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Strigolactone analogues and mimics derived from phthalimide, saccharine, *p*-tolylmalondialdehyde, benzoic and salicylic acid as scaffolds

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ABSTRACT

A series of new strigolactone (SL) analogues is derived from simple and cheap starting materials. These SL analogues are designed using a working model. The first analogue is a modified Nijmegen-1, the second contains saccharin as substituent (bio-isosteric replacement of a carbonyl in Nijmegen-1 by a sulfonyl group) and the third one is derived from *p*-tolylmalondialdehyde. These new SL analogues are appreciably to highly active as germination stimulants of seeds of *Striga hermonthica* and *Orobanche cernua*. The SL analogue derived from saccharin is the most active one.

A serendipitous and most rewarding finding is that the compound obtained by a direct coupling of saccharin with the chlorobutenolide exhibits a high germination activity especially towards *O. cernua* seeds. Two other SL mimics are obtained from benzoic and salicylic aid by a direct coupling reaction with chlorobutenolide, both of them are very active germinating agents. These SL mimics represent a new type of germination stimulants. A tentative molecular mechanism for the mode of action of these SL mimics has been proposed.

1. Introduction

Strigolactones (SLs) are currently in the focus of interest as they are considered as a new type of plant hormones.[1–6] Natural strigolactones have been isolated from root exudates of various plants, especially those that are parasitised by the noxious weeds *Striga* and *Orobanche* spp. The first natural SL that has been isolated is strigol (**1**)[7] (Fig. 1). Full details of the structure of this germination stimulant for seeds of the just mentioned weeds were described about 20 years after its initial isolation.[8] Up to now several natural germination stimulants have been isolated and identified from the root exudates of both host and nonhost plants.[7,9–14] The structures of SLs invariably contain four rings, the main structural differences are encountered in the AB part, whereas all stimulants contain the same D-ring.[12–15] It is highly relevant to mention that these stimulants occur only in minute amounts in root exudates. The production of strigol per plant is estimated to be ca. 25–30 pg per day.[16,17] As a consequence the isolation and identification of natural SLs is extremely difficult. The natural life cycle of the parasitic weeds *Striga* and *Orobanche* spp. involves the germination of their seeds induced by a SL exuded by the roots of the host plant. This is followed by the formation of a radicle from the seed and its subsequent attachment to the host roots allowing the developing parasite to take nutrients, hormones and water from the host plant which, as a consequence, will suffer and not be able to grow normally.[18] Infestation of cereal crops (main staple food) by *Striga* results in considerable crop losses, which is considered to be the major obstacle to food production in Central and Southern Africa.[19] The estimated loss of cereal crop amounts to of million of tons each year.[19,20] Weed pest control is therefore of utmost importance.[19,20] The concept of suicidal germination is an appealing approach to control these noxious weeds.[15,21–25] In this approach the soil is treated with stimulant in absence of a host, hence the germinated seeds cannot develop and will die. The natural stimulants cannot be used for this purpose as their synthesis[26] is far too laborious and expensive. Therefore, analogues have been prepared in which the bioactivity is predominantly retained. The first successful series of analogues are the GR compounds,[27] as is exemplified by GR 24 (**3**)[28] (Fig. 1).

An extensive study of structure–activity relationship revealed that the bioactiphore resides in the CD part of the molecule.[29,30] In addition, a molecular mechanism has been proposed for the initial reaction of the stimulants with protein receptor in the seeds (Scheme 1).[29,30] In essence this is nucleophilic addition to the α, β-unsaturated unit followed by a retro reaction with the concurrent elimination of the D-ring. Combining the information of the bioactiphore and the molecular mechanism leads to a working model for designing new germination stimulants as shown in Figure 2.[15]

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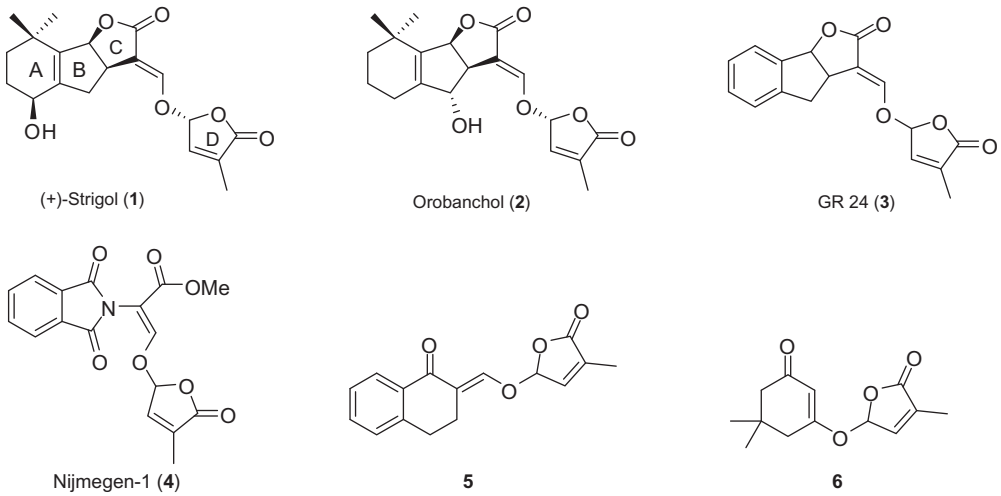
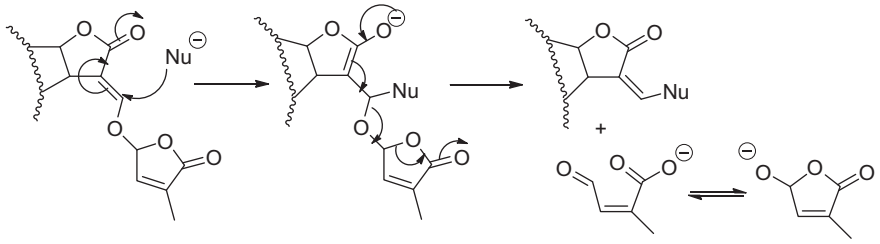


Figure 1. Chemical structures of some natural and synthetic SLs.



Scheme 1. Molecular mechanism for the triggering of germination by SLs.

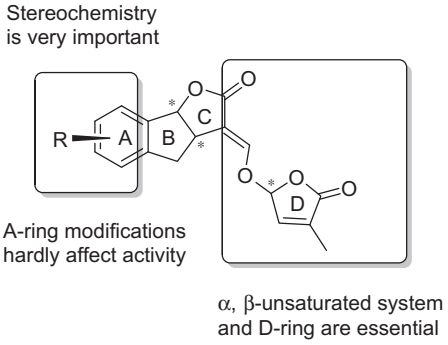


Figure 2. Working model for designing SL analogues.

In the early stages of developing this model Nijmegen-1 was designed as a potential stimulant.[31] Gratifyingly, this compound showed an appreciable stimulation activity towards seeds of both parasitic weeds.[31] Even more rewarding was the successful application of Nijmegen-1 in the field in controlling *Orobanche* in tobacco crops.[15] Recently, we reported the synthesis and bioactivity of a series of ketone derived SL analogues.[23,32] The best performing ketone SL analogue is **5**, which is readily prepared from 1-tetralone in two steps. A second series of SL analogues was obtained from simple cyclic keto enols in a single step operation.[24] The SL analogue **6** from dimedone was one of the most active stimulants. Both series of SL analogues were designed on the basis of the model[15] shown in Figure 2.

In this paper, we report the design of three new SL analogues. The first one is a structural modification of the phthalimide scaffold of Nijmegen-1. The second one is an isosteric replacement of one of the carbonyl groups in Nijmegen-1 by a sulfonyl group which leads to a saccharine derived analogue. The third one is derived from a 1,3-dialdehyde leading to the enal derived SL analogue.

The second part of the paper deals with a serendipitous finding of a new type of stimulant which contains no α,β-unsaturated unit, but the D-ring only. These new SL mimics are derived from saccharine, as well as from benzoic and salicylic acid.

2. Results and discussion

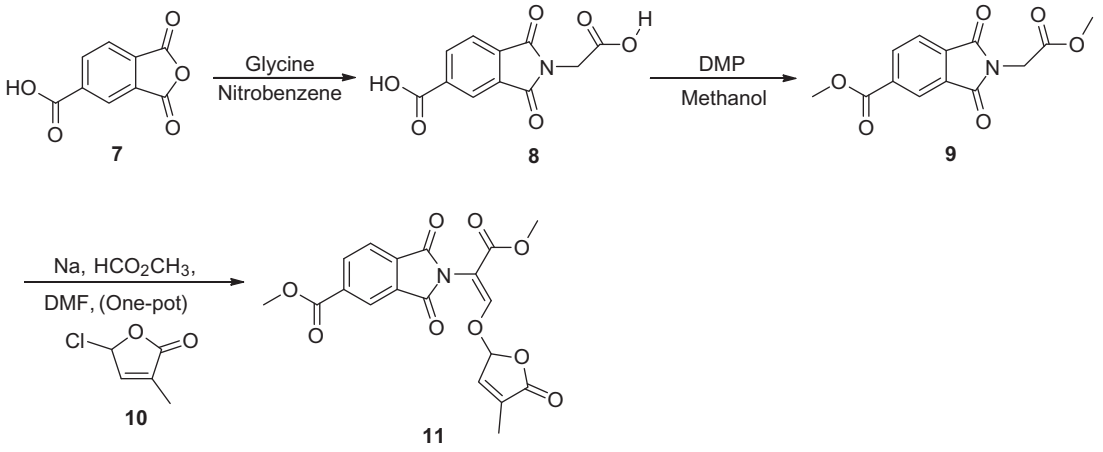
2.1. Synthesis of SL analogues and mimics

The synthesis of compound **11** was achieved by first treating trimellitic anhydride **7** with glycine[33] followed by subsequent esterification of both carboxylic acid groups of **8** using dimethoxypropane and methanol. Hydroxymethylidenation of thus obtained **9** with methyl formate, using metallic sodium, gave the expected hydroxymethylene compound as its sodium enolate. This salt was treated in situ with chlorobutenolide **10**. Using this one-pot procedure the desired product **11** was obtained in a modest yield (Scheme 2).

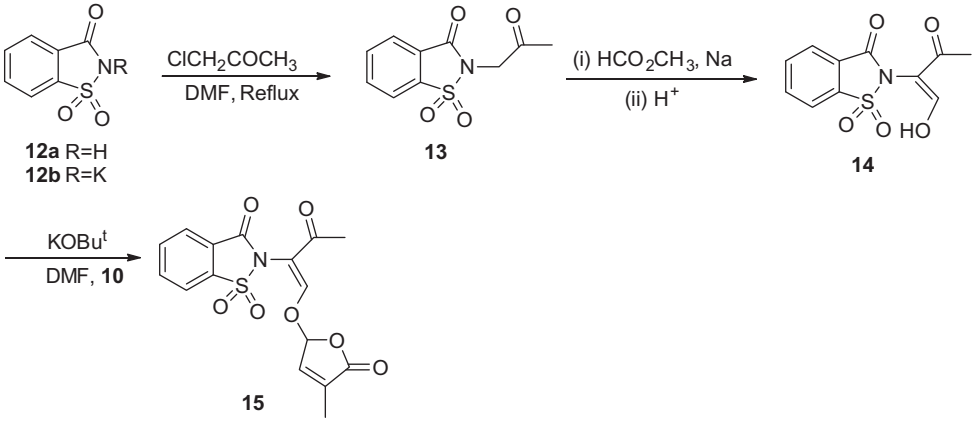
A saccharin SL analogue was obtained by first reacting of potasium saccharinate 12b with chloroacetone to give acetonylsaccharin 13 in 86% yield.[34] Subsequent hydroxymethylidenation with methyl formate using metallic sodium gave crystalline enol **14** and a colourless oily byproduct which could not be identified. Coupling of **14** with chlorobutenolide **10** gave the desired saccharin derived SL analogue **15** in 43% yield (Scheme 3).

The first SL analogue (**17**) having an enal as the α,β-unsaturated unit was obtained from 2-*p*-tolyl-malondialdehyde **16** by a one-pot procedure as shown in Scheme 4 in 52% yield.

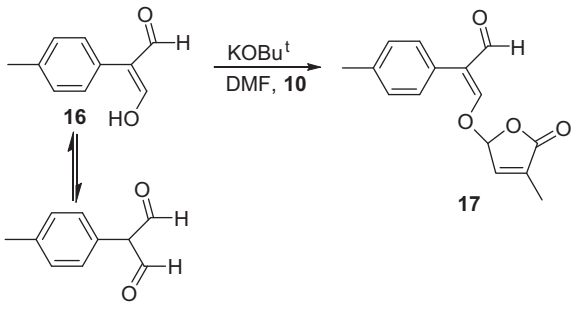
For the sake of comparison and curiosity, the saccharin derivative (**18**) in which the D-ring is coupled directly to saccharin was also



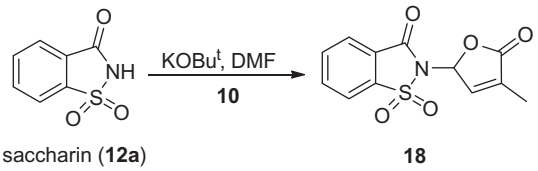
Scheme 2. Synthesis of modified Nijmegen-1.



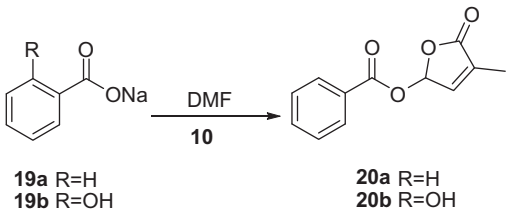
Scheme 3. Synthesis of a SL analogue from acetonyl saccharin.



Scheme 4. Synthesis of a SL analogue from *p*-tolylmalondialdehyde.



Scheme 5. Synthesis of SL mimic from saccharin.



Scheme 6. Synthesis of SL mimics from benzoic and salicylic acid.

prepared. The coupling reaction took place in the presence of potassium *tert*-butoxide and DMF as the solvent. The desired product **18** was obtained as a crystalline product in 29% yield (Scheme 5).

As it was expected that direct coupling was a new option for preparing SL mimics (see Section 2.2) also benzoic and salicylic acid were directly coupled with the D-ring. In this manner the SL mimics **20a** and **20b** were obtained in a single step (Scheme 6).

The yields of these preparations of SL analogues and mimics have not been optimised. At this stage of the work, the bioactivities are most relevant.

2.2. Germination activity of the new SL analogues and mimics

The stimulatory germination activities of SL analogues **11**, **15** and **17** were assayed[35] using seeds of *Striga hermonthica* and *Orobanche cernua*. The germination results are collected in Tables 1 and 2. There is a considerable difference in the induction of germination for both seed types. It should be noted however, that the positive control, that is, the activity of GR 24, is different for both

Table 1
Germination stimulatory activity[a] of the new analogues and mimics towards the seeds of *S. hermonthica*

Entry	Starting material	D-ring derivative	% Germination ± SE[b] at a concentration of		
			1 mg/L	0.1 mg/L	0.01 mg/L
1		GR 24 (**3**)[d,f]	nd[e]	19.7 ± 1.4	43.2 ± 4.4
2	Methoxycarbonyl phthalimidoacetic ester	**11**	17.6 ± 3.6	35.4 ± 7.3	6.4 ± 1.4
	Acetonyl saccharin	**15**	64.2 ± 6.9	68.4 ± 1.7	55.9 ± 4.6
4	*p*-Tolylmalondialdehyde	**17**	0.0 ± 0.0[c]	9.6 ± 2.7	34.4 ± 3.5
5	Saccharin	**18**	0.0 ± 0.0[c]	18.5 ± 2.3	11.1 ± 2.1
6	Sodium benzoate	**20a**	2.7 ± 1.1[c]	8.7 ± 2.7	6.7 ± 2.4
7	Sodium salicylate	**20b**	2.6 ± 0.8[c]	6.9 ± 1.1	21.3 ± 3.5

[a] Activities are given as germination percentages obtained after treatment of the seeds with the stimulant solution. Germination percentages given are means ± SE of one representative experiment.
[b] Values are the mean germination percentages ± SE obtained by treatment of the seeds with GR 24 (**3**) in the same bioassay.
[c] No activity. Values are not significantly different from germination percentages obtained in the aqueous control.
[d] Equimolar mixture of two racemic diastereomers of GR 24 (**3**).
[e] Not determined (nd).
[f] The germination percentage of GR 24 (**3**) at 0.001 mg/L was 64.3 ± 7.5.

Table 2
Germination stimulatory activity[a] of the new analogues and mimics towards the seeds of *O. cernua*

Entry	Starting material	D-ring derivative	% Germination ± SE[b] at a concentration of		
			1 mg/L	0.1 mg/L	0.01 mg/L
1		GR 24 (**3**)[d,f]	nd[e]	85.7 ± 1.7	81.6 ± 3.5
2	Methoxycarbonyl phthalimidoacetic ester	**11**	74.5 ± 4.4	68.7 ± 13.0	6.9 ± 0.5
3	Acetonyl saccharin	**15**	84.9 ± 3.2	79.0 ± 3.5	54.5 ± 3.8
4	Arylmalondialdehyde	**17**	63.4 ± 5.1	89.0 ± 3.5	87.1 ± 5.0
5	Saccharin	**18**	70.2 ± 2.9	31.0 ± 5.3	3.7 ± 1.0[c]
6	Sodium benzoate	**20a**	87.3 ± 1.7	77.7 ± 5.9	20.6 ± 4.2
7	Sodium salicylate	**20b**	51.9 ± 10.6	81.7 ± 8.3	72.4 ± 4.0

[a] Activities are given as germination percentages obtained after treatment of the seeds with the stimulant solution. Germination percentages given are means ± SE of one representative experiment.
[b] Values are the mean germination percentages ± SE obtained by treatment of the seeds with GR 24 (**3**) in the same bioassay.
[c] No activity. Values are not significantly different from germination percentages obtained in the aqueous control.
[d] Equimolar mixture of two racemic diastereomers of GR 24 (**3**).
[e] Not determined (nd).
[f] The germination percentage of GR 24 (**3**) at 0.001 mg/L was 55.9 ± 5.1.

test runs, but the conclusions will be influenced only marginally by this difference.

For *S. hermonthica* the ester substituted Nijmegen-1 (**11**) is only moderately active at low concentration, but at 0.1 mg/L it is appreciably active when compared with GR24 at the same concentration. The saccharin derived SL analogue **15** shows a high activity. The SL analogue **17** obtained from the 1,3-dialdehyde **16** is reasonably active at low concentration when compared with GR24. In contrast, the *Orobanche* seeds respond much better to the newly prepared stimulants. Analogue **11** is almost as active as GR24, but SL analogue **15** derived from saccharin is the most active. Also the enal derived SL analogue **17** is very active as well.

The compound **18** derived from saccharin by direct coupling with the D-ring shows an unexpected high activity for *O. cernua* seeds at the concentration of 1 mg/L. Also the compounds obtained from benzoic acid and salicylic acid, **20a** and **20b**, respectively, are surprisingly active. Because of the germinating activities of compounds **18**, **20a** and **20b** we call them SL mimics. The activity of these SL mimics towards *S. hermonthica* seeds is much lower. By taking into account that the GR24 response is rather low, the SL analogues still seem appreciably active, especially for the saccharin derived mimic **18**.

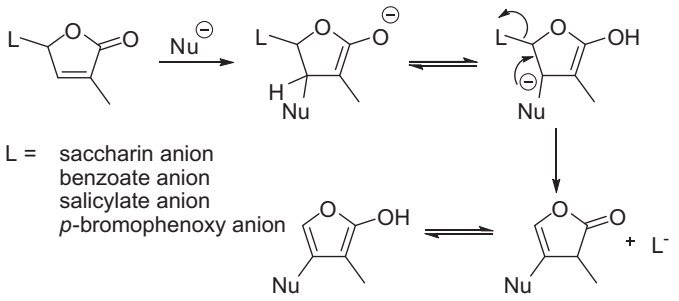
2.3. Discussion

The three newly SL analogues **11**, **15** and **17** based on the model compound shown in Figure 2, are all are active as germination agents. This observation is another demonstration of the validity of this model for the design of SL analogues. Modified Nijmegen-1 **11** has an α,β unsaturated ester unit, the saccharin derived SL analogues **15** has an α,β unsaturated ketone unit and the SL analogue **17** obtained from malondialdehyde has an α,β-unsaturated aldehyde moiety. In fact, the latter compound is the first SL analogue having an enal group connected to the D-ring. The saccharin SL analogue is of interest for two reasons. Firstly, it shows that the isosteric replacement of the carbonyl in the phthalomido scaffold by a sulfonyl group is allowed. Secondly, it throws some doubt on the claim that the phthalimido part of Nijmegen-1 is an essential molecular fragment for seed germination and development as was suggested by McCourt et al. on the basis of a molecular similarity study.[36]

These three new stimulating agents show a similar activity profile. The best performing stimulant for *both* seed types is saccharin derived compound **15**, as it is active over a wide concentration range. Therefore, this SL analogue is an interesting candidate for the use in the suicidal approach for reducing the devastating effect of the parasitic weeds. A drawback of this compound **15** is that its synthesis requires optimisation.

These results constitute another demonstration of the validity of the model shown in Figure 2 for designing SL analogues. A series of SL analogues with the enol ether unit conjugated with an ester[24,27,31,37], ketone[23,24,32,38] or aldehyde[38] are now available for further evaluation. For all these analogues the molecular mechanism shown in Scheme 1 is a conceivable mode of action. Recently it has been shown[23] that the same holds for SL imino analogues [ArC(CN)═N–O–D-ring where Ar is Ph or 2-pyridyl],[39] in spite of an earlier contrasting opinion.[39]

The germinating activity of the saccharin derivative **18** is a great surprise. The same holds for **20a** and **20b**. These compounds are lacking the α,β-unsaturated unit which is considered to be a prerequisite for active germination stimulants (see model compound, Fig. 2). The identification of these compounds as SL mimics is a clear case of serendipity. The intriguing question is whether the activity of these furanone derivatives can be explained by a modified molecular mechanism. As in the receptor site only nucleophilic functionalities are available for initiating a reaction, it is suggested that such a nucleophile adds to the furanone in a Michael fashion as shown in Scheme 7. Subsequent internal proton shift, followed by an elimination of the group at C-2, leads to a furanone with a β,γ-olefinic bond which is in equilibrium with a 2-hydroxylfuran. In essence, this tentative explanation is again an addition-elimination reaction. A condition for such a reaction to place is that the substituent at the 2-postion of the SL mimic is a good leaving group. In the case of the saccharin, benzoate and salicylate mimics this condition is fulfilled. It important to note that presence of the D-ring still is a prerequisite. Recently, germination was also observed for a 2-aryloxyfuranone.[40] Provided that this aryloxy group is a good leaving group, which is the case for the *p*-bromoophenoxy group, the same molecular mechanistic explanation as shown in Scheme 7 is conceivable. It is relevant to note here that alkoxy-2(5)-furanones are inactive as germination stimulants as has been previously described in great detail.[41] This is in agreement with the



Scheme 7. Tentative molecular mechanism for the mode of action of SL mimics.

tentative mechanism shown in Scheme 7. Alkoxy groups cannot serve as good leaving groups in this mode of action. Alternatively, a simple accommodation of the SL mimic in the receptor through dipole–dipole interactions can be envisaged, but then the difference between alkoxy and aryloxy furanones can not readily be explained.

Whether the SL analogues and SL mimics are interacting with the same or with different receptors is a question that cannot be answered in this stage. For the moment we hypothesise that both stimulants interact with the same receptor but with different modes of action. Both interaction modes are, to certain extend, related as both of them are similar addition–elimination reactions. It is worth noting that the enol unit, for example, OCH=CH–C(=O)–OMe, as present in the working model shown in Figure 2, may also serve as leaving group L in Scheme 7.

The interesting outcome of this study is that appropriately 2-substituted 2(5*H*)-furanones are new candidates for parasitic weed control using the suicidal approach.

Currently SLs receive much attention in the literature because of the newly discovered bioproperties. Natural SLs have been identified as the branching factor for arbusular mycorrhizal (AM) fungi[5,42] and as inhibitor of plant shoot branching.[43,44] SLs are now considered as a new class of plant hormones[1,2] for which more functions will be uncovered in the coming years. Interestingly, the synthetic SL analogue GR24 is also active for both functions.[5,42–44] Therefore, searching for other SL analogues for the new activities is another incentive for this study. Now there two options, namely SL analogues and SL mimics. It has been shown that some aryloxy-2-(5*H*)-furanones are active as branching inhibitors.[40] Predictably, the SL mimics described in this paper offer good prospects for being active as shoot branching inhibitors or as hyphal branching factors for AM fungi.

3. Experimental section

3.1. Synthesis

3.1.1. 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 focusing 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 precoated silica gel 60 F_{254} plates (0.25 mm) using the eluents indicated. Spots were visualised 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 at140 °C. Column chromatography was performed on silica gel (Kieselgel, Merck) using eluents indicated. All solvents were dried under standard conditions.

The chlorobutenolide 10 was prepared as previously described.[45]

3.1.2. 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).

3.1.2.1. Methyl 2-(2-methoxy-2-oxoethyl)-1,3-dioxo-5-isoindolinecarboxylate (9). Carboxyphthalimidoacetic acid 8[33] (7.0 g, 28 mmol) dissolved in a mixture of methanol (70 mL) and 2,2-dimethoxypropane (80 mL) containing *p*-toluenesulfonic acid (500 mg) was stirred at room temperature for 1 h. The temperature of the reaction mixture was then elevated to 60 °C and set aside for 18 h. The mixture was cooled to room temperature, concentrated in vacuo, and the resultant solid material was recrystallised from 2-propanol to give 9 as a pure white solid, 2.1 g, (27%), mp 125–130 °C. The spectroscopic data were in complete agreement with the assigned structure. ^{1}H NMR ($CDCl_3$, 100 MHz): δ 8.54–7.94 (m, 3H, ArH), 4.48 (s, 2H, NCH_2CO), 4.00 (s, 3H, CH_3O), 3.78 (s, 3H, OCH_3).

3.1.2.2. Methyl 2-{(*Z*)-1-(methoxycarbonyl)-2-[(4-methyl-5-oxo-2,5-dihydro-2-furanyl)oxy]-1-ethenyl}-1,3-dioxo-5-isoindolinecarboxylate (11). A stirred solution of the phthalimido ester 9 (600.0 mg, 2.17 mmol) in methyl formate (15 mL), whilst maintained under nitrogen, was treated with small pieces of metallic sodium (61.0 mg). The mixture was set aside for 24 h and then concentrated in vacuo. The residue was dissolved in anhydrous DMF (15 mL), cooled (−50 °C) and then treated dropwise with the solution of chlorobutenolide 10 (378.8 mg, 2.86 mmol) in DMF (5 mL). The mixture was allowed to warm to room temperature, set aside for 5 days. The mixture was concentrated in vacuo, the residue treated with a mixture of water (50 mL) and ethyl acetate (30 mL), the separated aqueous layer was extracted with ethyl acetate (2 × 20 mL), and the combined organic layers were washed with water, then dried ($MgSO_4$), and concentrated in vacuo. The resultant crude product gave compound **11** as a white foam (228.8 mg, 26.3%) after column chromatography (hexane/ethyl acetate, 1:1, v/v). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.53–7.93 (m, 4H, ArH + =CHO), 6.91 (s, 1H, CH=), 6.20 (s, 1H, OCHO), 4.00 (s, 3H, CH_3OCO), 3.79 (s, 3H, OCH_3) 1.98 (s, 3H, CH_3). ^{13}C NMR, ($CDCl_3$, 100 MHz): δ 169.9, 165.2, 162.9, 153.7, 140.9, 135.7, 132.3, 124.9, 123.9, 106.0, 100.1, 60.4, 52.9, 52.4, 31.8, 22.6, 14.2, 14.1, 10.7; MS [EI, *m*/*z*, rel. intensity (%)]: 402 ([M+1]$^+$, 0.8); 401 ([M]$^+$, 3.4); 370 ([M$^+$−31], [$C_{18}H_{12}NO_8$]$^+$, 10.4), 304 ([M$^+$−97], [$C_{14}H_{10}NO_7$]$^+$, 100), 97 ([M$^+$−304], [$C_5H_5O_2$]$^+$, 82.3); HRMS/EI: *m*/*z* calcd for $C_{19}H_{15}NO_9$: 401.0746. Found: 401.0745.

3.1.2.3. 2-(2-Oxopropyl)-2,3-dihydro-1*H*-1λ^6-benzo[d]isothiazole-1,1,3-trione (13). A stirred solution of saccharin (10.0 g, 54.64 mmol) in DMF (80 mL), whilst maintained under nitrogen, was treated with potassium *tert*-butoxide (6.13 g, 54.64 mmol) followed by chloroacetone (6.1 g, 65.6 mmol). The mixture was set aside for

15 min at room temperature and then heated at 125 °C for 2 h. The cooled mixture was concentrated in vacuo, the residue was dissolved in dichloromethane (80 mL) and washed with water (100 mL). The dichloromethane layer was dried ($MgSO_4$) and concentrated in vacuo. The residue was crystallised from hot methanol to give 16 (11.2 g, 86%) as a white solid, which was sufficiently pure for the subsequent step (mp 143–144 °C).[34] ^{1}H NMR ($CDCl_3$, 100 MHz): δ 8.14–7.86 (m, 4H, ArH.), 4.48 (s, 2H, NCH_2CO), 2.29 (s, 3H, $COCH_3$).

3.1.2.4. 2-[(*Z*)-1-Acetyl-2-hydroxy-1-ethenyl]-2,3-dihydro-1*H*-1λ^6-benzo[*d*]isothiazole-1,1,3-trione (14). A stirred, cooled (−10 °C) solution of acetonyl saccharin 16 (5.0 g, 20.9 mmol) in methyl formate (30 mL), whilst maintained under nitrogen was treated with small pieces of metallic sodium (481.2 mg, 20.9 mmol). The mixture was allowed to attain room temperature, set aside for 14 h, and then concentrated in vacuo. The residue was treated with a mixture of glacial acetic acid (2 mL) and 1 M HCl (5 mL), and extracted with ethyl acetate (50 mL), and the separated aqueous layer extracted with ethyl acetate (2 × 50 mL). The combined organic layers were washed with water, dried ($MgSO_4$) and concentrated in vacuo. The resultant crude material was purified by column chromatography (hexane/ethyl acetate, 2:1, v/v) to give compound 14 (1.92 g in 34.3%) as a yellow solid and an unidentified compound (1.50 g, 26.9%) as oil. Recrystallisation of 14 from di-isopropyl ether gave pure material, mp 150–151 °C; ^{1}H NMR ($CDCl_3$, 100 MHz): δ 14.93 (s, 1H, OH (hydrogen bonding), 8.19–7.69 (m, 4H, ArH), 6.07 (br s, 1H, OH), 2.44 (s, 3H, CH_3). MS [EI, *m*/*z*, rel. intensity (%)]: 267 ([M]$^+$, 0.2); 239 ([M$^+$−28], [$C_{10}H_9NO_4S$]$^+$, 100); HRMS/EI: *m*/*z* calcd for $C_{11}H_9NO_5S$: 267.0201. Found: 267.01993.

3.1.2.5. 2-{(*Z*)-1-Acetyl-2-[(4-methyl-5-oxo-2,5-dihydro-2-furanyl)oxy]-1-ethenyl}-2,3-dihydro-1*H*-λ^6-benzo[*d*]isothiazole-1,1,3-trione (15). Compound **14** (300.0 mg, 1.12 mmol) was added to a stirred, cooled (−40 °C) solution of potassium *tert*-butoxide (126.1 mg, 1.12 mmol) in dry DMF (8 mL), whilst maintained under nitrogen. The mixture was stirred for 0.5 h at room temperature, cooled to −60 °C and then treated dropwise with a solution of the chlorobutenolide 10 (156.3 mg, 1.18 mmol) in DMF (4 mL). The mixture was allowed to warm to room temperature and set aside for 24 h. The mixture was concentrated in vacuo, the residue treated with a mixture of water (50 mL) and ethyl acetate (30 mL), the separated aqueous layer was extracted with ethyl acetate (3 × 20 mL), and the combined organic layers were washed with water, then dried ($MgSO_4$), and concentrated in vacuo. The resultant crude product 18 was obtained as a yellow solid (423.0 mg). Recrystallisation from di-isopropyl ether gave pure product 15 as yellowish crystals (176.5 mg, 43.3%), mp 245–253 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.87–7.77 (m, 4H, ArH + =-CHO), 6.88 (m, 1H, CH=), 6.58 (s, 1H, OCHO), 2.33 (s, 3H, $COCH_3$), 1.93 (s, 3H, CH_3). ^{13}C NMR, ($CDCl_3$, 100 MHz): δ 194.1 (C=O), 174.7 (C=O), 171.4 (C=O), 165.6 (C=O), 140.0, 138.5, 135.9, 134.5, 133.9, 129.4, 128.2, 123.6, 89.9, 88.0, 22.4, 10.8; MS [EI, *m*/*z*, rel. intensity (%)]: 335 ([M+1]$^+$, 2.4); 335 ([M]$^+$, 6.1); 335 ([M$^+$−18], 7.7), 211 ([M$^+$−152], 3.2), 97 ([M$^+$−266], 60.2), 98 ([M$^+$−265], 100); Anal. Calcd for $C_{16}H_{13}NO_7S$: C, 52.89; H, 3.61; N, 3.85; S, 8.85. Found: C, 53.45; H, 3.87; N, 4.20; S, 9.02. HRMS/EI: *m*/*z* calcd for $C_{15}H_{13}NO_6S$: 335.0464. Found: 335.04631.

3.1.2.6. *E*-3-[(4-Methyl-5-oxo-2,5-dihydro-2-furanyl)oxy]-2-(4-methylphenyl)-2-propenal (17). A stirred mixture of *p*-tolylmalondialdehyde 1**6** (200.0 mg, 1.23 mmol), DBU (206.5 mg, 1.36 mmol) and the chlorobutenolide **10** (163.4 mg, 1.23 mmol) in dry dichloromethane (10 mL), whilst maintained under nitrogen, was set aside at room temperature for 24 h, after which the mixture was processed in the manner described above to give compound **17** as white crystals (165.0 mg, 51.8%), mp 119–121 °C, after column chromatography (heptane/ethyl acetate, 1:1, v/v), ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.48 (s, 1H, COH (aldehydic proton), 7.30–7.18 (m, 4H, ArH + CH=), 6.94 (s, 1H, CH=), 6.23 (s, 1H, OCHO), 2.35 (s, 3H, Ar–CH_3), 2.01 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 190.4 (C=O), 170.1 (C=O), 161.4, 141.0, 138.1, 135.9, 129.2, 128.9, 126.2, 125.7, 100.5, 21.3, 10.7; MS [CI, *m*/*z*, rel. intensity (%)]: 259 ([M+1]$^+$, 77.4); 258 ([M]$^+$, 15.5); 241 ([M$^+$+1]−18, [$C_{15}H_{12}O_3$]$^+$, 58.2), 161 ([M$^+$−97], [$C_{10}H_{19}O_2$]$^+$, 18.6), 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, 69.83; H, 5.37.

3.1.2.7. 2-(4-Methyl-5-oxo-2,5-dihydro-2-furanyl)-2,3-dihydro-1*H*-1λ^6-benzo[*d*]isothiazole-1,1,3-trione (18). A stirred solution of saccharin (250.0 mg, 1.34 mmol) in anhydrous DMF (10 mL) was treated with potassium *tert*-butoxide (153.3 mg, 1.34 mmol), followed by the chlorobutenolide 10 (252.8 mg, 1.91 mmol), whilst being maintained under nitrogen. The mixture was set-aside at room temperature for ∼67 h and then processed in a manner similar to that described above. The resultant crude material was triturated with a mixture of heptane/ethyl acetate (1:1, v/v) to give pure 19 as a white solid, 111.5 mg (29.3%), mp 173–176 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.09–7.77 (m, 4H, ArH + =CH), 6.82 (m, 1H, OCHO), 2.09 (m, 3H, CH_3); MS [EI *m*/*z*, rel. intensity (%)]: 280 ([M+1]$^+$, 2.8); 279 ([M]$^+$, 16.1); 250 ([M$^+$−29], 81.3); 183 ([M$^+$+1]−97, [C_7H_4 NO_3 S]$^+$, 32.9); 97 ([M$^+$+1]−183, [$C_5H_5O_2$]$^+$, 100); HRMS/EI: *m*/*z* calcd for $C_{12}H_9NO_5$ S: 279.0201. Found: 279.02006.

3.1.2.8. 4-Methyl-5-oxo-2,5-dihydro-2-furanyl benzoate (20a). A stirred mixture of sodium benzoate **19a** (429.2 mg, 3.0 mmol) and the chlorobutenolide **10** (470.2 mg, 3.55 mmol) in an anhydrous DMF (15 mL), whilst maintained under nitrogen, was set aside at room temperature for 18 h. Then TLC analysis indicated the total consumption of **10**. The mixture was concentrated in vacuo, the residue treated with a mixture of water (50 mL) and ethyl acetate (30 mL), the separated aqueous layer was extracted with ethyl acetate (2 × 20 mL), and the combined organic layers were washed with water, then dried ($MgSO_4$), and concentrated in vacuo. The resultant crude product was crystallised from di-isopropyl ether to give **20a** as pure white crystals, 387.8 mg (59.3%), mp 96–102 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.06–7.47 (m, 5H, ArH), 7.13 (m, 1H, CH=), 7.04 (m, 1H, OCHO), 2.04 (s, 3H, CH_3). ^{13}C NMR, ($CDCl_3$, 400 MHz): δ 171.0 (C=O), 164.7 (C=O), 142.1, 134.6, 134.0, 130.1, 128.6, 128.4, 93.0, 10.7; MS [EI *m*/*z*, rel. intensity (%)]: 219 ([M+1]$^+$, 8.4); 218 ([M]$^+$, 61.3); 189 [M$^+$−29], 54.1, 105 [M$^+$−113], 100, 97 ([M$^+$−121], [$C_5H_5O_2$]$^+$, 90.6); Anal. Calcd for $C_{12}H_{10}O_4$: C, 66.05; H, 4.62. Found: C, 65.51; H, 4.59.

3.1.2.9. 4-Methyl-5-oxo-2,5-dihydro-2-furanyl salicylate (20b). A stirred mixture of sodium salicylate **19b** (600.0 mg, 3.75 mmol) and compound **10** (541.6 mg, 4.09 mmol) in anhydrous DMF (15 mL), whilst maintained under nitrogen, was set-aside at room temperature for ∼65 h. Then the mixture was processed in the same manner as described above. The resultant solid was crystallised from di-isopropyl ether to give **20b** as pure white crystals, 365.4 mg (41.6%), mp 80–81 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 10.28 [s, 1H, OH (hydrogen bonding)], 7.81–7.00 (m, 4H, ArH + =CH + O-CHO), 2.04 (m, 3H, CH_3); ^{13}C NMR, ($CDCl_3$, 100 MHz), δ 170.8 (C=O), 168.4 (C=O), 162.2, 141.6, 136.9, 135.0, 130.1, 119.5, 117.9, 110.9, 92.8, 10.7; MS [EI *m*/*z*, rel. intensity (%)]: 235 ([M+1]$^+$, 11.2); 234 ([M]$^+$, 73.9); 138 ([M$^+$+1]−97, [$C_7H_5O_3$]$^+$, 91.2); 97 ([M$^+$−137, [$C_5H_5O_2$]$^+$, 100); HRMS/EI: *m*/*z* calcd for $C_{12}H_{10}$ O_5: 234.0528. Found: 234.05289.

3.2. Germination

The bioassays were carried out as described previously.[23,24,35]

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