaction, giving 2-amino-4-(4-*β-*D-allopyranoside-phenyl)-6-3(4)-substituted phenylpyrimidines (**2a–2h**), which were characterized by IR, ^{1}H NMR, and HR-MS. The target compounds were evaluated by the spontaneous locomotor activity test, showing that all of them had good calming activity; compound **2f** was found to have the greatest.*

Keywords: helicid, guanidine hydrochloride, pyrimidine, calming activity.

Helicid (4-formylphenyl-β-D-allopyranoside, $C_{13}H_{16}O_7$) is a pure natural compound extracted from the fruit of *Helicia nilagirica* Beed, which has been successfully used in the treatment of patients with insomnia in China. It has good biological activities, such as calming and sleep-inducing activity for the treatment of neurasthenia and neurasthenia syndrome caused by headache, poisoning, or rheumatism [1–3]. However, it also has some disadvantages, such as slow action and low biological utilization. Therefore, in order to obtain helicid analogues with better therapeutic effect and low side effect, we try our best to search for a ‘‘superhelicid’’.

Our attention was focused on the restructuring of the formyl group of aromatic ring of 4-formylphenyl-β-D-allopyranoside. 1-Substituted *N*-methyl-series derivatives [4, 5], benzimidazole derivatives [6, 7], and oxadiazoline and isoxazole derivatives [8, 9] were obtained by the Mannich reaction, condensation reaction, and 1,3-dipolar cycloaddition reaction, respectively. Because pyrimidines have the basic nucleus in nucleic acids and have been associated with a number of biological activities [10–12], the pyrimidine ring was introduced into the structure of helicid in order to further improve the biological activities of helicid.

Styryl-acetophenone, guanidine hydrochloride, and KOH can be directly generate the pyrimidine ring [13, 14]. In this paper, the extension of the formyl conjugate carbon chain was performed by the Shmidt-Claisen reaction to get α,β-unsaturated carbonyl derivatives **1a–1h**. Because helicid is similar to the acetal structure, which is more stable in the base solution than in the acid solution [15], guanidine hydrochloride was neutrallized with KOH solution, and then pyrimidine derivatives **2a–2h** were obtained through 1,4-Michael reaction.



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

a: $R_1 = R_2 = H$; **b:** $R_1 = CH_3$, $R_2 = H$; **c:** $R_1 = CH_2CH_3$, $R_2 = H$; **d:** $R_1 = OCH_3$, $R_2 = H$
e: $R_1 = F$, $R_2 = H$; **f:** $R_1 = Cl$, $R_2 = H$; **g:** $R_1 = Br$, $R_2 = H$; **h:** $R_1 = H$, $R_2 = Cl$

College of Chemistry, Sichuan University, Chengdu, 610064, P. R. China, fax: 86 028 85503392, e-mail: chuandayouji217@163.com. Published in Khimiya Prirodnykh Soedinenii, No. 5, pp. 657–659, September–October, 2010. Original article submitted April 23, 2009.

0009-3130/10/4605-0779

TABLE 1. Sedative-hypnotic Activities for Target Compounds Evaluated Using the Spontaneous Locomotor Activity Test

Compound	Number of movements per minute					
	initial	after 15 min	after 30 min	after 60 min	after 90 min	after 120 min
Saline	217.67±8.24	181.83±36.20	170.33±32.05	163.67±29.18	165.33±31.83	160.67±29.43
Diazepam	209.00±9.72	3.17±2.79***	3.17±1.90***	0.67±0.49**	0.00±0.00**	4.00±2.02**
Helicid	214.14±92.87	168.27±31.00	136.01±66.92	149.35±42.75	139.61±52.14	146.26±30.63
2a	218.1±17.71	171.00±25.85	112.50±18.14	136.17±35.75	92.67±33.44*	99.00±34.19*
2b	222.50±15.58	88.33±24.08**	119.00±34.12	107.33±26.92	168.67±26.94	112.83±37.86
2c	214.67±14.91	95.50±21.15**	135.83±13.88	119.83±18.61	148.50±20.99	110.17±27.92
2d	213.00±10.49	122.50±27.98	69.33±18.05**	161.67±24.40	110.00±28.48	107.83±36.20
2e	213.33±24.78	202.50±17.21	146.00±34.73	68.17±29.55**	138.83±34.65	52.67±20.74**
2f	207.50±6.30	111.00±8.03*	43.83±15.83**	72.00±28.53	90.33±21.63	14.17±7.88***
2g	208.83±13.04	141.17±29.44	50.00±28.24*	77.50±36.04	90.83±24.41	59.50±28.76**
2h	215.00±15.61	154.50±13.95	76.00±25.49*	84.33±19.89	109.67±33.68	59.67±23.54**

Values are means ± S. *P < 0.05, **P < 0.01, ***P < 0.001 compared with saline.
Dose of compound: 200 mg·kg^{-1}; diazepam, 20 mg·kg^{-1}.

The results of spontaneous locomotor activity test are shown in Table 1. Compared with saline helicid, all the compounds tested showed good calming activities. Moreover, compounds **2e**, **2f**, **2g**, and **2h** significantly altered the spontaneous locomotor activity. After 30 min the motor activity of derivatives **2e**, **2f**, **2g**, and **2h** decreased from 213.33 ± 24.78, 207.50 ± 6.30, 208.83 ± 13.04, and 215.00 ± 15.61 movements/min to 146.00 ± 34.73, 43.83 ± 15.83, 50.00 ± 28.24, and 76.00 ± 25.49 movements/min, respectively. After 120 min the motor activity of derivatives **2e**, **2f**, **2g**, and **2h** decreased from 146.00 ± 34.73, 43.83 ± 15.83, 50.00 ± 28.24, and 76.00 ± 25.49 movements/min to 52.67 ± 20.74, 14.17 ± 7.88, 59.50 ± 28.76, and 59.67 ± 23.54 movements/min, respectively. In particular, compound **2f** was found to be the best. In general, the order of calming activities of the 2-amino-4-(4-β-D-allopyranoside-phenyl)-6-3(4)-substituted-aryl-pyrimidines at the -6-[3(4)-substituted] phenyl position was electron-drawing groups > electron-donating groups. Comparison of substituents at the 3 and 4 positions showed that 4-substituents were better. Compounds **2a–2h** were characterized and evaluated for their calming activities in mice. So further modification of helicid should be worthwhile.

EXPERIMENTAL

Materials see [16].

Mice (Kunming strain) weighting 17–22 g were obtained from the West China School of Pharmacy at Sichuan University (Chengdu China). All samples were dissolved in 0.9% NaCl solution to form different concentrations of solutions for later use.

Compounds **1a**, **1b**, **1d**, **1f**, and **1g** were prepared according to [17], while compounds **1c** and **1e** were prepared according to [18].

***E*-4-β-D-Allopyranoside-cinnamic-3-chlorophenyl Ketone (1h)**. To a solution of helicid (1.420 g, 5 mmol) in 30 mL of anhydrous ethanol, 10% NaOH aqueous solution were added in a period of about 30 min under an ice bath, and then 3-substituted hypnone (2.310 g, 5.5 mmol) was added. This reaction was maintained at 0°C for 10 h, monitored by TLC. The solution was neutralized with diluted hydrochloric acid after the reaction ended, then concentrated to half of the original volume; then water (30 mL) was added. The solid was filtered and recrystallized from ethanol–H_2O (5:1) to give a pale yellow solid.

Yield 80%, mp 90–94°C, $[\alpha]_D^{25}$ –28.10° (*c* 0.017 mol/L, C_2H_5OH). IR (KBr, cm^{-1}): 3380, 3068, 2892, 1653, 1596, 1508, 1423, 1215, 1172, 1078, 1033, 975, 809, 693, 788, 614. ^{1}H NMR (400 MHz, DMSO-d_6, δ, J/Hz): 4.50–5.20 (6H, m), 3.35–3.85 (4H, br, 4OH), 5.22–5.23 (1H, m, OCHO), 7.81 (1H, d, J = 16.0, CH=CHCO), 7.30–7.70 (4H, m, Ar-H), 7.60–8.00 (4H, m, m-Cl-Ar-H). HR-MS-ESI calcd for $C_{21}H_{21}O_7Na$ $[M + Na]^+$: 443.0866, found: 443.0867.

General Procedure for 2-Amino-4-(4-β-D-allopyranoside-phenyl)-6-3(4)-substituted-phenyl-pyrimidines (2a–2h). Guanidine hydrochloride (1.43 g, 15 mmol) was dissolved in 10 mL of water, then neutralized with 20% KOH aqueous solution, which was then heated to 50–60°C for 0.5 h. Then an ethanol solution of **1a** (5 mmol) was added, and the mixture

stirred at reflux temperature for 7–8 h. The reaction was monitored by TLC, concentrated under reduce pressure in a rotary evaporator, and the crude product was purified by flash chromatography on silica gel (MeOH–$CHCl_3$, 1:6, v/v) to afford the pure compound **2a**.

2-Amino-4-(4-β-D-allopyranoside-phenyl)-6-phenylpyrimidine (2a). This compound was prepared from compound **1a**. Yellow solid, yield 64%, mp 150–152°C, $[\alpha]_D^{25}$ –27.61° (*c* 0.016 mol/L, C_2H_5OH). IR (KBr, cm^{-1}): 3361, 2920, 1609, 1570, 1539, 1511, 1452, 1365, 1231, 1179, 1079, 1036, 916, 830, 771, 695. ^1H NMR (400 MHz, DMSO-d_6, δ): 3.95 (2H, s, NH_2), 4.55–5.30 (6H, m), 3.30–3.80 (4H, br, 4OH), 5.21–5.24 (1H, m, OCHO), 6.80 (1H, s, 5-H), 7.10–7.80 (4H, m, Ar-H), 8.10–8.30 (5H, m, Ar-H). HR-MS-ESI calcd for $C_{22}H_{24}N_3O_6$ $[M + H]^+$: 426.1660, found: 426.1642.

2-Amino-4-(4-β-D-allopyranoside-phenyl-6-(4-methylphenyl)pyrimidine (2b). This compound was prepared from compound **1b**. Yellow solid, yield 62%, mp 150–153°C, $[\alpha]_D^{25}$ –28.35° (*c* 0.021 mol/L, C_2H_5OH). IR (KBr, cm^{-1}): 3381, 2920, 1609, 1579, 1538, 1509, 1445, 1366, 1233, 1132, 1080, 1036, 916, 815, 754, 653. ^1H NMR (400 MHz, DMSO-d_6, δ): 2.31 (3H, s, CH_3), 3.96 (2H, s, NH_2), 4.55–5.25 (6H, m), 3.35–4.00 (4H, br, 4OH), 5.24–5.26 (1H, m, OCHO), 6.7 (1H, s, 5-H), 7.10–7.60 (4H, m, Ar-H), 8.20–8.30 (4H, m, *p*-CH_3-Ar-H). HR-MS-ESI calcd for $C_{23}H_{26}N_3O_6$ $[M + H]^+$: 440.1816, found: 440.1802.

2-Amino-4-(4-β-D-allopyranoside-phenyl)-6-(4-ethylphenyl)pyrimidine (2c). This compound was prepared from compound **1c**. Yellow solid, yield 61%, mp 152–154°C, $[\alpha]_D^{25}$ –27.41° (*c* 0.025 mol/L, C_2H_5OH). IR (KBr, cm^{-1}): 3401, 2920, 1641, 1571, 1536, 1512, 1453, 1367, 1232, 1180, 1081, 1037, 915, 831, 771, 574. ^1H NMR (400 MHz, DMSO-d_6, δ): 1.25 (3H, t, CH_3), 2.35 (2H, q, CH_2), 3.98 (2H, s, NH_2), 4.60–5.30 (6H, m), 3.40–3.80 (4H, br, 4OH), 5.24–5.26 (1H, m, OCHO), 6.50 (1H, s, 5-H), 7.10–7.40 (4H, m, Ar-H), 8.10–8.30 (4H, m, *p*-CH_2CH_3-Ar-H). HR-MS-ESI calcd for $C_{24}H_{28}N_3O_6$ $[M + H]^+$ 454.1973, found: 454.1992.

2-Amino-4-(4-β-D-allopyranoside-phenyl)-6-(4-methoxyphenyl)pyrimidine (2d). This compound was prepared from compound **1d**. Yellow solid, yield 63%, mp 149–151°C, $[\alpha]_D^{25}$ –28.13° (*c* 0.026 mol/L, C_2H_5OH). IR (KBr, cm^{-1}): 3353, 2925, 1622, 1569, 1538, 1511, 1461, 1365, 1232, 1153, 1078, 1034, 916, 828, 787, 695. ^1H NMR (400 MHz, DMSO-d_6, δ): 3.20 (3H, s, OCH_3), 3.90 (2H, s, NH_2), 4.55–5.25 (6H, m), 3.35–4.00 (4H, br, 4OH), 5.22–5.24 (1H, m, OCHO), 6.70 (1H, s, 5-H), 7.10–7.60 (4H, m, Ar-H), 8.15–8.35 (4H, m, *p*-OCH_3-Ar-H). HR-MS-ESI calcd for $C_{23}H_{26}N_3O_7$ $[M + H]^+$: 456.1765, found: 456.1767.

2-Amino-4-(4-β-D-allopyranoside-phenyl)-6-(4-fluorophenyl)pyrimidine (2e). This compound was prepared from compound **1e**. Yellow solid, yield 65%, mp 152–153°C, $[\alpha]_D^{25}$ –27.55° (*c* 0.022 mol/L, C_2H_5OH). IR (KBr, cm^{-1}): 3365, 2923, 1604, 1575, 1540, 1508, 1446, 1366, 1229, 1180, 1080, 1037, 911, 823, 755, 639. ^1H NMR (400 MHz, DMSO-d_6, δ): 3.95 (2H, s, NH_2), 4.40–5.10 (6H, m), 3.25–3.80 (4H, br, 4OH), 5.25–5.27 (1H, m, OCHO), 6.75 (1H, s, 5-H), 7.10–7.40 (4H, m, Ar-H), 8.15–8.35 (4H, m, *p*-F-Ar-H). HR-MS-ESI calcd for $C_{22}H_{23}FN_3O_6$ $[M + H]^+$: 444.1565, found: 444.1556.

2-Amino-4-(4-β-D-allopyranoside-phenyl)-6-(4-chlorophenyl)pyrimidine (2f). This compound was prepared from compound **1f**. Yellow solid, yield 66%, mp 152–154°C, $[\alpha]_D^{25}$ –26.34° (*c* 0.020 mol/L, C_2H_5OH). IR (KBr, cm^{-1}): 3377, 2922, 1613, 1584, 1536, 1511, 1445, 1364, 1231, 1179, 1084, 1038, 910, 819, 775, 637. ^1H NMR (400 MHz, DMSO-d_6, δ): 3.90 (2H, s, NH_2), 4.55–5.25 (6H, m), 3.35–4.0 (4H, br, 4OH), 5.22–5.24 (1H, m, OCHO), 6.70 (1H, s, 5-H), 7.10–7.60 (4H, m, Ar-H), 8.15–8.35 (4H, m, *p*-Cl-Ar-H). HR-MS-ESI calcd for $C_{22}H_{23}ClN_3O_6$ $[M + H]^+$: 460.1270, found: 460.1248.

2-Amino-4-(4-β-D-allopyranoside-phenyl)-6-(4-bromophenyl)pyrimidine (2g). This compound was prepared from compound **1g**. Yellow solid, yield 65%, mp 153–155°C, $[\alpha]_D^{25}$ –28.00° (*c* 0.017 mol/L, C_2H_5OH). IR (KBr, cm^{-1}): 3369, 2921, 1610, 1586, 1538, 1511, 1445, 1363, 1231, 1178, 1080, 1036, 912, 817, 754, 619. ^1H NMR (400 MHz, DMSO-d_6, δ): 3.95 (2H, s, NH_2), 4.50–5.20 (6H, m), 3.35–3.85 (4H, br, 4OH), 5.22–5.24 (1H, m, OCHO), 6.80 (1H, s, 5-H), 7.10–7.80 (4H, m, Ar-H), 8.15–8.35 (4H, m, *p*-Br-Ar-H); HR-MS-ESI calcd for $C_{22}H_{23}BrN_3O_6$ $[M + H]^+$: 504.0765, found: 504.0754.

2-Amino-4-(4-β-D-allopyranoside-phenyl)-6-(3-chlorophenyl)pyrimidine (2h). This compound was prepared from compound **1h**. Yellow solid, yield 61%, mp 152–153°C, $[\alpha]_D^{25}$ –26.62° (*c* 0.019 mol/L, C_2H_5OH). IR (KBr, cm^{-1}): 3380, 2921, 1570, 1571, 1538, 1512, 1444, 1361, 1231, 1179, 1079, 1036, 907, 827, 788, 614. ^1H NMR (400 MHz, DMSO-d_6, δ): 3.95 (2H, s, NH_2), 4.50–5.20 (6H, m), 3.35–3.85 (4H, br, 4OH), 5.24–5.26 (1H, m, OCHO), 6.80 (1H, s, 5-H), 7.10–7.60 (4H, m, Ar-H), 8.15–8.35 (4H, m, m-Cl-Ar-H). HR-MS-ESI calcd for $C_{22}H_{23}ClN_3O_6$ $[M + H]^+$: 460.1270, found: 460.1275.

Biological Activity Tests. All the target compounds were tested for sedative-hypnotic activities by the spontaneous locomotor activity method. All samples were dissolved in saline (200 mg/kg). Diazepam was dissolved in saline (20 mg/kg) for use later. Sixty-six mice were randomized into 11 groups of 6 mice each (3 male and 3 female). In order to maintain suitable environmental conditions throughout the experiments, all mice were placed in a recorder for 5 min before the experiments. Group A received saline by injection, group B received diazepam (20 mg/kg, i.p.), group C received 4-formylphenyl-β-D-

allopyranoside (20 mg/kg, i.p.), and groups **2a**–**2h** received the synthesized compounds (200 mg/kg, i.p.). When testing, the prepared solutions were injected into the mouse stomach with a syringe in a volume of 0.2 mL/10 g body weight, and the spontaneous activity was recorded for 5 min after 0, 15, 30, 60, 90, and 120 min. The data were recorded as number of movements per minute.

ACKNOWLEDGMENT

We are grateful to the Analytical & Testing Center of Sichuan University, P. R. China for providing analytical data, and Mr. Bao (College of Pharmacy, Sichuan University) for completing the sedative-hypnotic test.

REFERENCES

1. Jiangsu Medical College, Chinese Dictionary, Shanghai Science and Technology Press, Shanghai, 1985, p. 1046.
2. Z. Y. Wu, *Yunnan List of Seed Plants*, Yunnan People′s Publishing House, Kunming, 1985, p. 322.
3. W. S. Chen, S. D. Lu, and B. Eberhard, *Liebigs Ann. Chem*., **10**, 1893 (1981).
4. Q. L. Zhu, Q. Tang, Y. Li, and S. F. Yin, *Chin. J. Org. Chem*., **26**, 1264 (2006).
5. Q. L. Zhu, Y. Li, J. L. Li, and S. F. Yin, *Huaxi Pharm. J.*, **23** (1), 012 (2008).
6. H. J. Yang, C. Hu, Y. Li, and S. F. Yin, *Chin. J. Org. Chem*., **28** (5), 899 (2008).
7. C. Hu, H. J. Yang, Y. Li, and S. F. Yin, *Chin. J. Org. Chem*., **29** (1), 89 (2009).
8. M. Liu, Y. Li, and S. F. Yin, *Chin. J. Org. Chem*., **28** (2), 348 (2008).
9. J. L. Li, S. F. Yin, Y. L i, and B. Fan, Chinese Patent, 2007100494459 (2007).
10. K. L. Sayle, J. Bentley, F. T. Boyle, A. H. Calvert, Y. Z. Cheng, N. J. Curtin, J. A. Endicott, B. T. Golding, L. Z. Wang, and R. J. Griffin, *Bioorg. Med. Chem. Lett*., **13**, 3079 (2003).
11. M. A. Ghannoum, K. Abu-Elteen, and N. R. El-Rayyes, *Microbios*, **60**, 23 (1989).
12. N. Ingarsal, G. Saravanan, P. Amutha, and S. Nagarajan, *Eur. J. Med. Chem*., **42**, 517 (2007).
13. A. L. Marzinzik and E. R. Felder, *J. Org. Chem*., **63**, 723 (1998).
14. L. Varga, T. Nagy, I. Kovesdi, J. Benet-Buchkholz, G. Dorman, L. Urge, and F. Darvas, *Tetrahedron*, **59**, 655 (2003).
15. Q. Y. Xing, R. Q. Xu, Z. Zhou, and W. W. Pei, *Basic Organic Chemistry*, Higher Education Press, Beijing, 1994, p. 974.
16. L. Fu, G. P. Chen, Y. Li, and S. F. Yin, *Chem. Nat. Comp*., **46**, (2010).
17. B. Fan, J. L. Li, Y. Li, and S. F. Yin, *Chin. J. Org. Chem*., **27**, 1150 (2007).
18. L. Fu, D. Ye, Y. L i , and S. F. Yin, Chinese Patent, 2008100454988 (2008).