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Synthesis and biological evaluation of potential substrates for the isolation of the strigol receptor

J an W illem J . F. Thuring, Rolf K eltjens, Gerard H . L . N efkens and Binne Z wanenburg*

NSR-Centre for M olecular Structure, D esign and S ynthesis, D epartment of O rganic C hemistry, U niversity of N ijmegen, T oernooiveld, 6525 E D N ijmegen, T he N etherslands

A series of analogues derived from (+)-strigol, which is a germination stimulant for seeds of the parasitic weeds Striga and Orobanche, has been prepared. For the isolation and characterization of the strigol receptor, labelled analogues are required in which a photoreactive function may be incorporated. The synthetic strategy allows for the synthesis of a range of A-ring substituted analogues of GR24 (which is a strigol analogue), including fluorescent dansyl GR24. Bioassays reveal that the stimulatory activity of these analogues in the seed germination of Striga hermonthica is retained.

Introduction

Parasitic weeds belonging to the genera Striga and Orobanche severely reduce yields of economically important crops in tropical and semitropical areas of the eastern hemisphere. The parasitic process begins with the seed germination of the weeds induced by a stimulant which is present in the root exudate of the host plant. Following the isolation and identification of the naturally occurring germination stimulant (+)-strigol (Fig. 1), several structure-bioactivity studies have been conducted, which revealed that the bioactivophore resides in the C4D-fragment and the vinyl ether moiety.

In particular, GR24 (Fig. 1) turned out to be a highly potent synthetic strigol analogue. Based on the structural requirements for retaining stimulatory activity a tentative molecular mechanism for germination has been proposed which is depicted in Scheme 1.

A series of analogues derived from (+)-strigol, which is a germination stimulant for seeds of the parasitic weeds Striga and Orobanche, has been prepared. For the isolation and characterization of the strigol receptor, labelled analogues are required in which a photoreactive function may be incorporated. The synthetic strategy allows for the synthesis of a range of A-ring substituted analogues of GR24 (which is a strigol analogue), including fluorescent dansyl GR24. Bioassays reveal that the stimulatory activity of these analogues in the seed germination of Striga hermonthica is retained.

Results and discussion

Strategy

In designing potential labelled germination stimulants, GR24 (Fig. 1) was used as the lead molecule. The bioactivity of GR24 is very high and its preparation is well documented. Since the CD-part as well as the connecting enol ether unit are

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essential for full biological activity. It was suggested that the tag and/or photoreactive group can best be incorporated into the A-ring of the stimulant molecule. Amino tricyclic lactone 1 and amino GR24 2 were selected as appropriate compounds to serve this purpose as a wide range of transformations are feasible with these synths, such as acylation, sulfonylation or alkylation, whereby coupling with an external tag can be achieved. The strategy for the synthesis of labelled GR24 analogues is outlined in Scheme 2. The label may be introduced either before (route I) or after (route II) coupling with the D-ring.

**Synthesis**

In our strategy the main issue is the introduction of an amino function into the A-ring of tricyclic lactone 3 in a regiocontrolled manner (Scheme 3).

This was achieved via nitration of 3 under relatively mild conditions (Scheme 3), to give two regioisomeric lactones 4a and 4b in a ratio of 9:1 in excellent yield, which could readily be separated. The structure of 4a was deduced unambiguously from a 2D-NOESY experiment. The preferred formation of 4a can be explained by the fact that C-7 is the least electron-deficient carbon atom in the aromatic ring. The nitration under similar conditions took place also starting from GR24 to give 7-nitro GR24 5a and its 5-nitro isomer 5b in a ratio of 9:1 (Scheme 4).

However, several attempts to obtain amino GR24 2 by reduction of the nitro function in 5 were unsuccessful. In contrast, reduction of the nitro group in 4a was accomplished employing aqueous Cu(OAc)₂ and NaBH₄ in methanol in yields in the range 34-95%. A more reliable procedure (Scheme 3) involves the use of Sn–HCl under reflux, which gave the 7-amino tricyclic lactone 1a in a reproducible yield of 99% (77% after recrystallization). The preparation of amino GR24 2a from amino tricyclic lactone 1a involves a four-step procedure as is depicted in Scheme 5.

The amino group in 1a was first protected as a Schiff base by reaction with benzaldehyde, then followed by formylation, coupling with bromo butenolide 7, similar to that described for GR24, and finally deprotection. Crude 7-benzalimino GR24 was isolated in an overall yield of 78%, based on 1a. Deprotection was not as straightforward as expected. Several conventional methods, such as 5% oxalic acid, failed to give the desired result and only starting imine was recovered. However, during purification of 7-benzalimino GR24 by flash chromatography, the deprotection took place on the silica gel column and amino GR24 2a was isolated in an overall yield of 27%. The diastereoisomers could not be separated, neither by flash chromatography nor by recrystallization, in contrast to GR24.

The key intermediate amino GR24 2a was then utilized for further derivatization (Scheme 6), especially for the purpose of receptor identification.

Diazotation and substitution in the presence of NaN₃ gave 8, which is thermally rather unstable in high yield. A applying the appropriate sulfonyl chloride, mesyl GR24 9 and dansyl GR24 10 were synthesized in high yields. The alternative procedure for the preparation of the labelled GR24 analogues 8, 9 and 10, involving modification of the amino function of 1a into the azido, mesylamino and dansylamino group, respectively, fol-
of results obtained in different test series, which is important, assay. This reference to GR24 enables a comparison between Striga hermonthica and dansyl GR24 moving diastereoisomers were used, whereas amino GR24 was tested as a mixture of diastereoisomers. It was shown for GR24 that the activity of the most active (fast-moving) diastereoisomer is not seriously influenced by the presence of the less active diastereoisomer. 

The data in Tables 1 and 2 reveal that compounds 2a, 5a and 10 behave quite differently towards seeds of Striga hermonthica and Orobanche crenata. In the case of Striga hermonthica, the biological activity of the substituted GR24 derivatives is relatively little affected as compared to GR24. The activity of amino GR24 2a is about one order of magnitude lower than GR24, whereas nitro GR24 5a and dansyl GR24 10 possess comparable concentration-dependent activities. Interestingly, the intrinsic activity of dansyl GR24 10 is considerably higher than that of GR24, which becomes apparent at the higher concentrations. In contrast, dansyl GR24 10 is completely inactive in the stimulation of Orobanche crenata seeds. Evidently, substituents in the A-part of GR24 have a negative effect on the bioactivity in the case of this parasitic species. The difference in response exerted by A-ring analogues 2a, 5a and 10 on seeds of Striga hermonthica and Orobanche crenata is rather unexpected, since previous studies revealed that structural modifications in the BC-part, enol ether moiety 25 and D-ring 26 generally give similar results for both parasitic species. Nevertheless, the prospects of incorporating a tag in the A-ring of GR24 with the aim of identifying the receptor protein is very promising for Striga hermonthica. The remarkable activity of the bulky dansyl derivative 10 suggests a large degree of structural freedom in the A-part with retention of full biological activity.

### Conclusion

In this synthetic study the preparation of amino GR24 2a is described. This compound is a versatile synthon for the preparation of a range of A-ring analogues of GR24. The synthesis of the fluorescent GR24 analogue 10 has been accomplished. Application of the thus developed synthetic strategy provides a feasible approach to the incorporation of radioactive tags and radioactive labels in GR24 derivatives.

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**Table 1** Germination percentages for seeds of Striga hermonthica after exposure to solutions of 2a, 5a and 10 at different concentrations

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>% Germination ± SE at 1 mg l⁻¹</th>
<th>% Germination ± SE at 0.01 mg l⁻¹</th>
<th>% Germination ± SE at 0.001 mg l⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>70.0 ± 1.9</td>
<td>57.8 ± 3.0</td>
<td>19.4 ± 1.3</td>
</tr>
<tr>
<td>2</td>
<td>5a</td>
<td>54.1 ± 4.3</td>
<td>59.3 ± 4.6</td>
<td>44.1 ± 2.2</td>
</tr>
<tr>
<td>3</td>
<td>2a</td>
<td>46.9 ± 3.8</td>
<td>17.5 ± 2.2</td>
<td>7.1 ± 1.9</td>
</tr>
</tbody>
</table>

* *Germination percentages are the mean of two replicate tests. In each test the percentage was determined 12 times by counting the number of germination seeds. 

**Table 2** Germination percentage for seeds of Orobanche crenata after exposure to 2a, 5a and 10 at different concentrations

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>% Germination ± SE at 1 mg l⁻¹</th>
<th>% Germination ± SE at 0.1 mg l⁻¹</th>
<th>% Germination ± SE at 0.01 mg l⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>1.5 ± 0.6</td>
<td>0.0 ± 0.0</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>2</td>
<td>5a</td>
<td>56.7 ± 3.5</td>
<td>9.3 ± 1.6</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>3</td>
<td>2a</td>
<td>33.6 ± 3.5</td>
<td>2.7 ± 1.0</td>
<td>0.0 ± 0.0</td>
</tr>
</tbody>
</table>

* *Germination percentages are the mean of two replicate tests. In each test the percentage was determined 12 times by counting the number of germination seeds. 

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**Biological activity**

The stimulatory activity of nitro GR24 5a, amino GR24 2a and dansyl GR24 10 was determined using seeds of Striga hermonthica and Orobanche crenata. The germination percentages are collected in Tables 1 and 2, together with those obtained for GR24 under the same conditions in the same bioassay. This reference to GR24 enables a comparison between results obtained in different test series, which is important, because the response of seeds of parasitic weeds, in particular of Striga hermonthica, varies considerably from test to test. For bioassays of nitro GR24 5a and dansyl GR24 10, the fast-moving diastereoisomers were used, whereas amino GR24 2a was tested as a mixture of diastereoisomers. It was shown for GR24 that the activity of the most active (fast-moving) diastereoisomer is not seriously influenced by the presence of the less active diastereoisomer. 

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**Scheme 6** Reagents and conditions: a, X = NHMs; c, y = 78%. 9, X = NHMs, c, y = 89%. 10, X = NHDans, c, y = 89%. 8, X = NMe₂, c, y = 88%.
photoreactive units in the G.R 24 molecule. The germination stimulatory activity of G.R 24 analogues 2a, 5a and 10 is relatively little affected for seeds of Striga hermonthica, whereas it is considerably reduced for seeds of Orobanche crenata. Therefore Striga hermonthica is an attractive target to perform protein fishing experiments.

**E Experimental**

**Synthesis**

**Nomenclature.** We have used the AUTONOM 1.0 program, provided by the Beilstein Institute and Springer Verlag, Weinhein, BRD.

**General remarks.** 100 M H 2 H N M R. Spectra and 400 M H z 1H N M R. spectra were recorded on a Bruker A 100 spectrometer and a Bruker A M-400 spectrometer, respectively (M eqSi as internal standard). All coupling constants are given as J in H z, unless indicated otherwise. IR Spectra were recorded on Perkin-Elmer 298 1R spectrophotometer. For mass spectra a double focussing VG 7070E mass spectrometer was used. GC-MS Experiments were run on a Varian Saturn 2 GC-M S ion-trap system. Separation was carried out on a fused-silica capillary column (DB-5, 30 m x 0.25 mm). H elum was used as carrier gas, and electron impact (EI) was used as ionization mode. GC-MS was conducted using a DB-210 fused silica capillary gas chromatograph, using a capillary column (25 m) of HP-1 and nitrogen (2 ml min⁻¹, 0.5 atm) as the carrier gas. Melting points were measured with a Reichert Thermopan microscope and are uncorrected. Elemental analyses were performed at the Department of Micro-analysis of this laboratory. Fluorescence measurements were performed using a Perkin-Elmer luminescence spectrometer LS50B. Solvents were dried using the following methods: dichloromethane was distilled from P 2O 5 diethyl ether distilled from NaH; hexane was distilled from pure N, 6.37. C 119 H 220 O 25 N 6.39) 3a(H) (100 M H z; CDCl 3) 2.42 (1 H, dd, J 4.8, 5.0, 3-H, 5.0, 3-H), 2.76–3.38 (4 H, m, 3-H, 4-H and 3a-H), 3.70 (2 H, br s, NH 11.25, 7-H), 7.83 (1 H, d, J 7.7, 7-H), 8.25 (1 H, d, J 7.7, 6-H) m/z 219 (M⁺, 28%), 128 (100, C 8 H 14 N 2 O), and 83.5 (1 H, d, J 2.1, 8-H); m/z 219 (M⁺, 39%), 175 (100, C 8 H 14 N 2 O). Compounds 4b, R 0.35 (hexane-ethyl acetate: 1:1) mp 137–140°C (from hexane-ethyl acetate) (Found: C, 59.93; H, 4.02; N, 6.34. C 119 H 220 O 25 N 6.39) J 4.8, 5.0, 3-H) 2.76–3.38 (4 H, m, 3-H, 4-H and 3a-H), 3.70 (2 H, br s, NH 11.25, 7-H), 7.83 (1 H, d, J 7.7, 7-H), 8.25 (1 H, d, J 7.7, 6-H) m/z 219 (M⁺, 28%), 128 (100, C 8 H 14 N 2 O) and 115 (54, C 6 H 10 N 2 ).

7-Amino-3,3a,4,8b-tetrahydroindeno[1,2-b]furandione 4a (1.00 g, 4.57 mmol) and methyl formate (0.84 ml, 13.7 mmol) in THF (35 ml) with stirring at −78°C under nitrogen. The mixture was allowed to warm to room temperature and then stirred for 1 h. After the THF had been removed in vacuo, the residue was dissolved in DMF (35 ml) and the solution was cooled to

7-ido-3-(4-methyl-5-oxo-2,5-dihydrofuran-2-yloxymethylene)-3,3a,4,8b-tetrahydroindeno[1,2-b]furan-2-one 11

Sodium nitrate (33 mg, 0.48 mmol) was added to a solution of a freshly prepared imine (210 mg, 270 mmol) in THF (25 ml) and then concentrated by removal of the solvent in vacuo. The residue was dissolved in dichloromethane and saturated aqueous NaHCO₃ (10 ml) and then protected with 2-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate. Flash chromatography (SiO₂, hexane-ethyl acetate, 1 : 1) afforded two diastereoisomers in 57% yield. The fast-moving diastereoisomer of 11 (R, 0.25, hexane-ethyl acetate, 1 : 1) was crystallized from hexane-ethyl acetate to give colourless crystals, mp 189–191 °C (from hexane-ethyl acetate); the 1 H NMR spectrum (CDCl₃, 100 MHz) was identical with the 1 H NMR spectrum of the fast-moving diastereoisomer of 11 (vide supra; m/z 424 (M+ + 6%), 327 (M+ + 4%), 97 (C₂H₇OH) and 97 (100, C₇H₇O₂); Found (HRMS): m/z 423.9810. Calc. for C₁₇H₁₄O₄: 423.9804.

7-Amino-3-(4-methyl-5-oxo-2,5-dihydrofuran-2-yloxymethylene)-3,3a,4,8b-tetrahydroindeno[1,2-b]furan-2-one 2a

A solution of the amino tricyclic lactone 1a (440 mg, 2.33 mmol) in THF (25 ml) was added potassium tert-butoxide (287 mg, 2.56 mmol) in small portions at 0 °C under nitrogen. The mixture was then brought to room temperature and then concentrated by removal of the THF in vacuo. The residue was dissolved in DMF (20 ml) and the solution was cooled to −60 °C when it was treated with the bromo butenolide 2b (287 mg, 2.56 mmol) and benzaldehyde (247 mg, 2.33 mmol) in ethyl acetate (20 ml), in the presence of molecular sieves 4 Å, for 12 h at room temperature. A filter this M gSO₄ was added to the mixture which was then filtered over Hyflo and then concentrated by removal of the solvent in vacuo to give the imine 6 (645 mg, 100%) as a pale yellow solid, which was used immediately in the coupling reaction; 6 (100 MHz; CDCl₃) 2.36 (1 H, dd, 1) 18.1, 5.4, 3-H); 2.70–3.56 (4 H, m, 3-H, 4-H and 3a-H), 3.75 (1 H, d, 1) 4.0, 8.8, 4-H); 6.70 (1 H, d, 1) 7.8, 8-H); 7.20–7.30 (3 H, m, Ph), 7.43–7.53 (3 H, m, Ph), 7.64–7.94 (2 H, m, Ph) and 8.44 (1 H, s, N–H).

To a solution of the freshly prepared imine 6 (645 mg, 2.33 mmol) and methyl formate (0.5 ml, 7 mmol) in THF (25 ml) was added potassium tert-butoxide (287 mg, 2.56 mmol) in small portions at 0 °C under nitrogen. The mixture was allowed to warm to room temperature at which time it was stirred for 18 h and then concentrated by removal of the THF in vacuo. The residue was dissolved in DMF (20 ml) and the solution was cooled to −60 °C when it was treated with the bromo butenolide 7 (494 mg, 2.70 mmol) in DMF (3 ml), added gradually to a solution of a mixture of the diastereoisomers of 7-amino GR24 8 (247 mg, 2.33 mmol) in ethyl acetate (20 ml), in the presence of molecular sieves 4 Å, for 12 h at room temperature. A filter this M gSO₄ was added to the mixture which was then filtered over Hyflo and then concentrated by removal of the solvent in vacuo to give the imine 6 (645 mg, 100%) as a pale yellow solid, which was used immediately in the coupling reaction; 6 (100 MHz; CDCl₃) 2.36 (1 H, dd, 1) 18.1, 5.4, 3-H); 2.70–3.56 (4 H, m, 3-H, 4-H and 3a-H), 3.75 (1 H, d, 1) 4.0, 8.8, 4-H); 6.70 (1 H, d, 1) 7.8, 8-H); 7.20–7.30 (3 H, m, Ph), 7.43–7.53 (3 H, m, Ph), 7.64–7.94 (2 H, m, Ph) and 8.44 (1 H, s, N–H).

To a solution of the freshly prepared imine 6 (645 mg, 2.33 mmol) and methyl formate (0.5 ml, 7 mmol) in THF (25 ml) was added potassium tert-butoxide (287 mg, 2.56 mmol) in small portions at 0 °C under nitrogen. The mixture was allowed to warm to room temperature at which time it was stirred for 18 h and then concentrated by removal of the THF in vacuo. The residue was dissolved in DMF (20 ml) and the solution was cooled to −60 °C when it was treated with the bromo butenolide 7 (494 mg, 2.70 mmol) in DMF (3 ml), added gradually under nitrogen. The mixture was brought to room temperature and then left at room temperature for 18 h after which it was concentrated by removal of the solvent in vacuo. The residue was dissolved in dichloromethane and saturated aqueous NaHCO₃. The aqueous phase was separated and extracted with dichloromethane (3×) and the combined organic layers were washed with saturated aqueous NaHCO₃ (1x), dried over anhydrous sodium sulfate and then concentrated by removal of the solvent in vacuo. The residue was dissolved in dichloromethane and saturated aqueous NaHCO₃. The aqueous phase was separated and extracted with dichloromethane (2x) and the combined organic layers were washed with saturated aqueous NaHCO₃ (1x), dried over anhydrous sodium sulfate and then concentrated by removal of the solvent in vacuo. The residue was dissolved in dichloromethane and saturated aqueous NaHCO₃. The aqueous phase was separated and extracted with dichloromethane (2x) and the combined organic layers were washed with saturated aqueous NaHCO₃ (1x), dried over anhydrous sodium sulfate and then concentrated by removal of the solvent in vacuo. The residue was dissolved in dichloromethane and saturated aqueous NaHCO₃. The aqueous phase was separated and extracted with dichloromethane (2x) and the combined organic layers were washed with saturated aqueous NaHCO₃ (1x), dried over anhydrous sodium sulfate and then concentrated by removal of the solvent in vacuo.
Slow-moving diastereoisomer of 8, R, 0.19 (hexane-ethyl acetate 1:1), mp 164–167°C; the 1H NMR spectrum (CDCl₃, 100 MHz) was identical with the 1H NMR spectrum of the fast-moving diastereoisomer of 8, m/z 339 (M⁺, 2%), 313 (1, C₈H₉NO₂), 214 (2, C₆H₅NO2) and 97 (100, C₄H₄O₂) [Found (HR MS): m/z 339.0856. Calc. for C₁₇H₁₇NO₄S: 339.0855].

An analytically pure sample of the fast-moving diastereoisomer of 10 (264 mg, 89%) as pale yellow solids with R, 0.2 and 0.17 (hexane-ethyl acetate, 1:1). A spectrophotometric sample of the fast-moving diastereoisomer of 10 was obtained by recrystallization from diisopropyl ether–dichloromethane, mp 186–188°C (from diisopropyl ether–dichloromethane) (Found: C, 63.50; H, 4.83; N, 5.10; S, 5.83. C₈H₉NO₂S requires C, 63.73; H, 4.79; N, 5.13; S, 5.83).

A mixture of slow- and fast-moving 7-amino GR24 (2a (170 mg, 0.543 mmol) and dansyl chloride (36.6 mg, 0.320 mmol) and pyridine (10 ml). Work-up and purification of the crude product was purified by flash chromatography (hexane–ethyl acetate, 1:1) to provide two diastereoisomers of 10 (18 mg, 78%) as a mixture of inseparable diastereoisomers.

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