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New strigolactone mimics: Structure–activity relationship and mode of action as germinating stimulants for parasitic weeds

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ABSTRACT

Strigolactones (SLs) are new plant hormones with varies important bio-functions. This Letter deals with germination of seeds of parasitic weeds. Natural SLs have a too complex structure for synthesis. Therefore, there is an active search for SL analogues and mimics with a simpler structure with retention of activity. SL analogues all contain the D-ring connected with an enone moiety through an enol ether unit. A new mechanism for the hydrolysis SL analogues involving bidentate bound water and an α,β-hydrolase with a Ser-His-Asp catalytic triad has been proposed. Newly discovered SL mimics only have the D-ring with an appropriate leaving group at C-5. A mode of action for SL mimics was proposed for which now supporting evidence is provided. As predicted an extra methyl group at C-4 of the D-ring blocks the germination of seeds of parasitic weeds.

Strigolactones (SLs) are new plant hormones that are currently much in focus.[1–10] The predominant activities of these SLs are stimulation of germination of seeds of parasitic weeds,[1–10] branching factor of AM fungi,[4,11,12] and inhibition of shoot branching and bud outgrowth.[13–15] This Letter deals primarily with the activity of SLs as germination stimulants.

Naturally occurring SLs are present in the root exudates of many plants, especially host plants for the parasitic weeds. They invariably contain three annulated rings, the ABC scaffold, connected with a butenolide ring (D-ring) via an enol ether unit.[1,2,7–10] Typical examples are (+)-strigol (**1**)[16] and (−)-*ent*-2′-*epi*-orobanchol (**2**)[17–19] (Fig. 1). For practical application these natural SLs have a too complex structure, and therefore, SL analogues have been developed with a much simpler structure but with retention of the essential bioactivity.[9,10] The most well known analogue is GR24 (**3**)[9,10,20,21] (Fig. 1).

For germination stimulants a model compound was used for the design and preparations of new bioactive analogues. This model (Fig. 2) was based on a structure–activity analysis combined with a tentative mode of action for germination.[9,10,22] It was shown that the bioactiphore of SLs resides in the CD part of the SL molecules, implying that the α,β-unsaturated carbonyl moiety connected via an enol ether unit with the D-ring is predominantly responsible for the bioactivity.[9,10,22] Illustrative examples of such SL analogues designed on the basis of the modelcompound shown in Figure 2 are Nijmegen-1 (**4**)[23] and analogues derived from tetralone (**5**),[24,25] hydroxy coumarin (**6**)[26] and saccharin (**7**)[27] (Fig. 1).

Although the full details of the germination process are not known yet, it is assumed that on a molecular level germination is triggered by an initial reaction of water with the enol ether moiety in a Michael fashion whereby the water molecule is bound in a bidentate manner.[10] In a subsequent retro-Michael reaction a cleavage process gives the hydroxy butenolide and the formylated ABC scaffold.[9,10,22] There is some evidence that this hydrolytic cleavage is catalyzed by an α/β hydrolase having a Ser-His-Asp canonical catalytic triad at the active site that is capable of accommodating an SL molecule.[28] This newly proposed mechanism of hydrolytic cleavage reaction is depicted in Figure 3, whereby histidine serves as the base to initiate the Michael addition of water.

Recently, we reported our serendipitous finding that compounds lacking the enol ether unit, such as the saccharine D-ring derivative **8** and the aroyloxy substituted butenolides **9** (Fig. 4) which are not in accordance with the model shown in Fig. 2, are active as germinating agents for parasitic weeds.[27] These compounds are named as SL mimics.

A structurally related SL mimic is butenolide **10**, which shows a moderate germination activity at a relative high concentration.[29]

In order to rationalize the activity of the SL mimics a mode of action shown in Figure 5 was tentatively proposed.[27] An essential feature of this proposal is a proton shift prior to the elimination

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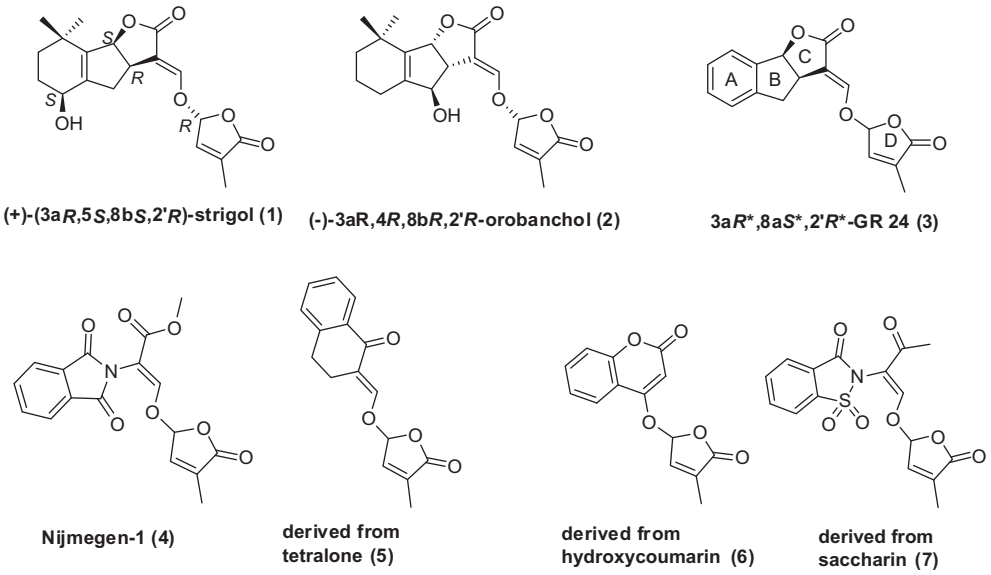

Figure 1. Structures of some natural SLs, analogue GR24 and newly designed SL analogues.

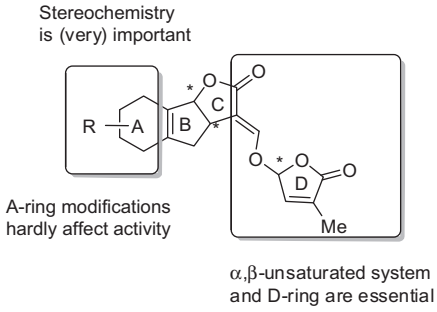

Figure 2. Model for the design of new bioactive SL analogues with germinating activity.

of the leaving group L. In the SL mimics a benzoate and a saccharide ion serve as L.

In this Letter we provide supporting evidence for this mode of action. By replacing the hydrogen atom at C-4 by a methyl group a modified SL mimic is obtained in which the essential proton transfer cannot take place anymore and accordingly these modified SL mimics are predicted to be inactive as germinating agents.

The synthesis of such C-4 methyl containing SL mimics is straightforward. A set of 4 SL mimics with a C-4 hydrogen and 3 mimics with a C-4 methyl was prepared as shown in Figure 6. The SL mimics **9** were readily obtained by a coupling of bromo-butenolide with an appropriate sodium carboxylate.[30] Coupling of a suitable acid chloridewith 3,4-dimethyl hydroxy butenolide in the presence of pyridine resulted in the SL mimics **11**. [32] There

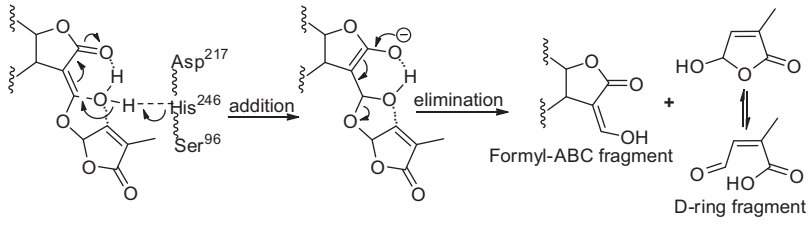

Figure 3. Proposal for the hydrolysis mechanism of SLs in the catalytic triad of Ser^{96}-His^{246}-Asp^{217}.

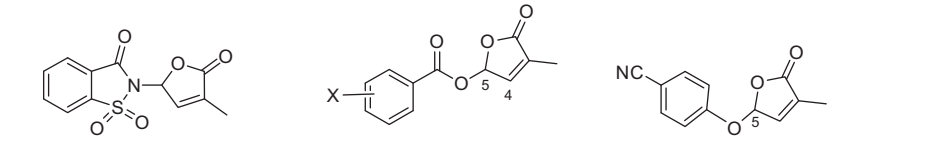

Figure 4. SL mimics.

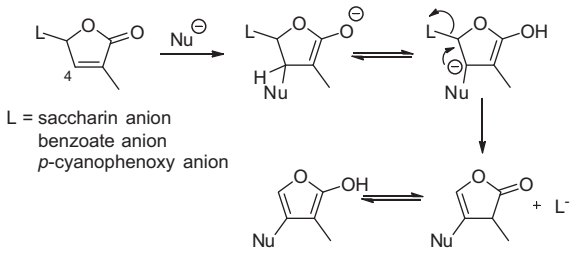

Figure 5. Mode of action proposed for SL mimics.

are a good reasons to believe that the SL mimics **9** and **11** are sufficiently stable in aqueous solution. It was shown previously that enantiopure 4-methyl-5-oxo-2,5-dihydro-2-furanyl acetate (SL mimic **9a** with an acetate instead of a benzoate) retains its optical activity in phosphate buffers of pH 6.7 and 8.[34] In addition, aqueous 30 μM solution of **9c** and **11d** were monitored with time using HPLC. No change was observed after 5 days; hence, untimely hydrolysis of these SL mimics does not occur. The mimics **9d** and **11c** slowly hydrolyze with an estimated half life of 3 days.[35]

These seven compounds were assayed as germinating agents for seeds of *Striga hermonthica*, *Phelipanche ramosa* (France) and *P. ramosa* (Italy), using GR 24 as positive and water as negative control.[36, 37] The results of these bioassays are collected in Figure 7. The SL mimics **9a**, **b**, **c** and **d** show a good response for *Striga* seeds although the required concentrations are higher than needed for germination of both *P. ramosa's*. The activity of **9d** is different for thetwo types of *ramosa's* for an unknown reason. The data clearly demonstrate that the 3,4-dimethyl butenolides **11a**, **11b** and **11c** are practically inactive at concentrations at which the SL mimics with one methyl group at C-3 only are highly active. This observation is in full accordance with the prediction on the basis of the mode of action shown in Figure 5. The SL mimic[29] **10** also fits in this picture. It should be noted that mimics in which the cyano group in **10** is replaced by a weaker electron-withdrawing group are inactive towards *S. hermonthica*.[29]

There is a considerable differencebetween SL analogues having an enol ether unit and SL mimics that lack such a unit. When an extra methyl group is introduced in the butenolide ring in analogue Nijmegen-1 (**4**) the germination activity is hardly affected,[38] whereas an extra methyl in mimics **9** results in a dramatic loss of activity. This difference in behavior strongly suggests that analogues and mimics have different receptor sites as is reflected by their different modes of action. The SL mimics cannot undergo a hydrolytic process as described above for SL analogues. Consequently, the bioactiphore for SL analogues and SL mimics must be different. For SL mimics the unsaturated lactone, that is, the butenolide ring, is the only conceivable reactive functionality that can serve as bioactiphore, whereby substituents and functional groups are decisive for the bioactivity. This is exemplified by the SL mimics describedin this Letter.

In this preliminary study only two types of seeds have been bioassayed. It is well known that the response of seeds of different parasitic weeds to SL analogues can be quite different.[18,39] That possibly may also be the case for SL mimics.

The new SL mimics **9** are of interest for the reduction of seeds banks of parasitic weeds using the concept of suicidal germination.[9,10] It is well documented that parasitic weeds are causing severe damage to important food crops especially in African countries.[40] Reduction of seeds banks of these weeds is an option to eradicate these noxious parasites.[9,10,40,41,42]

The results described above demonstrate that small changes in the molecular structure of a SL mimic may have an enormous impact on the bioactivity.

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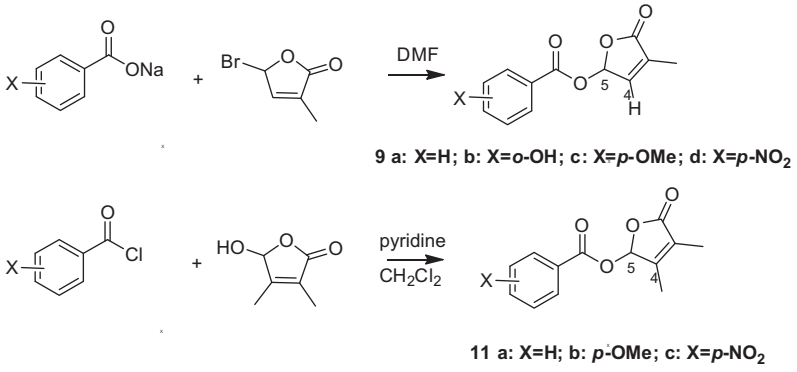

9 a: X=H; b: X=*o*-OH; c: X=*p*-OMe; d: X=*p*-NO_2

11 a: X=H; b: *p*-OMe; c: X=*p*-NO_2

Figure 6. Synthesis of SL mimics.

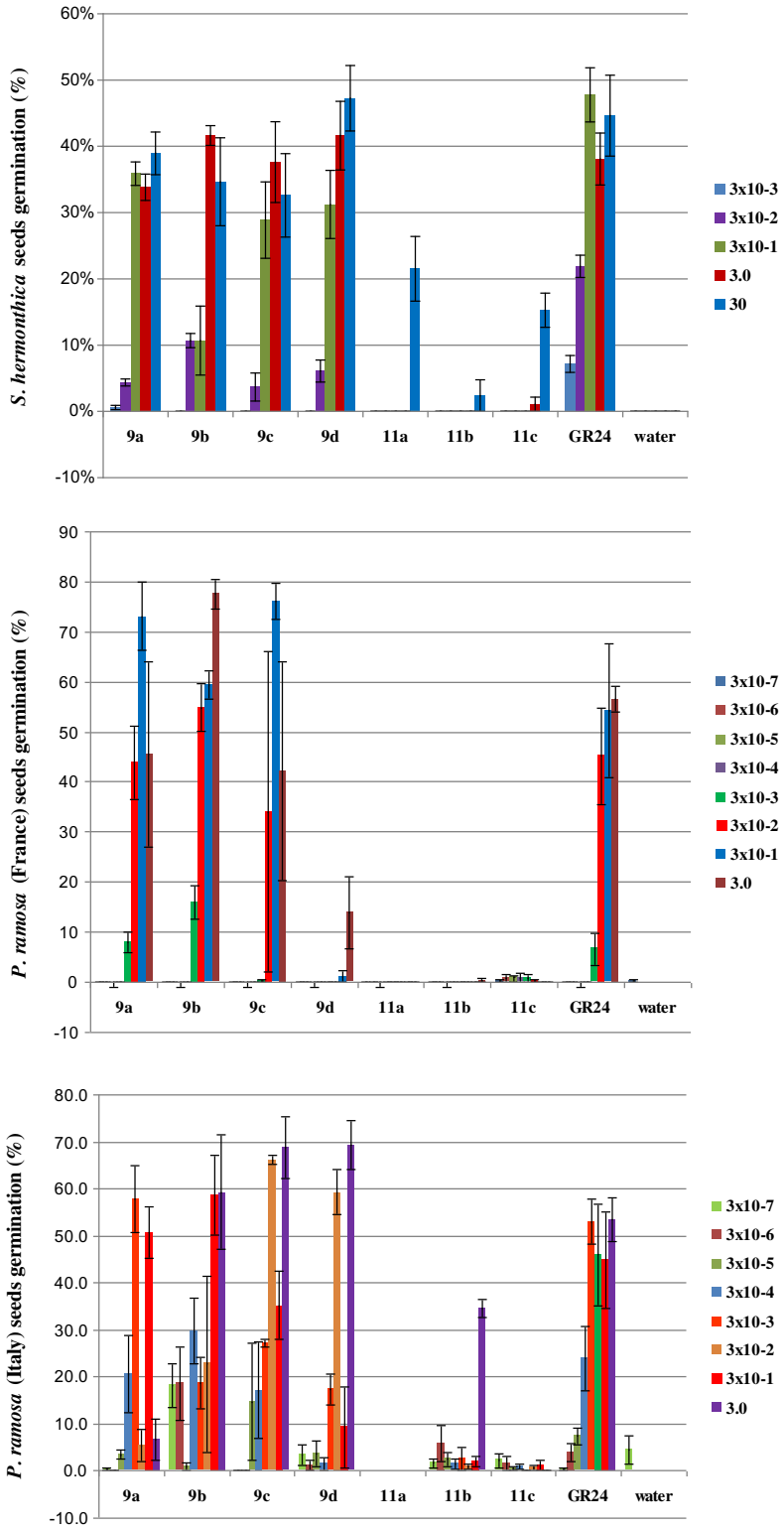

Figure 7. Bioassays of *Striga hermonthica* and *Phelipanche ramosa* collected in France and in Italy. Seed germination induced by SL mimics **9a**, **9b**, **9c**, **9d**, **11a**, **11b** and **11c** in the concentration range 3×10^{-7}, 3×10^{-6}, 3×10^{-5}, 3×10^{-4}, 3×10^{-3}, 3×10^{-2}, 3×10^{-1}, 3.0 and 30 μM. The SL analogue GR24 and dematerialized water were used as positive and negative control, respectively ($n = 6$). Bars represent means ± s.e.

17. This is the revised structure[18] of this SL in root exudate of Red Clover. It is a stereoisomer of the originally proposed (3a*S*,4*S*,8b*S*,2′*R*)-orobanchol.[19]
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30. Compounds **9a** and **9b** were also prepared previously.[27] *3-Methyl-bromo-2(5H)-furanone*. To a solution of 3-methyl-2(*5H*)-furanone (9.81 g, 0.10 mol) in dry 1,2-dichloroethane (150 ml) was added *N*-bromosuccinimide (22.25 g, 0.125 mol) and dibenzoyl peroxide (0.1 g) and the mixture was refluxed under Dean–Stark conditions. The turbid mixture slowly became clear and turned brown when the reaction progressed. After completion of the reaction (3 h, monitored by TLC), the mixture was cooled to ambient temperature and diluted with heptane (150 ml) whereby succinimide precipitated. The obtained suspension was passed through Celite and the Celite bed was washed with heptane/1,2-dichloroethane (50 ml). Removal of solvents in vacuo afforded a thick yellow oil which was immediately purified by flash chromatography (silica gel; heptane/ethyl acetate 19:1) to give pure product (12.1 g, yield 68%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.22 (s, 1H), 6.86 (s, 1H), 2.03 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.7, 147.6, 130.7, 74.89, 10.5. For an alternative synthesis, see: Ref. 31. *4-Methyl-5-oxo-2,5-dihydro-2-furanyl benzoate* (**9a**). To a stirred solution of bromobutenolide (0.48 g, 2.71 mmol) in anhydrous DMF (4 ml) was added solid sodium benzoate (**5**) (0.325 g, 2.26 mmol) under argon and the mixture was set aside at room temperature for 18 h. Sodium benzoate slowly dissolved when the reaction progressed and ultimately a clear solution was obtained. TLC indicated complete consumption of brombutenolide. The mixture was concentrated in vacuo, the residue was treated with a mixture of water (20 ml) and ethyl acetate (2 × 20 ml), and the combined organic layers were washed with water, brine, dried (Na_2SO_4) and concentrated in vacuo. The resultant crude product was crystallized from ethyl acetate/heptane to afford pure **9a** as colorless tiny crystals (0.395 g, 81%). For physical and spectral data, see Ref. 27. *4-Methyl-5-oxo-2,5-dihydro-2-furanyl salicylate* (**9b**). Prepared as described for **9a** starting from sodium salicylate (0.448 g, 2.8 mmol). The crude product was purified by chromatography (silica gel, heptane/ethyl acetate 19:1) to give pure **9b** (0.493 g, 70%). For physical and spectral data, see Ref. 27. *4-Methyl-5-oxo-2,5-dihydro-2-furanyl-4′-methoxybenzoate* (**9c**). To a cooled (−50 °C) and stirred suspension of potassium *tert*-butoxide (0.393 g, 3.5 mmol) in dry DMF (8 ml) a solution of 4-methoxybenzoic acid (0.487 g, 3.2 mmol) in dry DMF(8 ml) was gradually added under argon. After stirring for 30 min. at room temperature, the mixture was cooled (−50 °C) and a solution of bromobutenolide (0.531 g, 3.0 mmol) in DMF (8 ml) was gradually added. Then the mixture was allow warmed to ambient temperature and stirred until all bromobutenolide had reacted (3 h, monitored by TLC, heptane/ethyl acetate 6:4), whereon acetic acid (0.5 ml) was added and the mixture was concentrated in vacuo. The residue was quenched with water (20 ml) and extracted with ethyl acetate (3 × 15 ml). The combined organic layers were washed with saturated $NaHCO_3$, water, brine, dried (Na_2SO_4) and then concentrated in vacuo. The resultant crude product was purified by flash chromatography (silica gel, heptane/ethyl acetate 19:1) to give pure **9c** (0.512 g, 69 % yield), mp 103.2–103.6. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.06 (d, 2H, *J* = 8.4 Hz), 7.11 (m, 1H), 7.02 (m, 1H), 6.93 (m, 2H), 3.87 (s, 3H), 2.03 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.2, 164.4, 164.2, 142.4, 134.4,132.2, 120.6, 113.9, 92.9, 55.5, 10.7. *m*/*z* 248.06833, calc. for $C_{13}H_{12}O_5$: 248.06847. *4-Methyl-5-oxo-2,5-dihydro-2-furanyl-4′-nitrobenzoate* (**9d**). Prepared as described for **9c** starting from *p*-nitrobenzoic acid (0.535 g, 3.2 mmol). Yield 0.648 g, 82%, colorless tiny crystals, mp 141.8–142.1. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.38 (d, 2H, *J* = 8.4 Hz), 8.23 (d, 2H, *J* = 8.4 Hz), 7.13 (s, 1H), 7.06 (s, 1H), 2.06 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.7, 163.0, 151.1, 141.5, 135.1, 133.7, 131.2, 123.7, 93.3, 10.7. *m*/*z* 263.04395, calc. for $C_{12}H_9NO_6$: 263.04432.
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32. *3,4-Dimethyl-5-oxo-2,5-dihydro-2-furanyl-benzoate* (**11a**). To a solution of 5-hydroxy-3,4-dimethylbutenolide[33] (0.385 g, 3.0 mmol) and benzoyl chloride (0.492 g, 3.5 mmol) in dry dichloromethane (10 ml), was added dry pyridine (0.5 ml) and the mixture was stirred at ambient temperature until all the butenolide had reacted (3 h, monitored by TLC, heptane/ethyl acetate 7:3). The mixture was quenched with water, acidified with cold aqueous 1 N HCl and extracted with ethyl acetate (2 × 20 ml). The organic layer was washed with water, brine, dried (Na_2SO_4) and concentrated in vacuo. The resultant crude product was crystallized from ethyl acetate/heptane to yield pure **11a** as a colorless solid (0.512 g, 73%), mp 86.7–86.8 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.06 (m, 2H), 7.49 (m, 1H), 7.45 (m, 2H), 7.00 (s, 1H), 2.04 (s, 3H), 1.92 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.6, 165.0, 153.6, 134.0, 130.0, 128.6, 128.4, 127.2, 94.2, 11.4, 8.6. *m*/*z* 232.07394, calcd for $C_{13}H_{12}O_4$: 232.07356. *3,4-Dimethyl-5-oxo-2,5-dihydro-2-furanyl-4′-methoxybenzoate* (**11b**). Prepared as described for **11a** starting from hydroxybutenolide (0.385 g, 3.0 mmol) and 4-methoxybenzoyl chloride (0.546 g, 3.2 mmol). Yield 0.632 g, 80%, colorless tiny crystals, mp 141.1–141.4. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.01 (d, 2H, *J* = 7.2 Hz), 6.98 (s, 1H), 6.93 (d, 2H, *J* = 7.2 Hz); 73.87 (s, 3H), 2.03 (s, 3H), 1.91 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.7, 164.6, 164.2, 153.7, 132.2, 127.0, 120.6, 113.8, 94.0, 55.5, 11.4, 8.5. *m*/*z* 262.08439, calcd for $C_{14}H_{14}O_5$: 262.08412. *3,4-Dimethyl-5-oxo-2,5-dihydro-2-furanyl-4′-nitrobenzoate* (**11c**). Prepared as described for **11a** starting from hydroxybutenolide (0.385 g, 3.0 mmol) and 4-nitrobenzoyl chloride (0.576 g, 3.1 mmol). Yield 5.34 g, 64%), slightly yellow crystals, mp 145.1–145.3. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.33 (d, 2H, *J* = 7.2 Hz), 8.23 (d, 2H, *J* = 7.2 Hz), 7.00 (s, 1H), 2.06 (s, 3H), 1.94 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.2, 163.3, 152.9, 133.8, 131.7, 131.2, 127.8, 124.1, 123.7, 94.5, 11.5, 8.6. *m*/*z* 277.06022, calcd for $C_{13}H_{11}NO_6$: 277.05864.
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35. It is important to note that the effective contact time of seeds with stimulant solution is much shorter than the half life of the stimulant in water. The stimulant, after the diffusion controlled penetration of into the seed, initiates the germination process ultimately leading to extrusion of a radical. This relatively slow process takes a few days. The germinated seeds are counted.
36. Solutions of mimics **9** and **11** were obtained by dissolving ca 5 mg (0.02 mmol) of stimulant in 25 ml of analytical grade acetone. Of this stock solution 1 ml was diluted with demineralized water in a measuring flask of 25 ml. This solution of 30 μM was further diluted to the indicated test concentrations (Fig. 7). Bioassays were performed using standard protocols.[37] Germinated *Striga* seeds were counted after 48 h of incubation at 30 °C. *Pelipanche* seeds were incubated for 5 days at 25 °C and then germinated seeds were counted. The batch of seeds of *P. ramosa* (Italy) was probably not homogeneous, although no anomalies were observed during visual inspection. At lower concentrations the response was too low for **9a** and **9b**.
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