Synthesis and Evaluation of New Halogenated GR24 Analogs as Germination Promotors for Orobanche cumana

Yuchao Chen1,2,3†, Yi Kuang4†, Liyang Shi4†, Xing Wang4, Haoyu Fu4, Shengxiang Yang4*, Diego A. Sampietro5*, Luqi Huang1,2* and Yuan Yuan2*

1School of Pharmacy, Anhui University of Chinese Medicine, Hefei, China, 2State Key Laboratory of Dao-di Herbs Breeding Base, National Resources Center for Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, China, 3Agricultural Biotechnology Center, Ningxia Academy of Agriculture and Forestry Sciences, Yinchuan, China, 4Zhejiang Provincial Collaborative Innovation Center for Bamboo Resources and High-Efficiency Utilization, Zhejiang A&F University, Lin’an, China, 5LABIFITO, National University of Tucumán, Tucumán, Argentina

Orobanche and Striga are parasitic weeds extremely well adapted to the life cycle of their host plants. They cannot be eliminated by conventional weed control methods. Suicidal germination induced by strigolactones (SLs) analogs is an option to control these weeds. Here, we reported two new halogenated (+)-GR24 analogs, named 7-bromo-GR24 (7BrGR24) and 7-fluoro-GR24 (7FGR24), which were synthesized using commercially available materials following simple steps. Both compounds strongly promoted seed germination of Orobanche cumana. Their EC50 values of 2.3 ± 0.28 × 10⁻⁸ M (7BrGR24) and 0.97 ± 0.29 × 10⁻⁸ M (7FGR24) were 3- and 5-fold lower, respectively, than those of (+)-GR24 and rac-GR24 (EC50 = 5.1 ± 1.32–5.3 ± 1.44 × 10⁻⁸ M; p < 0.05). The 7FGR24 was the strongest seed germination promoter tested, with a stimulation percentage of 62.0 ± 9.1% at 1.0 × 10⁻⁸ M and 90.9 ± 3.8% at 1.0 × 10⁻⁶ M. It showed higher binding affinity (IC50 = 0.189 ± 0.012 μM) for the SL receptor ShHTL7 than (+)-GR24 (IC50 = 0.248 ± 0.032 μM), rac-GR24 (IC50 = 0.319 ± 0.032 μM), and 7BrGR24 (IC50 = 0.521 ± 0.087 μM). Molecular docking experiments indicated that the binding affinity of both halogenated analogs to the strigolactone receptor OsD14 was similar to that of (+)-GR24. Our results indicate that 7FGR24 is a promising agent for the control of parasitic weeds.

Keywords: strigolactones, Orobanche cumana, parasitic weeds, GR24 analogs, suicidal germination

INTRODUCTION

The parasitic weeds Orobanche spp. (broomrapes) and Striga spp. (witchweeds) can feed through haustoria invading the roots of host plants (Musselman, 1980). They parasitize major crops, including maize (Zea mays), sorghum (Sorghum bicolor), rice (Oryza sativa), tomato (Lycopersicon esculentum), tobacco (Nicotiana tabacum), and sunflower (Helianthus annuus). Orobanche and Striga species infest more than 60 million hectares of farmland worldwide, resulting in the...
loss of billions of dollars each year (Chesterfield et al., 2020). For instance, approximately 1.34 million hectares of rain-fed rice field in Africa are infested with *Striga*, resulting in crop losses of more than USD100 million. These weeds cause economic pressure on millions of smallholder farmers (Parker, 2012; Rodenburg et al., 2016). These parasitic weeds are expanding their geographical range. *Orobanche cumana* was first reported on sunflowers in central Russia at the end of the 19th century. It spread over east Europe in a few decades along with the successful expansion of sunflower harvests. It is currently found in most of the main sunflower-producing countries in Eurasia, from Spain to China and is regarded as the most important biotic constraint for sunflower production (Rubiales, 2020).

*Orobanche* and *Striga* weeds are not effectively controlled by conventional methods, such as breeding resistant varieties, rotation, and herbicides (Hearne, 2009). Their plants produce tens of thousands of tiny seeds that remain viable and dormant for over 10 years and lead to the formation of extensive seed stocks in the soil (Musselman, 1980). The seeds only germinate in response to specific germination signals, known as strigolactones (SLs), which are released in the rhizosphere by the host plants. Strigol was the first SL identified (Cook et al., 1967). Since then, more than 20 SLs have been isolated from host crop plants, including sorghum, maize, rice, and tobacco (Hauck et al., 1992; Siame et al., 1993; Xie et al., 2013). Molecules of these natural SLs are composed of a tricyclic lactone ring (ABC-ring) and a butenolide ring (D-ring) that are connected by an enol-ether linkage, where the bioactivephor for germination resides in the CD part (Zwanenburg et al., 2009; Zwanenburg and Blanco-Ania, 2018). *Orobanche* and *Striga* weeds need their plant hosts to survive. Hence, the application of SLs to soils infested with parasitic weeds is a promising alternative to stimulate suicidal seed germination before the crop is planted (Zwanenburg et al., 2016). However, natural SLs found in root exudates are available at picogram levels and have an unstable structure (Yoneyama et al., 2013). Therefore, synthetic analogs, such as GR24, GR7, GR5, Nijmegen-1, and T-010, were synthesized. They offer interesting prospects for eliminating parasitic weeds through suicidal germination (Zwanenburg and Blanco-Ania, 2018). However, most of the synthetic SL analogs promote less seed germination than natural SLs. Thus, modification of synthetic SL analogs for commercial application toward controlling parasitic weeds remains highly desirable. Here, we reported the synthesis of new SL analogs and their effect on seed germination of parasitic weeds.

**MATERIALS AND METHODS**

**General Experimental Procedure**

All reactions requiring anhydrous or inert conditions were carried out under a positive atmosphere of argon in oven-dried glassware. Solutions or liquids were introduced into round-bottomed flasks using oven-dried syringes through rubber septa. All reactions were stirred magnetically using Teflon-coated stirring bars. If needed, reactions were warmed using an electrically heated silicon oil bath. Organic solutions obtained after aqueous workup were dried over MgSO₄. The removal of solvents was accomplished using a rotary evaporator at water aspirator pressure. GR24 stands for rac-GR24, which was purchased from Shanghai Yuanye Biotechnology (Shanghai, China). Chemicals for the syntheses were purchased from Sigma-Aldrich (Shanghai, China).

NMR spectra were recorded on Bruker ADVANCE III (400 MHz) spectrometers (Karlsruhe, Germany) for 1H NMR and 13C NMR. CD₃OD and CDCl₃ were used as solvents for the NMR analysis, with tetramethylsilane as the internal standard. Chemical shifts were reported upfield to TMS (0.00 ppm) for 1H NMR and relative to CDCl₃ (77.3 ppm) for 13C NMR. Optical rotation was determined using a Perkin Elmer 343 polarimeter. HPLC analysis was conducted on an Agilent 1260 series instrument (California, America). Column chromatography was performed using silica gel Merck 60 (230–400 mesh). All new products were further characterized by HRMS. A positive ion mass spectrum of the sample was acquired on a Thermo LTQ-FT mass spectrometer (MA, United States) with an electrospray ionization source.

**Synthesis of (+)-GR24**

Small portions of potassium tert-butoxide (0.85 g, 7.56 mmol) were added to a solution of compound D (1.1 g, 6.3 mmol) and methyl formate (0.82 ml, 9.45 mmol) in anhydrous THF (15 ml) at 0°C under nitrogen (Figure 1). The reaction mixture was stirred at 25°C until completion. THF was removed in vacuo. The resulting solid was solubilized in 20 ml anhydrous DMF under N₂. Bromobutenolide (1.67 g, 9.45 mmol) was added to this solution and the reaction mixture was stirred overnight. The reaction was quenched with saturated aqueous ammonium chloride (20 ml). The reaction mixture was diluted with ethyl acetate (50 ml) and washed with water (3 × 30 ml). The organic extract was then washed with brine, dried with Na₂SO₄, and the solvent removed under vacuum. The residue was finally purified by silica gel column chromatography (eluent: petroleum ether/ethyl acetate = 3:1, v/v) to give the (+)-GR24.

**Synthesis of (−)-epi-GR24**

The synthetic protocol described for (+)-GR24 was carried out starting with compound E (1.11 g, 6.3 mmol; Figure 2) to yield a residue. The residue was finally purified by silica gel column chromatography (eluent: petroleum ether/ethyl acetate = 3:1, v/v) to give the (−)-epi-GR24.

**Synthesis of 7-bromo-GR24 and 7-fluoro-GR24**

Compound A (10 mmol of a ketone) and 15 mmol of glyoxyl acid were added to a round bottomed flask (Figure 3). Then, the mixture was stirred at 95°C for 3 h. The reaction mixture was dissolved in acetic acid (15 ml) and water (5 ml). Zinc dust (15 ml) was added to the solution for 1 h and the mixture was stirred for an additional 3 h. The mixture was diluted with ethyl acetate and then filtered through celite. The filtrate
was extracted with ethyl acetate, washed with brine, dried over Na₂SO₄, and concentrated under vacuum. The residue was purified by chromatography on silica gel using hexane:ethyl acetate (2:1, v/v) and 0.5% acetic acid as an eluent, resulting in 70% yield of compound B. Compound B (5 mmol) was dissolved in 15 ml of anhydrous MeOH. Then, 15 mmol of NaBH₄ were added in small portions at 0°C under nitrogen. The reaction mixture was stirred at 25°C until the completion of the reaction. We carefully added 20 ml of distilled water to the mixture. The solution was extracted three times with ethyl acetate and the combined organic phase was dried over Na₂SO₄, filtered, and concentrated under vacuum. The resulting solid was solubilized in 20 ml anhydrous MeOH. TsOH (0.1 mmol) was added to this solution and the reaction mixture was stirred at 75°C for 6 h. MeOH was removed in vacuo. Then, 20 ml of distilled water was added. The solution was extracted with ethyl acetate three times and the combined organic phase was dried over Na₂SO₄, filtered, and concentrated under vacuum. The residue was purified by chromatography on silica gel using hexane:ethyl acetate (3:1, v/v) and 0.5% acetic acid as eluents. It yielded 95% of compound C.

The protocol described for (+)-GR24 was performed starting with compound C (1.1 g, 6.3 mmol) to finally obtain a residue that was subjected to column chromatography, generating pure 7-bromo-GR24 (7BrGR24) and 7-fluoro-GR24 (7FGR24).
Germination Assays
Seeds of *O. cumana* were kindly provided by Professor Yongqing Ma (North-west Agriculture & Forest University, Yangling, China). The assay was carried out in petri dishes according to a method previously reported by Kang et al. (2020). Prior to use, the seeds were sterilized for 8 min in 1% sodium hypochlorite, soaked in 75% ethanol for another 1 min, rinsed five times with sterile distilled water, and finally left to air dry on a clean bench. A sterile filter paper disk of 6 mm in diameter was placed in each petri dish and wetted with 200 μl of sterile distilled water. Then, aqueous solutions of the tested compounds (100 μl per filter paper) were added. The sterile seeds were distributed on the petri dishes at a density of approximately 65 seeds per dish. Finally, the sealed petri dishes were stored in the dark and incubated at 25°C for 14 days. After the incubation, the percentage of germination was calculated according to a method previously reported by Tsuchiya et al. (2015).

Yoshimulactone Green Assay
The assay was carried out according to a method previously reported by Tsuchiya et al. (2015). The stock solutions of yoshimulactone green (YLG), rac-GR24, (+)-GR24, (-)-epi-GR24 and the new SL analogs dissolved in DMSO (1 ml, 1 mM). Then, stock solutions were diluted with sterile distilled water to final concentrations of 50 μM (YLG), and 20, 5, 2.5, 1, 0.5, and 0.1 μM [rac-GR24, (+)-GR24, (-)-epi-GR24, and the new SL analogs]. Protein coding sequences for SLH7 were inserted into KpnI and HindIII sites of the pET32α(+) vector (Invitrogen, CA, United States) and transformed into the Escherichia coli strain BL21(DE3; TransGen Biotech, Beijing, China). Briefly, a single colony on the plate was inoculated into a 25 ml sterilized LB medium containing 0.28 mM ampicillin for 12 h. Then, the culture was centrifuged for 10 min at 12,000 rpm at 4°C for 10 min. The supernatant was filtered with a 0.45 μm filter and the filtrate was added into a Ni-NTA column (TransGen Biotech, Beijing, China), which had been equilibrated with PBS buffer. After the Ni-NTA column was washed three times with the PBS buffer, the column was eluted with a 15 ml gradient of 20, 50, 100, and 300 mM imidazole prepared in the PBS buffer. Fractions from 100 mM eluent were pooled for the YLG assay.

The volume of each reaction solution (200 μl) contained 5 μl of YLG (50 μM), 10 μl of a dilution of an SL analog, 15 μl of ShHTL7 protein (1.5 mg/ml), and 170 μl of PBS buffer. Reactions were carried out for 3 h in the dark in a water bath at 26°C. The blank control contained water (10 μl) instead of a dilution of the SL analog. Then, the reaction solutions were added to a 96-well black plate (Nest, Wuxi, China) and its fluorescent intensity was measured by SpectraMax i3 ( Molecular Devices, CA, United States) at an excitation wavelength of 480 nm and emission wavelength of 520 nm. Relative fluorescence units (FU) were calculated as (k−k’)/k, where k and k’ are the fluorescence intensities of the blank control and a dilution of the SL analog, respectively. FU were used to calculate IC_{50} values with probit tests using SPSS 21.0 software.

Molecular Docking Experiment
The molecular modeling computational study was performed using Autodock vina 1.1.2 software. The crystal structure of rice DWARF14 (OsD14; PDB: 5DJ5) was used for the docking study. The grid box was set as a 20×20×20 Å three cube and its center was set at the position of the original ligand GR24. The molecular modeling computational study was performed using Autodock vina 1.1.2 software. The crystal structure of rice DWARF14 (OsD14; PDB: 5DJ5) was used for the docking study. The grid box was set as a 20×20×20 Å three cube and its center was set at the position of the original ligand GR24.

Statistical Analysis
Data of seed germination and fluorescence-based comparison assays were subjected to the ANOVA and differences among means were evaluated by the least significant difference test ($p<0.05$).

RESULTS AND DISCUSSION
Synthesis of SL Analogs
The main features of the synthesized compounds were as follow: (+)-GR24: White solid, 0.65 g, 35% yield, [α]_D^{20} = +449 (c = 0.50, CHCl_3). 1H NMR (400 MHz, CDCl_3) δ 7.51–7.49 (m, 3H), 7.35 (m, 3H), 7.00 (s, 1H), 6.21 (s, 1H), 5.95 (d, J = 7.9 Hz, 1H), 3.97–3.92 (m, 3H), 3.11 (dd, J = 16.9, 9.4 Hz, 1H), 3.11 (dd, J = 16.9, 3.2 Hz, 1H), 2.03 (t, J = 1.4 Hz, 3H). 13C NMR (100 MHz, CDCl_3) δ 171.44, 170.37, 151.27, 142.66, 141.16, 138.82, 135.83, 130.04, 127.49, 126.42, 125.18, 113.14, 100.71, 85.99, 83.85, 37.31, 10.73.

(-)-epi-GR24: White solid, 0.56 g, 30% yield, [α]_D^{20} = −290 (c = 0.50, CHCl_3). 1H NMR (400 MHz, CDCl_3) δ 7.51–7.49 (m, 3H), 7.35–7.23 (m, 3H), 6.99 (s, 1H), 6.21 (s, 1H), 5.96 (d, J = 8.0 Hz, 1H), 3.97–3.92 (m, 3H), 3.42 (dd, J = 16.9, 9.3 Hz, 1H), 3.10 (dd, J = 16.9, 3.1 Hz, 1H), 2.03 (t, J = 1.4 Hz, 3H). 13C NMR (100 MHz, CDCl_3) δ 171.42, 170.40, 151.29, 142.66, 141.16, 138.82, 135.76, 130.05, 127.45, 126.42, 125.18, 113.14, 100.71, 85.99, 83.85, 37.31, 10.73.

7BrGR24 and 7FGR24 were assayed at concentrations of 1.0 × 10^{-3}, 1.0 × 10^{-4}, 2.0 × 10^{-4}, 1.0 × 10^{-5}, and 1.0 × 10^{-6} M. Three petri dishes were used for each concentration, and assays were carried out three times. The compounds (+)-GR24, (-)-epi-GR24, and rac-GR24 were used as the positive control, and the filter paper disk added with 100 μl of sterile distilled water was used as the negative control. The EC_{50} values of the tested compounds were calculated with probit tests using SPSS 21.0 software.
3.39 (1H, dd, J = 17.1, 9.3 Hz, H-4β), 3.08 (1H, dd, J = 17.0, 3.1 Hz, H-4α), 2.07 (1H, s, H-7). 13C NMR (100 MHz, CDCl3) δ 170.8 (C-2), 169.9 (C-5), 151.1 (C-6), 141.4 (C-8a), 141.1 (C-3), 140.7 (C-4a), 136.0 (C-8), 134.4 (C-4), 129.4 (C-5), 126.3 (C-7), 121.0 (C-6), 112.5 (C-3), 100.3 (C-2'), 85.2 (C-8b), 39.2 (C-4), 36.8 (C-3a), 10.8 (C-7). HR-ESI-MS (m/z): calcd. For C17H23BrNaO3 398.9839; found 398.9835 [M+Na]+.

7FGR24: White solid, mp 183–186°C, 0.69 g, 35% yield. 1H NMR (400 MHz, CDCl3) δ 7.49 (1H, d, J = 2.0 Hz, H-8), 7.43 (1H, dd, J = 8.4, 4.4 Hz, H-6), 7.01 (1H, m, H-5), 6.97 (1H, m, H-6), 6.88 (1H, m, H-3), 6.22 (1H, s, H-2), 5.88 (1H, d, J = 7.9 Hz, H-8b), 4.01 (1H, m, H-3a), 3.40 (1H, dd, J = 17.2, 9.3 Hz, H-4β), 3.07 (1H, dd, J = 17.2, 3.1 Hz, H-4α), 2.01 (1H, s, H-7). 13C NMR (100 MHz, CDCl3) δ 171.2 (C-2), 170.4 (C-5'), 165.1 (C-7), 151.4 (C-6'), 145.3 (C-8a), 141.2 (C-3'), 135.8 (C-4a), 134.8 (C-4'), 127.9 (C-5), 115.1 (C-8), 112.8 (C-6), 111.9 (C-3), 100.7 (C-2'), 85.0 (C-8b), 39.4 (C-4), 37.3 (C-3a), 10.7 (C-7). HR-ESI-MS (m/z): calcd. For C17H23F3NaO3 339.0639; found 339.0642 [M+Na]+.

### Seed Germination Assay

Table 1 shows the impact of (+)-GR24, (-)-epi-GR24, rac-GR24, 7BrGR24, and 7FGR24 on the seed germination of *O. cumana*. (+)-GR24 and rac-GR24 showed a similar stimulatory effect (EC50 = 5.1 ± 1.32–5.3 ± 1.44 × 10−8 M), whereas (-)-epi-GR24 had no effect. These results suggested strong stereospecificity in the SL perception of *O. cumana*. (+)-GR24 and (-)-epi-GR24 were diasteroisomers with opposite stereochemistry in the C-ring that is β- and α-oriented, respectively, and had the same 2’R configuration of the D-ring. Indeed, parasitic plant species also vary considerably in their germination responses to different SLs (Wang and Bouwmeester, 2018; Bouwmeester et al., 2021). In general, the 2’R configuration of the D-ring has been confirmed essential for SLs germination activity, and stereochemistry in the C-ring is considered to be closely related to the activity (Thuring et al., 1997a; Xie et al., 2010). The use of (+)-GR24 and (-)-epi-GR24 in germination tests could provide profound clues about the general stereochimical adaptation of parasitic weeds for the perception of strigol-like (β-oriented C-ring) and orobanchol-like (α-oriented C-ring) SLs, respectively. These are the two families of natural canonical SLs currently known (Scaffidi et al., 2014; Ueno et al., 2014; Xie, 2016).

### Table 1

| Compounds          | Concentration (M) | EC50 (10−8 M) |
|--------------------|-------------------|--------------|
|                    | 1.0 × 10−9        | 1.0 × 10−8    | 2.0 × 10−8    | 1.0 × 10−7    | 1.0 × 10−6    |
| (+)-GR24           | 7.3 ± 5.9%        | 21.5 ± 13.4% | 41.1 ± 10.1% | 66.6 ± 9.1%  | 81.5 ± 3.9%  | 5.1 ± 1.32   |
| (-)-epi-GR24       | 0°                | 0°           | 0°           | 0°           | 0°           | –            |
| 7-bromo-GR24        | 16.9 ± 4.1%       | 39.6 ± 5.0%  | 49.1 ± 6.6%  | 69.6 ± 3.3%  | 83.8 ± 2.5%  | 2.3 ± 0.28   |
| 7-fluoro-GR24       | 11.2 ± 7.8%       | 62.0 ± 9.1%  | 63.8 ± 4.9%  | 84.2 ± 8.6%  | 90.9 ± 3.8%  | 0.97 ± 0.29  |
| rac-GR24           | 15.3 ± 8.5%       | 27.2 ± 4.2%  | 47.1 ± 5.0%  | 58.5 ± 10.7% | 75.2 ± 5.7%  | 5.3 ± 1.44   |

Different lowercase letters in the same column indicate significant differences between means, according to the least significant difference test (p < 0.05). Values represent means ± SD (n = 3).
group on the A-ring enhanced the germination-stimulating activity on *O. minor*, where a hydroxyl group is preferable at C-9 instead of at C-5 (Kim et al., 2010). Furthermore, the 6-methyl substituent on (+)-GR24 resulted in higher percentages of germinated *O. crenata* seeds (Wigchert and Zwanenburg, 1999). Moreover, bulky side groups joined to the A-ring also reduced the activity of SL analogs more than small groups (Cohen et al., 2013). Accordingly, the germination-stimulating activity of SLs depended on both the position and size of the substituent on A-ring. Although, a few of reports declared the introduction of substituent such as iodine atom to the A-ring at the C-7 reduced the activity of SL analogs on *O. crenata* and *Pisum sativum* (Thuring et al., 1997c; Boyer et al., 2012). In our results, 7FGR24 showed higher activity. It might be due to the fact that both the A-ring halogenation at the 7-C position, which was far from the GR24 bioactiphore and the small size of the fluorine atom likely favored a high affinity of 7FGR24 to the active site of SLs receptors of *O. cumana*.

**YLG Assay**

(+)-GR24 and its halogenated analogs showed binding affinity to ShHTL7, an SL receptor found in the parasitic plant *S. hermonthica*, with a high affinity to SLs (Tsuchiya et al., 2015). Binding affinity was tested by an *in vitro* fluorescence-based competition assay involving YLG. The YLG was a small probe that emits fluorescence only after the hydrolysis, which was catalyzed by ShHTL7. A decrease in FUs showed the competition for receptor binding between a fixed YLG concentration and increasing concentrations of the SL analog. The halogenated GR24 analogs 7BrGR24 and 7FGR24 tested at concentrations between 2.5 and 20 μM showed approximately 0.1 FU, which were similar to those recorded for (+)-GR24 and rac-G2R24 (Figure 4). The FU of 7FGR24 were below 0.34 as in the case of (+)-GR24 and rac-GR24, even at a concentration range from 0.5 to 1.0 μM. Moreover, 7FGR24 tested at 0.1 μM was 0.72 FU, which was significantly lower than 0.81 and 0.92 FU recorded for rac-GR24 and (+)-GR24, respectively (*p < 0.05*). Probit analysis based on FUs indicated that 7FGR24 was the strongest competitor tested (IC$_{50}$ = 0.189 ± 0.012 μM), followed by (+)-GR24 (IC$_{50}$ = 0.248 ± 0.032 μM), whereas rac-GR24 and 7BrGR24 had a lower affinity for ShHTL7 with IC$_{50}$ values of 0.319 ± 0.032 and 0.521 ± 0.087 μM, respectively. Consistent with this, the substituent at C-8 also showed higher affinity for ShHTL7 (Tsuchiya et al., 2015). Although, the 7BrGR24 posed lower affinity, as compared to (+)-GR24, which was inconsistent with seed germination activity. The discrepancy could be due to the fact that the that ShHTL7 protein was derived from a *Striga* ssp. not an *Orobanchaceae* ssp., both of which could respond differently to 7BrGR24.

![Graphs of Relative Fluorescence Unit (FU) values for rac-GR24, (+)-GR24, 7FGR24, and 7BrGR24 analogs.](#)
Molecular Docking Assays

Rice DWARF14 (OsD14) was selected for docking studies in order to understand how the SL analogs interacted with the SL receptor. SLs receptors were AtD14 paralogs forming part of the α, β-fold hydrolases family, which not only binded to the SL molecules but also cleaved them into their ABC-ring and the D-ring parts (Hamiaux et al., 2012). They were structurally similar and had a conserved catalytic pocket consisting of a triad of serine, histidine, and aspartate (Yao et al., 2016). The docking analyses indicated that (+)-GR24 and the halogenated (+)-GR24 analogs could smoothly enter the binding pocket of the OsD14 protein (Figure 5). Their D-rings acquire the same orientation predicted for (+)-GR24 during its interaction with the receptor (Trott and Olson, 2010; Figure 5D). As shown in Figure 5B, the carbonyl oxygen in the D-ring of 7FGR24 formed hydrogen bonding forces with Ser97 and Hip247, which were part of the OsD14 catalytic triad. The polar connection between the hydroxyl hydrogen atom in Ser97 and the carbonyl oxygen in the D-ring of SLs was a key step required for the successful hydrolysis of SLs (Kagiyama et al., 2013). Further predictions obtained for these two hydrogen bonds in the enzyme-catalyzed reactions revealed that their distances and positions were similar to those expected for the ligation of (+)-GR24 (Figure 5C). This should be responsible for the high biological activity observed in 7FGR24. It was worth noting that, the fluorine atom could modify physicochemical properties of the GR24 analog, such as pKa and lipophilicity, improving its permeability through cell membranes (Purser et al., 2008). Moreover, as shown in Figure 5F, the posture of the D ring in 7FGR24 was obviously more similar to the original ligand of the crystal structure-GR24, which meant that 7FGR24 could be more conducive to hydrolysis, compared to 7BrGR24. Furthermore, these different postures could be related to the distinct atomic radii and electronegativities of observed between atoms F and Br.

In addition, we also conducted docking experiments on (-)-epi-GR24, which was inactive on seed germination. The (-)-epi-GR24 had two main binding poses differing from each other in the location of the D-ring. One pose showed the D-ring into the active site, while the other revealed the ABC-ring positioned into the active pocket with its D-ring in an outer location (Figure 5G). In both cases, D-ring orientation was different from the expected during (+)-GR24-OsD14 interaction. Binding energies calculated for the poses of (-)-epi-GR24 were near to the binding energy predicted for (+)-GR24. Hence, both bindings of (-)-epi-GR24 were possible, although, the D-ring would be not properly oriented for the interaction.
for the hydrolytic cleavage at the enol–ether bond catalyzed by OsD14. In addition, the docking analyses for (+)-GR24 and 7BrGR24 were similar to those obtained for 7FGR24.

**CONCLUSION**

Two halogenated (+)-GR24 analogs (7BrGR24 and 7FGR24) were synthesized through a relatively short number of synthetic steps and their promotive effect were tested on seed germination of *O. cumana*. Both stimulated its germination and showed a binding affinity for the SL receptor protein ShHTL7. However, 7FGR24 was the strongest germination promoter tested and had the highest binding affinity to ShHTL7. Molecular docking assays supported structural features of 7FGR24, which explained the higher activity compared to that of rac-GR24 and (+)-GR24. Our results indicate that 7FGR24 is a promising agent for the control of parasitic weeds.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

**AUTHOR CONTRIBUTIONS**

YY, LH, and SY conceived and designed the experiments. YK, LS, and XW designed and synthesized the analogs. YC, YK, LS, LH, and YY wrote the manuscript and respective parts. YY and SY supervised the study. All authors contributed to the article and approved the submitted version.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2021.725949/full#supplementary-material

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