A new class of conjugated strigolactone analogues with fluorescent properties: synthesis and biological activity†

Chaitali Bhattacharya,[a] Paola Bonfante,[b] Annamaria Deagostino,[c] Yoram Kapulnik,[a] Paolo Larini,‡[c] Ernesto G. Occhiato,[d] Cristina Prandi*[c] and Paolo Venturello[c]

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A new class of strigolactone analogues has been synthesized. They differ from known molecules, both of natural and synthetic origin, in two main features. The conjugated system extends from the enol ether bridge to the A ring, the B ring is a heterocycle while the C ring is a cyclic ketone instead of a γ-lactone. The key step of the synthesis is a Nazarov cyclization on activated substrates. Bioassays using *Orobanche* seeds have revealed that all the molecules strongly stimulate germination; in particular the oxygen containing analogues are the most active. Interestingly, some of the new molecules show fluorescent properties.

Introduction

Strigolactones are a group of sesquiterpene lactones which were isolated more than 40 years ago when, in 1966, Cook *et al.* reported the isolation of (+)-strigol from the root exudates of cotton (*Gossypium hirsutum* L.),[1] and found that they act as a germination stimulant for seeds of the parasitic weeds *Striga* (witchweed) and *Orobanche* (broomrape).[2] In more recent times, the strigolactone 5-deoxy-strigol has been isolated from the root exudates of the legume *Lotus japonicus* and identified as a bioactive molecule that induces an extensive hyphal branching in arbuscular mycorrhizal (AM) fungi which live in symbiosis with many plants.[3] Given the presence of strigolactones in the root exudates of many dicotyledon and monocotyledon species, it appears that these molecules are widespread throughout the soil and play multiple roles in plant biology, regulating the interactions between host plants and AM fungi and between host plants and parasitic weeds. Since the same plant signalling molecules are perceived by both beneficial fungal symbionts and by harmful parasitic weeds, it has been suggested that species of *Striga* exploited a communication system that was already active in the very ancient plant–AM association.[4] Therefore, this offers us an interesting example of the evolution of molecular dialogues between plants and microbes.[5] Recently, it has been proposed that strigolactones act as a new endogenous hormone class controlling shoot branching in a wide range of plants. This discovery further widens the action spectrum of strigolactones in plants thus confirming their function in underground communication.[6] In recent years several efficient stereocontrolled syntheses have been devised due to the renewed interest towards these attractive allelochemicals. Since the first published total syntheses of (*rac*)-strigol in the mid 1970s,[7] several natural[8] and structural analogues have been synthesised (Chart 1). Zwanenburg's group has given a significant contribution to deepening the structure–reactivity insight; in particular, the analogues GR7,[9] GR24[10] and Nijmegen-1[11] exhibit remarkable bioactivity, and at this time GR24 is universally used as a reference compound in bioactivity tests. Recent improvements in the enantioselective syntheses of strigolactones[12] have allowed an efficient evaluation of the biological activity of enantiopure compounds to be made. Synthetic strategies aimed at controlling the configuration of the C-2′ of the D-ring, generally considered to be critical for the germination activity of strigolactones, are particularly worthwhile. On the basis of the biological assays it was discovered that the enantiomer with the same C_2' stereocenter configuration as the natural compounds showed an enhanced activity.[13] A comprehensive set of structure–activity studies enabled Zwanenburg's group to propose a tentative molecular mechanism at the strigol receptor (Scheme 1).[14]

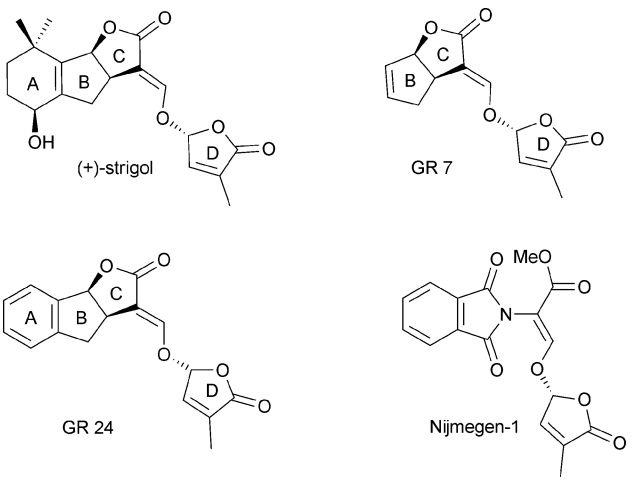

Chart 1 Synthetic analogues of strigolactones

[a]*Department of Agronomy and Natural Resources Inst. of Plant Sciences ARO, the Volcani Center Bet Dagan, 50250, Israel*
[b]*Dipartimento di Biologia Vegetale dell'Università, Istituto per la Protezione delle Piante - CNR Sezione di Torino Viale Mattioli 25, 10125, Torino, Italy*
[c]*Dipartimento di Chimica Generale e Chimica Organica dell'Università, Via Pietro Giuria, 7, I-10125, Torino, Italy. E-mail: cristina.prandi@unito.it.; Fax: +39 01167607642; Tel: +39 0116707641*
[d]*Dipartimento di Chimica Organica "U. Schiff", Università di Firenze, Via della Lastruccia 13, I-50019, Sesto, Fiorentino, Italy*

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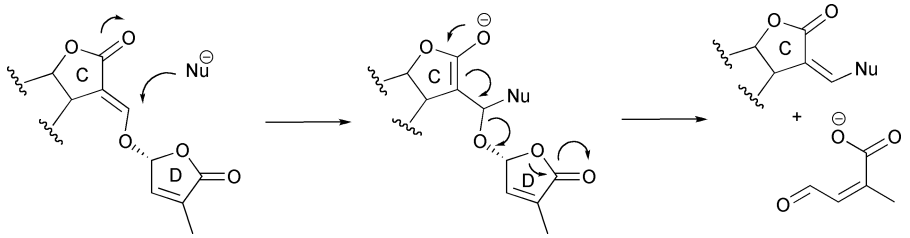
‡ This work has been partially taken from Paolo Larini's PhD thesis.



Scheme 1 Proposed mechanism of binding at the receptor.

The initial binding of the strigolactone at the receptor site is followed by a Michael nucleophilic addition to the enol ether moiety, that leads, through a cascade series of events, to the final opening of ring D. The tricyclic core of the molecule remains covalently bonded to the receptor, thus inducing an irreversible change that was proposed to be the first signal leading to the germination of the parasitic weeds (or to the hyphal branching in AM fungi, Scheme 1). To confirm the hypothesized mechanism, the strigol analogue carba GR24, containing a saturated carbon chain instead of the reactive enol ether bridge between ring C and D, was synthesized and proved to be completely inactive for the stimulation of seed germination.[15] More recently, some imino analogs of strigolactones in which there is no longer an active electrophilic site were prepared and their stimulating activity tested.[16] Surprisingly, in some cases the activity is retained, thus confirming that the issue of which mechanism is active at the receptor site is still open to investigation.

Remarkably, in recent years some biologically active strigolactone analogues functionalized with affinity tags or photoaffinity labels have been designed and synthesized,[17] hence signifying that this important class of molecules could be used in a wider scope as a valuable tool to identify and isolate the strigolactone receptor.

Due to renewed interest in these attractive molecules, we planned to synthesize a new class of analogues according to a design aimed at introducing two elements of innovation. The first is a structural feature, namely the extension of the conjugated system to the ABC framework across a heterocyclic B ring, the second principally concerns the species' reactivity. Actually, in naturally occurring strigolactones and in all major analogues (Fig. 1) the C ring is a lactone in which the carboxylic group is conjugated with the enol-ether bridge that links the C ring with the D ring. With the aforementioned strigolactone action

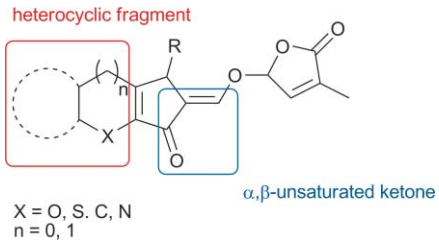


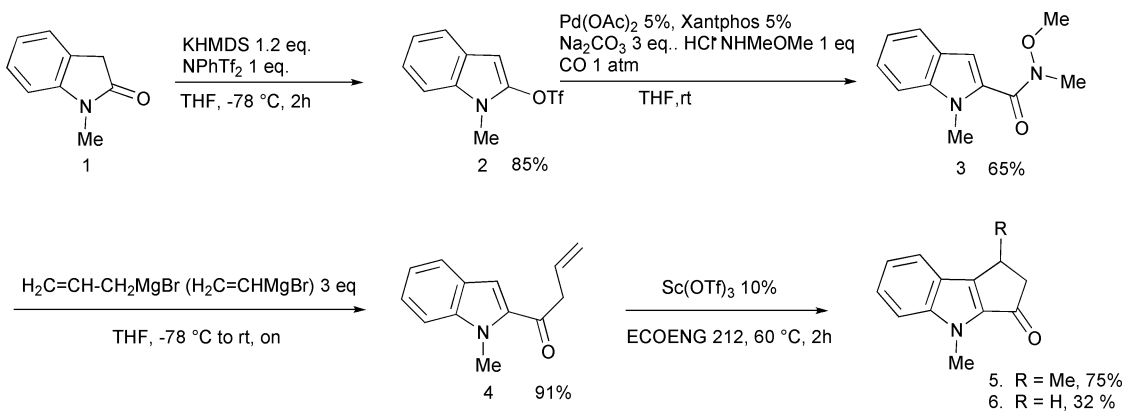
Fig. 1 New class of heterocyclic strigolactone analogues.

mechanism in mind (Scheme 1) together with the grounding that α,β-unsaturated ketones are more reactive towards nucleophiles than α,β-unsaturated esters,[18] we reasoned that the replacement of the carboxyl moiety (present on the C ring) with a carbonyl moiety could have an enhanced effect on biological activity (Fig. 1).[19] This article gives full accounts of the synthesis of a number of the featured analogues and the preliminary results on their germination activity with *Orobanche aegyptiaca* seeds.

Results and discussion

Synthesis

In order to obtain our target compounds we initially focused our attention on setting up a general and feasible synthetic sequence that could eventually be applied to a number of heterocyclic molecules. Moreover, due to the structural diversity of the ABC systems obtainable according to our design, we first decided to perform biological activity tests on diastereomeric or racemic mixtures and thereafter to plan the enantioselective syntheses of the most promising molecules. To this purpose we chose the commercially available 1-methylindolin-2-one (Scheme 2, **1**) as



Scheme 2 Synthesis of the tricyclic core ABC through the Weinreb amide path.

starting material and relied on our past experience in heterocycle derived vinyl triflate chemistry[20] to design the further synthetic steps.

To obtain the core ABC nucleus (Fig. 2), two main routes have been envisaged, and the first one is depicted in Scheme 2. The first synthetic step consists of the generation of triflate **2** starting from indanone **1**. Derivative **2** is quite stable: it can be purified by column chromatography and then coupled under carbonylative conditions in the presence of methyl methoxy amine hydrochloride according to a reliable procedure which has recently been set up in our laboratory,[21] thus obtaining the corresponding Weinreb amide **3** in 65% yield after purification. The Weinreb amide can be successfully coupled with allyl magnesium bromide affording dienone **4** which possesses the suitable electronic arrangement to undergo an acid catalyzed Nazarov reaction. The Nazarov reaction is here exploited to build up the C ring of the final structure and is the key step in our synthetic sequence. It has been previously employed in the construction of the B ring by Zwanenburg.[22,8c,8d] In the present case we have dedicated some effort to trying innovative experimental conditions in order to create milder conditions and higher reaction yields. The use of ionic liquids, in this case 1-ethyl-3-methyl-imidazolium ethylsulfate (ECOENG 212) proved itself able to enhance the reaction rate probably because the cationic intermediate arising from the conrotatory 4π cyclization benefits from a strong polar medium. The cyclization proceeds through the preliminary isomerization of the terminal double bond to afford the tricyclic structure **5** in 75% yield after purification. In addition, we reacted the Weinreb amide **3** with vinyl magnesium bromide but unfortunately the corresponding divinyl ketone undergoes Nazarov cyclization with a moderate yield (32%) due to the formation of polymerized byproducts. Moreover, the core of our target molecules can be obtained resorting to a further synthetic route. As shown in Scheme 3, a simple and mild Suzuki coupling between triflate **2** and dienyl boronates **9** or **10**[23] afforded indolyl functionalized dienes **11** or **12** that are suitable to undergo a Nazarov cyclization under extremely mild conditions.

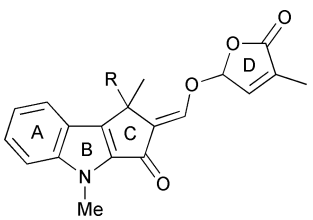
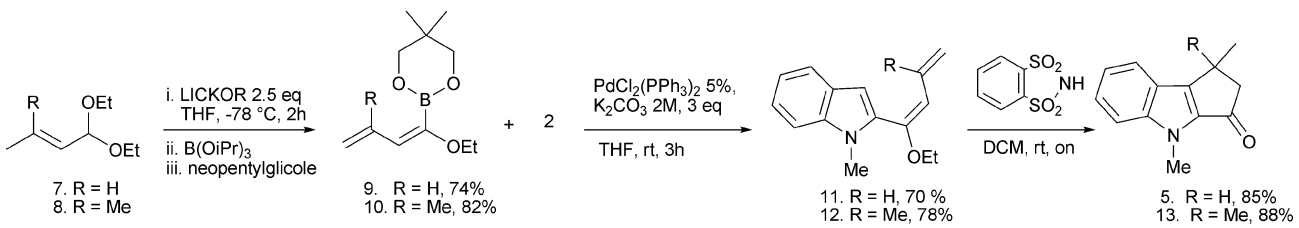


Fig. 2 Indolyl derivative analogues.

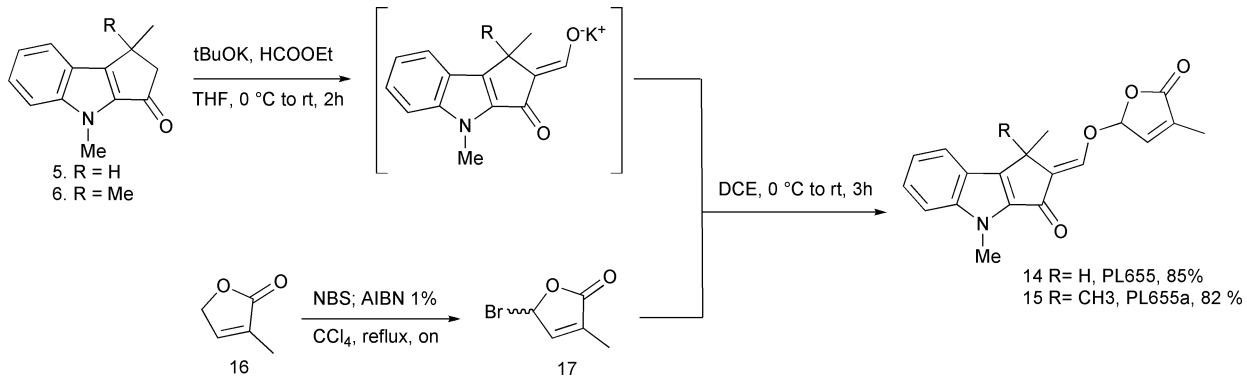
Actually, when the reaction is carried out with a catalytic amount of *o*-benzenedisulfonimide the cyclic products **5** and **13** were obtained in excellent yields. The syntheses were completed by a slight modification of the well known procedure used to link the D ring to the ABC nucleus (see Experimental Section). Thus, the potassium enolate of **5** or **13** was treated with ethyl formate and successively with racemic bromofuranone **17**. This was in turn obtained resorting to an allylic bromination on butenolide **16** in an almost quantitative yield.[24] The one step procedure afforded (±) **14** as an inseparable mixture of diastereoisomers and (±) **15**, in excellent yields after purification. The *E* stereochemistry of the enol ether double bond was determined by NOESY experiments, and it is consistent with the literature data.[5] Afterwards, the same synthetic sequence was applied to the syntheses of the strigolactone heterocycle analogues depicted in Chart 2. The carba derivative PLC655 was obtained starting from commercial 5-methyl-3,4-dihydronaphthalen-1(2*H*)-one. Besides, we were guided in the design of the molecules, in which the A ring is missing, by the thought that this part of the molecule is the furthest removed from the bioactiphore in the receptor site, indeed the literature data strongly support this assertion and significant variations on the A ring are generally well tolerated without affecting the biological activity. Compound GR7, a strigolactone with no A-ring is in fact one of the most potent analogues.[8a,8b] In Scheme 5, the synthetic sequence leading to bicyclic derivatives is represented. In each case we choose to start from easily available starting products. So, in a typical procedure δ-valero lactam previously protected on nitrogen **18**, δ-valero lactone **19** or thiolactone **20** were converted into the corresponding triflates **21–23** and thus coupled with the dienyl boronate **9**. The coupling products **24–26** were then subjected to a Nazarov cyclization with Amberlyst to afford the bicyclic systems **27–29** that in turn were reacted with racemic bromo butenolide **17**. For all the products, the synthetic sequence represented in Scheme 2, passing through the Weinreb amide, can be applied as well. Hence, the aza- **30**, oxy- **31** and thio- **32** derivatives of the PL65 series have been obtained in 20, 65 and 71% overall yield respectively as diastereomeric mixtures.[25]

Biological activity

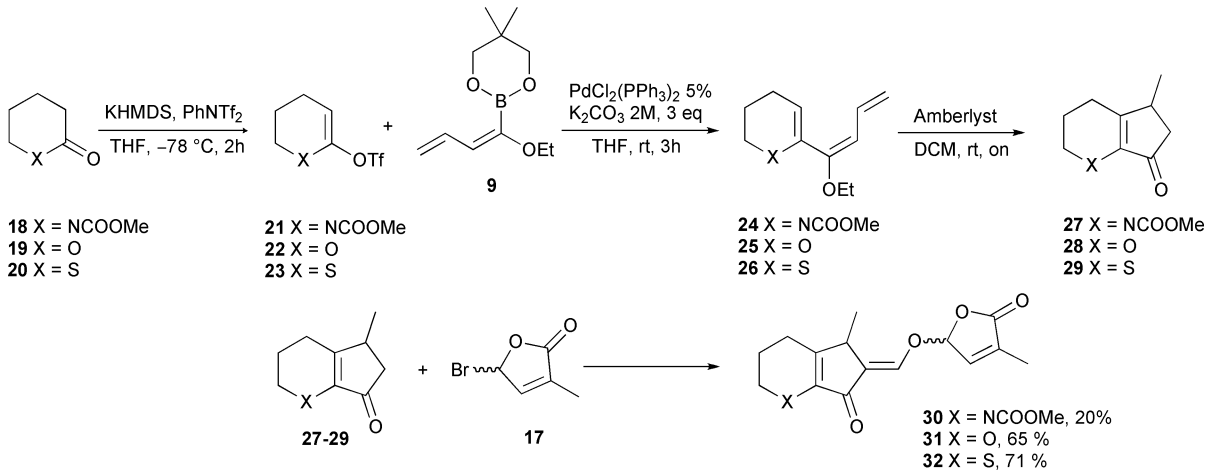
The stimulation activity of the PL series analogues was tested using *Orobanche aegyptiaca* seeds. In all the germination assays, the germination rate was analyzed under *in vitro* conditions after one week of incubation in the presence of the tested compound and in three independent experiments performed on 50–70 seeds. A diastereomeric mixture of GR24 was always included as a positive control and an aqueous solution of 0.1% acetone was included as negative control. The feature that characterizes all these new molecules is mainly the fact that, with respect to the natural and to the synthesized analogues so far, the C ring is a ketone instead of the more familiar lactone and the conjugation extends from



Scheme 3 Synthesis of the tricyclic core ABC through the dienyl boronate path.



Scheme 4 Linkage of the butenolide to the tricyclic core.



Scheme 5 Synthesis of PLN65, PLO65 and PLS65.

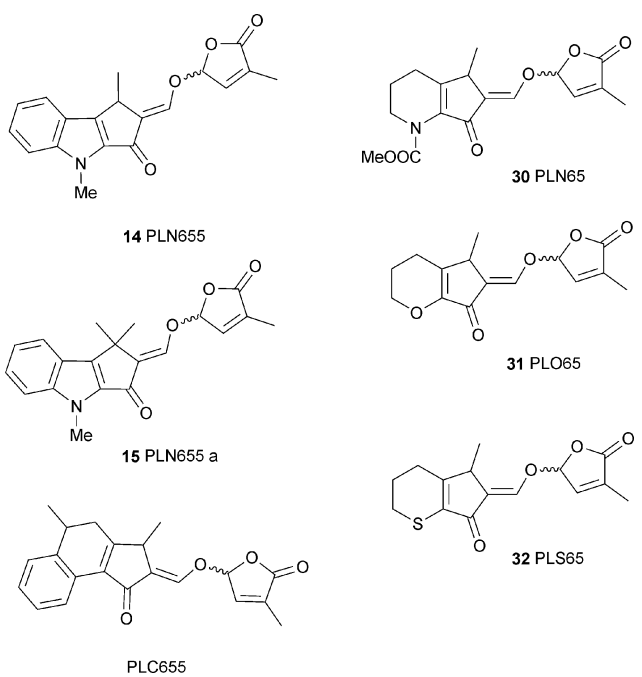


Chart 2 PL series of strigolactone analogues

the enol ether bridge to the A (PLN655, PLN655a, PLC655) or B ring (PLN65, PLO65, PLS65). Moreover, the B ring is a five or six membered cycle, which is in turn an aza-, carba-, oxa- or thio-derivative.

Moreover, in three cases (PLN65, PLO65, PLS65) the A ring is missing. When present (PLN655, PLN655a, PLC655) it is an aromatic ring. As can be deduced from the data reported in Table 2 and in Fig. 3, the efficiency of the PL series molecules (PLN655, PLC655, PLO65, and PLS65) is generally higher than the reference compound GR24.[26] These data could be better highlighted considering the ED_{50} parameter (Table 1), that is to say the concentration that induces one-half the maximal response. Among the examined compounds PLO is the most effective molecule, there is a marginal difference between PLC655 and PLN665, while PLS

Table 1 The ED_{50} (median efficiency dose) values

Entry	Compound	ED_{50} Values
1	PLO65	3.14×10^{-10}
2	PLN655	1.15×10^{-9}
3	PLC655	6.94×10^{-9}
4	PLS65	1.73×10^{-8}
6	GR24	2.15×10^{-7}

Table 2 Germination percentages for seeds of *Orobanche aegyptiaca* after exposure to solutions of the strigolactone analogues PLN655, PLC655, PLO65, PLS65 relative to the control GR24[a]

		% germination ± SE at a concentration of					
Entry	Compound	10^{-4}mol L^{-1}	10^{-6}mol L^{-1}	10^{-8}mol L^{-1}	10^{-10}mol L^{-1}	10^{-12}mol L^{-1}	10^{-14}mol L^{-1}
1	PLN655	89.1 ± 1.4	83.3 ± 5.6	81.2 ± 2.4	28 ± 5.5	7.5 ± 2.3	6.9 ± 2.1
2	PLC655	76.9 ± 2.7	87.0 ± 4.3	57.2 ± 3.1	8.6 ± 0.3	9.2 ± 0.7	5.3 ± 1.5
3	PLO65	96.2 ± 0.4	80.8 ± 4.5	77.4 ± 3.4	40.2 ± 7.4	23.4 ± 5.5	10.6 ± 1.9
4	PLS65	93.3 ± 2.3	84.2 ± 5.6	52.2 ± 4.1	14.0 ± 4.7	—	—
5	GR24[b]	82.2 ± 2.7	70.0 ± 5.2	17.2 ± 4.5	3.7 ± 2.3	8.1 ± 1.8	—
6	Water	3.09 ± 1.83					

[a] Data presented the average mean ± of SE of four experiments. [b] Equimolar mixture of two racemic diastereoisomers.

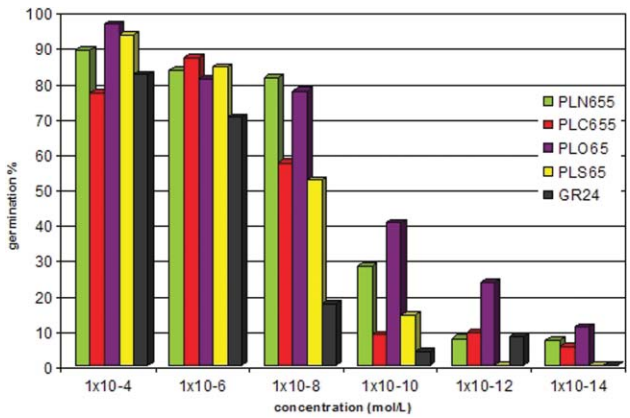


Fig. 3 Bar representation of the germination percentages for seeds of *Orobanche aegyptiaca* after exposure to different concentration of PL series molecules.

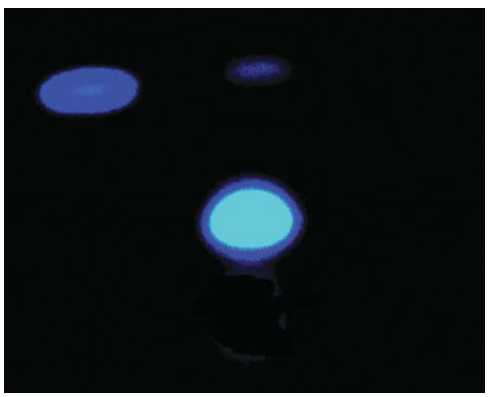


Fig. 4 TLC plate showing the progress of reaction depicted in Scheme 4.

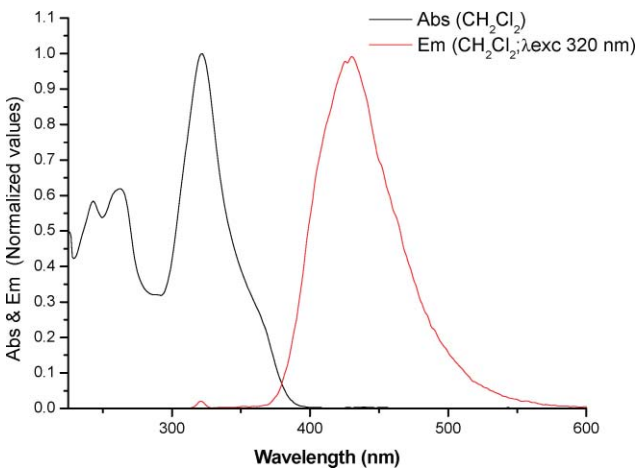


Fig. 5 Absorption (black) and emission (red) spectra of PLN655a in DCM at 25 °C.

seems to be less reactive, even if the germination activity remains quite interesting with respect to GR24. If some general outlines could be drawn from these preliminary experiments, we could conclude that the change in functional group at the reactive site of the molecule has been completely effective.

In any case, a heteroatomic B ring or the presence of the A ring seems to be less important, thus confirming the literature data. It is worth emphasizing at this stage of our studies that all the molecules have been used in the biological assays as racemic or diastereomeric mixtures of racemic compounds, so that it could be hypothesized that the effectiveness of the enantiopure molecules could be even greater.

In the course of the syntheses we realized that a number of the prepared molecules showed a luminescent behaviour when subjected to UV radiation (360 nm, Fig. 4). In view of possible future exploitation in the field of bioimaging investigations, a preliminary study of the fluorescent properties of **13** and **15** has been undertaken. The exploitation of bioactive molecules with a "built in label" would avoid the use of external fluorescent probes whose introduction could presumably affect the bioactivity.

The absorption and emission spectra for compound PLN655a are reported in Fig. 5. As can be observed, the absorption spectrum shows a maximum at 320 nm while the fluorescent emission spectrum exhibits a peak at 425 nm, thus indicating a wide Stokes' shift.

The fluorescent quantum yield (Φ_f) and lifetimes (τ) have been determined in dichloromethane at 25 °C upon excitation at the selected wavelengths. The results are reported in Table 3.

Table 3 Selected photochemical properties

Entry	Sample	λ_{ex}/nm	λ_{em}/nm	Φ_f[a]	τ_1/ns	τ_2/ns
1	**14** (DCM)	320	425	0.04	0.66 (97.5%)	4.18 (2.5%)
2	**5** (DCM)	300	380	0.01	0.64 (75.5%)	1.57 (24.5%)

[a] Fluorescence quantum yields (Φ_f) were determined at 25 °C, using both rhodamine and anthracene as the standards, upon selection of λ_{ex} and λ_{em} (maximum of emission) as the excitation and emission wavelengths.

The fluorescence intensity decay for the sample was reasonably fitted to a biexponential, indicating emission from a singlet excited state in each case.

Conclusions

In conclusion, in this article we reported the synthesis of a number of strigolactone analogues, featuring an unprecedented extended conjugated system. Moreover, what is hypothesized to be the reactive site of the molecule has been changed from a common α,β-unsaturated lactone to an α,β-unsaturated cyclic ketone. These compounds have been tested in some preliminary bioassays towards parasitic plants seeds (*Orobanche aegyptiaca*) as germinating factors. These tests have shown that all the molecules possess remarkable activity and. From these, PLO65 is clearly the most active. Further perspectives will involve the synthesis of enantiopure versions of the most active strigolactone analogues. Finally, due to the interesting luminescent properties of some of these molecules, the design and synthesis of similar fluorescent analogues will be undertaken in order to exploit them in bioimaging studies.

Experimental

General remarks

Chromatographic separations were carried out on silica gel using flash-column techniques; R_f values refer to TLC carried out on 0.25 mm silica gel plates (Merck F254), with the same eluent indicated for the column chromatography. ^{1}H NMR spectra and NOESY 2D experiments were recorded at 200 MHz, ^{13}C NMR spectra at 50 MHz. MS spectra were recorded at an ionizing voltage of 70 eV. THF was distilled from Na/benzophenone. Triflates **2**, **21–23**, boronates **9**, **10** ethoxydienyl heterocycles **24–26** and bicycles **27–29** have been prepared as previously reported.[18,19]

Synthesis

***N*-Methoxy-*N*,1-dimethyl-1*H*-indole-2-carboxamide (3).** An oven-dried three necked round bottomed flask with one tap was equipped with a magnetic stir bar and was filled with argon. All solid reagents were added by briefly removing the rubber septum under a flow of argon: $Pd(OAc)_2$ (0.02 equiv., 0.02 mmol, 4.50 mg), Xantphos (0.02 equiv., 0.02 mmol, 5.8 mg), *N*-methoxy-*N*-methyl amine hydrochloride (1 equiv., 1 mmol, 97 mg), Na_2CO_3 (3 equiv. 3 mmol, 318 mg) and THF (10 mL). Then, the reaction was purged for *ca*. 10 min with CO(g). A solution of triflate **2** (1 equiv., 1 mmol, 279 mg) in 3 mL of THF was then added. A balloon filled with CO(g) was connected to the reaction vessel and the reaction mixture was stirred to rt until the triflate was completely consumed as judged by TLC analysis. The reaction mixture was then diluted with EtOAc (*.ca* 10 mL), filtered through a celite plug (eluting with ethyl acetate) and concentrated under reduced pressure. The crude material obtained was purified by flash chromatography with petroleum ether–EtOAc 1 : 1 ($R_f = 0.22$), 142 mg (65%) of a yellow oil was recovered ^{1}H NMR (200 MHz, $CDCl_3$): ppm 7.78 (d, $J = 8.2$ Hz, 1H), 7.46 (m, 2H), 7.28 (m, 2H), 3.95 (s, 3H), 3.68 (s, 3H), 3.45 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 162.8 (s), 138.3 (s), 129.5 (s), 126.2 (d), 123.9 (s), 121.9 (d), 120.1 (d), 109.8 (d), 106.9 (d) 61.2 (q), 33.8 (q), 31.6 (q); MS (*m*/*z*): 218 (M^+, 32), 158 (100), 130 (17), 89 (40). Anal. Calcd for $C_{12}H_{14}N_2O_2$: C, 66.04; H, 6.47; N, 12.84; found C, 66.16; H, 6.45; N, 12.76%.

1-(1-Methyl-1*H*-indol-2-yl)prop-2-en-1-one (4). An oven-dried Schlenk flask equipped with a magnetic stir bar was flame dried and kept under an argon atmosphere. A solution of allylmagnesium bromide (2 mmol) in anhydrous THF (5 mL) was introduced and refrigerated to −78 °C with an acetone–liquid nitrogen bath. A solution of the Weinreb amide 3 (1 mmol, 218 mg) was then slowly added. The reaction mixture was stirred and, if necessary the temperature was progressively raised to rt until the amide was completely consumed as judged by TLC analysis. The reaction mixture was then diluted with ethyl acetate (~10 mL), washed twice with brine, the organic layers were then dried over sodium sulfate and concentrated under reduced pressure. The crude materials so obtained were judged pure enough to be directly used in the successive cyclization reaction. We obtained 181 mg (91%) of a yellow oil ^{1}H NMR (200 MHz, $CDCl_3$): ppm 7.78 (d, $J = 8.2$ Hz, 1H), 7.46—7.15 (m, 4H), 6.25 (m, 1H), 5.65 (m, 1H), 5.60 (m, 1H), 4.15 (s, 3H), 3.75 (d, $J = 5.5$. Hz, 2H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 191.6 (s), 140.0 (s), 134.1 (s), 131.3 (s), 125.8 (d), 125.6 (d), 122.7 (d), 120.6 (d), 118.5 (t), 111.5 (d), 110.2 (d), 44.8 (q), 32.0 (t). MS (*m*/*z*): 199 (M^+, 100), 182 (35), 167 (19), 130 (28). Anal. Calcd for $C_{13}H_{13}NO$: C, 78.36; H, 6.58; N, 7.03; found C, 78.36; H, 6.52; N, 7.06%.

General procedure for the synthesis of 11, 12, 24, 25, and 26. To a solution of the corresponding crude triflate (1.0 mmol) in THF (10 mL) were added, under a nitrogen atmosphere, $(Ph_3P)_2PdCl_2$ (35 mg, 0.05 mmol), (*E*)-2-(1-ethoxybuta-1,3-dienyl)-5,5-dimethyl-1,3,2-dioxaborinane **9** or (*E*)-2-(1-ethoxy-3-methylbuta-1,3-dienyl)-5,5-dimethyl-1,3,2-dioxaborinane **10** (1.0 mmol), and a 2 M aqueous K_2CO_3 solution (1 mL). The mixture was stirred for 3 h at room temperature. H_2O (25 mL) was then added, the mixture extracted with Et_2O (3 × 20 mL) and dried over anhydrous Na_2CO_3. Evaporation of the solvent afforded a yellow oil which was purified by chromatography.

*(*E*)-2-(1-Ethoxybuta-1,3-dienyl)-1-methyl-1H-indole (**11**).* (EtOAc–petroleum ether, 1 : 4, 1% Et_3N, R_f 0.80) 159 mg, (70%) as a yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.6 (d, $J = 5.2$ Hz, 1 H), 7.39–6.90 (m, 3H), 6.62 (s, 1H), 6.55 (dt, $J = 16.1$, 10.5 Hz, 1H), 5.9 (d, $J = 10.5$ Hz, 1H), 5.35 (dd, $J = 16.1$, 1.8 Hz, 1H), 4.90 (dd, $J = 10.5$, 1.8 Hz, 1H), 3.85 (q, $J = 6.5$ Hz, 2 H), 3.75 (s, 3 H), 1.26 (t, $J = 6.5$ Hz, 3 H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 147.4 (s), 139.8 (s), 137.1 (s), 134.5 (s), 122.0 (d), 121.8 (d), 120.9 (d), 119.5 (d), 114.8 (t), 111.4 (d), 109.4 (d), 104.1 (d), 63.5 (t), 30.1 (q), 14.7 (q). MS *m*/*z* 227 (M+, 35), 212 (100), 197 (80), 89 (40). Anal. Calcd for $C_{15}H_{17}NO$: C, 79.26; H, 7.54; N, 6.16; Found C, 79.29; H, 7.51; N, 6.11%.

*(*E*)-2-(1-Ethoxy-3-methylbuta-1,3-dienyl)-1-methyl-1H-indole (**12**).* (EtOAc–petroleum ether, 1 : 4, 1% Et_3N, R_f 0.80) 188 mg, (78%) as a yellow oil.^{1}H NMR (200 MHz, $CDCl_3$): δ 7.6 (d, $J = 5.2$ Hz, 1 H), 7.39–6.90 (m, 3H), 6.62 (s, 1H), 5.9 (t, $J = 1.8$ Hz, 1H), 4.8 (br s, 2H), 3.85 (q, $J = 6.5$ Hz, 2 H), 3.75 (s, 3 H), 1.75 (d, $J = 1.8$ Hz, 3H), 1.26 (t, $J = 6.5$ Hz, 3 H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 147.4 (s), 139.8 (s), 137.1 (s), 134.5 (s), 127.2 (s), 122.0 (d), 120.9 (d), 119.5 (d), 114.8 (t), 111.4 (d), 109.4 (d), 104.1 (d), 63.5 (t), 30.1 (q), 21.7 (q), 14.7 (q). MS *m*/*z* 241 (M^+, 55), 212 (100), 197 (80), 89 (40). Anal. Calcd for $C_{16}H_{19}NO$: C, 79.63; H, 7.94; N, 5.80. Found C, 79.79; H, 7.91; N, 5.91%.

General procedure for the synthesis of 5, 13. To a solution of **11** or **12** (1 mmol) in 2 mL of DCE *o*-benzenedisulfonimide was added (30% mol) and the reaction mixture was stirred in an open air vessel until at 80 °C TLC and GC analyses showed no further reaction progress. The crude reaction mixture was treated with Et_2O–H_2O (1 : 1, 20 mL) and the aqueous phase extracted with Et_2O (20 mL); combined organic extracts were dried over anhydrous Na_2CO_3. Evaporation of the solvent afforded the crude products **5** and **13**, which were purified by flash chromatography (Et_2O–petroleum ether 1 : 4).

*1,4-Dimethyl-1,2-dihydrocyclopenta[b]indol-3(4H)-one (**5**).* (EtOAc–petroleum ether, 1 : 2, 1% Et_3N, R_f 0.7) 169 mg, 85% as a yellow solid. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.65 (d, $J = 8.1$ Hz, 1 H), 7.45–7.25 (m, 2 H), 7.15 (t, $J = 5.1$ Hz, 1H), 3.85 (s, 3 H), 3.55 (m 1H), 3.26 (dd, $J = 10.8$, 2 Hz, 1H), 2.45 (d, $J = 10.8$ Hz, 1H), 1.55 (d, $J = 6.5$ Hz, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 194.1 (s), 149.2 (s), 144.7 (s), 137.9 (s), 126.4 (d), 122.4 (s), 121.7 (d), 120.0 (d), 110.8 (d), 50.5 (t), 29.8 (d), 27.8 (q), 20.9 (q); MS *m/z* 199 (M^+, 100), 184 (45), 131 (28), 89 (58). Anal. Calcd for $C_{13}H_{13}NO$: C, 78.36; H, 6.58; N, 7.03; O, 8.03; Found: C, 78.54; H, 6.51; N, 7.05%. IR (CCl_4) 2975, 2912, 2856, 1689, 1349, 1152, 1076 cm^{-1}. Mp 81–83 °C.

*1,1,4-Trimethyl-1,2-dihydrocyclopenta[b]indol-3(4H)-one (**13**).* (EtOAc–petroleum ether, 1 : 4, 1% Et_3N, R_f 0.80) 140 mg, (88%) as a yellow solid. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.65 (d, $J = 8.1$ Hz, 1 H), 7.45–7.15 (m, 3 H), 3.85 (s, 3 H), 2.80 (s, 2H), 1.55 (s, 6 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 194.1 (s), 153.0 (s), 145.0 (s), 137.2 (s), 126.7 (d), 122.2 (d), 121.9 (s), 120.3 (d), 111.3 (d), 58.5 (t), 35.5 (s), 30.3 (q), 29.4 (q); MS *m/z* 213 (M^+, 28), 198 (100), 154 (18), 115 (17), 77 (20). Anal. Calcd for $C_{14}H_{15}NO$: C, 78.84; H, 7.09; N, 6.57. Found: C, 78.74; H, 7.07; N, 6.45%. IR (CCl_4) 2977, 2934, 2866, 1690, 1349, 1152, 1076 cm^{-1}. Mp 86–88 °C.

General procedure for the synthesis of sorgolactone analogues 14, 15, 30, 31, 32. To a cooled (0 °C) and stirred solution of the appropriate ketone (1 mmol) in anhydrous THF (10 mL) and under a stream of argon were added 10 equiv of ethyl formate (10 mmol, 740 mg) and 1.2 equiv of potassium *tert*-butoxide (1.2 mmol, 134 mg). The reaction was stirred until a TLC control showed the disappearance of the starting material (usually after 3 hours at room temperature). At this point, THF was removed using a stream of argon gas, and the thus-obtained formylated potassium salt was dissolved in DME (10 mL) and refrigerated at 0 °C. Then, a solution of racemic bromobutenolide **17** (1 mmol, 176 mg) in THF (3 mL) was added. The reaction was then stirred overnight at room temperature. The mixture was quenched with H_2O and diluted with ethyl acetate. The aqueous phase was extracted twice with ethyl acetate and the combined organic layers were washed with brine (2 times), dried over K_2CO_3 and concentrated under reduced pressure. The crude products were purified by flash chromatography.

*(E)-1,4-Dimethyl-2-((4-methyl-5-oxo-2,5-dihydrofuran-2-yloxy)methylene)-1,2-dihydrocyclopenta[b]indol-3(4H)-one (**14**).* mixture of diastereoisomers (1 : 1): EtOAc–petroleum ether, 1 : 1, 1% Et_3N, R_f 0.25) 274 mg, (85%) as a yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.85 (d, $J = 5.2$ Hz, 1H), 7.40 (s, 3H), 7.15 (t, $J = 5.2$ Hz, 1H), 6.95 (s, 1H), 6.20 (s, 1H), 4.20–4,10 (m, 1H), 3.95 (s, 3H), 2.05 (s, 3H), 1.55 (two d superimposed, $J = 4.5$ Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 182.7 (s), 170.5 (s), 145.9 (d), 144,3 (d), 142.8 (d), 139,4 (s), 135.4 (s), 129.4 (s), 129.3 (s), 126.3 (d), 122.0 (s), 121.5 (d), 120.2 (d), 110. 8 (d), 100.6 (d), 30.6 (d), 30.0 (q), 18.4 (q), 10.6 (q). MS *m/z* 323 (M^+, 23), 226 (57), 81 (50), 69 (100). IR (CCl_4) 2985, 2915, 2856, 1782, 1693, 1640, 1493, 1349, 1162, 1001 cm^{-1}. Anal. Calcd for $C_{19}H_{17}NO_4$:C, 70.58; H, 5.30; N, 4.33; Found C, 70.49; H, 5.31; N, 4.33%.

*(E)-1,1,4-Trimethyl-2-((4-methyl-5-oxo-2,5-dihydrofuran-2-yloxy)methylene)-1,2-dihydrocyclopenta[b]indol-3(4H)-one (**15**).* EtOAc–petroleum ether, 1 : 1, 1% Et_3N, R_f 0.25) 276 mg, (82%) as a yellow solid. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.85 (d, $J = 5.2$ Hz, 1H), 7.40 (s, 3H), 7.15 (t, $J = 5.2$ Hz, 1H), 6.95 (br s, 1H), 6.20 (br s, 1H), 3.95 (s, 3H), 2.10 (s, 3H), 1.60 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 182.4 (s), 170.4 (s), 147.7 (d), 145.3 (s), 144.4 (s), 141.2 (d), 138.0 (s), 135.4 (s), 133.5 (s), 126.3 (d), 121.6 (d), 121.1 (d), 121.1 (s), 110.9 (d), 100.7 (d), 38.4 (s), 30.0 (q), 25.9 (q), 10.6 (q). MS *m/z* 337 (M^+, 19), 241 (55), 96 (35), 69 (100). IR (CCl_4) 2978, 2905, 2876, 1797, 1695, 1655, 1495, 1340, 1152, 1051 cm^{-1}. Anal. Calcd for $C_{20}H_{19}NO_4$: C, 71.20; H, 5.68; N, 4.15; Found C, 71.28; H, 5.63; N, 4.23%. Mp 115–117 °C.

*(E)-Methyl 5-methyl-6-((4-methyl-5-oxo-2,5-dihydrofuran-2-yloxy)methylene)-7-oxo-2,3,4,5,6,7-hexahydro-1H-cyclopenta-[b]pyridine-1-carboxylate (**30**).* Mixture of diastereoisomers (1 : 1): EtOAc–petroleum ether, 1 : 1, 1% Et_3N, R_f 0.30 to give 66 mg, (20%) as a yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.31 (s, 1H), 6.94 (br s, 1H), 6.15 (br s, 1H), 3.75 (t, $J = 3.5$ Hz, 2H), 3.95 (s, 3H), 3.3 (q, $J = 3.1$ Hz, 1H), 2.21 (m, 2H), 2.05 (s, 3H), 1.75 (m, 2H), 1.20 (two d superimposed, $J = 4.5$ Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 185.3 (s), 171.9 (s), 170.3 (d), 149.2 (s), 147.3 (d), 142. 2 (s), 133.3 (s), 131.4 (s), 115. 3 (s), 94.4 (d), 52.3 (s), 43.1 (t), 24.2 (t), 22.1 (t), 21.3 (d), 19.4 (q), 15.2 (q). MS *m/z* 333 (M^+, 9), 274 (43), 161 (35), 113 (15), 69 (100). IR (CCl_4) 2985, 2915, 2856, 1773, 1695, 1625, 1487, 1351, 1160, 998 cm^{-1}. Anal. Calcd for $C_{17}H_{19}NO_6$: C, 61.25; H, 5.75; N, 4.20; Found C, 61.39; H, 5.71; N, 4.33%.

*(E)-5-Methyl-6-((4-methyl-5-oxo-2,5-dihydrofuran-2-yloxy)-methylene)-3,4,5,6-tetrahydrocyclopenta[b]pyran-7(2H)-one (**31**).* Mixture of diastereoisomers (1 : 1): EtOAc–petroleum ether, 1 : 1, 1% Et_3N, R_f 0.38 to give 179 mg, (65%) as a yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.31 (s, 1H), 6.94 (br s, 1H), 6.15 (br s, 1H), 4.2 (m, 2H), 3.25 (m, 1H), 2.45-2.15 (m, 2H), 2.05 (s, 3H, superimposed on m, 2H), 1.25 (two d superimposed, $J = 4.5$ Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 187.9 (s), 170.4 (s), 151.4 (s), 145.5 (s), 142.4 (s), 141.0 (d), 135.6 (d), 121.3 (s), 100.5 (d), 66.7 (t), 34.4 (d), 21.4 (t), 20.8 (t), 16.3 (q), 10.6 (q). MS *m/z* 276 (M^+, 2), 179 (100), 151 (22), 123 (18), 97 (16), 69(19). IR (CCl_4): 2928, 1780, 1700, 1649, 1591, 1346, 1178, 1135, 990 cm^{-1}. Anal. Calcd for $C_{15}H_{16}O_5$: C, 65.21; H, 5.84; Found C, 65.24; H, 5.81%.

*(E)-3-Methyl-5-((5-methyl-7-oxo-3,4-dihydrocyclopenta[b]-thiopyran-6(2H,5H,7H)-ylidene)methoxy)furan-2(5H)-one (**32**).* Mixture of diastereoisomers (1 : 1): EtOAc–petroleum ether, 1 : 1, 1% Et_3N, R_f 0.41 to give 207 mg, (71%) as a yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.31 (s, 1H), 6.93 (br s, 1H), 6.14 (br s, 1H), 3.3 (m, 1H), 2.95 (t, $J = 4.5$ Hz, 2H), 2.55–2.35 (m, 2H), 2.05 (s, 3H, superimposed on m, 2H), 1.25 (two d superimposed, $J = 3.5$ Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 191.9 (s), 170.4 (s), 160.8 (s), 145.8 (d), 141.1 (d), 135.4 (s), 133.8 (s), 121.3 (s), 100.6 (d), 40.1 (d), 25.4 (t), 24.4 (t), 22.1 (t), 15.9 (q), 10.5 (q). MS *m/z* 292 (M^+, 2), 263 (100), 235 (68), 193 (85), 165 (32), 137 (16). IR (CCl_4) 2928, 1780, 1700, 1649, 1591, 1346, 1178, 1135,

990 cm^{-1}. Anal. Calcd for C, 61.62; H, 5.52; S, 10.97; Found C, 61.53; H, 5.49; S, 10.95%.

(E)-5-((3,5-Dimethyl-1-oxo-4,5-dihydro-1H-cyclopenta[a]-naphthalen-2(3H)-ylidene)methoxy)-3-methylfuran-2(5H)-one (PLC655). Mixture of diastereoisomers: EtOAc–petroleum ether, 1 : 1, 1% Et_3N, R_f 0.55 to give 129 mg, (42%) as a yellow oil. ^1H NMR (200 MHz, $CDCl_3$): δ 8.05 (d, $J = 5.5$, 1H), 7.65–7.55 (m, 3H), 7.32 (s, 1H), 6.98 (br s, 1H), 6.18 (br s, 1H),), 2.05–1.95 (m, 2H), 2.05 (s, 3H), 1.55 (m, 2H), 1.25 (two d superimposed, $J = 4.5$ Hz, 3H), 0.95 (d, $J = 4.5$ Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 193.8 (s), 156.8 (s), 147.4 (s), 143.3 (s), 141.6 (d), 138.5 (d), 131.7 (s), 129.4 (d), 128.3 (s), 126.3 (s), 124.1 (d), 122.8 (d), 100.7 (s), 35.07 (d), 25.4 (d), 24.4 (t), 22.1 (q), 15.9 (q), 15.5 (q), 10.5 (q). MS *m/z* 308 (M^+, 2), 263 (100), 235 (68), 193 (85), 165 (32), 137 (16). Anal. Calcd for $C_{21}H_{20}O_4$: C, 74.98; H, 5.99; Found C, 74.45; H, 5.68%.

Bioassays

Plant material. Seeds of *Orobanche aegyptiaca* were collected from field grown tomato in the West Galilee region of Israel. The seeds were stored in glass vials in the dark at room temperature until use in germination tests. Preparation of test solutions: the compound to be tested was weighted out very accurately and dissolved in 1 mL of MeOH and then diluted with demineralized water to reach the desired concentrations. All solutions were prepared just before use.

All bioassays were performed at the Department of Agronomy and Natural Resources of Plant Sciences Institute, ARO, the Volcani Center Bet Dagan, 50250, Israel. For surface sterilization all seeds were exposed for 5 min to 50% (v/v) aqueous solutions of commercial bleach (2% hypochlorite). Subsequently, the seeds were thoroughly rinsed with demineralized water and air-dried. For preconditioning the seeds were spread on a glass fiber filter paper disk (9 mm diameter, approximately 50–70 seeds per disk). These discs were placed on a filter paper, wetted with demineralized water in Petri dishes, and stored at 25 °C in the dark for 6 days. Thereafter, the preconditioned seeds were placed in a new Petri plate and were allowed to dry completely in the laminar flow. These seeds were then subjected to the test solution, for each treatment three replicates were taken into account. Synthetic strigolactone GR24 and an aqueous solution of 0.1% acetone was included as positive and negative control, respectively. After 7 days the germination percentage of these seeds was calculated using a binocular microscope. Seeds were considered to be germinated if the radicle protruded through the seed coat.

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