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Article

Strigolactone Analogues Derived from Dihydroflavonoids as Potent Seed Germinators for the Broomrapes

Yunyao Kang, Zhili Pang, Niuniu Xu, Fangjie Chen, Zhong Jin,* and Xiaohua Xu*

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ABSTRACT: The broomrapes (*Orobanche* and *Phelipanche* spp.) and witchweeds (*Striga* spp.) are a class of parasitic weeds, which are distributed widely in the tropical, subtropical, and temperate areas of the globe. Since they have completely consistent lifecycles with the host plants, it is difficult to control them selectively through using the conventional herbicides. Inducing suicidal germination of these weed seeds by small molecular signaling agents proved to be a promising strategy for the management of parasitic weeds. As a class of naturally occurring terpenoid metabolites, strigolactones (SLs) show significant biological activities including stimulation germination of weed seeds, inhibition of shoot-branching, and so on. However, the widespread application of these natural SLs is greatly limited by their extremely low natural abundance and complex molecular structures. Design and synthesis of the simplified analogues as natural SLs alternatives provide a viable avenue for the efficient control of these parasitic weeds. We herein disclose the development of a novel class of SLs analogues derived from dihydroflavonoids as potent seed germinators of parasitic weeds. It was shown that one of them displayed a higher potential toward the seed germination of the broomrapes than the positive control GR24. The structure−activity relationship of these SLs analogues was further validated on the basis of the binding affinity experiment to strigolactone receptor protein *HTL7* by using a YLG fluorescent probe method.

KEYWORDS: *strigolactones, parasitic weeds, seed germination, dihydroflavonoids, Orobanche*

INTRODUCTION

The broomrapes (*Orobanche* and *Phelipanche* spp.) and witchweeds (*Striga* spp.) belong to a class of parasitic angiosperms.[1,2] They can directly invade to the roots of the host plants, such as corn, sorghum, millet, rice, and sugar cane, thereby competing for the nutritional uptake with the host plants. Commonly, *Striga* species own the functional chloroplasts and belong to a type of the hemiparasites. The low photosynthesis efficiency of *Striga* spp. makes them difficult to survive without host plants,[2] whereas holoparasites, *i.e.*, *Orobanche* spp., depend completely on the supply of nutrient from their host plants.[3] *Striga* spp. are widely distributed in the continent of Africa. According to the FAO (Food and Agriculture Organization of the United Nations) report from 2006, about six million hectacres (ha) of maize growing in eastern, western, and southern Africa were infested with *Striga* spp.[4] In western Kenya, approximate 76% of lands that grow maize and sorghum are infested with *S. hermonthica*, leading to the serious loss of crop yields every year.[5] Because the requisite temperature for seed germination of the broomrapes is normally not higher than that of *Striga* spp. seeds, the broomrapes can survive in the most subtropical and temperate zones including Asia, Australia, Mediterranean, and eastern Europe, besides Africa.[6,7] As far as we know, there are 11 species of *Orobanche* distributed in China, and *O. cumana* and *O. aegyptiaca* were found to be among the most harmful species toward the crops.[7,8] In the Xinjiang Uygur Autonomous Region of China, the infestation of *O. aegyptiaca* has caused 20−70% yield loss of melon and watermelon.[8] Although *Orobanche* spp. and *Striga* spp. have caused great harm to the crops, it was demonstrated to be hard to control them by common herbicides given that the lifecycles of these parasitic weeds are almost in accord with those of the host plants.[9] It was reported by the USDA (United States Department of Agriculture) staff that *Striga* spp. could be successfully controlled through using ethylene gas to induce the suicidal germination of parasite seeds.[10] However, it is still impractical in the developing countries considering the high expense of this methodology. Moreover, this method was also shown to be less effective to some types of the broomrapes.[6]

As mentioned above, one of the promising strategies to control *Orobanche* and *Striga* is to induce the suicidal germination of their seeds. The seed germination of the parasitic weeds could be efficiently stimulated by a class of specifically natural secondary metabolites such as strigol, orobanchol, solanacol, *etc.*, namely, strigolactones (SLs) (Scheme 1a).[11] Since strigol was first isolated from cotton roots in 1966, up to 20 strigolactones have been isolated and identified to date. As a class of naturally occurring plant signaling molecules, these compounds generally show extensive biological activities, including seed germination, shoot-branching inhibition, and root-colonization by symbiotic arbuscular mycorrhizal fungi.[12,13] Structurally, these natural

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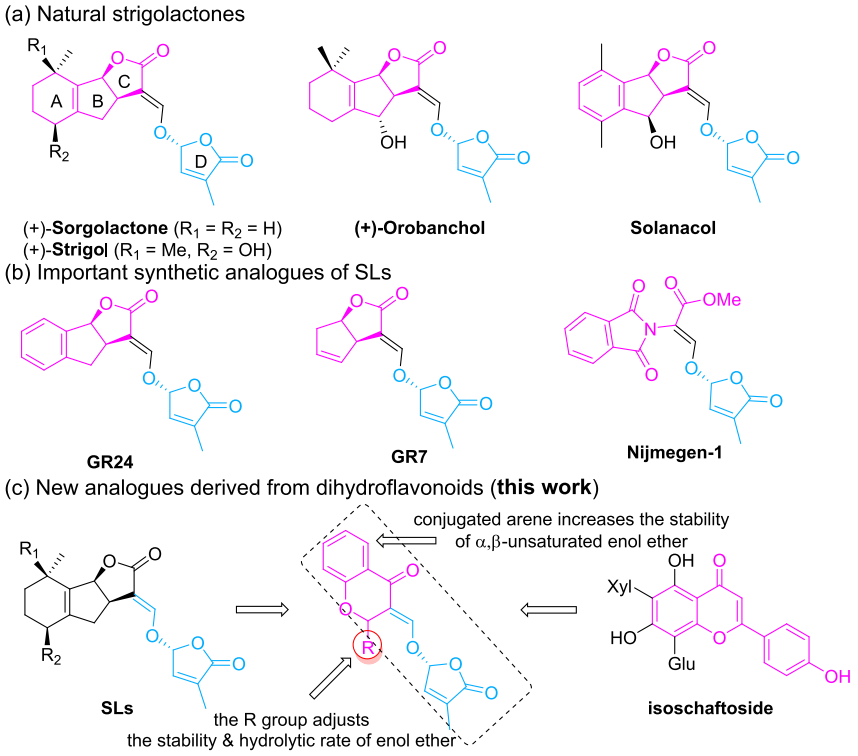
Scheme 1. Natural Strigolactones and Related Synthetic Analogues



lactones contain a fused three-ring (ABC ring) skeleton, connected with a butenolide ring (D ring) by an enol ether unit.[11] Because of the extremely low natural abundance and complicated molecular structures, the large-scale syntheses and application of these SLs as herbicides have yet to be fulfilled.[14,15] The structure−activity relationship (SAR) of SLs on seed germination was first deduced by Zwanenburg et al.,[16] and they demonstrated that the *syn*-fused lactone C/D ring moiety in their scaffolds is essential for their seed germination activity. To date, a wide range of SL analogues incorporating this active pharmacophore core, as GR24, GR7, and Nijmegen-1 (Scheme 1b), has been exploited as potent herbicides against the parasitic weeds.[17,18] Regrettably, most of synthetic analogues exert germination activity toward parasitic weeds no better than natural SLs. In addition, it is also noted that not all seeds of parasitic weeds respond to GR24 and its analogues, *e.g.*, *O. crenata*, *O. fetida*, *O. hederae*, and *O. densiflora*.[19] As a consequence, the development of novel SL analogues against those irresponsive parasitic weeds still remains highly desirable.

To the best of our knowledge, in this class of compounds, *α,β*-unsaturated enol ether connected with a lactone D ring is the indispensable active pharmacophore core for seed germination activity.[19] The strigolactone signaling mechanism study on the *α,β* hydrolase (At)D14 revealed that the generation of the active form of strigolactone involved a D14-triggering open-to-closed transition state pathway to release the active lactone D ring.[20] Consequently, the stability of *α,β*-unsaturated enol ether and hydrolytic rate of the lactone D ring highly correlated with the biological activity of SL analogues. Besides alkaloids and terpenes, flavones and the related derivatives belong to an important class of natural products that generally exhibits diverse biological activities including anti-oxidant, anti-inflammatory, anti-microbial, and anti-tumor activities.[21−23] It was shown that isoschaftoside, a di-*C*-glycosylflavone isolated from the root extract and root exudate of *Desmodium uncinatum* Jacq.,[24] could effectively prevent parasitism of *Striga hermonthica* (Del.) Benth. (witchweed) through an allelopathic mechanism (Scheme 1c). As our ongoing research toward the development of novel herbicides against the parasitic weeds, we envisioned that the hybrid compounds derived from flavonoids and active pharmacophore core of SLs might share both biological modes of action, serving as a new class of herbicides against the parasitic broomrapes and witchweeds. The incorporation of *α,β*-unsaturated enol ether in the 3-position of dihydroflavone scaffold would improve the stability of active pharmacophore core due to the presence of conjugated arene. Meanwhile, 2-substituted group in dihydroflavone might also adjust the hydrolytic rate of enol ether and hemiacetal of D ring. Herein, we report synthesis and seed germination assay toward the parasitic weeds of this type of new SL analogues derived from dihydroflavones. Of particular note, seed germination assay showed that some of synthetic SL analogues revealed seed germination activity toward the broomrapes 10 times higher than that of the positive control GR24. Moreover, the binding affinity of these dihydroflavonoid-derived SL analogues to the strigolactone receptor *HTL7* was also studied by using a YLG fluorescent probe method.

MATERIALS AND METHODS

Materials and Instruments. The seeds of *O. cumana* and *O. aegyptiaca* were kindly provided by Professor Yongqing Ma (Northwest Agriculture & Forest University, Yangling, China). Prior to the use, the seeds were sterilized by 1% (w/w) sodium hypochlorite (NaClO) for 3 min and then soaked in 75% (v/v) ethanol for another 3 min. After the surface sterilization, all seeds were thoroughly rinsed with sterile water and finally dried in air above a clean bench.

Both the expression and purification of *HTL7* protein and synthesis of Yoshimulactone Green (YLG) were performed according to the

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J. Agric. Food Chem. XXXX, XXX, XXX−XXX

previously reported methods.[25,26] Water for *HTL7* inhibition assay was distilled twice prior to use. Fluorescence measurements were carried out on a Varian Cary Eclipse spectrofluorometer (Varian Co.). ^{1}H and ^{13}C NMR and HRMS spectra of all synthesized compounds were recorded on a Bruker AvanceII400 MHz spectrometer and Agilent 6520 Q-TOF LC/MS, respectively. The starting dihydroflavone derivatives **1a−17a** were prepared from the corresponding aromatic aldehydes according to the previously reported methods.[27,28] The dihydroflavone derivatives **18a−24a** and the corresponding enol intermediates were synthesized according to the previously described methods.[29−31] The enol intermediate **25b**,[31] 5-bromo-3-methyl-2(5*H*)-furanone,[32] and *rac*-GR24[33,34] were prepared according to the previously reported procedures.

Synthesis of Strigolactone Analogues. To a solution of *N*,*N*-dimethylformamide dimethyl acetal (DMFDMA) (21 mmol) in toluene (15 mL) at room temperature was added the corresponding dihydroflavone (7 mmol). The resulting mixture was then warmed to reflux overnight and monitored by TLC. After complete consumption of the starting materials, toluene was removed under reduced pressure and the residue was purified by silica gel column chromatography (eluent: petroleum ether/ethyl acetate = 2:1, v/v) to give the desired enamine intermediates **1b−17b**.[35]

The enamine (2 mmol) thus obtained was dissolved in a mixture of THF (5 mL), acetic acid (5 mL), and H_2O (5 mL) at room temperature. The mixture was then stirred vigorously and monitored by TLC. After the completion of hydrolysis, the solution was extracted with ethyl acetate exhaustively. The combined organic phases were washed with saturated $NaHCO_3$ aqueous solution, and then, the solvent was evaporated under reduced pressure. The crude enol obtained was subjected to the next reaction without further purification.

To a solution of the crude enol intermediate in THF (15 mL) was added *t*-BuOK (2.2 mmol) at 0 °C. The mixture was stirred for 15 min at 0 °C, and a solution of 5-bromo-3-methyl-2(5*H*)-furanone (3 mmol) in THF (5 mL) was added dropwise. The reaction mixture was stirred overnight, and the solvent was then evaporated under reduced pressure. The resulting residue was finally purified by silica gel column chromatography (eluent: petroleum ether/ethyl acetate/dichloromethane = 7:1:2, v/v/v) to give the desired SL analogues **1−25**.

Compound ***1***. R_f = 0.35 (petroleum ether/ethyl acetate/dichloromethane = 5:1:2, v/v/v). Yellow oil, 290 mg, 34% yield from the intermediate enamine **1b** (680 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86−7.89 (1H, m, H-5), 7.77−7.81 (1H, m, H-1″), 7.43 (1H, t, J = 8.0 Hz, H-7), 7.22−7.33 (5H, m, H-2′, 3′, 4′, 5′, 6′), 6.89−7.00 (3H, m, H-6, 8, 3″), 6.47−6.51 (1H, m, H-2″), 6.24 (1H, s, H-2), 2.01 (3H, s, 4″-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.35, 181.30, 170.31, 170.27, 159.59, 159.55, 151.00, 150.95, 141.10, 141.02, 138.69, 138.32, 136.14, 136.08, 135.86, 135.82, 128.59, 128.27, 128.22, 127.12, 127.06, 126.64, 126.60, 122.00, 121.78, 121.70, 121.63, 118.61, 116.57, 100.76, 74.91, 74.80, 10.71. HR-ESI-MS: m/z ($[M + H]^+$) calcd for $C_{21}H_{16}O_5$ 349.1076, found 349.1070.

Compound ***2***. R_f = 0.30 (petroleum ether/ethyl acetate/dichloromethane = 5:1:2, v/v/v). White solid, 230 mg, 28% yield from the intermediate enamine **2b** (670 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93−7.96 (1H, m, H-5), 7.71−7.72 (1H, m, H-1″), 7.38−7.43 (1H, m, H-7), 7.22−7.29 (1H, m, H-3′), 7.13−7.18 (1H, m, H-4′), 6.95−7.07 (3H, m, H-6, 5′, 6′), 6.81−6.88 (2H, m, H-8, 3″), 6.69 (1H, s, H-2″), 6.15−6.16 (1H, m, H-2), 1.99 (3H, m, 4″-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.32, 181.29, 170.31, 170.22, 160.99 (d, J_{C-F} = 249 Hz), 160.92 (d, J_{C-F} = 248 Hz), 159.50, 159.45, 151.09, 150.91, 141.01, 140.92, 136.24, 136.19, 135.92, 135.89, 130.71 (d, J_{C-F} = 9 Hz), 130.68 (d, J_{C-F} = 8 Hz), 128.76 (d, J_{C-F} = 4 Hz), 128.71 (d, J_{C-F} = 4 Hz), 127.07, 127.03, 126.35 (d, J_{C-F} = 14 Hz), 126.06 (d, J_{C-F} = 14 Hz), 124.13, 124.09, 124.05, 121.83, 121.75, 121.56, 121.37, 118.52, 118.49, 115.96 (d, J_{C-F} = 22 Hz), 115.83 (d, J_{C-F} = 22 Hz), 115.23, 115.19, 100.63, 100.55, 70.48, 70.45, 70.41, 70.38, 10.83, 10.80. HR-ESI-MS: m/z ($[M + H]^+$) calcd for $C_{21}H_{15}FO_5$ 367.0982, found 367.0976.

Compound ***3***. R_f = 0.22 (petroleum ether/ethyl acetate/dichloromethane = 7:1:2, v/v/v). White solid, 260 mg, 45% yield from the intermediate enamine **3b** (470 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.94−7.97 (1H, m, H-5), 7.73 (1H, s, H-1″), 7.36−7.41 (2H, m, H-7, 3′), 7.19−7.27 (2H, m, H-4′, 6′), 7.11 (1H, t, J = 8.0 Hz, H-5′), 7.00 (1H, t, J = 8.0 Hz, H-6), 6.73−6.87 (3H, m, H-8, 2″, 3″), 6.12 (1H, s, H-2), 1.98 (3H, m, 4″-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.57, 181.51, 170.28, 170.19, 159.46, 159.42, 151.22, 140.97, 140.84, 136.49, 136.24, 136.19, 135.88, 135.82, 134.63, 134.51, 130.20, 130.15, 130.01, 128.98, 128.87, 127.10, 127.04, 126.96, 126.87, 121.89, 121.80, 121.64, 121.46, 118.64, 115.56, 115.45, 100.50, 73.22, 73.04, 10.82, 10.78. HR-ESI-MS: m/z ([M + H]+) calcd for $C_{21}H_{15}ClO_5$383.0686, found 383.0684.

Compound ***4***. R_f = 0.31 (petroleum ether/ethyl acetate/dichloromethane = 5:1:2, v/v/v). Yellow solid, 270 mg, 46% yield from the intermediate enamine **4b** (490 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98 (1H, dd, J = 4.0, 8.0 Hz, H-5), 7.77 (1H, s, H-1″), 7.58−7.63 (1H, m, H-7), 7.43 (1H, t, J = 8.0 Hz, H-3′), 7.15−7.29 (3H, m, H-4′, 5′, 6′), 7.01−7.05 (1H, m, H-6), 6.79−6.91 (2H, m, H-8, 3″), 6.70−6.73 (1H, m, H-2″), 6.14 (1H, s, H-2), 2.01 (3H, m, 4″-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.57, 181.49, 170.29, 170.20, 159.42, 159.35, 151.29, 140.98, 140.84, 138.13, 137.88, 136.24, 136.19, 135.86, 135.78, 133.55, 133.32, 130.43, 130.38, 129.16, 129.03, 127.62, 127.52, 127.11, 127.05, 124.71, 124.59, 121.92, 121.83, 121.65, 121.49, 118.68, 118.66, 115.70, 115.58, 100.47, 75.41, 75.19, 10.83, 10.78. HR-ESI-MS: m/z ($[M + H]^+$) calcd for $C_{21}H_{15}BrO_5$ 427.0181, 429.0161, found 427.0171, 429.0155.

Compound ***5***. R_f = 0.35 (petroleum ether/ethyl acetate/dichloromethane = 5:1:2, v/v/v). Yellow solid, 210 mg, 30% yield from the intermediate enamine **5b** (590 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 (1H, dd, J = 1.6, 8.0 Hz, H-5), 7.84−7.89 (1H, m, H-1″), 7.73−7.74 (1H, m, H-7), 7.38−7.42 (1H, m, H-3′), 7.19 (2H, m, H-5′, 6′), 6.93−7.02 (2H, m, H-6, 4′), 6.86−6.89 (1H, m, H-3″), 6.76−6.83 (1H, m, H-8), 6.52 (1H, m, H-2″), 6.11 (1H, m, H-2), 1.98 (3H, m, 4″-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.55, 181.48, 170.31, 170.20, 159.31, 159.24, 151.35, 141.16, 141.02, 140.92, 140.85, 140.38, 140.13, 136.24, 136.19, 135.85, 135.78, 130.57, 130.53, 128.72, 128.57, 128.47, 128.38, 127.12, 127.06, 121.96, 121.87, 121.70, 121.54, 118.72, 118.69, 115.96, 115.85, 100.46, 100.43, 100.12, 100.02, 99.92, 79.40, 79.14, 10.84, 10.80. HR-ESI-MS: m/z ($[M + H]^+$) calcd for $C_{21}H_{15}IO_5$ 475.0043, found 475.0031.

Compound ***6***. R_f = 0.22 (petroleum ether/ethyl acetate/dichloromethane = 5:1:2, v/v/v). Yellow oil, 520 mg, 39% yield from the intermediate enamine **6b** (1.1 g). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85−7.89 (1H, m, H-5), 7.53−7.55 (1H, m, H-1″), 7.28 (1H, t, J = 8.0 Hz, H-7), 7.12−7.19 (1H, m, H-6′), 7.02−7.08 (1H, m, H-6), 6.87 (1H, t, J = 8.0 Hz, H-5′), 6.65−6.79 (4H, m, H-8, 3′, 4′, 3″), 6.56−6.60(1H, m, H-2″), 6.00−6.04 (1H, m, H-2), 3.68 (3H, m, 2′-OMe), 1.88 (3H, m, 4″-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.77, 181.70, 170.36, 170.24, 159.97, 159.94, 157.80, 157.74, 150.16, 149.78, 141.15, 141.08, 135.79, 135.70, 135.56, 130.19, 130.16, 128.85, 128.77, 127.63, 127.30, 126.76, 126.67, 121.45, 121.27, 121.15, 121.04, 120.27, 120.24, 118.27, 118.21, 116.35, 116.23, 111.29, 111.22, 100.60, 100.44, 72.32, 72.15, 55.57, 55.53, 10.70. HR-ESI-MS: m/z ($[M + H]^+$) calcd for $C_{22}H_{18}O_6$ 379.1182, found 379.1178.

Compound ***7***. R_f = 0.27 (petroleum ether/ethyl acetate/dichloromethane = 5:1:2, v/v/v). Yellow oil, 330 mg, 42% yield from the intermediate enamine **7b** (650 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85−7.88 (1H, m, H-5), 7.76−7.81 (1H, m, H-1″), 7.43 (1H, t, J = 8.0 Hz, H-7), 7.15−7.20 (1H, m, H-6′), 6.88−7.02 (5H, m, H-6, 8, 2′, 5′, 3″), 6.76 (1H, t, J = 8.0 Hz, H-4′), 6.44−6.48 (1H, m, H-2″), 6.24 (1H, s, H-2), 3.73 (3H, m, 3′-OMe), 2.00 (3H, s, 4″-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.32, 181.26, 170.33, 170.25, 159.88, 159.86, 159.62, 150.94, 141.06, 140.95, 140.31, 139.94, 136.19, 136.14, 136.10, 136.04, 129.69, 127.29, 127.22, 122.11, 121.91, 121.84, 121.75, 119.00, 118.89, 118.64, 116.74, 116.69, 113.81, 113.42, 112.83, 112.29, 100.84, 100.76, 74.79, 74.71, 55.30, 10.86. HR-ESI-MS: m/z ($[M + H]^+$) calcd for $C_{22}H_{18}O_6$ 379.1182, found 379.1184.

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J. Agric. Food Chem. XXXX, XXX, XXX−XXX

Compound ***8***. R_f = 0.22 (petroleum ether/ethyl acetate/dichloromethane = 5:1:2, v/v/v). Yellow oil, 190 mg, 24% yield from the intermediate enamine **8b** (660 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.22 (1H, m, H-2′), 8.10 (1H, t, J = 7.2 Hz, H-4′), 7.88 (2H, m, H-5, 6′), 7.58−7.66 (1H, m, H-1″), 7.44−7.51 (2H, m, H-7, 5′), 6.97−7.07 (3H, m, H-6, 8, 3″), 6.54 (1H, s, H-2″), 6.28−6.32 (1H, m, H-2), 2.03 (3H, s, 4″-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.54, 180.51, 170.21, 170.08, 159.08, 159.03, 152.02, 151.62, 148.56, 144.17, 141.23, 140.85, 140.77, 136.62, 136.58, 136.43, 136.29, 132.46, 132.39, 129.88, 129.83, 127.43, 127.40, 123.36, 123.35, 122.44, 122.37, 121.92, 121.89, 121.85, 121.70, 118.66, 118.61, 115.79, 115.70, 100.98, 100.80, 73.98, 10.90. HR-ESI-MS: m/z ([M + H]$^+$) calcd for $C_{21}H_{15}NO_7$ 394.0927, found 394.0916.

Compound ***9***. R_f = 0.20 (petroleum ether/ethyl acetate/dichloromethane = 7:1:2, v/v/v). Yellow oil, 140 mg, 23% yield from the intermediate enamine **9b** (510 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86−7.89 (1H, m, H-5), 7.75−7.80 (1H, m, H-1″), 7.41 (1H, t, J = 8.0 Hz, H-7), 7.23−7.26 (2H, m, H-2′, 6′), 6.89−6.98 (3H, m, H-6, 3′, 5′), 6.77−6.80 (2H, m, H-8, 3″), 6.42−6.45 (1H, m, H-2″), 6.23 (1H, s, H-2), 3.73 (3H, m, 4′-OMe), 2.00 (3H, m, 4″-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.58, 181.53, 170.36, 170.33, 159.61, 159.56, 159.54, 159.50, 150.76, 150.74, 141.10, 140.98, 136.16, 136.10, 136.03, 135.97, 130.74, 130.31, 128.21, 128.15, 127.19, 127.12, 122.10, 121.87, 121.71, 121.62, 118.72, 116.74, 113.99, 113.96, 100.77, 100.73, 74.70, 74.62, 55.32, 55.31, 10.85. HR-ESI-MS: m/z ([M + H]$^+$) calcd for $C_{22}H_{18}O_6$ 379.1182, found 379.1181.

Compound ***10***. R_f = 0.20 (petroleum ether/ethyl acetate/dichloromethane = 7:1:2, v/v/v). Brown oil, 250 mg, 35% yield from the intermediate enamine **10b** (590 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87−7.89 (1H, m, H-5), 7.76−7.81 (1H, m, H-1″), 7.44 (1H, t, J = 8.0 Hz, H-7), 7.26−7.31 (2H, m, H-3′, 5′), 6.90−7.00 (5H, m, H-6, 8, 2′, 6′, 3″), 6.43−6.46 (1H, m, H-2″), 6.24 (1H, s, H-2), 2.02 (3H, m, 4″-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.23, 181.19, 170.24, 170.20, 162.68 (d, J_{C-F} = 246 Hz), 159.39, 159.36, 150.96, 140.94, 140.85, 136.29, 136.21 (d, J_{C-F} = 4 Hz), 128.64 (d, J_{C-F} = 8 Hz), 128.59 (d, J_{C-F} = 8 Hz), 127.30, 127.23, 121.97, 121.89, 118.67, 116.55, 115.63 (d, J_{C-F} = 22 Hz), 115.61 (d, J_{C-F} = 22 Hz), 100.81, 100.74, 74.44, 74.38, 10.87. HR-ESI-MS: m/z ([M + H]+) calcd for $C_{21}H_{15}FO_5$ 367.0982, found 367.0975.

Compound ***11***. R_f = 0.25 (petroleum ether/ethyl acetate/dichloromethane = 5:1:2, v/v/v). Brown oil, 210 mg, 33% yield from the intermediate enamine **11b** (530 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 (1H, d, J = 8.0 Hz, H-5), 7.76−7.82 (1H, m, H-1″), 7.43−7.46 (1H, m, H-7), 7.25 (4H, s, H-2′, 3′, 5′, 6′), 6.90−7.01 (3H, m, H-6, 8, 3″), 6.42−6.46 (1H, m, H-2″), 6.24 (1H, s, H-2), 2.02 (3H, s, 4″-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.13, 181.09, 170.23, 170.18, 159.35, 159.33, 151.10, 140.91, 140.80, 137.26, 136.88, 136.34, 136.27, 136.23, 134.34, 134.31, 128.91, 128.89, 128.24, 128.19, 127.35, 127.28, 122.07, 121.98, 121.85, 118.66, 116.36, 116.33, 100.80, 100.75, 74.39, 74.34, 10.90. HR-ESI-MS: m/z ([M + H]$^+$) calcd for $C_{21}H_{15}ClO_5$ 383.0686, found 383.0682.

Compound ***12***. R_f = 0.20 (petroleum ether/ethyl acetate/dichloromethane = 5:1:2, v/v/v). Yellow oil, 160 mg, 37% yield from the intermediate enamine **12b** (360 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11−8.14 (2H, m, H-3′, 5′), 7.82−7.89 (2H, m, H-5, 1″), 7.46−7.50 (3H, m, H-7, 2′, 6′), 7.00−7.05 (2H, m, H-6, 3″), 6.93−6.96 (1H, m, H-8), 6.54 (1H, d, J = 4.0 Hz, H-2″), 6.27−6.30 (1H, m, H-2), 2.03 (3H, m, 4″-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.55, 180.52, 170.08, 170.02, 159.12, 159.10, 151.66, 151.55, 147.89, 146.00, 145.67, 140.73, 140.66, 136.57, 136.51, 136.48, 136.42, 127.63, 127.49, 127.43, 123.98, 123.96, 122.46, 122.39, 121.98, 121.77, 118.58, 118.56, 115.87, 115.85, 100.92, 100.79, 74.15, 75.11, 10.91. HR-ESI-MS: m/z ([M + H]$^+$) calcd for $C_{21}H_{15}NO_7$ 394.0927, found 394.0922.

Compound ***13***. R_f = 0.25 (petroleum ether/ethyl acetate/dichloromethane = 5:1:2, v/v/v). Yellow oil, 220 mg, 56% yield from the intermediate enamine **13b** (330 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82−7.88 (2H, m, H-5, 1″), 7.43−7.54 (5H, m, H-7, 2′, 3′, 5′, 6′), 6.98−7.03 (2H, m, H-6, 3″), 6.90−6.94 (1H, m, H-8), 6.50 (1H, d, J = 6.4 Hz, H-2″), 6.25−6.28 (1H, m, H-2), 2.01 (3H, m, 4″-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.93, 180.88, 170.19, 170.13, 159.34, 159.30, 151.43, 142.74, 142.40, 140.86, 140.77, 136.44, 136.37, 136.26, 127.40, 127.33, 127.09, 127.05, 125.73, 125.70, 125.67, 122.20, 122.12, 122.01, 121.80, 118.61, 116.12, 116.10, 100.87, 100.79, 74.42, 74.35, 10.86. HR-ESI-MS: m/z ([M + H]$^+$) calcd for $C_{22}H_{15}F_3O_5$ 417.0950, found 417.0943.

Compound ***14***. R_f = 0.30 (petroleum ether/ethyl acetate/dichloromethane = 5:1:2, v/v/v). Yellow oil, 210 mg, 40% yield from the intermediate enamine **14b** (430 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.91−7.94 (1H, m, H-5), 7.72−7.74 (1H, m, H-1″), 7.33−7.37 (1H, m, H-7), 6.93−7.01 (3H, m, H-6, 6′, 3″), 6.77−6.85 (3H, m, H-3′, 5′, 8), 6.55 (1H, s, H-2″), 6.15 (1H, m, H-2), 2.54 (3H, s, 2′-CH_3), 2.24 (3H, m, 4′-CH_3), 1.98 (3H, m, 4″-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 182.19, 170.38, 170.05, 159.38, 159.36, 150.80, 150.14, 141.10, 140.90, 138.81, 137.85, 137.72, 136.16, 136.08, 136.03, 135.82, 133.71, 133.41, 131.98, 131.94, 127.48, 127.33, 127.10, 127.06, 126.40, 126.32, 121.95, 121.77, 121.62, 121.54, 118.53, 118.49, 116.38, 116.30, 100.76, 100.42, 73.51, 73.25, 21.13, 19.79, 19.76, 10.84. HR-ESI-MS: m/z ([M + H]$^+$) calcd for $C_{23}H_{20}O_5$ 377.1389, found 377.1390.

Compound ***15***. R_f = 0.25 (petroleum ether/ethyl acetate/dichloromethane = 4:1:2, v/v/v). Yellow oil, 180 mg, 46% yield from the intermediate enamine **15b** (320 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86−7.89 (1H, m, H-5), 7.74−7.77(1H, m, H-1″), 7.41−7.45 (1H, m, H-7), 6.90−6.99 (3H, m, H-6, 8, 6′), 6.84 (1H, s, H-3″), 6.72−6.77 (1H, m, H-2′), 6.66−6.68 (1H, m, H-5′), 6.36−6.39 (1H, m, H-2″), 6.23 (1H, m, H-2), 5.90 (2H, m, −OCH_2O−), 2.01 (3H, m, 4″-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.41, 181.37, 170.31, 170.24, 159.44, 159.39, 150.92, 150.76, 148.14, 148.12, 147.75, 141.03, 140.92, 136.22, 136.15, 136.08, 132.59, 132.21, 127.26, 127.20, 122.10, 121.88, 121.84, 121.76, 120.54, 120.49, 118.73, 116.70, 108.21, 108.18, 107.51, 107.46, 101.33, 100.76, 100.74, 74.78, 74.74, 10.88. HR-ESI-MS: m/z ([M + H]$^+$) calcd for $C_{22}H_{16}O_7$ 393.0974, found 393.0969.

Compound ***16***. R_f = 0.27 (petroleum ether/ethyl acetate/dichloromethane = 5:1:2, v/v/v). Yellow oil, 230 mg, 40% yield from the intermediate enamine **16b** (460 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89−7.92 (1H, m, H-5), 7.74−7.77 (1H, m, H-1″), 7.42−7.46 (1H, m, H-7), 7.20−7.22 (1H, m, H-3′), 6.84−7.02 (5H, m, H-6, 8, 1′, 2″, 3″), 6.63 (1H, d, J = 8.0 Hz, H-2′), 6.24 (1H, s, H-2), 2.02 (3H, m, 4″-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.97, 180.92, 170.27, 170.21, 159.06, 159.04, 150.95, 150.75, 142.42, 142.20, 141.00, 140.90, 136.25, 136.16, 136.11, 127.24, 127.19, 126.84, 126.73, 126.65, 126.53, 126.48, 122.16, 122.12, 122.08, 121.96, 118.92, 116.68, 116.63, 100.79, 100.76, 71.74, 71.60, 10.90, 10.88. HR-ESI-MS: m/z ([M + H]$^+$) calcd for $C_{19}H_{14}O_5S$ 355.0640, found 355.0630.

Compound ***17***. R_f = 0.30 (petroleum ether/ethyl acetate/dichloromethane = 5:1:2, v/v/v). Yellow oil, 80 mg, 13% yield from the intermediate enamine **17b** (500 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 (1H, d, J = 8.0 Hz, H-5), 7.69−7.71 (1H, m, H-1″), 7.40−7.45 (1H, m, H-7), 7.34 (1H, s, H-3′), 7.01 (1H, t, J = 8.0 Hz, H-6), 6.90−6.92 (2H, m, H-8, 3″), 6.41 (1H, d, J = 4.0 Hz, H-2″), 6.19−6.23 (3H, m, H-2, 1′, 2′), 2.01 (3H, m, 4″-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.11, 170.27, 159.15, 159.08, 151.44, 151.22, 151.10, 150.94, 143.55, 143.50, 141.01, 140.91, 136.23, 136.12, 136.07, 127.11, 127.08, 122.00, 121.93, 121.76, 118.54, 114.57, 114.53, 110.42, 110.35, 109.98, 109.65, 100.76, 100.67, 69.13, 69.06, 10.88. HR-ESI-MS: m/z ([M + H]$^+$) calcd for $C_{19}H_{14}O_6$ 339.0869, found 339.0862.

Compound ***18***. R_f = 0.20 (petroleum ether/ethyl acetate/dichloromethane = 7:1:2, v/v/v). White solid, mp 123−124 °C, 100 mg, 31% yield from the intermediate enol (210 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 (1H, dd, J = 8.0, 1.2 Hz, H-5), 7.57 (1H, s, H-1″), 7.45 (1H, t, J = 8.0 Hz, H-7), 7.02 (1H, t, J = 8.0 Hz, H-6), 6.95 (1H, s, H-3″), 6.94 (1H, d, J = 8.0 Hz, H-8), 6.21 (1H, s, H-2″), 5.03 (2H, d, J = 1.2 Hz, H-2a, 2b), 2.02 (3H, s, 4″-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.81, 170.36, 161.44, 149.62, 141.08, 135.97,

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J. Agric. Food Chem. XXXX, XXX, XXX−XXX

Table 1. Seed Germination Activity towards *O. aegyptiaca*[a]

compound number	test concentration (mg/L) 0.2	2.0	20	EC_{50} (M)
1	74.8 ± 8.6%	91.2 ± 5.1%	91.0 ± 5.0%	2.12×10^{-7}
2	36.9 ± 8.0%	85.1 ± 6.0%	92.1 ± 4.6%	n.d.
3	36.1 ± 9.6%	71.1 ± 8.3%	85.4 ± 5.1%	n.d
4	26.4 ± 9.5%	41.9 ± 9.0%	76.2 ± 7.9%	n.d
5	18.9 ± 7.6%	46.2 ± 13.2%	82.9 ± 6.7%	n.d
6	52.0 ± 12.4%	83.0 ± 5.2%	91.0 ± 4.0%	6.71×10^{-7}
7	41.6 ± 9.6%	78.7 ± 10.1%	91.8 ± 6.3%	n.d
8	24.7 ± 9.4%	49.4 ± 11.4%	73.2 ± 6.9%	n.d
9	59.3 ± 10.4%	86.4 ± 2.7%	90.5 ± 5.7%	4.84×10^{-7}
10	36.2 ± 12.6%	67.1 ± 8.6%	86.8 ± 4.1%	n.d
11	28.4 ± 6.0%	60.5 ± 6.9%	81.9 ± 5.9%	n.d
12	26.9 ± 10.6%	66.4 ± 11.9%	81.0 ± 8.4%	n.d
13	67.0 ± 10.0%	55.4 ± 12.0%	74.2 ± 11.7%	n.d
14	41.7 ± 12.8%	75.8 ± 6.7%	88.2 ± 4.8%	n.d
15	38.3 ± 13.6%	82.8 ± 9.1%	91.1 ± 5.0%	n.d
16	57.8 ± 8.3%	83.8 ± 8.6%	86.6 ± 5.1%	6.69×10^{-7}
17	17.0 ± 7.2%	41.9 ± 7.6%	74.6 ± 9.6%	n.d
18	75.2 ± 4.8%	79.4 ± 6.6%	85.0 ± 5.5%	3.31×10^{-8}
19	**79.4 ± 5.9%**	**87.0 ± 5.4%**	**83.6 ± 4.2%**	$\mathbf{3.49 \times 10^{-9}}$
20	70.9 ± 10.3%	75.2 ± 5.9%	82.8 ± 4.7%	2.33×10^{-8}
21	72.9 ± 9.1%	80.6 ± 7.7%	80.4 ± 6.7%	3.50×10^{-8}
22	75.0 ± 8.6%	79.0 ± 5.8%	79.3 ± 9.7%	5.18×10^{-8}
23	72.3 ± 9.6%	82.6 ± 5.9%	80.2 ± 4.9%	2.92×10^{-7}
24	83.8 ± 6.5%	92.5 ± 4.9%	94.8 ± 5.6%	8.99×10^{-8}
25	31.9 ± 7.3%	53.7 ± 7.2%	66.4 ± 7.8%	n.d
GR24	82.9 ± 4.6%	95.5 ± 3.6%	93.1 ± 7.5%	5.70×10^{-8}

[a]The seed germination rate for negative control (distilled water) was lower than 5%. [b]n.d: not determined.

135.80, 127.43, 122.12, 121.84, 118.06, 114.12, 100.76, 64.32, 10.84. HR-ESI-MS: m/z ($[M + H]^+$) calcd for 273.0763, found 273.0761.

Compound ***19***. R_f = 0.20 (petroleum ether/ethyl acetate/dichloromethane = 7:1:2, v/v/v). Yellow oil, 40 mg, 9% yield from the intermediate enol (290 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.92 (1H, d, J = 8.0 Hz, H-5), 7.51−7.55 (1H, m, H-1″), 7.46 (1H, t, J = 8.0 Hz, H-7), 6.98−7.03 (1H, m, H-6), 6.95−6.96 (1H, m, H-3″), 6.90−6.93 (1H, m, H-8), 6.17−6.22 (1H, m, H-2″), 5.51−5.57 (1H, m, H-2), 2.03 (3H, s, 4″-CH_3), 1.41−1.44 (3H, m, 2-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.42, 181.33, 170.42, 170.37, 159.45, 159.41, 149.36, 149.20, 143.10, 141.08, 136.12, 136.07, 136.00, 127.20, 127.16, 121.50, 121.43, 118.86, 118.72, 100.90, 100.74, 71.07, 70.99, 20.63, 20.60, 10.90. HR-ESI-MS: m/z ($[M + H]^+$) calcd for 287.0919, found 287.0917.

Compound ***20***. R_f = 0.25 (petroleum ether/ethyl acetate/dichloromethane = 7:1:2, v/v/v). Yellow oil, 100 mg, 10% yield from the intermediate enol (660 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.91 (1H, dt, J = 8.0, 1.2 Hz, H-5), 7.55−7.59 (1H, m, H-1″), 7.45 (1H, t, J = 8.0 Hz, H-7), 7.00 (1H, td, J = 8.0, 2.0 Hz, H-6), 6.94−6.96 (1H, m, H-3″), 6.91−6.94 (1H, m, H-8), 6.18−6.22 (1H, m, H-2″), 5.27−5.30 (1H, m, H-2), 2.03 (3H, s, 4″-CH_3), 1.82−1.91 (1H, m, 2-CH_2−), 1.56−1.61 (1H, m, 2-CH_2−), 0.94 (3H, m, 2-CH_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.65, 181.56, 170.42, 170.37, 159.56, 159.50, 149.85, 149.68, 143.10, 141.11, 141.08, 136.10, 136.08, 136.03, 135.97, 127.20, 127.15, 121.62, 121.44, 121.36, 118.60, 117.96, 117.94, 100.94, 100.72, 96.94, 75.94, 75.86, 27.62, 10.90, 10.80, 9.95, 9.92. HR-ESI-MS: m/z ($[M + H]^+$) calcd for 301.1076, found 301.1072.

Compound ***21***. R_f = 0.20 (petroleum ether/ethyl acetate/dichloromethane = 7:1:2, v/v/v). Yellow oil, 320 mg, 29% yield from the intermediate enol (780 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.91 (1H, d, J = 8.0 Hz, H-5), 7.53−7.57 (1H, m, H-1″), 7.45 (1H, t, J = 8.0 Hz, H-7), 6.98−7.02 (1H, m, H-6), 6.94−6.95 (1H, m, H-3″), 6.91 (1H, d, J = 8.0 Hz, H-8), 6.18−6.22 (1H, m, H-2″), 5.39 (1H, m, H-2), 2.03 (3H, s, 4″-CH_3), 1.80−1.87 (1H, m, 2-CH_2−), 1.36−1.55 (3H, m, 2-CH_2CH_2−), 0.89 (3H, q, J = 8.0 Hz, 2-$(CH_2)_2CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.66, 181.57, 170.41, 170.35, 159.57, 159.52, 149.58, 149.41, 141.09, 141.07, 136.13, 136.08, 136.03, 135.98, 127.20, 127.15, 121.63, 121.46, 121.42, 121.35, 118.62, 118.19, 100.91, 100.68, 74.43, 74.35, 36.46, 36.41, 18.64, 13.79, 13.73, 10.89. HR-ESI-MS: m/z ($[M + H]^+$) calcd for 315.1232, found 315.1228.

Compound ***22***. R_f = 0.20 (petroleum ether/ethyl acetate/dichloromethane = 7:1:2, v/v/v). Yellow oil, 350 mg, 28% yield from the intermediate enol (890 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 (1H, dt, J = 8.0, 1.6 Hz, H-5), 7.52−7.56 (1H, m, H-1″), 7.42−7.47 (1H, m, H-7), 6.97−7.01 (1H, m, H-6), 6.93−6.95 (1H, m, H-3″), 6.91 (1H, d, J = 8.0 Hz, H-8), 6.17−6.22 (1H, m, H-2″), 5.34−5.38 (1H, m, H-2), 2.01−2.03 (3H, m, 4″-CH_3), 1.78−1.87 (1H, m, 2-CH_2−), 1.49−1.56 (1H, m, 2-CH_2−), 1.23−1.41 (4H, m, 2-$CH_2CH_2CH_2$−), 0.81−0.87 (3H, m, 2-$(CH_2)_3CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.65, 181.56, 170.41, 170.37, 159.51, 159.46, 149.64, 149.46, 141.12, 141.08, 136.06, 136.05, 136.01, 135.89, 127.16, 127.11, 121.56, 121.40, 121.32, 118.60, 118.59, 118.15, 118.12, 100.88, 100.68, 74.58, 74.51, 34.01, 27.43, 22.31, 22.27, 14.06, 14.02, 10.88, 10.86. HR-ESI-MS: m/z ($[M + H]^+$) calcd for 329.1389, found 329.1386.

Compound ***23***. R_f = 0.20 (petroleum ether/ethyl acetate/dichloromethane = 7:1:2, v/v/v). Yellow oil, 290 mg, 20% yield from the intermediate enol (1.07 g). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89 (1H, d, J = 8.0 Hz, H-5), 7.52−7.56 (1H, m, H-1″), 7.44 (1H, t, J = 8.0 Hz, H-7), 6.89−7.00 (3H, m, H-6, 8, 3″), 6.16−6.22 (1H, m, H-2″), 5.33−5.37 (1H, m, H-2), 2.01 (3H, s, 4″-CH_3), 1.83 (1H, m, 2-CH_2−), 1.51 (1H, m, 2-CH_2−), 1.36 (2H, brs, 2-CH_2CH_2−), 1.23 (4H, brs, 2-$(CH_2)_2$−CH_2CH_2−), 0.83 (3H, m, 2-$(CH_2)_4CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.60, 181.51, 170.39, 170.36, 159.48, 159.44, 149.69, 149.48, 142.38, 141.14, 141.10, 136.02, 135.97, 135.80, 127.10, 127.06, 121.54, 121.39, 121.35, 121.29, 118.57,

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J. Agric. Food Chem. XXXX, XXX, XXX−XXX

118.11, 118.05, 100.88, 100.69, 98.39, 74.56, 74.51, 34.22, 31.32, 31.27, 24.95, 22.57, 22.55, 14.05, 14.02, 10.82. HR-ESI-MS: m/z ([M + H]$^+$) calcd for 343.1545, found 343.1540.

Compound ***24***. R_f = 0.25 (petroleum ether/ethyl acetate/dichloromethane = 7:1:2, v/v/v). Brown oil, 290 mg, 17% yield from the intermediate enol (1.16g). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89 (1H, d, J = 8.0 Hz, H-5), 7.60 (1H, s, H-1″), 7.45 (1H, t, J = 8.0 Hz, H-7), 6.99 (1H, t, J = 8.0 Hz, H-6), 6.95 (1H, s, H-3″), 6.88 (1H, d, J = 8.0 Hz, H-8), 6.17 (1H, s, H-2″), 2.03 (3H, s, 4″-CH_3), 1.64 (3H, s, 2-CH_3), 1.62 (3H, s, 2-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 182.48, 170.40, 159.67, 150.70, 140.99, 136.05, 136.00, 127.19, 121.34, 121.29, 120.67, 118.57, 101.20, 79.82, 27.71, 10.91. HR-ESI-MS: m/z ([M + H]$^+$) calcd for $C_{17}H_{16}O_5$ 301.1076, found 301.1071.

Compound ***25***. R_f = 0.55 (dichloromethane/ethyl acetate = 50:1, v/v). White solid, mp 104−105 °C, 30 mg, 2.5% yield from the intermediate enol (700 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43 (1H, d, J = 4.0 Hz, H-1″), 6.91 (1H, s, H-3″), 6.13 (1H, s, H-2″), 4.52 (2H, s, H-2), 3.97 (2H, t, J = 4.0 Hz, H-6), 2.52 (2H, td, J = 8.0, 2.4 Hz, H-5), 2.01 (3H, s, 4″-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 195.58, 170.39, 149.41, 141.07, 135.97, 116.97, 100.68, 65.27, 64.71, 39.38, 10.87. HR-ESI-MS: m/z ([M + H]$^+$) calcd for 225.0763, found 225.0764.

Seed Germination Assay. The pretreated seeds of *O. aegyptiaca* were evenly spread on each sterile water-wetted filter paper disk (6 mm diameter, approximately 25−65 seeds per disk) in Petri dishes. The sealed Petri dishes were stored in the dark at room temperature for 3−7 days. Then, a piece of the glass fiber filter paper disk was placed in Petri dish and a solution of tested compound in acetone (25 μL) was added. After complete evaporation of acetone at room temperature, the filter paper disk with preincubated seeds was coated on it. Subsequently, another part of sterile water (25 μL) was added on each disk and a piece of sterile water-wetted filter paper was placed on the center of Petri dishes to preserve moisture. Finally, the sealed Petri dishes were stored in the dark and incubated at room temperature for 9−10 days. After the incubation, the percentage ratios of germination were calculated with the assistance of a microscope. In each concentration of tested compound, four disks of pretreated seeds were used and each assay was carried out three times. *rac*-GR24 was used as the positive control, and the disks that were added with 25 μL of sterile water was used as negative control. The EC_{50} values of the tested compounds were calculated with SPSS 19.0. The filter paper disks used in seed germination assay were sterilized prior to the use.

The germination activity assay of *O. cumana* seeds was carried out by Prof. Yongqing Ma at the Forestry Institute of Northwest Agriculture & Forest University, and the test method was slightly modified on the basis of aforementioned method for *O. aegyptiaca* seeds. The pretreated *O. cumana* seeds were evenly spread to sterile water-wetted glass fiber filter paper disks (approximately 25−65 seeds per disk) in Petri dishes and then preincubated in the dark at room temperature for 3−7 days. After the incubation, the glass fiber filter paper disks with preincubated *O. cumana* seeds were placed in Petri dishes and a solution (20 μL) of tested compound (first dissolved in acetone and then diluted with water to the content of acetone lower than 5% (v/v)) was added directly. A piece of wet filter paper was placed on the center of Petri dish to preserve moisture. Finally, the sealed Petri dishes were stored in the dark and incubated at room temperature for 9−10 days, and the percentage of germination was determined by using a microscope. For the seed germination data of all new compounds, see Table 1 and Figure 1.

***HTL7* Inhibition Assay.** The competition assay of tested compounds were performed according to the previously reported method.[26] Prior to the test, YLG stock solution (5 mM in DMSO) was diluted with water to 50 μM, while the stock solution of GR24 (10 mM in DMSO) and tested compounds (1 mM in DMSO) were diluted with water to 1000, 333.33, 111.11, 37.037, 12.346, 4.115, 1.372, and 0.457 μM, respectively.

The solutions of YLG (50 μM, 4 μL), tested compound (5 μL), HEPES buffer (100 mM, 150 mM NaCl, pH 7, 88 μL), and *HTL7* protein solution (1.8 mg/mL in HEPES buffer, 3 μL) were successively added to the wells of a 96-well plate in turn. For the blank test, water (5 μL) was used instead of the solution of the tested compound. The fluorescent intensity was measured by spectrofluorometer (excitation wavelength, 480 nm; emission wavelength, 520 nm, the excitation and emission slit widths were set as 10 and 20 nm, respectively). The fluorescence intensity change rate was calculated with Origin, where the lg[c(μM)]-(k'/k)% plot (k' and k were the fluorescence intensity change rates of the test compound and blank control, respectively) was achieved by the Growth/Sigmoidal-DoseResp curve fitting method of Origin. Finally, the IC_{50} value and R^2 were obtained from the above plot.

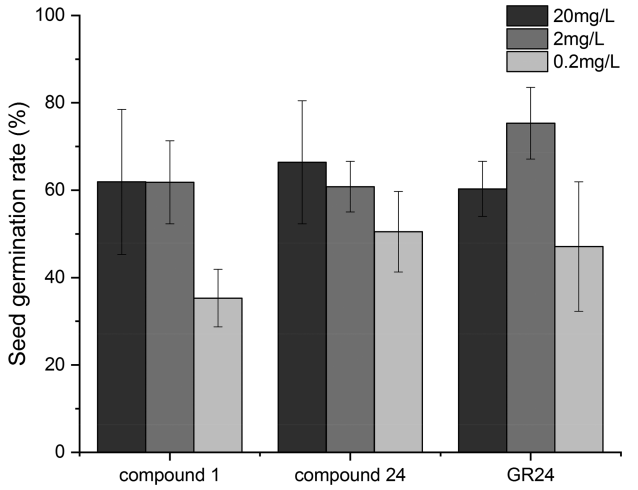

Figure 1. Seed germination activity toward *O. Cumana*. The seed germination rate for negative control (distilled water) was lower than 10%.

Docking Experiment. The molecular modeling computational study was performed using Autodock vina 1.1.2 software. The crystal structure of rice DWARF14 (PDB: 5DJ5) was used for the docking study. The grid box was set as a 20 × 20 × 20 Å^3 cube, and the center of it was set at the position of original ligand GR24 (x = −30.80, y = 14.65, z = −21.05). The docking results were visualized by Pymol and Discovery studio.

Stability Test. The stability of the strigolactone analogues is an important factor affecting their seeds germination activity toward the parasitic weeds. The hydrolytic stability of synthesized analogues was tested under pH 5 and 8 conditions, respectively. Compound **24** was selected as the representative test sample. First, it was dissolved in methanol to give a stock solution (100 mM). This stock solution (10 μL) was then added to a mixture solution (1 mL) of phosphate buffer (pH 8)/methanol (1:1) and phosphate citrate buffer (pH 5)/methanol (1:1), respectively. The stability of compound **24** was measured by HPLC/UV (SHIMADZU 228-45041-91 reservoir tray, C_{18} column, 5u, 250 mm × 4.6 mm, eluent, 50% methanol in water, wavelength, 254 nm) after 1, 2, 3, 4, 5, 6, 7, and 8 days. The stability test of the control GR24 was performed in a similar manner.

RESULTS AND DISCUSSION

Synthesis of Strigolactone Analogues. First, 2-aryl-4-chromanones **1a−17a** were synthesized from 2-hydroxyacetophenone and corresponding aromatic aldehyde *via* a Mannich reaction by using I_2/aniline catalytic system, originally developed by Yao et al., and a sequential Aldol reaction.[27,28] Condensation of these dihydroflavonoids **1a−17a** with an excess of *N*,*N*-dimethylformamide dimethyl acetal (DMFDMA) gave the corresponding enamine intermediates **1b−17b** in modest to good yield.[35] All of these enamines were isolated as the single *E*-isomers based on the analysis of their NOESY spectra. Subsequently, the hydrolysis of the enamine intermediates in the presence of acetic acid successfully

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J. Agric. Food Chem. XXXX, XXX, XXX−XXX

Scheme 2. Synthesis of SL Analogues 1−25

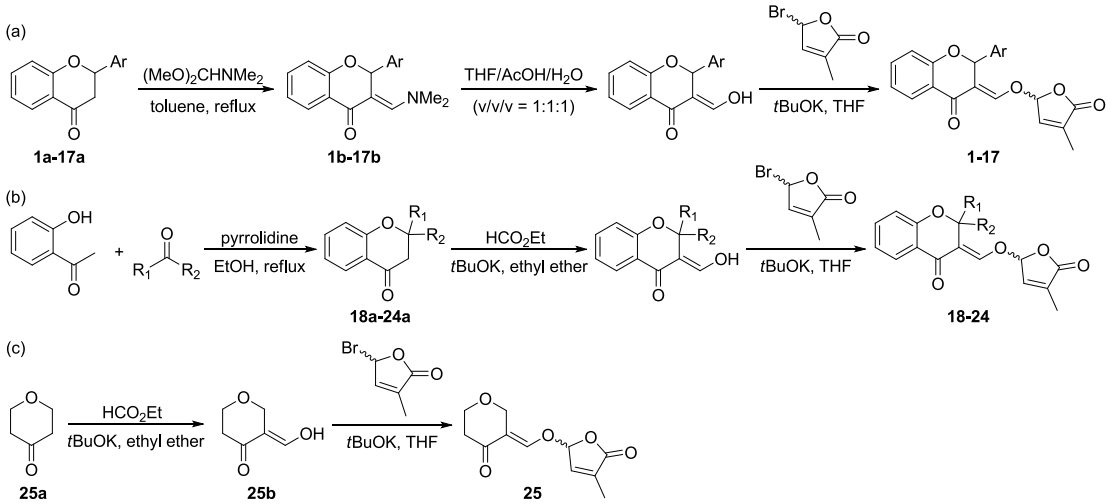
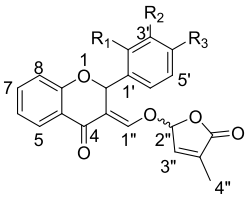
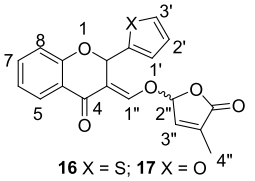


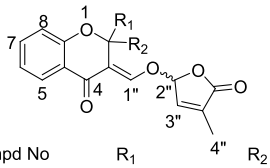
Chart 1. Structures of SLs 1−25



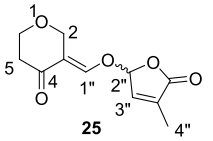
Compd No	R_1	R_2	R_3
1	H	H	H
2	F	H	H
3	Cl	H	H
4	Br	H	H
5	I	H	H
6	OCH_3	H	H
7	H	OCH_3	H
8	H	NO_2	H
9	H	H	OCH_3
10	H	H	F
11	H	H	Cl
12	H	H	NO_2
13	H	H	CF_3
14	CH_3	H	CH_3
15	H	-OCH_2O-	



16 X = S; **17** X = O



Compd No	R_1	R_2
18	H	H
19	H	Me
20	H	Et
21	H	*n*Pr
22	H	*n*Bu
23	H	*n*Pent
24	Me	Me



25

provided the precursor enols, which were found not to be stable enough on silica gel column chromatography and thus were directly subjected to condensation with 5-bromo-3-methyl-2(5*H*)-furanone without further purification. Finally, the target SL analogues **1**−**17** were obtained in good yields (Scheme 2a). These new SL analogues were found to be stable when kept in −20 °C for months.

For the synthesis of 2-alkyl-substituted 4-chromanone derivatives **18a**−**24a**, pyrrolidine-catalyzed cyclization of 2-hydroxyacetophenone with aliphatic aldehydes or ketones readily afforded the desired products *via* a known Mannich procedure.[29] The coupling of compounds **18a**−**24a** with ethyl formate using potassium *tert*-butanoate (*t*BuOK) as the base successfully yielded the enol intermediates, which were subsequently converted into the target SL analogues **18**−**24** upon condensation with 5-bromo-3-methyl-2(5*H*)-furanone in the presence of *t*-BuOK (Scheme 2b). The *E*- configuration was determined to be major isomers for these SLs based on the analysis of NOESY spectra. The control compound **25** was also prepared from commercially available dihydro-2*H*-pyran-4(3*H*)-one **25a** in a similar manner (Scheme 2c). For the structures and synthetic routines of these novel SL analogues, see Scheme 2 and Chart 1. Their spectroscopic characterization data are listed in the Materials and Methods section.

Seed Germination Assay. The synthetic SL analogues derived from 2-aryl and 2-alkyl dihydroflavone derivatives were first evaluated for their seed germination activity of *O. aegyptiaca*. As shown in Table 1, for all 2-(hetero)aryl substituted SL analogues, except compounds **4** (*o*-bromo), **8** (*m*-nitro), **13** (*p*-trifluoromethyl), and **17** (2-furyl), most

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J. Agric. Food Chem. XXXX, XXX, XXX−XXX

compounds induced more than 80% germination rates at a concentration of 20 mg/L, whereas compounds **1** (phenyl), **2** (*o*-fluoro), **6** (*o*-methoxy), and **16** (2-thienyl) still afforded more than 80% germination rates at lower concentration of 2.0 mg/L. These compounds showed an EC_{50} value arranged from 2.12−6.71 × 10^{-7} M (compounds **1**, **6**, **9**, and **16**, Table 1). Even at a concentration of as low as 0.2 mg/L, compound **1** achieved up to 74% germination rate and an EC_{50} value of 2.12 × 10^{-7} M.

In comparison to 2-aryl substituted SL analogues, the SL analogues **18**−**24** derived from 2-mono- and 2-dialkyl substituted dihydroflavonoids generally displayed remarkably higher seed germination activity toward *O. aegyptiaca*. It was shown that, at a lower dosage of 0.2 mg/L, all these 2-alkyl substituted SL analogues revealed germination rates of more than 70% toward *O. aegyptiaca* seeds. The steric effect of 2-alkyl substituent was found to play an important role on seed germination activity. The larger the alkyl group, the higher EC_{50} values were observed (from 2.33 × 10^{-8} to 2.92 × 10^{-7} M). Of particular note, compound **19** (2-methyl) showed up to 10 times higher seed germination activity than GR24 under the same assay conditions, which showed the lowest EC_{50} value of 3.49 × 10^{-9} M toward *O. aegyptiaca* seeds. As far as we know, related to other known SL analogues derived from 3-methyl-2(5*H*)-furanone, *i.e.*, gibberellic acid hybrid strigolactone mimics,[36] this compound displays a higher seed germination activity toward *O. aegyptiaca*. It is noteworthy that compound **24** bearing two methyl groups at the C2 position also reveals a remarkable EC_{50} value of 8.99 × 10^{-8} M. While for the control compound **25** without a conjugated benzene ring, modest germination rates were provided, suggesting that the aryl ring in the dihydroflavonoids is indispensable for their seed germination potential toward parasitic weeds.

Compounds **1** and **24** were selected to be further evaluated for their seed germination activity against *O. cumana*. As illustrated in Figure 1, these new SL analogues displayed remarkable germination activity in comparison to the control *rac*-GR24 at every tested concentration. At a concentration of 20 mg/L, both test samples showed a more than 60% germination rate. Even at a lower concentration of 0.2 mg/L, compound **24** still displays more than 50% germination rate toward *O. cumana* seeds.

***HTL7* Inhibition Assay.** Yoshimulactone Green (YLG), a small-molecule fluorescence turn-on probe, can activate strigolactone signaling and illuminate signal perception by the strigolactone receptors of the parasitic plant *Striga hermonthica*, *ShHTLs*, thereby elucidating the regulatory dynamics for strigolactone signal transduction in *Striga*.[25,26] Among all subgroups of the strigolactone receptor *ShHTLs*, *ShHTL6* and *ShHTL7* showed indiscriminately high affinity to all of the tested SLs, making them ideal targets to detect binding affinity of structurally diverse strigolactone analogues. In particular, *HTL7* was found to be sensitive to the structurally diverse molecules at the levels of picomolar when heterologously expressed in *Arabidopsis*.[37] As a consequence, the inhibition assay of these novel strigolactones toward *HTL7* receptor were performed using YLG fluorescent probe method.

As shown in Table 2, for 2-aryl substituted dihydroflavonoid-derived analogues **1**, **6**, **9**, and **16**, the substituent pattern in the aryl ring showed no prominent effect on the binding affinity toward *HTL7* proteins. Meanwhile, from all tested 2-alkyl substituted dihydroflavonoid-derived analogues (compounds **18**−**24**, Table 3), compound **24** was found to show the highest binding activity toward *HTL7* protein receptor (IC_{50} = 1.06 μmol/L), while the IC_{50} value for the control GR24 was 1.98 μmol/L. As seen in Table 3, the obvious decline in binding affinity toward *HTL7* proteins was observed with the bulkiness of the 2-alkyl group in the dihydroflavonoids. Related to the 2-aryl group, the 2-alkyl group on the C2 position of dihydroflavonoid provided lower IC_{50} values.

Table 2. *HTL7* Inhibition Activity of Compounds 1, 6, 9, and 16 (first batch)

compound number	R	IC_{50} (μmol/L)	R^2
1	phenyl	5.11274	0.9981
6	2-OMe-C_6H_4	5.44412	0.99503
9	4-OMe-C_6H_4	5.26619	0.99252
16	2-thienyl	6.69811	0.9987
GR24		0.84217	0.99876

Table 3. *HTL7* Inhibition Activity of Compounds 18-24 (second batch)

compound number	R	IC_{50} (μmol/L)	R^2
18	H	1.79543	0.99456
19	Me	2.19524	0.99844
20	Et	2.88826	0.99272
21	*n*-Pr	2.63495	0.99127
22	*n*-Bu	5.5582	0.99124
23	*n*-Pent	7.76506	0.99619
24	Me, Me	1.06094	0.9918
GR24		1.98125	0.99472

Molecular Docking Experiment. The interaction of SLs with a protein receptor is an essential step for stimulating the seed germination of parasitic weeds. There is distinct evidence that the D14 protein is involved in this process.[11] The result of this interaction is the detachment of the D-OH ring, which leads to the change of the enzyme pocket conformation and then subsequent cascade reactions, finally giving rise to the germination of parasitic weed seeds.[19] In order to rationalize the seed germination activity of these dihydroflavonoid-derived strigolactone analogues, the molecular docking of compounds **18**, **19**, and **20** into the catalytic site of OsD14 hydrolase (PDB: 5DJ5) was thus performed (Figure 2).

The formed polar contact between carbonyl group in D ring and Ser 97 is required for the successful hydrolysis of the strigolactone analogues.[38] As shown in Figure 2A,B, there was no hydrogen bond formed between compound **19** and Ser 97, but the orientation of the D ring in compound **19** was the same as that of GR24. Moreover the electrostatic interaction was formed between compound **19** and His 247, which belongs to the catalytic triad of OsD14. The $\pi-\pi$ interaction and covalent bonding were also formed between the aromatic ring and Tyr 159 and Trp155, respectively (Figure 2D). The above interactions make compound **19** bound firmly in the catalytic site of OsD14 with the right orientation. Additionally, the molecular docking of compound **18** in OsD14 protein was also performed. As shown in Figure 2C, the orientation of the D ring in compound **18** was not same as that of compound **19**. This result indicates that the C2 substituent group in the dihydroflavonoid scaffold has the potential to adjust the orientation of D ring. Moreover, the docking result of compound **20** was also similar to that of compound **19**,

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J. Agric. Food Chem. XXXX, XXX, XXX−XXX

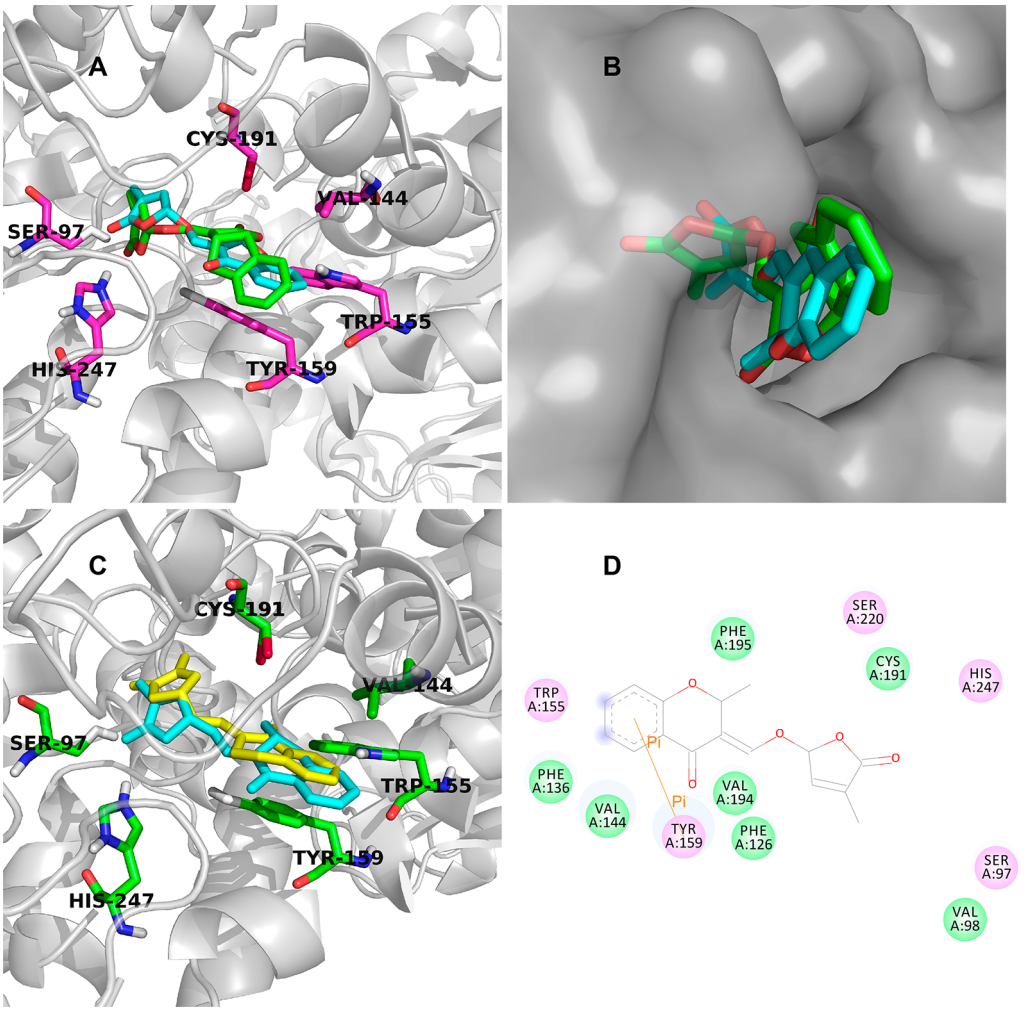

Figure 2. Docking modes of compounds **18** and **19** with OsD14. (A) Close-up view of compound **19** as blue sticks bound in the catalytic site of OsD14. (B) View from the top of the catalytic pocket with compound **19** shown as blue sticks. (C) Close-up view of compounds **18** (yellow sticks) and **19** (blue sticks) bound in the catalytic site of OsD14. (D) 2D view of compound **19** in the catalytic site of OsD14. Note: The green sticks shown in A and B are GR24, which is the original ligand of OsD14.

which might account for better germination activity of compounds **19** and **20** in comparison with compound **18**.

Hydrolytic Stability. Due to the presence of the labile groups including enol ether, hemiacetal, and α,β-unsaturated lactones, the stability of both natural and synthetic strigolactone analogues plays a vital role in stimulating the potential suicidal germination of parasitic plants. Generally, regardless of the presence of additional stability-enhancing agents and microorganisms, the pH values of the soil have an important impact on the hydrolysis rate of SLs. As illustrated in Figure S53, these dihydroflavonoid-derived SLs were found to be stable at acidic conditions. For instance, about 45% of compound **24** was hydrolyzed in an acidic (pH 5) solution of water/methanol (1/1, v/v) after 8 days (see Figure S54 of the Supporting Information). Comparatively, the control GR24 was only hydrolyzed no more than 10%. On the contrary, complete hydrolysis within 1 day was observed for both compound **24** and GR24 at alkaline conditions (water/methanol, 1/1, v/v, pH 8). By comparison with the resorcinol-type mimics,[37] these compounds are shown to be prone to hydrolysis to release the active D ring. These results suggest that this class of dihydroflavonoid-derived SLs might completely decompose in the soil and could be used as the eco-friendly herbicides or promising lead compounds against parasitic weeds.

In summary, to explore novel herbicides against parasitic weeds, such as broomrapes (*Orobanche* and *Phelipanche* spp.) and witchweeds (*Striga* spp.), a series of strigolactone analogues derived from dihydroflavonoids were synthesized. Their suicide-inducing seed germination potential toward the broomrapes was evaluated, and the preliminary structure–activity relationship was also discussed. The 2-alkyl group in the dihydroflavone skeleton was found to play a key role in their seed germination activity. From these, compound **19** bearing a methyl group displayed 10 times higher germination activity than that of the control GR24. This result was further demonstrated by the strigolactone receptor *HTL7* inhibition assay. In light of their hydrolytic stability, the SL analogues described herein were shown to be promising candidates for further study as suicidal germinators against parasitic weeds.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.9b08044.

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J. Agric. Food Chem. XXXX, XXX, XXX−XXX

Figures of NMR spectra, NOSEY spectra, stability test, and seed germination activity (PDF)

AUTHOR INFORMATION

Corresponding Authors

Zhong Jin − *State Key Laboratory of Elemento-organic Chemistry, College of Chemistry, Nankai University, Tianjin 300071, China*; orcid.org/0000-0001-5722-7175; Email: zjin@nankai.edu.cn

Xiaohua Xu − *State Key Laboratory of Elemento-organic Chemistry, College of Chemistry, Nankai University, Tianjin 300071, China*; Email: xiaohuaxu@nankai.edu.cn

Authors

Yunyao Kang − *State Key Laboratory of Elemento-organic Chemistry, College of Chemistry, Nankai University, Tianjin 300071, China*

Zhili Pang − *State Key Laboratory of Elemento-organic Chemistry, College of Chemistry, Nankai University, Tianjin 300071, China*

Niuniu Xu − *State Key Laboratory of Elemento-organic Chemistry, College of Chemistry, Nankai University, Tianjin 300071, China*

Fangjie Chen − *College of Forestry, Northwest Agriculture & Forest University, Xianyang 712100, Shanxi Province, China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jafc.9b08044

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Notes

The authors declare no competing financial interest.

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