Bioorganic & Medicinal Chemistry 19 (2011) 5006–5011

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Single step synthesis of strigolactone analogues from cyclic keto enols, germination stimulants for seeds of parasitic weeds

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ARTICLE INFO

Article history:
Received 10 May 2011
Revised 17 June 2011
Accepted 18 June 2011
Available online 25 June 2011

Keywords:
Strigolactone
Parasitic weeds
Bioassay
Striga
Orobanche
Germination

ABSTRACT

The single step synthesis of a newly designed series of strigolactones (SLs) from cyclic keto enols is described. The germinating activity of these SL analogues towards seeds of the parasitic weeds *Striga* and *Orobanche* spp. is reported. The first of these SL analogues are derived from the hydroxyl γ-pyrones kojic acid and maltol, the second type from hydroxyl α-pyrones, namely, 4-hydroxy-6-methyl-2*H*-pyran-2-one and 4-hydroxy-coumarin and the third type from 1,3-diketones, namely, 1,3-cyclohexane-dione (dimedone) and tricyclic 1,3-dione. All keto enols are coupled in a single step with the appropriate D-ring precursor in the presence of a base to give the desired SL analogues. All SL analogues are acceptably biologically active in inducing the germination of seeds of *Striga hermonthica* and *Orobanche cernua*. Most interesting are the analogues derived from 4-hydroxy coumarin and dimedone, as they have a remarkably high biological activity towards the seeds of parasitic weeds at relatively low concentrations, comparable with that of the general standard stimulant GR24.

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1. Introduction

Important food crops such as maize, sorghum and millet and rice in third world countries are enormously damaged by the parasitic weeds, *Striga* and *Orobanche* spp.[1,2] The seeds of these weeds germinate by a chemical signal which is present in the root exudates of the host plants. The concentration of these stimulants in root exudates is extremely low, which hampers their isolation and characterization.[3–9] These compounds which are collectively named as strigolactones (SLs), are structurally similar, all having four rings, as shown in the formula of the first isolated stimulant, strigol (**1**)[3] (Fig. 1).

Extensive structure–activity studies revealed that the bioactiphore for germination resides in the C–D part of these molecules.[10–18] A tentative molecular mechanism[12] for understanding the interaction of the stimulant molecule in the receptor site involves an addition–elimination reaction initiated by a Michael addition of a nucleophilic group as is depicted in Scheme 1.

The working model for the design of stimulant molecules shown in the Figure 2, is constructed by combining the structural information of the bioactiphore and the features of the molecular mechanism.[13,18]

Natural stimulants can only be obtained from plant roots in minute amounts. Their structures are too complicated for a gram scale synthesis.[19] Therefore, there has been a continuous search for structurally simplified strigolactones that can be synthesized relatively easy. The first series developed was the GR family,[10,11] typically represented by GR 24 (**2**) and GR 7 (**3**). SL analogues lacking the B-ring[16] or having a modified C-ring[16] have been described. Also analogues with an imino bioisosteric unit[20] replacing the enol ether and with a heterocyclic ABC moiety[21] have been reported. Nijmegen-1[22] which has an appreciable stimulant activity has been designed on the basis of the working model. This molecule contains all essential structural elements for activity, namely, the D-ring which is invariably present in all natural strigolactones, and the enone moiety necessary for the addition–elimination reaction to take place. It has been argued that the molecular mechanism shown in Scheme 1 is not appropriate for imino bioisosters.[20] However, recently is was shown that these compounds can also be fitted into this mechanism.[23] In a previous paper,[23] we designed a series of cyclic ketone derived SL analogues with considerable stimulant activity. The analogue **5** obtained from 1-tetralone has an activity comparable to that of GR 24, the world wide used standard stimulant.[10,24]

This paper deals with the design of a new series of SL analogues derived from readily available starting materials, all being cyclic keto enols. The enol is then coupled with the D-ring in a single step, thereby giving structures containing the structural elements required for activity, namely the enone, the vinyl ether and the D-ring unit. The prime objective of this work is to check the

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doi:10.1016/j.bmc.2011.06.057

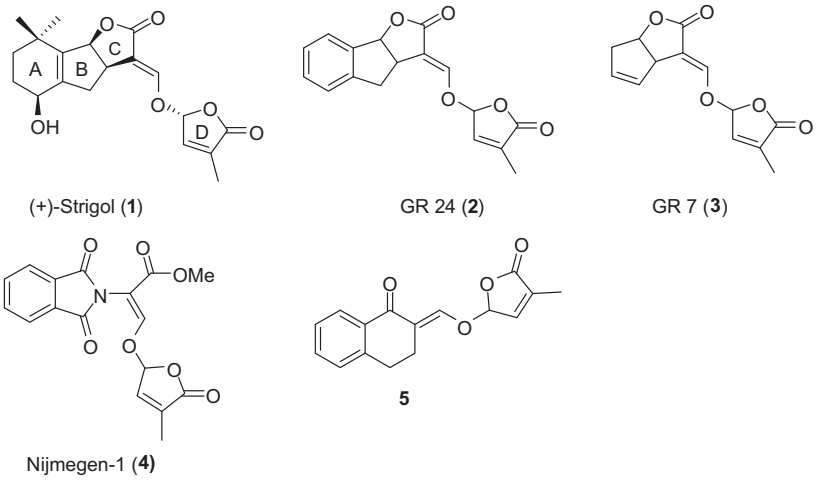
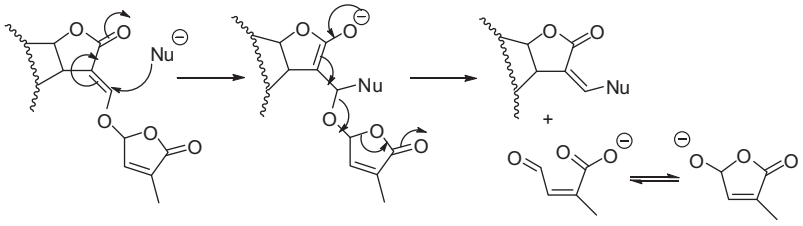


Figure 1. Some natural and synthetic strigolactones.



Scheme 1. Molecular mechanism for the initiation of germination.[12]

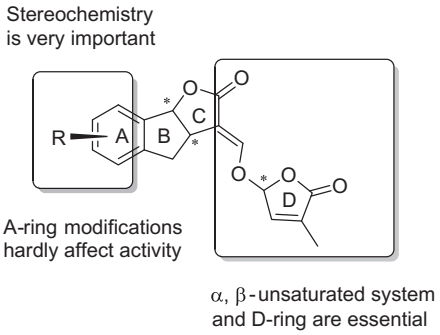


Figure 2. The working model for designing stimulant molecules.[18]

validity of the working model for designing new synthetically readily accessible stimulant molecules. When sufficiently active the new stimulants may be candidates for the use in parasitic weed control applying the concept of suicidal germination,[18,25–27] that is treatment of seeds of the parasite in the absence of a suitable host. Due to lack of nutrients and water, the germinated seeds will die after a few days. We already demonstrated that Nijmegen-1 can successfully be used for this purpose.[18]

2. Results and discussion

2.1. Synthesis of new SL analogues

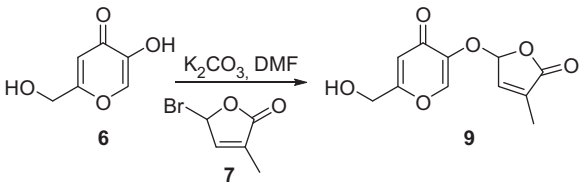
The first keto enol that was selected for this project is the hydroxyl γ-pyrone **6** (Kojic acid) which is commercially available for a reasonable price. Coupling of **6** with bromo butenolide **7** was achieved by using potassium carbonate as the base, giving SL analogue **9** in 37% yield. Structural analysis revealed that coupling had taken place at the enolic hydroxyl group (Scheme 2).

The second hydroxy γ-pyrone chosen was maltol **10**. This starting material is naturally occurring and can be isolated from pine needles and larch bark. The coupling of maltol (**10**) with bromo butenolide **7** using potassium carbonate as the base resulted in the desired crystalline compound **11** in 65% yield (Scheme 3).

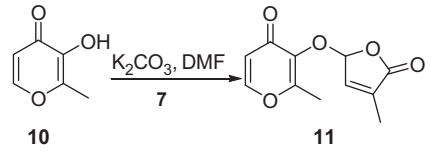
Two hydroxy α-pyrones, both having the required enolone unit, were included in this study, namely the commercially available, 4-hydroxy-6-methyl-2*H*-pyran-2-one (**12**) and 4-hydroxy-coumarin (**14**). The coupling with halo butenolide in the presence of potassium carbonate afforded in a single step the desired SL analogues **13** and **15**, respectively, in good yields (Schemes 4 and 5). The structures of these products were ascertained by spectroscopic analysis.

The third type of enol ketones constitutes the 1,3-diketones **16** and **18** (dimedone) and the rather unusual tricyclic 1,3-dione **20**. All three diketones are predominantly present in the enol form. Coupling with the D-ring precursor in a single step proceeded smoothly in the same manner described above to give the desired SLs shown in the Schemes 6 and 7.

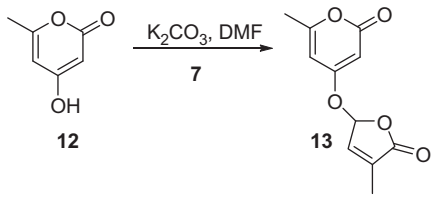
The yields for the coupling reactions have not been optimized. At this stage of the work, the bioactivity is the most relevant issue.



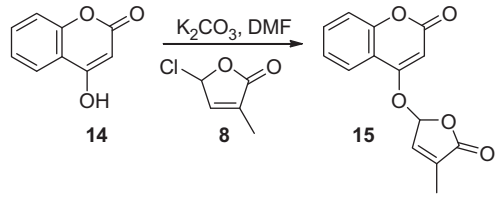
Scheme 2. Conversion of Kojic acid into SL analogue **9**.



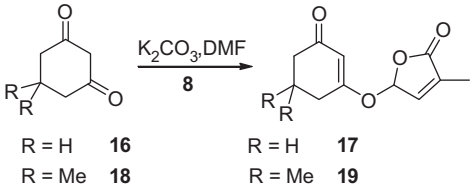
Scheme 3. Synthesis of SL analogue **11** from maltol.



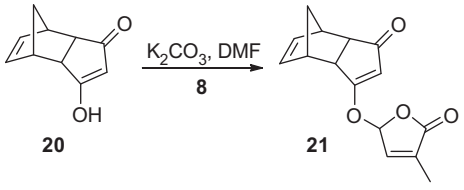
Scheme 4. Synthesis of SL analogue **13** from 4-hydroxy-6-methyl-2*H*-pyran-2-one.



Scheme 5. Single step conversion of hydroxy coumarin into SL analogue **15**.



Scheme 6. SL analogues derived from cyclohexane-1,3-diones.



Scheme 7. An SL analogue derived from a tricyclic 1,3-dione.

2.2. Germination activity of the newly prepared SL analogues

Seeds of *Striga hermonthica* (from *Sorghum bicolor* (L.) Moench) were harvested in Sudan in 1987. *Orobanche cernua* seeds were harvested in Spain in 1994 and were stored in the dark at room temperature until required. The bioassay experiments were carried out according to the standardized procedure.[28] An aqueous solution of acetone (0.1% v/v) was used as a negative control and the diastereomeric mixture of GR 24 (**2**) as a positive control. The results of the bioassays with *S. hermonthica* are shown as bar graph representations in Figure 3. The germination activities towards *O. cernua* seeds are presented in Figure 4.

The bioassays of the newly prepared SL analogues **9**, **11**, **13** and **15** reveal that all these compounds have an appreciable activity in stimulating the germination of *Striga* seeds. Analogues **13** and **15** give the best performance. The analogue **19** derived from dimedone has a very good activity, in fact comparable with that of GR 24. Remarkably, the analogue **21** from tricyclic dione is also very active. The response of the newly prepared stimulants towards *Orobanche* seed shows a very similar profile. In general, the dose–response curve of the best performing newly prepared stimulants may have an optimum at a higher concentration than GR 24. The most promising stimulants for *Striga* and *Orobanche* seeds that came out of this study are the SL analogues **13** derived from 4-hydroxy-6-methyl-2*H*-pyran-2-one, **15** obtained from 4-hydroxycoumarin and **19** prepared from dimedone, the latter being the best. Especially, SL analogue **19** from dimedone is a potential candidate for parasitic weed control using the concept of suicidal germination.

2.3. Discussion

All new strigolactone analogues derived from cyclic keto enols were prepared in a single step by coupling the selected keto enols with the halo butenolide in the presence of a base. All these compounds have appreciable germination activity towards seeds of *Striga* and *Orobanche*, hence the working model[18] for designing new stimulants (Fig. 2) is proven to be valid. The analogues **9** and **11** are the least active ones, particularly for *Orobanche* seeds. This may be attributed to the fact that these structures are in fact cross-conjugates which necessitates the addition–elimination mechanism (see Scheme 1) to take a slightly deviant course. This implies that **9** and **11** are not ideally suited for the molecular model. Among these newly prepared stimulants, the most promising ones are those prepared from hydroxy coumarin and dimedone: SL analogues **15** and **19**, respectively. It should be noted that especially the activity towards *Orobanche* seeds is remarkably high. The best performing stimulants are synthetically readily accessible in a single step synthetic operation. The application protocol for the suicidal germination approach in combating plagues of parasitic

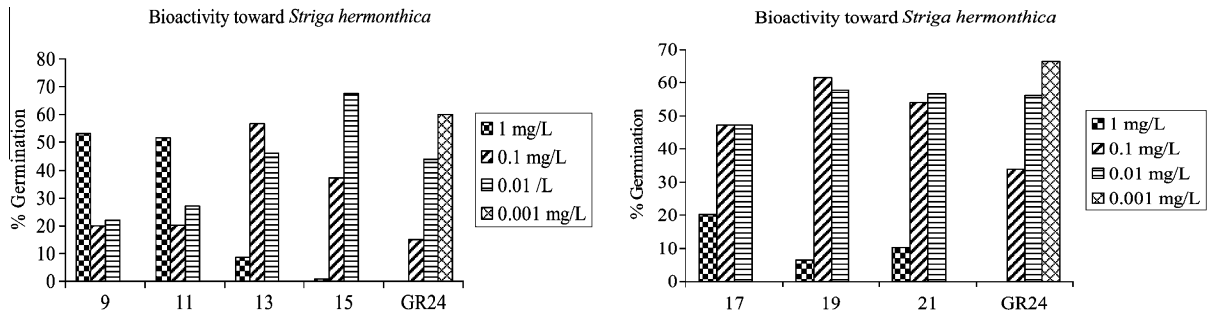


Figure 3. Bar graph representation of percentages of the germinated seeds of *S. hermonthica* after exposure to various concentrations of analogues **9**, **11**, **13**, **15** **17**, **19** and **21** with GR 24 as a positive control.

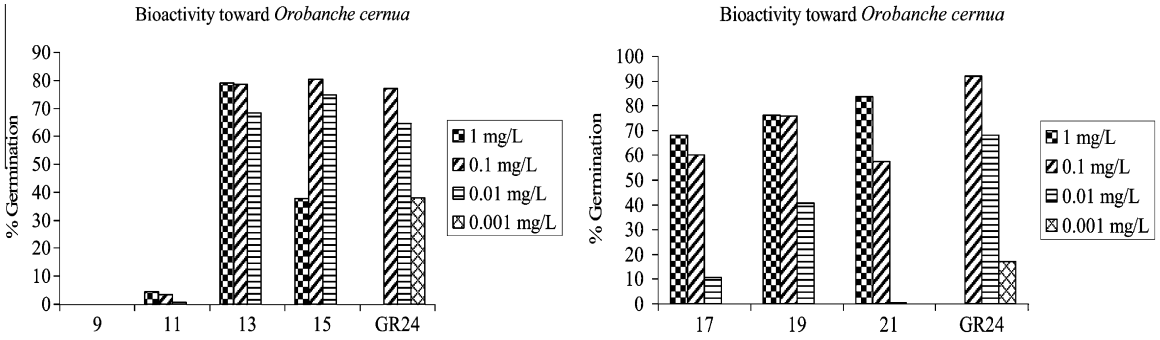


Figure 4. Bar representation of the percentages of the germinated seeds of *O. cernua* after exposure to various concentrations of analogues **9**, **11**, **13**, **15** **17**, **19** and **21** with GR 24 as a positive control.

weeds involves formulation of the stimulants as an emulsion in order to avoid untimely decomposition in the soil and slowing down of the leaching of the stimulants to lower soil layers where it will not be effective. In addition, factors such as bioavailability[18,29], optimum concentration[18,30] of stimulant and optimum stimulation time[18,29] have to be taken into account. The idea of suicidal germination was coined for the first time as early as 1976[25] and applied in soil boxes using aqueous solution of the GR 7, but discontinued due to untimely decomposition of the stimulant. Several discouraging reports[31–33] about the soil stability of GR 24 and GR 7 appeared and therefore, the suicidal approach was not considered as vey realistic.[26,27] Recently, this approach was reconsidered using formulated Nijmegen-1 for the reduction of seed banks of *Orobanche ramosa* in tobacco.[18] The results are very encouraging. Hence, this is a stimulus for the design and synthesis of new SL analogues with high germinating activity. This paper constitutes a contribution to this objective.

It is relevant to note that SLs recently received much attention in the literature because of the newly discovered bioproperties. SLs have been identified as the branching factor for arbusular mycorrhizal (AM) fungi[34] and as inhibitor of plant shoot branching.[35] It has been suggested that SLs constitute a new class of plant hormones[36] for which more functions will be uncovered in the coming years. It was found that GR 24 is also active for both functions. Therefore, searching for other SL analogues for the new activities is another incentive for this study. It remains to be seen whether the some model compound as shown in Figure 2 is applicable for these new activities.

3. Experimental section

3.1. Synthesis

General remarks. IR spectra were recorded using a Perkin-Elmer 298 infrared spectrophotometer and a Bio-Rad FTS-25 instrument. ^{1}H NMR spectra were recorded on a Bruker AC 100 spectrometer (100 MHz), AC 300 (300 MHz), and a Bruker AM-400 (400 MHz), respectively, using Me_4Si (TMS) as the internal standard. ^{13}C NMR spectra were recorded on a Bruker AC 300 (operating at 75 MHz) and AM 400 (operating at 100 MHz) spectrometers with $CDCl_3$ (77.0 ppm), acetone-d_6 (29.206 and 206 ppm) as standards. Melting points were determined with a Reichert Thermopan microscope and are uncorrected. Elemental analyses were conducted on a Carlo-Erba instruments CHNSO EA 1108 elemental analyzer. Mass spectra were recorded using a double focussing VG 7070E mass spectrometer in the mode indicated, or a Varian Saturn 2 GC–MS ion-trap system. GC–MS separations were carried out on a fused-silica capillary column (DB-5, 30 m × 0.25 mm) and helium was used as the carrier gas. GLC was conducted with a Hewlet–Packard HP 5890 gas chromatograph using a capillary column (25 m) HP-1 with nitrogen (2 mL/min, 0.5 atm) as the carrier gas. Thin layer chromatography (TLC) was carried out on Merck pre-coated silica gel 60 F_{254} plates (0.25 mm) using the eluents indicated. Spots were visualized using a UV lamp, or with a potassium dichromate spray (prepared from 7.5 g of $K_2Cr_2O_7$ in 250 mL H_2O containing 12.5 mL 1 M H_2SO_4) followed by heating at 140 °C. Column chromatography was performed on silica gel (Kieselgel, Merck) using eluents indicated. All solvents were dried under standard conditions. Solvents like DMF, DME and THF were distilled using standard purification procedures.

The halo butenolides **7** and **8** were prepared as previously described.[37,38]

Nomenclature. The IUPAC nomenclature has been used for all compounds. The systematic names were generated using the ACD/Name programme provided by Advanced Chemistry Development Inc. (Toronto, Canada).

3.1.1. 2-(Hydroxymethyl)-5-[(4-methyl-5-oxo-2,5-dihydro-2-furanyl)oxy]-4H-pyran-4-one (9)

A stirred mixture of 5-hydroxy-2-(hydroxymethyl)-4*H*-pyran-4-one (**6**) (400.0 mg, 2.82 mmol), anhydrous potassium carbonate (428.2 mg, 3.10 mmol) and bromo butenolide **7** (548.3 mg, 3.10 mmol) in dry DMF (4 mL), while maintained under nitrogen, was set aside at room temperature for 18 h until TLC indicated the total consumption of **7**. The mixture was treated with a mixture of water (50 mL) and dichloromethane (25 mL), the separated aqueous layer was extracted with dichloromethane (2 × 15 mL), the combined organic layers were washed with brine, dried (Na_2SO_4), filtered and then concentrated in *vacuo*. The resultant crude product was purified by column chromatography (hexane/ethyl acetate, 1:1, v/v) to give **9**, 245.7 mg (36.6%) as a white solid. An analytical sample was obtained by recrystallization from di-isopropyl ether, mp 91–94 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.93 (s, 1H, OCH=CO), 7.09 (s, 1H, =CH), 6.58 (s, 1H, =CH), 6.37 (s, 1H, OCHO), 4.51 (s, 2H, CH_2), 3.10 (s, 1H, OH), 1.97 (s, 3H, CH_3). ^{13}C NMR, ($CDCl_3$, 100 MHz): δ 174.6 (C=O), 171.2 (C=O), 167.8, 147.5, 144.0, 142.4, 134.6, 113.3, 99.3, 60.7, 10.6; MS [EI *m*/*z*, rel. intensity (%)]: 239 ([M+1]$^+$, 1.1); 238 ([M]$^+$, 6.6); 97 ([M$^+$−141], [$C_5H_5O_2$]$^+$, 100); Anal. Calcd for $C_{11}H_{10}O_6$: C, 55.47; H, 4.23. Found: C, 55.35; H, 4.17.

3.1.2. 2-Methyl-3-[(4-methyl-5-oxo-2,5-dihydro-2-furanyl)oxy]-4H-pyran-4-one (11)

A stirred mixture of 3-hydroxy-2-methyl-4*H*-pyran-4-one (**10**) (200.0 mg, 1.59 mmol), anhydrous potassium carbonate (241.3 mg, 1.75 mmol) and bromo butenolide **7** (309.0 mg, 2.33 mmol) in dry DMF (2 mL) was maintained under nitrogen at room temperature for 5 h and then processed in the manner described above. The crude product was purified by column chromatography (hexane/ethyl acetate, 1:1, v/v) (306.7 mg (65%) and then

recrystallized (di-isopropyl ether) to give **11** as white crystals, 207.8 mg, mp 104 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.68 (d, 1H, J = 5.7 Hz, CH=), 7.14 (m, 1H, CH=), 6.48 (m, 1H, OCHO), 6.41 (d, 1H, J = 5.7 Hz, =CH), 2.35 (s, 3H, CH_3), 1.99 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.9 (C=O), 171.5 (C=O), 161.3, 154.1, 143.0, 141.8, 134.1, 117.3, 100.3, 15.2, 10.6; MS [EI m/z, rel. intensity (%)]: 222 ([M]$^+$), 8.9; 223 ([M]$^+$+1), 0.1; 126 ([M]$^+$−97, [$C_6H_5O_3$]$^+$), 44.5; 97 ([M$^+$−125], [$C_5H_5O_2$]$^+$), 100); Anal. Calcd for $C_{11}H_{10}O_5$: C, 59.46; H, 4.54. Found: C, 59.22; H, 4.41.

3.1.3. 6-Methyl-4-[(4-methyl-5-oxo-2,5-dihydro-2-furanyl)oxy]-2H-pyran-2-one (13)

Treatment of 4-hydroxy-6-methyl-2*H*-pyran-2-one (**12**) (200.0 mg, 1.59 mmol) with anhydrous potassium carbonate (241.3 mg, 1.75 mmol) and bromo butenolide **7** (309.0 mg, 2.33 mmol) in dry DMF (4 mL) in the manner described above, followed by column chromatography (hexane/ethyl acetate, 2:1, v/v) gave **13** (121.4 mg, 58.8%). Recrystallization (di-isopropyl ether) gave an analytically pure sample (107.9 mg) as white crystals, mp 125–127 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.97 (s, 1H, CH=), 6.31 (s, 1H, OCHO), 5.84 (s, 1H, CH=CO), 5.77 (s, 1H, CH=C), 2.24 (s, 3H, CH_3), 2.02 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.1 (C=O), 167.7 (C=O), 163.8, 163.4, 140.8, 135.3, 99.4, 96.2, 91.5, 20.0, 10.6; MS [EI m/z, rel. intensity (%)]: 222 ([M]$^+$, 7.6); 193 ([M$^+$−29], 2.8, [$C_{10}H_{10}O_4$]$^+$); 97 ([M$^+$−125], [$C_5H_5O_2$]$^+$, 100); Anal. Calcd for $C_{11}H_{10}O_5$: C, 59.46; H, 4.54. Found: C, 59.42; H, 4.29.

3.1.4. 4-[(4-Methyl-5-oxo-2,5-dihydro-2-furanyl)oxy]-2H-chromen-2-one (15)

The reaction of 4-hydroxy-coumarin (**14**) (400.0 mg, 2.47 mmol) with anhydrous potassium carbonate (412.5 mg, 2.71 mmol) and chloro butenolide **8** (359.6 mg, 2.71 mmol) in dry DMF (4 mL) was conducted in the manner described above, followed by recrystallization from di-isopropyl ether to give **15**, 315.4 mg (49.5%) as pure yellowish crystals, mp 177–182 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.74–7.27 (m, 4H, ArH protons), 7.11 (m, 1H, CH=), 6.49 (s, 1H, =CHCO), 6.11 (s, 1H, OCHO), 2.09 (s, 3H, CH_3). ^{13}C NMR, ($CDCl_3$, 100 MHz): δ 170.0 (C=O), 163.0 (C=O), 161.7, 153.4, 140.7, 135.6, 132.9, 124.1, 122.8, 116.9, 114.7, 96.6, 94.2, 10.6; MS [EI m/z, rel. intensity (%)]: 258 ([M]$^+$), 12.9; 259 ([M]$^+$+1), 1.9; 97 ([M$^+$−161], [$C_5H_5O_2$]$^+$, 100); Anal. Calcd for $C_{14}H_{10}O_5$: C, 65.11; H, 3.90. Found: C, 64.74; H, 3.83.

3.1.5. 3-Methyl-5-[(3-oxo-1-cyclohexenyl)oxy]-2(5H)-furanone (17)

Treatment of 1,3-cyclohexanedione (**16**) (300.0 mg, 2.68 mmol) with potassium carbonate (407.2 mg, 2.95 mmol) and chloro butenolide **8** (390.3 mg, 2.95 mmol) in dry DMF (3 mL) was conducted in the manner as described above, to give the desired compound **17** as white crystals, 90.9 mg (16.3%) after column chromatography (hexane/ethyl acetate, 1:1, v/v). An analytical sample was obtained by recrystallization from di-isopropyl ether, mp 120–124 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.94 (s, 1H, CH=), 6.24 (s, 1H, OCHO), 5.73 (s, 1H, =CH), 2.46–2.28 (m, 6H, 2 × CH_2), 2.01 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 199.0 (C=O), 174.1 (C=O), 170.5, 141.2, 134.9, 106.3, 95.8, 36.6, 28.2, 20.9, 10.6; MS [EI m/z, rel. intensity (%)]: 209 ([M+1]$^+$, 2.9); 208 ([M]$^+$, 21.7); 97 ([M$^+$−111], [$C_5H_5O_2$]$^+$, 100); Anal. Calcd for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81. Found: C, 63.26; H, 5.65.

3.1.6. 5-[(5,5-Dimethyl-3-oxo-1-cyclohexenyl)oxy]-3-methyl-2(5H)-furanone (19)

Method A. 5,5-Dimethyl-1,3-cyclohexanedione (**18**) (300.0 mg, 2.14 mmol) was treated with potassium carbonate (325.8 mg, 2.36 mmol) and chloro butenolide **8** (312.3 mg, 2.36 mmol) in dry DMF (3 mL) in the manner described above. The crude product was purified by column chromatography (hexane/ethyl acetate, 1:1, v/v) to give compound **19** as white crystals, 78.5 mg (14.5%). An analytical sample was obtained by recrystallization from di-isopropyl ether, mp 96–99 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.94 (s, 1H, CH=), 6.24 (s, 1H, OCHO), 5.73 (s, 1H, =CH), 2.30 (s, 2H, CH_2), 2.26 (s, 2H, CH_2), 2.02 (s, 3H, CH_3), 1.09 (s, 6H, 2 × CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 199.0 (C=O), 172.4 (C=O), 170.5, 141.2, 134.9, 105.1, 95.9, 50.6, 42.1, 32.5, 28.5, 27.9, 10.6; MS [EI m/z, rel. intensity (%)]: 237 ([M+1]$^+$, 1.0); 236 ([M]$^+$, 8.9); 97 ([M$^+$−139], [$C_5H_5O_2$]$^+$, 100); Anal. Calcd for $C_{13}H_{16}O_4$: C, 66.09; H, 6.83. Found: C, 66.11; H, 6.71%.

Method B. A stirred mixture of 5,5-dimethyl-1,3-cyclohexanedione (**18**) (300.0 mg, 2.14 mmol), DBU (358.8 mg, 2.36 mmol) and chloro butenolide **8** (283.9 mg, 2.14 mmol) in dichloromethane (15 mL) while maintained under nitrogen was set aside at room temperature for 5 h, until TLC indicated the total consumption of the chloro butenolide **8**. The mixture was processed in the manner described above. Column chromatography (hexane/ethyl acetate, 2:1, v/v) gave compound **19** as white crystals, 73.3 mg (14%), mp 98–100 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.94 (s, 1H, CH=), 6.24 (s, 1H, OCHO), 5.73 (s, 1H, =CH), 2.30 (s, 2H, CH_2), 2.26 (s, 2H, CH_2), 2.02 (s, 3H, CH_3), 1.09 (s, 6H, 2 × CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 199.0 (C=O), 172.4 (C=O), 170.5, 141.2, 134.9, 105.1, 95.9, 50.6, 42.1, 32.5, 28.5, 27.9, 10.6; MS [EI m/z, rel. intensity (%)]: 237 ([M+1]$^+$, 1.0); 236 ([M]$^+$, 8.9); 97 ([M$^+$−139], [$C_5H_5O_2$]$^+$, 100); Anal. see above.

3.1.7. 3-Methyl-5-[(5-oxotricyclo[5.2.1.0^{2,6}]deca-3,8-dien-3-yl)oxy]-2(5H)-furanone (21)

The tricyclic keto-enol **20** (400.0 mg, 2.47 mmol) was treated with potassium carbonate (375.4 mg, 2.72 mmol) and chloro butenolide **8** (359.9 mg, 2.72 mmol) in dry DMF (4 mL) at room temperature for 18 h. When TLC indicated total consumption of chloro butenolide **8**, the mixture was treated with ice water (50 mL) and dichloromethane (20 mL), the separated aqueous layer was extracted with dichloromethane (2 × 15 mL), the combined organic layers washed with brine, dried (Na_2SO_4) and concentrated in *vacuo*. Column chromatography (hexane/ethyl acetate, 1:1, v/v) of the resultant material, followed by recrystallization from di-isopropyl ether gave **21** (112.6 mg, 18%) as white crystals, mp 168–171 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.93 (s, 1H, =CH), 6.13 (s, 1H, OCHO), 6.03 (s, 1H), 5.89 (s, 1H), 5.36 (s, 1H), 3.27 (m, 1H), 3.22 (br s, 1H), 3.02 (br s, 1H), 2.97 (m, 1H), 2.03 (s, 3H, CH_3), 1.75 (d, J = 8.5 Hz, 1H), 1.5 (d, J = 8.5 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 205.4 (C=O), 186.1 (C=O), 170.4, 140.8, 135.2, 133.8, 131.9, 110.3, 97.7, 52.0, 51.3, 46.2, 44.1, 43.6, 10.6; MS [EI m/z, rel. intensity (%)]: 259 ([M+1]$^+$, 2.0); 258 ([M]$^+$, 13.3); 230 ([M$^+$−28], [$C_{14}H_{14}O_3$]$^+$, 3.9); 161 ([M$^+$−97], [$C_{10}H_9O_2$]$^+$, 63.3); 97 ([M$^+$−161], [$C_5H_5O_2$]$^+$, 100); Anal. Calcd for $C_{15}H_{14}O_4$: C, 69.76; H, 5.46. Found: C, 69.87; H, 5.28.

3.2. Bioassays[28]

3.2.1. Seeds

Seeds of *S. hermonthica* (from *Sorghum bicolor* (L.) Moench) were harvested in the Sudan in 1987. *O. cernua* seeds were harvested in Spain in 1994. Seeds were stored in the dark at room temperature until required.

3.2.2. Preparation of test solutions

The compounds to be tested were accurately weighed (*ca.* 1 or *ca.* 2 mg) using a 5 decimal balance, dissolved in analytical grade acetone (1 mL) and then diluted with distilled water to a volume of 50 mL. Aliquots of these analytical grade solutions were further diluted with distilled water to obtain test solutions containing 1, 0.1 and 0.01 mg/L test compound and 0.01% (v/v) acetone,

respectively. Distilled water was boiled for a minimum of 30 min and then cooled to room temperature before use.

3.2.3. Bio-assays

For surface sterilization, seeds of *S. hermonthica* and *O. cernua* were exposed to an aqueous solution of sodium hypochlorite (2% active chlorine) for 5 min with agitation. The seeds were then rinsed thoroughly with demineralised water and dried on a blotting paper for *ca.* 30 min. For conditioning, the sterilized seeds were spread on glass fiber filter paper disks (8 mm diameter; approximately 30–70 seeds per disk) in Petri dishes, wetted with demineralised water, stored in the dark for 14 days at 20 °C for *Orobanche* seeds, at 30 °C for *Striga* seeds. The conditioning water was then removed and replaced by 100 μL of test solution per disk. After incubation for 24 h (*Striga*), and 5 days (*Orobanche*) in the dark at the temperatures indicated, the germination percentage was determined using a microscope. Seeds were considered to have germinated if the radical protruded through the seed coat. In each test series, aqueous solutions containing 0.01% (v/v) acetone were used as negative controls. Test solutions of the stimulant GR 24 (**2**) (concentrations of 0.1 and 0.01 and 0.001 mg/L) were used as positive controls. In each test, the germination percentages were determined on 9 disks per treatment. All bio-assays were compared with that of GR 24 in the same run, thus giving a reliable relative activity with respect to this positive control. All assays were carried out in triplicate; no statistical analyses were performed as for that more concentrations of the stimulants should have been investigated.

Acknowledgements

We thank H. Amatdjais, P. van Galen, and A. Swolfs for conducting elemental analysis, mass, and 400 MHz ^{1}H NMR measurements, respectively, Henk Regeling for his experimental advice and Dr. G.J.F. Chittenden for helpful discussions.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.06.057.

References and notes

1. Parker, C. *Pest Manag. Sci.* **2009**, *65*, 453. and refs cited therein.
2. Hearne, S. J. *Pest Manag. Sci.* **2009**, *65*, 603. and refs cited therein.
3. (a) Cook, C. E.; Whichard, L. P.; Turner, B.; Wall, M. E.; Egley, G. H. *Science* **1966**, *154*, 1189; (b) Cook, C. E.; Whichard, L. P.; Wall, M. E.; Egley, G. H.; Coggon, P.; Luhan, P. A.; Mcphail, A. T. *J. Am. Chem. Soc.* **1972**, *94*, 6189.
4. Hauck, C.; Müller, S.; Schildknecht, H. *J. Plant physiol.* **1992**, *139*, 474.
5. Siame, B. K.; Wood, K.; Ejeta, G.; Weerasuriya, Y.; Butler, L. G. *J. Agric. Food Chem.* **1993**, *41*, 1486.
6. Yokota, T.; Sakai, H.; Okuno, K.; Yoneyama, K.; Takeuchi, Y. *Phytochemistry* **1998**, *49*, 1967.
7. Koichi, Y.; Xie, X.; Yoneyama, K.; Takeuchi, Y. *Pest Manag. Sci.* **2009**, *65*, 467.
8. Xie, X.; Yoneyama, K.; Yoneyama, K. *Annu. Rev. Phytopathol.* **2010**, *48*, 93.
9. Yoneyama, K.; Awad, A. A.; Xie, X.; Yoneyama, K.; Takeuchi, Y. *Plant Cell Physiol.* **2010**, *51*, 1095.
10. Johnson, A. W.; Gowda, G.; Hassanali, A.; Knox, J.; Monaco, S.; Razavi, Z.; Roseberry, G. *J. Chem. Soc., Perkin Trans. I* **1981**, 1734.
11. Hassanali, A. Strigol Analogues: Synthetic Achievements and Prospects. In *Striga, Biology and Control*; Ayensu, E. S., Doggett, H., Keynes, K. D., Marton-Lefevre, J., Musselman, L. J., Parker, C., Peckering, A., Eds.; ICSU Press: Paris, 1984; pp 125–132.
12. Mangnus, E. M.; Zwanenburg, B. *J. Agric. Food Chem.* **1992**, *40*, 1066.
13. B. Zwanenburg, E.M. Mangnus, J.W.J.F. Thuring, Strigol Analogues: Design Synthesis and Biological Activity, in: A.H. Pieterse, J.A.C. Verkley, S.J. Terborg (Eds.), Proceedings of the Third International Workshop on Orobanche and Related Striga Research, Royal Tropical Institute, Amsterdam, (1994) 187-197.
14. Kranz, A.; Samson-Schulz, E.; Hennig, L.; Welzel, P.; Muller, D.; Mayer-Figge, H.; Sheldrick, W. S. *Tetrahedron* **1996**, *52*, 14827.
15. Mangnus, E. M.; Zwanenburg, B. *Recl. Trav. Chim. Pays-Bas* **1992**, *111*, 155.
16. Mangnus, E. M.; Van Vliet, L. A.; Vandenput, D. A. L.; Zwanenburg, B. *J. Agric. Food Chem.* **1992**, *40*, 1222.
17. Thuring, J. W. J. F.; Nefkens, G. H. L.; Zwanenburg, B. *J. Agric. Food Chem* **1997**, *45*, 1409.
18. Zwanenburg, B.; Mwakaboko, A. S.; Reizelman, A.; Anilkumar, G.; Sethumadhavan, D. *Pest Manag. Sci* **2009**, *65*, 478.
19. For some total syntheses of natural SLs, see: (a) MacAlpine, G. A.; Raphael, R. A.; Shaw, A.; Taylor, A.; Wild, H. J. *J. Chem. Soc., Chem. Perkin Trans* **1976**, 410; (b) Sugimoto, Y.; Wigchert, S. C. M.; Thuring, J. W. J. F.; Zwanenburg, B. *J. Org. Chem* **1998**, *63*, 1259; (c) Matsui, J.; Yokota, T.; Bando, M.; Takeuchi, Y.; Mori, K. *Eur. J. Org. Chem.* **1999**, 2201; (d) Reizelman, A.; Scheren, M.; Nefkens, G. H. L.; Zwanenburg, B. *Synthesis* **2000**, 1944.
20. Kondo, Y.; Tadokoro, E.; Matsuura, M.; Iwasaki, K.; Sugimoto, Y.; Miyake, H.; Takikawa, H.; Sasaki, M. *Biosci., Biotechnol., Biochem.* **2007**, *71*, 2781.
21. Bhattacharya, C.; Bonfante, P.; Deagostino, A.; Kapulnik, Y.; Larini, P.; Occhiato, E. G.; Prandi, C.; Venturello, P. *Org. Biomol. Chem.* **2009**, *7*, 3413.
22. Nefkens, G. H. L.; Thuring, J. W. J. F.; Beenakkers, M. F. M.; Zwanenburg, B. *J. Agric. Food Chem.* **1997**, *45*, 2273.
23. Mwakaboko, A. S.; Zwanenburg, B. *Plant Cell Physiol* **2011**, *52*, 699.
24. For improved syntheses GR 24, see: (a) Mangnus, E. M.; Dommerholt, F. J.; de Jong, R. L. P.; Zwanenburg, B. *J. Agric. Food Chem.* **1992**, *40*, 1230; (b) Malik, H.; Rutjes, F. P. J. T.; Zwanenburg, B. *Tetrahedron* **2010**, *66*, 7198.
25. Johnson, A. W.; Roseberry, G.; Parker, C. *Weed Res.* **1976**, *16*, 223.
26. Eplee, R. E.; Norris, R. S. Chemical Control of Striga. In *Parasitic Weeds in Agriculture Vol 1 Striga*; Musselman, L. J., Ed.; CRC Press: Baca Raton, FL, USA, 1987; pp 173–182.
27. Parker, C.; Riches, C. R. *Parasitic Weeds of the World, Biology and Control*; CAB International: Wallingford, Oxon, UK, 1993.
28. Mangnus, E. M.; Stommen, P. L. A.; Zwanenburg, B. *J. Plant Growth Regul* **1992**, *11*, 91.
29. Rubiales, D.; Fernández-Aparicio, M.; Wegmann, K.; Joel, D. M. *Weed Res.* **2009**, *49*, 23.
30. Wigchert, S. C. M.; Kuiper, E.; Boelhouwer, G. J.; Nefkens, G. H. L.; Verkleij, J. A. C.; Zwanenburg, B. *J. Agric. Food Chem.* **1999**, *47*, 1705.
31. Babiker, A. G. T.; Hamdoun, A. M.; Rudwan, A.; Mansi, N. G.; Faki, H. H. *Weed Res.* **1987**, *27*, 173.
32. Babiker, A. G. T.; Ibrahim, N. E.; Edwards, W. G. *Weed Res.* **1988**, *28*, 1.
33. A.R. Saghir, Dormancy and germination of Orobanche seeds in relation to control methods, in: S.J. Terborg, (Ed.), Biology and Control of Orobanche Proceedings of a workshop in Wageningen, 1986, sage, Thenetherlands, pp. 25-34.
34. (a) Akiyama, K.; Matsuzaki, K.-I.; Hayashi, H. *Nature* **2005**, *435*, 824; (b) Akiyama, K.; Ogasawara, S.; Ito, S.; Hayashi, H. *Plant Cell Physiol.* **2010**, *51*, 1104; (c) Bouwmeester, H. J.; Roux, C.; Lopez-Raez, J. A.; Bécard, G. *Trends Plant Sci.* **2007**, *12*, 224.
35. (a) Gomez-Roldan, V.; Fermas, S.; Brewer, P. B.; Puech-Pages, V.; Dun, E. A.; Pillot, J.-P.; Letisse, F.; Matusova, R.; Danoun, S.; Portais, J.-C.; Bouwmeester, H.; Becard, G.; Beveridge, C. A.; Rameau, C.; Rochange, S. F. *Nature* **2008**, *455*, 189; (b) Umehara, M.; Hanada, A.; Yoshida, S.; Akiyama, K.; Arite, T.; Takeda-Kamiya, N.; Magome, H.; Kamiya, Y.; Shirasu, K.; Yoneyama, K.; Kyozuka, J.; Yamaguchi, S. *Nature* **2008**, *455*, 195.
36. (a) Tsuchiya, Y.; McCourt, P. *Curr. Opin. Plant Biol.* **2009**, *12*, 556; (b) Chen, C. Y.; Zou, J. H.; Zhang, S. Y.; Zaitlin, D.; Zhu, L. H. *Sci. China Ser C-Life Sci.* **2009**, *52*, 693.
37. (a) Burness, D. M. *Org. Synth.* **1963**, *4*, 628; (b) Burness, D. M. *Org. Synth.* **1963**, *4*, 649.
38. (a) Nefkens, G. H. L.; Thuring, J. W. J. F.; Zwanenburg, B. *Synthesis* **1997**, 290; (b) Malik, H.; Rutjes, F. P. J. T.; Zwanenburg, B. *Synthesis* **2010**, 3271.