

## Antifeedant Activity of Some Polygodial Derivatives

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Polygodial (**1**) and its derivatives acetal **2** (propylene) and **3** (ethylene) were prepared and their antifeedant activity and toxic effects evaluated on several insect species with different feeding ecologies (*Spodoptera littoralis*, *Leptinotarsa decemlineata*, *Myzus persicae* and *Rhopalosiphum padi*) along with that of polygonone (**4**). We also tested their selective cytotoxic effects on insect-derived (*Spodoptera frugiperda* ovarian Sf9 cells) and mammalian Chinese hamster ovary (CHO) cells. The antifeedant activity of these compounds was consistent with the proposed mode of action for antifeedant drimanes, *i.e.* adduct formation with amino groups for *M. persicae* and *R. padi* (dialdehyde > ketoaldehyde > aldehydeacetal). This was not the case for *L. decemlineata*, and the cytotoxic effects on insect-derived Sf9 and mammalian CHO cells (aldehydeacetal > dialdehyde > ketoaldehyde).

**Key words:** Polygodial Derivatives, Antifeedant, Cytotoxic

### Introduction

Naturally occurring sesquiterpenoid dialdehydes of the drimane series, such as polygodial (**1**), warburganal (**5**) and muzigadial (**6**), have been thoroughly investigated due to their strong antifeedant activities, and considerable efforts have been devoted to the synthesis of these compounds (Fraga, 2001; Jansen and de Groot, 1991, 2004). The reactivity of the unsaturated dialdehyde functionality towards biological nucleophiles is considered to be responsible for the antifeedant activity of these compounds. Structure-activity studies of enedials with *Spodoptera* spp. suggest a mode of action including pyrrole formation, Michael addition of free -SH moieties and Van der Waals interactions of the A ring of the drimanes (Fritz *et al.*, 1989; Jansen and de Groot, 2004). However, the lack of correlation between reactivity towards nucleophiles of polygodial (**1**) and those of warburganal (**5**) suggests that their insect antifeedant action may depend on other properties (Jansen and de Groot, 2004; Jonassohn *et al.*, 1997), as indicated by the activity of keto-aldehydes and 3-hydroxydrimanes (Barrero *et al.*, 1995; Justicia *et al.*, 2005) which cannot form pyrrole derivatives.

This lack of correlation between reactivity towards nucleophiles and bioactivity prompted us to

evaluate the antifeedant and toxic effects of polygodial (**1**) and its synthetic derivatives **2** and **3**, along with the ketoaldehyde **4** on several insect species with different feeding ecologies (the polyphagous Lepidopteran *Spodoptera littoralis*, the Solanaceae-adapted Chrysomelid *Leptinotarsa decemlineata*, the polyphagous aphid *Myzus persicae* and the cereal-adapted aphid *Rhopalosiphum padi*). Additionally we studied their selective cytotoxic effects on *Spodoptera frugiperda* ovarian Sf9 and mammalian Chinese hamster ovary (CHO) cells.

### Results and Discussion

Acetals **2** and **3** were obtained by treatment of polygodial (**1**) with propylene and ethylene glycol, respectively, in the presence of *p*-toluenesulfonic acid. Polygonone (**4**) was also prepared from polygodial (**1**) in five steps using a sequence previously described (Cuellar *et al.*, 2003).

The antifeedant effects of terpenes **1–4** are shown in Table I. The *S. littoralis* feeding behaviour was moderately affected by **1**. Polygonone (**4**) was the strongest antifeedant to *L. decemlineata*, being 13 times more potent than **1**. Polygodial (**1**) strongly affected the *M. persicae* and *R. padi* settling behaviour, followed by **3** (*M. persicae*) and **4**

Table I. Antifeedant effects of the test compounds on *S. littoralis* larvae and adult *L. decemlineata* and settling inhibition of apterous *M. persicae* and *R. padi* adults.

| Compound                      | <i>S. littoralis</i> | <i>L. decemlineata</i> | <i>M. persicae</i> |                  | <i>R. padi</i>   |                  |
|-------------------------------|----------------------|------------------------|--------------------|------------------|------------------|------------------|
|                               |                      |                        | % C <sup>b</sup>   | % T <sup>b</sup> | % C <sup>b</sup> | % T <sup>b</sup> |
| <b>1</b>                      | 64 <sup>a</sup>      | 71 <sup>a</sup>        | 98                 | 2*               | 98               | 2*               |
| EC <sub>50</sub> <sup>c</sup> | nc                   | 8.34 (2.3, 30.1)       | 1.0 (0.6, 1.7)     |                  | 2.7 (1.9, 6.2)   |                  |
| <b>2</b>                      | 47 <sup>a</sup>      | 68 <sup>a</sup>        | 68                 | 32*              | 72               | 28*              |
| EC <sub>50</sub> <sup>c</sup> | nc                   | nc                     | nc                 |                  | nc               |                  |
| <b>3</b>                      | 7 <sup>a</sup>       | 48 <sup>a</sup>        | 87                 | 13*              | 68               | 32*              |
| EC <sub>50</sub> <sup>c</sup> | nc                   | nc                     | 12.1 (7.2, 20.2)   |                  | nc               |                  |
| <b>4</b>                      | 33 <sup>a</sup>      | 97 <sup>a</sup>        | 83                 | 17*              | 80               | 20*              |
| EC <sub>50</sub> <sup>c</sup> | nc                   | 0.62 (0.1, 2.3)        | 11.9 (6.2, 20.3)   |                  | 13.8 (9.3, 20.5) |                  |

<sup>a</sup> % FI =  $1 - (T/C) \cdot 100$ , where T and C are the consumption of treated and control leaf discs, respectively, at a dose of 50  $\mu\text{g}/\text{cm}^2$ .

<sup>b</sup> % C and % T are the percentage of aphids settled on control and treated leaf discs, respectively, at a dose of 50  $\mu\text{g}/\text{cm}^2$ .

<sup>c</sup> Effective antifeedant doses (EC<sub>50</sub>) and 95% confidence limits (lower, upper).

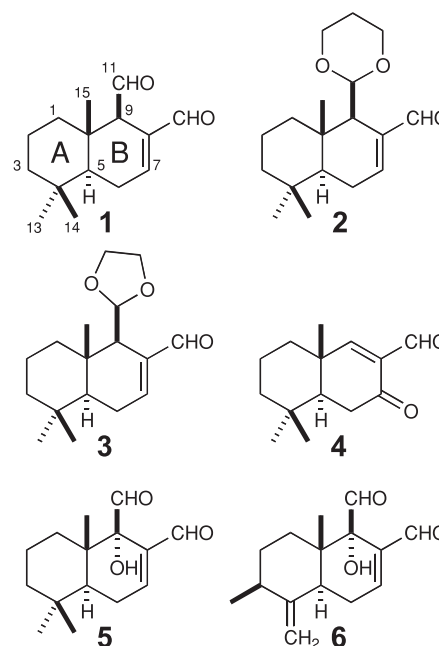
\*  $p < 0.05$ , Wilcoxon signed rank paired test.

nc, not calculated.

(*M. persicae* and *R. padi*), possibly due to the presence of a Michael acceptor group in these compounds as previously suggested (Fritz *et al.*, 1989; Messchendorp *et al.*, 2000).

Polygodial (**1**) is a well known insect antifeedant, acting on several insect species including the ones tested here. Specifically, **1** affected *S. littoralis* by variable responses (Barrero *et al.*, 1995; Ley *et al.*, 1987), inhibited *L. decemlineata* feeding (Gols *et al.*, 1996; Messchendorp *et al.*, 1998), deterred and affected the probing behaviour of *M. persicae* in choice tests (Hardie *et al.*, 1992; Messchendorp *et al.*, 1998; Powell *et al.*, 1993, 1995, 1996) and also acted on cereal aphids including *R. padi* (Powell *et al.*, 1997). However, this is the first report on the antifeedant action of derivatives **2–4**.

Substitution of the C-9 aldehyde group (as in acetals **2** and **3**, Fig. 1) reduced the antifeedant effects to *S. littoralis*, *M. persicae* and *R. padi*. A different structure-activity relationship (SAR) pattern was followed by *L. decemlineata* with ketoaldehyde **4**, being the most potent antifeedant, suggesting family- and/or species-related differences in the drimane molecular targets between these insects. Polygodial (**1**) and related compounds are probably sensory-mediated insect antifeedants (Messchendorp *et al.*, 2000). The pungency of **1** has been related to its ability to interact with vanilloid receptors on mammalian capsaicin-sensitive sensory neurons (Jansen and de Groot, 2004). The molecular mechanisms of detecting and

Fig. 1. Molecular structures of the compounds **1–6**.

avoiding plant pungent components are conserved between insects and mammals (Al-Anzi *et al.*, 2006) suggesting that the chemical transduction of pungent-related antifeedant effects may be neuro-receptor-mediated. Further research is needed to proof this hypothesis.

Table II. Biomass gain ( $\Delta B$ ) and consumption ( $\Delta I$ ) of orally injected *S. littoralis* L6 larvae, expressed as percent of the control, and cytotoxic effects on *S. frugiperda* Sf9 and mammalian CHO cells.

| Compound              | <i>S. littoralis</i> |              | EC <sub>50</sub> [ $\mu$ g/ml] <sup>c</sup> |                       |
|-----------------------|----------------------|--------------|---------------------------------------------|-----------------------|
|                       | $\Delta B^a$         | $\Delta I^b$ | Sf9                                         | CHO                   |
| <b>1</b>              | 96                   | 106          | 15.93 (9.50, 27.47)                         | 11.89 (9.84, 14.37)   |
| <b>2</b>              | 101                  | 114          | 30.61 (17.18, 54.52)                        | > 100                 |
| <b>3</b>              | 94                   | 117          | 16.52 (11.56, 23.63)                        | 17.52 (14.69, 20.90)  |
| <b>4</b>              | 86                   | 97           | 0.81 (0.31, 2.14)                           | 1.08 (0.48, 2.42)     |
| Rotenone <sup>d</sup> | 56*                  | 62*          | 0.23 (0.15, 0.30)                           | 38.14 (14.36, 101.16) |

<sup>a</sup>  $\Delta B$ , change in insect body weight (mg dry weight).<sup>b</sup>  $\Delta I$ , mass of food consumed (mg dry weight).<sup>c</sup> Effective antifeedant dose (EC<sub>50</sub>) and 95% confidence limits (lower, upper).<sup>d</sup> From González-Coloma *et al.* (2002).\* Significantly different from the control,  $p < 0.05$ , LSD test.

Molecular and species-dependent selectivity has been shown for other drimanes (Justicia *et al.*, 2005; Messchendorp *et al.*, 1998; Powell *et al.*, 1997). A saturated keto-aldehyde derivative of **1** was a more potent antifeedant to *S. littoralis* (Barrero *et al.*, 1995), however, polygonone (**4**) was not effective against this insect while being very active on *L. decemlineata*.

We tested the performance parameters (growth and ingestion) of orally injected *S. littoralis* larvae with the test compounds and found that none of them were effective (Table II). However, post-ingestive effects have been reported for 3 $\beta$ -hydroxycinnamolide and 3 $\beta$ -acetoxydrimenin against this insect (Justicia *et al.*, 2005) and suggested for synthetic analogues (lactones) of polygodial and warburganal on *Pieris brassicae* and *L. decemlineata* larvae (Messchendorp *et al.*, 2000).

Among the structurally related drimanes tested, **2** showed moderate selective cytotoxicity against insect-derived Sf9 cells, while polygonone (**4**) showed the strongest cytotoxicity against both cell lines with some selectivity towards Sf9 (10 times more sensitive than CHO) probably due to a combination of polarity and specific membrane factors (Table II). The lipid composition of insect cells significantly differs from that of mammalian cells. The plasma membranes of Sf9 cells contain 10 times less cholesterol than mammalian cells with a lower cholesterol to phospholipid ratio (Marheineke *et al.*, 1998). Compounds **1** and **3** were moderately cytotoxic to both cell lines, suggesting that the free dialdehyde group is not essential for this effect as shown for the antifungal activity of polygodial and related compounds (Brennan *et al.*, 2006). The lack of post-ingestive action of these

cytotoxic compounds suggests metabolic detoxification and/or assimilation by *S. littoralis*.

The fungicidal and bactericidal action of **1** has been attributed to its ability to act as a nonionic surfactant inhibiting the plasma membrane H<sup>+</sup>-ATPase by disrupting the hydrogen bonds at the lipid bilayer (Kubo *et al.*, 2001, 2005). The antifeedant effects against *L. decemlineata* and the cytotoxicity of compounds **1–4** (**4** > **1** > **2**, **3**) did not correlate with their potential reactivity (**1** > **4** > **2**, **3**); therefore these effects could be the consequence of the surfactant action of the drimanes tested. However, the antifeedant effects on *M. persicae* and *R. padi* did correlate with the reactivity of compounds **1–4**, suggesting family- and/or species-related differences in the drimane mode of action.

In summary, we tested the antifeedant properties of drimanes **1–4** against the insects *S. littoralis*, *L. decemlineata*, *M. persicae*, and *R. padi* and observed different activity levels. The antifeedant activity variation of these compounds is consistent with the proposed mode of action for antifeedant drimanes by adduct formation with amino groups on the insect molecular targets (Fritz *et al.*, 1989; Jansen and de Groot, 2004) for *M. persicae* and *R. padi* (dialdehyde > ketoaldehyde > aldehydeacetal) but is neither consistent for *L. decemlineata* nor for the cytotoxic effects on insect-derived Sf9 and mammalian CHO cells (aldehydeacetal > dialdehyde > ketoaldehyde).

## Experimental

### General

Melting points were determined on a Stuart Scientific SMP3 apparatus and are uncorrected. IR

Table III.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic data ( $\text{CDCl}_3$ , 400 MHz) of compounds **2** and **3** [ $\delta$  in ppm (multiplicities,  $J$  in Hz)].

| Position               | <b>2</b>            |                                                      | <b>3</b>            |                                                      |
|------------------------|---------------------|------------------------------------------------------|---------------------|------------------------------------------------------|
|                        | $\delta_{\text{C}}$ | $\delta_{\text{H}}$                                  | $\delta_{\text{C}}$ | $\delta_{\text{H}}$                                  |
| 1 $\alpha$             | 40.0                | 1.06 (ddd, 13.7, 9.6, 3.0)                           | 39.9                | 1.14–1.26 (m)                                        |
| 1 $\beta$              |                     | 1.90 (dddd, 9.6, 3.3, 3.2, 1.2)                      |                     | 2.02 (dddd, 13.3, 3.2, 3.1, 1.9)                     |
| 2 $\alpha$             | 18.5                | 1.41 (dddt, 14.6, 3.3, 3.1, 3.0)                     | 18.5                | 1.42–1.54 (m)                                        |
| 2 $\beta$              |                     | 1.48 (dtt, 14.6, 13.7, 3.3)                          |                     | 1.56 (dddd, 13.7, 13.5, 13.3, 3.3, 3.1)              |
| 3 $\alpha$             | 41.9                | 1.20 (ddd, 13.7, 13.3, 3.1)                          | 41.8                | 1.16 (ddd, 13.5, 13.3, 3.7)                          |
| 3 $\beta$              |                     | 1.32 (dtd, 13.4, 3.3, 1.2)                           |                     | 1.44 (dtd, 13.4, 3.3, 1.9)                           |
| 4                      | 32.9                |                                                      | 32.9                |                                                      |
| 5 $\alpha$             | 49.1                | 1.14 (dd, 11.5, 4.3)                                 | 49.0                | 1.20 (dd, 12.2, 4.7)                                 |
| 6 $\alpha$             | 23.9                | 2.13 (dtd, 22.6, 5.2, 4.3, 2.8)                      | 24.1                | 2.30 (dddd, 19.6, 5.9, 4.7, 2.4)                     |
| 6 $\beta$              |                     | 2.00 (ddd, 22.7, 11.5, 4.6, 2.5)                     |                     | 2.11 (dddd, 19.6, 12.3, 4.3, 2.3)                    |
| 7                      | 140.2               | 6.80 (ddd, 5.2, 2.8, 2.5)                            | 142.8               | 6.82 (ddd, 5.9, 2.5, 2.3)                            |
| 8                      | 137.1               |                                                      | 138.1               |                                                      |
| 9 $\alpha$             | 55.7                | 2.43 (dtd, 4.6, 2.8, 1.0)                            | 52.6                | 2.77 (dddd, 4.3, 2.5, 2.3, 1.0)                      |
| 10                     | 35.3                |                                                      | 35.1                |                                                      |
| 11                     | 101.0               | 5.26 (d, 1.0)                                        | 102.7               | 5.06 (d, 1.0)                                        |
| 12                     | 194.1               | 9.75 (s)                                             | 193.7               | 9.56 (s)                                             |
| 13                     | 14.5                | 0.83 (s) ( $\text{CH}_3$ )                           | 14.6                | 0.90 (s) ( $\text{CH}_3$ )                           |
| 14                     | 22.0                | 0.86 (s) ( $\text{CH}_3$ )                           | 22.0                | 0.93 (s) ( $\text{CH}_3$ )                           |
| 15                     | 33.2                | 0.83 (s) ( $\text{CH}_3$ )                           | 33.1                | 0.91 (s) ( $\text{CH}_3$ )                           |
| $\text{CH}_2$          | 25.4                | 1.08–1.19 (m) ( $\text{CH}_2$ )                      |                     |                                                      |
| $\text{O}-\text{CH}_2$ | 66.9                | 3.58–4.11 (m) ( $2 \times (-\text{CH}_2-\text{O})$ ) | 63.4                | 3.77–4.00 (m) ( $2 \times (-\text{CH}_2-\text{O})$ ) |
|                        | 67.3                |                                                      | 65.4                |                                                      |

spectra were recorded on a Bruker Vector 22-FT instrument in KBr discs. Optical rotations were obtained in an Optical Activity Ltd. instrument and their concentrations are expressed in g/100 ml. NMR spectra were recorded on a Bruker AVANCE 400 instrument (400.13 MHz for  $^1\text{H}$ , 100.03 MHz for  $^{13}\text{C}$ ) in  $\text{CDCl}_3$  with TMS as internal standard. Assignments were done by a combination of 1D and 2D NMR techniques, in each case, as needed. All  $^1\text{H}$  and  $^{13}\text{C}$  NMR data are summarized in Table III. Chromatographic separations were carried out on Merck silica gel 60 (230–400 mesh) using hexane/ethylacetate gradients of increasing polarity. Elemental analyses were obtained using a Fisons Instrument EA1108 micro-analyzer.

#### Compound isolation

Compounds **2**–**4** were prepared from natural polygodial (**1**) obtained from the crude hexane extract of the ground stem bark of *Drimys winteri* (Cortés and Oyarzún, 1981; Cuellar *et al.*, 2003).

#### (1*R*,4*aS*,8*aS*)-1-(1,3-Dioxan-2-yl)-5,5,8*a*-trimethyl-1,4,4*a*,5,6,7,8,8*a*-octahydronaphthalene-2-carbaldehyde (**2**)

A solution of polygodial (**1**) (500 mg, 2.14 mmol), propylene glycol (167 mg, 2.2 mmol), and *p*-tolu-

enesulfonic acid (4 mg) in dry benzene (40 ml) was refluxed for 15 h in a Dean-Stark apparatus. Chromatographic purification gave the compound **2** (280 mg, 45%), m.p. 155–157 °C (hexane/acetone). –  $[\alpha]_{\text{D}}^{18} + 66.8^\circ$  ( $c = 0.95$ ,  $\text{CHCl}_3$ ). – IR (KBr):  $\nu_{\text{max}} = 3420, 2960, 1680, 1620, 1140 \text{ cm}^{-1}$ . – Calcd. for  $\text{C}_{18}\text{H}_{28}\text{O}_3$ : C 74.73, H 9.04; found: C 74.50, H 9.03.

#### (1*R*,4*aS*,8*aS*)-1-(1,3-Dioxolan-2-yl)-5,5,8*a*-trimethyl-1,4,4*a*,5,6,7,8,8*a*-octahydronaphthalene-2-carbaldehyde (**3**)

A solution of polygodial (**1**) (500 mg, 2.14 mmol), ethylene glycol (137 mg, 2.2 mmol), and *p*-toluenesulfonic acid (3 mg) in dry benzene (30 ml) was refluxed for 12 h in a Dean-Stark apparatus. The reaction mixture was cooled, ethylacetate (60 ml) was added and the solution was washed with saturated sodium bicarbonate solution and dried. The solvent was evaporated and the residue was purified by column chromatography producing a white solid (415 mg, 70%), m.p. 146–148 °C (AcOEt). –  $[\alpha]_{\text{D}}^{18} + 56.3^\circ$  ( $c = 2.8$ ,  $\text{CHCl}_3$ ). – IR (KBr):  $\nu_{\text{max}} = 3012, 2929, 1695, 1622, 1135 \text{ cm}^{-1}$ . – Calcd. for  $\text{C}_{17}\text{H}_{26}\text{O}_3$ : C 73.34, H 9.41; found: C 73.26, H 9.82.

### Insect bioassays

*Spodoptera littoralis*, *Leptinotarsa decemlineata*, and the aphids *Myzus persicae* and *Rhopalosiphum padi* were reared on artificial diet and their respective host plants (*Solanum tuberosum*, *Capsicum annuum* and *Hordeum vulgare*) and maintained at  $(22 \pm 1)^\circ\text{C}$ ,  $> 70\%$  room humidity (r.h.) with a photoperiod of 16 h/8 h (L:D) in a growth chamber as described by Reina *et al.* (2001).

### Choice feeding assays

These experiments were conducted with sixth-instar *S. littoralis* larvae and adult *L. decemlineata*, *M. persicae* and *R. padi* (apterous). *Capsicum annuum*, *Solanum tuberosum* or *Hordeum vulgare* leaf discs/fragments ( $1.0\text{ cm}^2$ ) were treated on the upper surface with  $10\text{ }\mu\text{l}$  of the test substance. Two treated and two control discs were arranged alternatively on five agar-coated Petri dishes ( $9.0\text{ cm}$  diameter) with three insects (*S. littoralis* or *L. decemlineata*) which were allowed to feed in a growth chamber (environmental conditions as described above). Each experiment was repeated three times. Feeding was terminated after the consumption of 50–75% of the control discs. Percent feeding inhibition (% FI) was calculated as described by Reina *et al.* (2001). For the aphids, twenty  $2 \times 2\text{ cm}$  boxes with ten insects each were used for tests, and their settling inhibition index (% SI) was calculated as described by Gutierrez *et al.* (1997). Compounds with an FI  $> 70\%$  were tested in a dose-response experiment (dose series between  $50.00\text{--}0.08\text{ }\mu\text{g/cm}^2$ ) to calculate their relative potency ( $\text{EC}_{50}$  values, the effective dose for 50% feeding reduction) which was determined from linear regression analysis (STATGRAPHICS Plus) (% FI on log dose).

### Oral cannulation

This experiment was performed with preweighed newly molted *S. littoralis* L6 larvae. Each experiment consisted of 20 larvae orally dosed with  $40\text{ }\mu\text{g}$  of the test compound in  $4\text{ }\mu\text{l}$  of DMSO (treatment) or solvent alone (control) as described by Reina *et al.* (2001). At the end of the experiments (72 h), larval consumption and growth were calculated on a dry weight basis. An analysis of covariance (ANCOVA1) on biomass gains with

the initial biomass as covariate (covariate  $p > 0.05$ ) showed that initial insect weights were similar among all treatments. A second analysis (ANCOVA2) was performed on biomass gains with food consumption as covariate to test for post-ingestive effects (Reina *et al.*, 2001). The mitochondrial respiration inhibitor rotenone has been included as reference compound (González-Coloma *et al.*, 2002).

### Cytotoxicity

Sf9 cells derived from *S. frugiperda* pupal ovarian tissue (European Collection of Cell Cultures, ECCC) were maintained in TC-100 (from Sigma) insect cell medium supplemented with 10% fetal bovine serum, 1% L-glutamine and 1% penicillin/streptomycin at  $26^\circ\text{C}$ . Mammalian Chinese hamster ovary cells (CHO, a gift from Dr. Pajares, Instituto de C. Biomédicas, CSIC) were grown in RPMI 1640 (Gibco) medium supplemented as above at  $37^\circ\text{C}$  under a humidified atmosphere of 5%  $\text{CO}_2$ /95% air (Reina *et al.*, 2001). Cells were seeded in 96-well flat-bottom microplates with  $100\text{ }\mu\text{l}$  medium per well (initial densities of  $5 \cdot 10^4$  and  $10^4$  cells per well for the insect and mammalian cultures, respectively), and exposed for 48 h to serial dilutions of the test compounds in DMSO ( $< 1\%$  final content). Cell viability was analyzed using 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) (Sigma) by the MTT colourimetric assay method (Mossman, 1983). The purple coloured formazan precipitate was dissolved with  $100\text{ }\mu\text{l}$  of DMSO. Cell viability was calculated as the percent absorbance of the control (untreated cells). The active compounds were tested in a dose-response experiment to calculate their relative potency ( $\text{ED}_{50}$  values, the effective dose to give 50% cell viability) which was determined from linear regression analysis (% cell viability on log dose). The mitochondrial respiration inhibitor rotenone has been included as reference compound (González-Coloma *et al.*, 2002).

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