A New Series of Carlactonoic Acid Based Strigolactone Analogs for Fundamental and Applied Research

Muhammad Jamil1, Boubacar A. Kountche1, Jian You Wang1, Imran Haider1, Kun-Peng Jia1, Ikuo Takahashi2, Tsuyoshi Ota2, Tadao Asami2 and Salim Al-Babili1*

1 The BioActives Lab, Center for Desert Agriculture, Biological and Environment Science and Engineering (BESE), King Abdullah University of Science and Technology, Thuwal, Saudi Arabia, 2 Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan

Strigolactones (SLs) are a group of carotenoid derived plant hormones that play a key role in establishing plant architecture and adapting it to environmental changes, and are involved in plants response to biotic and abiotic stress. SLs are also released into the soil to serve as a chemical signal attracting beneficial mycorrhizal fungi. However, this signal also induces seed germination in root parasitic weeds that represent a major global threat for agriculture. This wide spectrum of biological functions has made SL research one of the most important current topics in fundamental and applied plant science. The availability of SLs is crucial for investigating SL biology as well as for agricultural application. However, natural SLs are produced in very low amounts, and their organic synthesis is quite difficult, which creates a need for efficient and easy-to-synthesize analogs and mimics. Recently, we have generated a set of SL analogs, Methyl Phenlactonoates (MPs), which resemble the non-canonical SL carlactonoic acid. In this paper, we describe the development and characterization of a new series of easy-to-synthesize MPs. The new analogs were assessed with respect to regulation of shoot branching, impact on leaf senescence, and induction of seed germination in different root parasitic plants species. Some of the new analogs showed higher efficiency in inhibiting shoot branching as well as in triggering parasitic seed germination, compared to the commonly used GR24. MP16 was the most outstanding analog showing high activity in different SL biological functions. In summary, our new analogs series contains very promising candidates for different applications, which include the usage in studies for understanding different aspects of SL biology as well as large scale field application for combating root parasitic weeds, such as Striga hermonthica that devastates cereal yields in sub-Saharan Africa.

Keywords: strigolactones, Striga, Phelipanche, tillering, senescence, strigolactone analogs

INTRODUCTION

Strigolactones (SLs) are well-known carotenoid derived metabolites that act as endogenous phytohormones as well as rhizospheric signaling molecules. As plant hormones, SLs are involved in controlling shoot branching/tillering, root architecture and contribute to further aspects of plant growth, as well as to pathogen defense and abiotic stress responses (Gomez-Roldan et al., 2008;
Africas. It has been reported that severe infestation of root parasitic plants of the genus Striga are considered as one of the major biotic constraints and are catalyzed by the 9-α/β-carotene isomerase (D27) (Alder et al., 2012; Bruno and Al-Babili, 2016; Abuauf et al., 2018) and the two carotenoid cleavage dioxygenases 7 (CCD7) (Alder et al., 2012; Bruno and Al-Babili, 2016; Abuauf et al., 2018). Canonical and non-canonical SLs differ in the structure of the moiety that is coupled to the α/β unsaturated system and D-ring of SL analogs are indispensable for their activity, while modifications of the ABC-ring impacted the level of their efficiency, as shown by testing the activity of the structurally less complex analogs GR5 and GR7 (Mangnus et al., 1992b; Zwanenburg et al., 2009, 2016c). Other simple analogs were developed by isosteric replacement leading to imino SL analogs (Kondo et al., 2007), CISA-1 (Rasmussen et al., 2013) and strigolactams (Lachia et al., 2015). We recently reported the synthesis of Nitro-Phenlactones (Jia et al., 2016) and a series of Methyl-Phenlactonoates (MPs) (Jamil et al., 2018, 2019), which were designed based on the structure of carlactone and carlactonico acid, respectively. In this paper, we further proposed a new series of carlactonico acid-based SL analogs. In the following, we describe the synthesis and evaluation of this new set of MPs, which is characterized by simple synthesis and includes quite highly efficient SL analogs.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Striga hermonthica seeds were provided by Prof. Abdel Gabar Babiker collected from Sorghum infested field near Wad Medani, Sudan. Seeds of Phelipanche aegyptiaca were provided by Prof. Mohamed Ewis Abdelaziz, Cairo University, Egypt. Seeds of the highly Striga susceptible rice cv IAC 165 are a gift from Dr. Jonne Rodenburg, Africa Rice, Tanzania. Dr. Junko Kyozuka, Tohoku University, Japan provided us seeds of the rice d10-1 and d3-1 mutants in the Shiokari background (Ishikawa et al., 2005; Arite et al., 2007, 2009; Hu et al., 2010). Rice seeds were germinated at 30°C on moist filter paper, and seedlings were grown at 30°C and 70% relative humidity with fluorescent white light (130–180 μM m⁻² s⁻¹) 12 h day/night period. Striga and Phelipanche seeds pre-conditioning was done under moist conditions in the dark at 30 and 22°C, respectively.

General Procedure for the Preparation of Esters and Amide

Phenylacetyl chloride (3 mmol) was added dropwise to the solution of pyridine (10 ml) and alcohol or phenol (10 mmol) with stirring in a 50 ml of round-bottom flask in an ice
bath (Figure 1). The mixture was stirred at room temperature overnight then poured into ice-cold water (10 ml). The organic layer was extracted with ethyl acetate (5 ml × 3), washed with saturated brine (20 ml), dried over anhydrous sodium sulfate, and filtered. The organic solvent was evaporated under reduced pressure and the residue was then purified on a silica gel (Wakosil® C-300HG) column eluted with ethyl acetate/n-hexane to give an ester (53–95% yield). The preparation of amide compound followed the same step as described above, except dimethyl amine was used as the starting material instead of alcohol (78% yield).

**General Procedure for the Preparation of MPs (MP16–MP25)**

To an ice-cold solution of the ester (2.47 mmol) obtained above, methyl formate (7.91 mmol), and trimethylamine (5.93 mmol) in dichloromethane (15 ml), titanium (IV) chloride (4.94 mmol) were added slowly and stirred for 10 min (Figure 1). The mixture was stirred further for 2 h at room temperature and then poured into ice-cold water (10 ml). The organic layer was extracted with ethyl acetate (5 ml × 3), washed with saturated brine (20 ml), dried over anhydrous sodium sulfate, and filtered. The organic solvent was evaporated under reduced pressure and the residue was then purified on a silica gel (Wakosil® C-300HG) column eluted with ethyl acetate/n-hexane to give aldehyde. All of the compounds thus obtained were used in the next step without measuring weight. To an ice-cold solution of the aldehyde obtained above in dichloromethane (13 ml), 5-bromo-3-methyl-2(5H)-furanone (2.47 mmol) and trimethylamine (3.71 mmol) was added and stirred overnight at room temperature and then poured into ice-cold water (10 ml). The organic layer was extracted with ethyl acetate (5 ml × 3), washed with saturated brine (20 ml), dried over anhydrous sodium sulfate, and filtered. The organic solvent was evaporated under reduced pressure and the residue was then purified on a silica gel (Wakosil® C-300HG) column eluted with ethyl acetate/n-hexane to give enol ether. MP17-MP25 (Figure 2) were prepared according to the method above (10–67% yield). The preparation of MP16 followed the same step as described above, except the amide compound was used as the starting material instead of ester (21% yield).

**1H, 13C NMR and HRMS spectra of all synthesized compounds were recorded on JEOL JNM-ECA500II 500 MHz spectrometer and ABSciex TripleTOF5600 Q-TOF LC/MSMS. Z/E stereochemistry was assigned based on NOE experiments. In case of the E-isomer (MP16) NOE between vinyl proton and aromatic proton was observed. In case of Z-isomer NOE between vinyl proton and aromatic proton was not observed and instead NOE between vinyl proton and methyl proton of carbomethoxy group was observed.**

The physico-chemical properties of all MPs are as under:

**MP16** (Z)-N,N-Dimethyl-3-((4-methyl-5-oxo-2,5-dihydrofuran-2-yl)oxy)-2-phenylacrylamide.

\[ ^{1}H \text{NMR (500MHz, CDCl}_3): \delta 7.27(5H, m), 6.93(1H, s), 6.77(1H, s), 6.09(1H, s), 3.03(3H, s), 2.91(3H, s), 1.97(3H, s). \]

\[ ^{13}C \text{-NMR (126 MHz, CDCl}_3): \delta 170.86, 167.15, 142.09, 139.30, 134.76, 132.93, 128.78, 127.58, 125.27, 121.11, 100.25, 37.54, 34.27, 10.51. \]

HRMS (m/z): [M+H]+ calcld. for C_{16}H_{18}NO_{4}, 288.1230; found, 288.1231. 21% Yield.

**MP17** (E)-Phenyl 3-((4-methyl-5-oxo-2,5-dihydrofuran-2-yl)oxy)-2-phenylacrylate.

\[ ^{1}H \text{NMR (500MHz, CDCl}_3): \delta 7.27(5H, m), 6.93(1H, s), 6.77(1H, s), 6.09(1H, s), 3.03(3H, s), 2.91(3H, s), 1.97(3H, s). \]

\[ ^{13}C \text{-NMR (126 MHz, CDCl}_3): \delta 170.86, 167.15, 142.09, 139.30, 134.76, 132.93, 128.78, 127.58, 125.27, 121.11, 100.25, 37.54, 34.27, 10.51. \]

HRMS (m/z): [M+H]+ calcld. for C_{16}H_{18}NO_{4}, 288.1230; found, 288.1231. 21% Yield.
FIGURE 2 | Structure of the developed methyl phenlactonoates (MP16-MP25) and the standard SL analog rac-GR24.

$^1$H NMR (500MHz, CDCl$_3$): $\delta$ 7.98(1H, s), 7.40(5H, m), 7.32(1H, m), 7.24(1H, $J = 7.5$Hz), 7.38(2H, d, $J = 5.5$Hz), 6.90(1H, s), 6.20(1H, s), 2.00(3H, s). $^{13}$C-NMR (126 MHz, CDCl$_3$) $\delta$: 170.32, 165.27, 154.13, 150.62, 141.17, 135.26, 131.14, 130.03, 129.25, 127.81, 125.64, 121.59, 114.93, 100.52, 10.49. HRMS (m/z): [M+H]$^+$ calcd. for C$_{20}$H$_{17}$O$_5$, 337.1071; found, 337.1069. 38% Yield.

**MP18** (E)-Benzyl 3-((4-methyl-5-oxo-2,5-dihydrofuran-2-yl)oxy)-2-phenylacrylate.

$^1$H NMR (500MHz, CDCl$_3$): $\delta$ 7.76(1H, s), 7.33(10H, m), 6.84(1H, s), 6.12(1H, s), 5.22(2H, s), 1.96(3H, s). HRMS (m/z): [M+Na]$^+$ calcd. for C$_{21}$H$_{18}$NaO$_5$, 373.1046; found, 373.1051. 67% Yield.

**MP19** (E)-Isobutyl 3-((4-methyl-5-oxo-2,5-dihydrofuran-2-yl)oxy)-2-phenylacrylate.

$^1$H NMR (500MHz, CDCl$_3$): $\delta$ 7.73(1H, s), 7.32(5H, m), 6.87(1H, s), 6.15(1H, s), 4.17(2H, t, $J = 7.0$Hz), 1.98(3H, s), 1.66(2H, m), 1.33(4H, m), 0.91(3H, t, $J = 7.5$Hz).

$^{13}$C-NMR (126 MHz, CDCl$_3$) $\delta$: 170.34, 166.58, 152.36, 141.31, 133.495, 131.52, 129.86, 127.52, 127.52, 115.52, 100.35, 64.68, 28.05, 27.86, 22.03, 13.72, 10.32. HRMS (m/z): [M+H]$^+$ calcd. for C$_{19}$H$_{23}$O$_5$, 331.1540; found, 331.1539. 42% Yield.

**MP20** (E)-Pentyl 3-((4-methyl-5-oxo-2,5-dihydrofuran-2-yl)oxy)-2-phenylacrylate.

$^1$H NMR (500MHz, CDCl$_3$): $\delta$ 7.73(1H, s), 7.33(5H, m), 6.87(1H, s), 6.15(1H, s), 1.98(3H, s), 1.66(2H, m), 1.33(4H, m), 0.91(3H, t, $J = 7.5$Hz).

$^{13}$C-NMR (126 MHz, CDCl$_3$) $\delta$: 170.34, 166.58, 152.36, 141.31, 133.495, 131.52, 129.86, 127.52, 127.52, 115.52, 100.35, 64.68, 28.05, 27.86, 22.03, 13.72, 10.32. HRMS (m/z): [M+H]$^+$ calcd. for C$_{21}$H$_{23}$O$_5$, 331.1540; found, 331.1539. 42% Yield.

**MP21** (E)-4-Chlorophenyl 3-((4-methyl-5-oxo-2,5-dihydrofuran-2-yl)oxy)-2-phenylacrylate.

$^1$H NMR (500MHz, CDCl$_3$): $\delta$ 7.96(1H, s), 7.30–7.40(7H), 7.07(2H, d, $J = 8.5$Hz), 6.90(1H, s), 6.20(1H, s), 1.99(3H, s).

$^{13}$C-NMR (126 MHz, CDCl$_3$) $\delta$: 170.23, 165.01, 154.44, 149.06, 141.07, 135.27, 130.91, 129.96, 129.23, 127.83, 127.91, 114.63, 100.49, 10.45. HRMS (m/z): [M+H]$^+$ calcd. for C$_{20}$H$_{16}$ClO$_5$, 371.0681; found, 371.0675. 11% Yield.

**MP22** (E)-3-Chlorophenyl 3-((4-methyl-5-oxo-2,5-dihydrofuran-2-yl)oxy)-2-phenylacrylate.

$^1$H NMR (500MHz, CDCl$_3$): $\delta$ 7.96(1H, s), 7.39(4H, d, $J = 4.5$Hz), 7.33(10H, m), 6.84(1H, s), 6.12(1H, s), 5.22(2H, s), 1.96(3H, s), 1.95(1H, m), 0.91(6H, d, $J = 6.0$Hz).

$^{13}$C-NMR (126 MHz, CDCl$_3$) $\delta$: 170.34, 166.12, 154.13, 150.62, 141.17, 135.26, 131.14, 130.03, 129.25, 127.81, 125.64, 121.59, 114.93, 100.52, 10.49. HRMS (m/z): [M+Na]$^+$ calcd. for C$_{20}$H$_{15}$ClNaO$_5$, 393.0500; found, 393.0498. 10% Yield.

**MP23** (E)-2-Chlorophenyl 3-((4-methyl-5-oxo-2,5-dihydrofuran-2-yl)oxy)-2-phenylacrylate.

$^1$H NMR (500MHz, CDCl$_3$): $\delta$ 8.03(1H, s), 7.45(3H, d, $J = 8.5$Hz), 7.38(2H, d, $J = 7.0$Hz), 7.25–7.34(2H, m), 6.90(1H, s), 6.21(1H, s), 2.00(3H, s).

$^{13}$C-NMR (126 MHz, CDCl$_3$) $\delta$: 170.34, 166.58, 152.36, 141.31, 133.495, 131.52, 129.86, 127.52, 127.52, 115.52, 100.35, 64.68, 28.05, 27.86, 22.03, 13.72, 10.32. HRMS (m/z): [M+H]$^+$ calcd. for C$_{20}$H$_{15}$ClNaO$_5$, 393.0500; found, 393.0498. 10% Yield.
After sterilization with 50% sodium hypochlorite, rice seeds (cv IAC-165) were surface sterilized with 50% sodium hypochlorite solution and 0.05% Tween-20, and germinated on moist filter paper in the dark at 30 °C for 7 days. After application of MP16 and GR24, each segment was put in a well (in 12-well plates) containing 4 ml sterile MilliQ water and GR24 were applied at 3.0 µM concentration (55 µl per disk) at 25 °C and for 1 week at 25°C (for *P. aegyptiaca*). Germination was recorded under a binocular microscope and used to determine the germination rate (%).

### Stability Measurement

Three selected MPs (MP16, MP18, MP21) and *rac*-GR24 were tested for their chemical stability at 21 ± 1°C in aqueous solution with a pH of 5.5–6.0, as described previously (Jamil et al., 2018). Compound solution (1 mg ml⁻¹) was prepared with 175 µl ethanol and 750 µl Mili-Q water. Thereafter, 25 µl Indanol (1 mg ml⁻¹, internal standard) was spiked in 975 µl previous prepared solution. The time course of degradation was monitored in about 50 ml aliquots by UPLC analysis using an Agilent HPLC ZORBAX Eclipse XDB-C18 column (3.5 µm, 4.6 × 150 mm) eluted first by 5% acetonitrile in water for 0.5 min then by a gradient from 5 to 100% acetonitrile within 18 min in water, and finally by 100% acetonitrile for 5 min. The column was operated at 40°C at 0.35 ml min⁻¹ flow rate. Compounds eluted from the column were detected with a photodiode array detector, and the relative quantity of non-degraded amount was calculated using Indanol as internal standard. Stability was monitored at 24 h intervals up to 3 weeks.

### In vitro YLG (Yoshimulactone Green) Assays

Purification of ShHTL7/OsD14 was carried out by adopting the procedure explained previously (Jamil et al., 2018). *In vitro* YLG hydrolysis assays were conducted as described before (Tsuzuki et al., 2015). About 3.0 µM of purified ShHTL7/OsD14 protein was used in a reaction buffer (1X PBS buffer, pH 7.3) with 0.1% dimethyl sulfoxide (DMSO) at a 100 µl volume on a 96-well black plate (Greiner). SL analogs (at range between 0.01 and 50 µM) were co-incubated with 1.0 µM of YLG (Tokyo Chemical Industry Co. Ltd., product number E1238) for 60 min at room temperature. In this competition assay, fluorescent intensity was measured by spectraMax i5 (Molecular Devices) at excitation at 480 nm and detection at 520 nm. The variation in fluorescence recorded over the course of 1 h of YLG incubation in protein-free buffer was subtracted from the data collected in presence of protein. IC₅₀ values were calculated by using Quest Graph™ IC₅₀ Calculator.

### Statistical Analyses

Standard procedure was adopted to collect data for each trait, which were analyzed statistically using statistical software package R (version 3.2.2). One-way analysis of variance (ANOVA) and LSD (Least significant difference) multiple range test were applied to investigate the effect of various MPs for applications. After pre-conditioning, as previously described (Jamil et al., 2012), *Striga* and *Phelipanche* seeds were first tested with each MP solutions at 2.5 µM concentration (55 µl per disk) and then at a concentration range from 10⁻⁵ M to 10⁻¹² M, to calculate EC₅₀. Sterile MilliQ water and GR24 were applied as a negative and a positive control, respectively. Treated seeds were incubated in dark for 24 h at 30°C (*for S. hermonthica*) and for 1 week at 25°C (*for P. aegyptiaca*). Germination was recorded under a binocular microscope and used to determine the germination rate (%).
RESULTS

Synthesis and Structure of MPs

Synthesis scheme and structure of the developed 10 analogs (MP16 to MP25) is shown in Figures 1, 2, respectively. Figure 2 also depicts the structure of rac-GR24 used in this study. Physicochemical properties of these compounds are described in the section General Procedure for the Preparation of MPs.

Effect of the New Analogs on Tillering of the Rice d10/ccd8 and d3 Mutants

To test their capability in regulating shoot branching, we applied the new analogs at a 2.5 µM concentration to hydroponically grown seedlings of the rice wild type (cv. Shiokari) and its mutants d10-1, a SL deficient, high-tillering dwarf mutant disrupted in CCD8 gene (Arite et al., 2007, 2009), and d3-1, a SL insensitive high-tillering dwarf mutant disrupted in D3 gene that mediates SL signal transduction (Ishikawa et al., 2005; Hu et al., 2010). All MPs showed statistically significant effect in inhibiting tillering of the SL deficient d10 mutant, compared to mock treatment (Figures 3A–C). Like the standard SL analog GR24, six MPs (MP16-MP21) restored wild-type tillering to the d10 mutant, decreasing the number of its tillers from 5 (untreated) to 1. As expected, neither MPs nor GR24 showed an effect on the tillering of the SL insensitive d3 mutant that retained an average of 4 tillers per plant regardless treatment (Supplementary Figure S1). Besides reducing the number of tillers, we observed that MP16 (applied at a 2.5 µM concentration) caused stunted growth and triggered senescence in rice seedlings, leading to reduced biomass and plant height (Supplementary Figure S2). This result indicated that MP16 might possess high activity in growth regulation and senescence.

Activity of MP16 in Dark-Induced Leaf Senescence

The high activity of MP16 led us to investigate its activity in triggering dark-induced leaf senescence in comparison to the standard SL analog GR24. MP16 and GR24 treated leaf segments showed a loss of the green color on the third day after application, which was 1 day earlier than mock control (Figure 4A). Accordingly, measurement of the chlorophyll content unraveled a significant reduction in GR24 and MP16 treated leaf segments on the third and fourth day of treatment (Figure 4B). In both GR24 and MP16 treated leaf segments, the ion leakage, an indicator of
the loss of membrane integrity, began to increase on the third day of treatment and continued to increase till day 6 (Figure 4C).

**Parasitic Seed Germination in Response to MPs Application**

Next, we tested the activity of the new SL analogs in inducing seed germination in root parasitic plants. For this purpose, we applied the compounds to pre-conditioned *S. hermonthica* seeds and determined the rate of germination (Figure 5A). Application of MP16 at a 2.5 µM concentration resulted in a high *Striga* germination rate (62%), which was comparable to that of GR24 (72%). Application of other MPs led generally to lower germination rate. We also tested germination inducing activity of all MPs on seeds of *Phelipanche aegyptiaca*. Application of MP16, MP18, and MP19 at 2.5 µM concentration caused highest germination rates (around 51%), which was statistically equal to that observed upon treatment with GR24 (43%) (Figure 5B). MP21, MP22, MP24, and MP25 showed the lowest activity in inducing *Phelipanche* seed germination. For a better quantification of *Striga* seed germination inducing activity, we determined the half maximal effective concentration (EC\(_{50}\)) of the different SL analogs (Figure 6). The standard analog GR24 showed the lowest EC\(_{50}\) value of 7.23 × 10\(^{-11}\) mol L\(^{-1}\) for *Striga* germination. MP16 was the second-best analog in this assay, with an EC\(_{50}\) value of 1.05 × 10\(^{-10}\) mol L\(^{-1}\), followed by MP18 (EC\(_{50}\) value of 6.44 × 10\(^{-9}\) mol L\(^{-1}\)). MP17 was the less active analog with the highest EC\(_{50}\) value of 4.35 × 10\(^{-6}\) mol L\(^{-1}\).

**Stability of SL Analogs**

Stability is an important feature that impacts biological activity of SL analogs. Hence, we determined the stability of MP16, MP18, the most active new MPs in *Striga* germination assays, and MP21, a less active MP, in aqueous solution (pH 5.5–6.0), in comparison with the standard SL analog GR24. The stability of MP16 and MP18 was in the range of that of GR24, while MP21 showed a higher degradation rate, compared to GR24 (Figure 7), which might be a reason for its low performance.

**ShHTL7/OsD14-Mediated YLG Hydrolysis Assays**

The high activity of MP16 in different bioassays led us to measure the rate of its hydrolysis by ShHTL7 (*Striga hermonthica* Hyposensitive to Light) (Toh et al., 2015), the most sensitive SL receptor in *Striga* seeds, and the rice SL receptor OsD14 (Arite et al., 2009; Yao et al., 2016). The hydrolysis rate of MP16 (IC\(_{50}\): 4.37 ± 0.21 µM) by ShHTL7 was lower than that of GR24 (IC\(_{50}\): 0.98 ± 0.26 µM) (Figure 8A). Similarly, in competition with YLG in OsD14-mediated hydrolysis assays, GR24 was hydrolyzed more efficiently (IC\(_{50}\): 1.04 ± 0.09 µM) than MP16 (IC\(_{50}\): 3.07 ± 0.54 µM) (Figure 8B).

**DISCUSSION**

SLs are an important plant hormone with an array of diverse functions including regulation of plant growth and development,
adaption to nutritional availability, contribution to biotic and abiotic stress responses, and communication with beneficial microorganisms and root parasitic plants (Cook et al., 1966; Gomez-Roldan et al., 2008; Ha et al., 2014; Al-Babili and Bouwmeester, 2015; Decker et al., 2017; Waters et al., 2017). However, the limited availability of SLs is a constraint in investigating their biology and a major obstacle on the way toward their application at a large scale in agriculture. The development of easy-to-synthesize, low cost SL analogs/mimics with improved bioactivity is a key in solving problem (Prandi and McErlean, 2019). Designing of SL analogs that exert particular SL functions would be also a decisive step in translating fundamental SL research into application (Zwanenburg and Pospisil, 2013).

Up-till now GR24, which requires a complex synthesis protocol (Mangnus et al., 1992a,b), is the widely used SL analog in labs. The demand for simple SL analogs has led to the development of compounds, such as AR36 (Boyer et al., 2014), 4-Br debranone (4BD) (Fukui et al., 2011, 2013) and Nijmegen-1 (Wigbert et al., 1999), which, however, are generally less active than GR24. Similarly, SL mimics were developed that are lacking the ABC scaffold but contain only D-ring with an appropriate substitute at C-5 (Zwanenburg et al., 2013; Boyer et al., 2014; Prandi and McErlean, 2019). SL mimics, such as Debranones (Fukui et al., 2011) and Para-bromo-phenyloxy butanolide (Vurro et al., 2019) T010 (Samejima et al., 2016), showed weak to moderate bioactivity. However, these SL mimics provide a clue to develop simple SL with easy synthesis (Zwanenburg et al., 2016b). The discovery of SL biosynthesis intermediate carlactone (Alder et al., 2012) paved the way for the discovery of some non-canonical SLs like methyl carlactonoate (Abe et al., 2014). The presence of non-canonical SLs as a separate family of SLs was further supported by the identification of heliolactone (Ueno et al., 2014), zealactone, zeapyranolactone (Charnikova et al., 2017, 2018), and avenanol (Yasui et al., 2017). Recently, we have developed MPs as analogs of non-canonical SLs, which are characterized by a simple structure and showed reasonable activity in different bioassays (Jamil et al., 2018). These encouraging results prompted us to develop a new series of MPs, with the aim of generating high-active and simple SL analogs. In this paper, we describe the synthesis and biological tests of these new MPs.

First, we evaluated the activity of these analogs (MP16–MP25) in regulating the growth and architecture of rice. Since shoot branching/tillering inhibition is the best known hormonal function of SLs (Gomez-Roldan et al., 2008; Umehara et al., 2008), we tested the effect of our MPs on this trait. For this purpose, we used the high tillering, SL deficient d10/CCD8 rice mutant. Six of the developed MPs (MP16 to MP21) were as efficient as GR24 in reducing the tiller number of this mutant (Figure 3). In addition to restoring wild-type tillering phenotype in d10 mutant, application of MP16 retarded the growth, lowered plant height and accelerated leaf senescence of treated seedlings, leading to noticeable decrease in dry biomass, which was more pronounced than that observed with other SL analogs including GR24 (Supplementary Figure S2). The high activity of MP16 makes it a very good candidate for application as structurally simple growth regulator and points to its being a very efficient SL analog. In addition, it effect on plant height in d10 background (Figure 3A), which contradicts that of other MPs and GR24, indicates that it might interfere with other SL-independent developmental processes. The retarded growth and senescence effect caused by MP16 further inspired us to test the effect of this analog on dark-induced leaf senescence activity (Yamada et al., 2014). Here again, we observed an activity similar to that of GR24, i.e., the application of MP16 accelerated the leaf senescence process, leading to a color loss after 2 days. Other senescence parameters, such as chlorophyll content and electrolytes leakage, were changed to a similar extent upon MP16 and GR24 application (Figures 4A–C).

Root parasitic plants, in particular S. hermonthica, are causing huge damage in African agriculture and are considered as a major threat to global food security. Infested fields have accumulated huge amounts of long-living and tiny seeds (Rubiales et al., 2009), which represent a major constraint in combating Striga and related species. Induction of suicidal germination of root parasitic seeds is a promising approach to combat these weeds in Africa and other parts of the world (Samejima et al., 2016; Zwanenburg et al., 2016a; Kountche et al., 2019). This approach...
FIGURE 6 | Half Maximal Effective Concentration (EC\textsubscript{50}) of MP16-MP25 for Striga seed germination. Preconditioned S. hermonthis seeds were treated with 55 µl of aqueous solutions of each MP, with various concentrations ranging from 10^{-5} to 10^{-12} M on a disk containing 50–100 pre-conditioned Striga seeds. GR24 is included as positive control.

requires efficient and easy-to-synthesize SL analogs and is a major potential application field for these chemistries. The results of Striga bioassays showed that application of 2.5 µM of MP16 caused 65% germination, which is in the range of the standard SL analog GR24 (Figure 5A). Among the previously described MPs (Jamil et al., 2018) MP1 was the most potent SL analog (EC\textsubscript{50} = 1.5 × 10^{-9} mol L^{-1}). This EC\textsubscript{50} value was about 17 times higher than that of GR24. In the present study, we show that EC\textsubscript{50} value of MP16 (EC\textsubscript{50} = 1.05 × 10^{-10} mol L^{-1}) is less than two times higher than that of GR24 (EC\textsubscript{50} = 7.23 × 10^{-11} mol L^{-1}). However, it can be speculated that the simple synthesis route of MP16 will more than compensate the lower activity of this compound, in comparison to GR24. In the near future, we are going to evaluate the efficiency of MP16 in heavily Striga-infested fields in Burkina Faso and Niger.

We also tested the utility of the new MPs in inducing seed germination of a further root parasitic weed, i.e., Phelipanche aegyptiaca. Here, we observed very high activity with MP16, MP18, and MP19, which was statistically equal to that of GR24 (Figure 5B). In contrast to P. aegyptiaca, we observed significant activity in inducing S. hermonthis seed germination only with MP16 (Figure 5A). This indicates that MPs vary in their efficiency in inducing seed germination of different parasitic weed species. The species-dependent distinction is likely caused by structural diversity of receptors involved in binding and perception of SLs or by differences in the uptake of particular MPs. Taken together, this result is a further indication for the application potential of the new MPs in combating different root parasitic species.

It is assumed that the hydrolysis rate of SLs by the Striga SL receptor ShHTL7 is an indicator for their activity in inducing seed germination. Therefore, we measured the hydrolysis of MP16 by conducting the competitive, ShHT7-mediated YLG hydrolysis assay. MP16 exhibited higher IC\textsubscript{50} value (IC\textsubscript{50} = 4.37 ± 0.21 µM) than GR24 (IC\textsubscript{50} = 0.98 ± 0.26 µM), which is in line with its lower activity. Similarly, despite the fact that MP16 showed a growth inhibitory and senescence effect on rice seedlings, the OsD14-mediated YLG hydrolysis assays showed it as a less preferred substrate (IC\textsubscript{50} = 3.07 ± 0.54 µM), compared to GR24.
(IC$_{50}$ = 1.04 ± 0.09 µM). This outcome reveals that some other elements, such as stability, transport and SL uptake could also be involved in the growth retarding activity of MP16 (Figure 8).

**CONCLUSION**

In conclusion, we have developed a new series of SL analogs that will help in understanding the different functions of SLs and have a large application potential in agriculture, particularly in combating root parasitic weeds.

**DATA AVAILABILITY STATEMENT**

All datasets generated for this study are included in the article/Supplementary Material.

**AUTHOR CONTRIBUTIONS**

SA-B and MJ conceived and designed the experiments. IT, TO, and TA designed and synthesized the analogs. BK, JW, IH, and K-PJ assisted and performed the experiments. MJ,
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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.2020.00434/full#supplementary-material

FIGURE S1 | Tillering inhibition of SL insensitive d3 rice mutant by MP16-MP25. (A) Tillering phenotype of d3 mutant in response to MPs. SL analogs were applied (2.5 μM) to 1 week old d3 rice seedlings grown hydroponically in 50 ml tube twice a week up to 3 weeks. (B) Number of tillers per plant counted after 3 weeks of MPs application. (C) Dry biomass of d3 rice seedlings measured after 3 weeks of MPs application. Data are means ± SE (n = 8). Means not sharing a letter in common differ significantly at F₀.₀₅.

FIGURE S2 | Effect of MPs on d10 rice plant height. All the MPs were applied (2.5 μM) to 1-week old hydroponically grown rice seedlings (d10/CCD8 mutant) twice a week up to 3 weeks. Plant height per plant were measured (n = 8) and compared by one-way ANOVA. Means not sharing a letter in common differ significantly at F₀.₀₅.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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