Research Article

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Tetraoxanes as a new class of efficient herbicides comparable with commercial products

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Abstract

BACKGROUND: Several 1,2,4,5-tetraoxanes were synthesised, and their herbicidal activity was tested against weeds and compared with the activity of commercial herbicides glyphosate and imazethapyr.

RESULTS: The compounds were prepared by reacting carbonyl compounds with hydrogen peroxide under acid catalysis, affording 1,1-dihydroperoxides (36–91%) that were further converted into 1,2,4,5-tetraoxanes (10–52%) under similar reaction conditions. All products were evaluated against *Sorghum bicolor* and *Cucumis sativus* at 0.0125–1.0 mM, and several tetraoxanes caused >70% inhibition of the growth of roots and aerial parts. The most active products were evaluated against the weeds *Sorghum arundinaceum*, *Euphorbia heterophylla*, *Brachiaria brizantha* and *Bidens pilosa*. Some compounds were highly effective (>80% inhibition at 1.0 mM) against the weeds, showing activity comparable with that of glyphosate or imazethapyr. One of the tetraoxanes was selective, being inactive against dicotyledonous species while inhibiting the roots and aerial parts of monocotyledonous species by 92.9–97.5%, which is comparable with the effect of glyphosate.

CONCLUSIONS: Tetraoxanes constitute a new class of effective herbicides with great potential for commercial development.
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Supporting information may be found in the online version of this article.

Keywords: 1,2,4,5-tetraoxanes; heterocyclic herbicides; plant growth regulators; peroxides; weeds

1 INTRODUCTION

During the last decades, the continuous use of herbicides has been one of the main strategies for controlling weeds in agriculture. Over the years, the world's agriculture has increased in productivity and acreage, accompanied with intensive use of agrochemicals, but many have been withdrawn from the market owing to unwanted properties such as persistence in the environment,[1] accumulation in the food chain[2] or development of resistance by the target species. In light of such problems, the agrochemical industries have been investing a great deal in the development of new molecules with new modes of action and better physicochemical characteristics, aiming to obtain products with better features and more environmentally friendly behaviours.[3–5]

In line with the new developments in this area, and within the framework of long-established research on substances with potential use as agrochemicals, especially for weed control, we have been using natural products isolated from microorganisms[6,7] and plants[8,9] as a model for the development of new biologically active compounds. This is a widely used strategy by companies for the development of new agrochemicals,[10] and also by governmental researchers in this field. Accordingly, a series of investigations have demonstrated[11–13] that artemisinin **1** (Fig. 1), extracted from the Chinese medicinal herb sweet wormwood (*Artemisia annua* L.), and its semi-synthetic analogues, which have successfully been used against malaria parasites, have a powerful inhibitory effect on plant growth. Some of the analogues, such as artesunate **2** and artemether **3**, are more potent than artemisinin **1**. The phytotoxic activity is dependent upon the presence of the peroxide moiety,[14–16] as deoxyartemisinin **4** is inactive.[17]

In view of the phytotoxic activities displayed by artemisinin **1** and other compounds possessing peroxide bridges, our research group has pioneered the discovery of phytotoxic ozonides (1,2,4-trioxolanes) possessing the general structure **5** and **6**[18,19] (Fig. 1). This work was inspired by our previous discovery of the antimalarial activity of ozonides,[20,21] that subsequently led other

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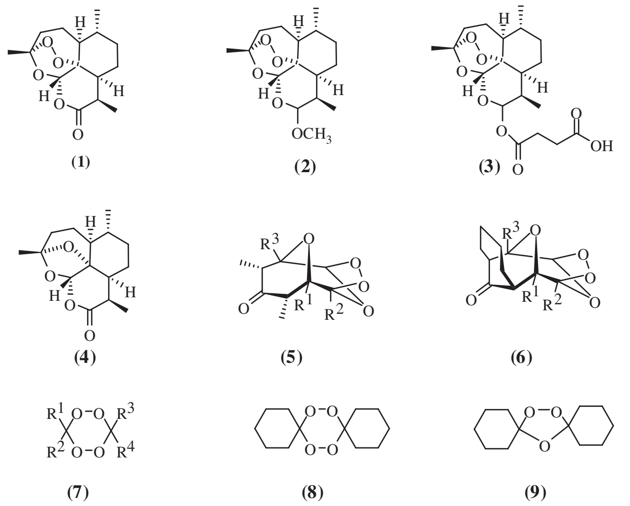

Figure 1. Structures of trioxolanes **1** to **6** and **9** and tetraoxanes **7** and **8**.

groups to develop several new compounds[22] that resulted in a commercial product to treat malaria.

Following our ongoing studies to develop new herbicides bearing a peroxide bridge, a series of 1,2,4,5-tetraoxanes, whose general structure **7** is depicted in Fig. 1, were synthesised. This class of compounds has proven antimalarial action,[23−25] but there are no reports in the literature about its phytotoxicity. Compound **8** has exhibited antimalarial activity, with IC_{50} in the 25 mM range, and is more stable and active than trioxolane **9**.[24]

Considering the reported phytotoxic activities of artemisinin **1**[26−31] and ozonides,[18−21] which are associated with the peroxide bridge, and the structural similarity between trioxolanes and tetraoxanes, in this work we describe the preparation of several tetraoxanes and report for the first time their phytotoxic activity against several weeds of economic importance.

2 MATERIALS AND METHODS

2.1 Experimental procedures

Reagents and solvents were purified, when necessary, according to procedures described in the literature.[32] Reagents were purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI) and utilised without further purification. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 300 instrument (300 and 75 MHz respectively), using deuterated chloroform ($CDCl_3$) and tetramethylsilane (TMS) as internal standards ($\delta = 0$). Infrared spectra were recorded using a Perkin-Elmer Paragon 1000 FTIR spectrophotometer as thin films on cesium iodide or potassium bromide plates, scanning from 4000 to 400 cm^{-1}. Mass spectrometry detection was performed using a Shimadzu LC-ESI-IT-TOFMS instrument working at high resolution and high mass accuracy (<5 ppm) under the following conditions: ESI ionisation at +4.5 kV and nebuliser gas at 1.5 L min^{-1}, curved desorption line (CDL) interface at 200 °C and drying gas at 100 kPa; octapole ion accumulation time 100 ms. Full scan mass spectra from *m/z* 100 to 1000 were acquired with a scan time of 0.2 s. Resolution was at least 10 000 full width at half maximum (FWHM) at *m/z* 500. Melting points were measured using a MQAPF-301 melting point apparatus (Microquimica, Brazil).

2.2 Synthetic procedures

2.2.1 *2,2-Dihydroperoxypropane (**10**)*

To a two-neck round-bottomed flask (50 mL) containing propanone (0.5 g, 8.6 mmol) dissolved in acetonitrile/dichloromethane (CH_3CN/CH_2Cl_2) solution (15.0 mL, 3:1 v/v) were added dropwise hydrogen peroxide (H_2O_2) 30% (8.8 mL, 86 mmol) and six drops of concentrated HCl. The reaction mixture was stirred at room temperature for about 6 h. The mixture was quenched with saturated aqueous sodium bicarbonate (25 mL) and CH_2Cl_2 (25 mL). The two layers were separated, and the aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and evaporated to dryness. The crude product **10** (580 mg, 62% of crude yield) was used in the further step without purification.[23]

The dihydroperoxides **10** to **13** and **15** to **20** were prepared employing a procedure similar to that described for compound **10**; the yields are presented in Table 1.

2.2.2 *1-(Dihydroperoxymethyl)-4-methoxybenzene (**21**)*

To a two-neck round-bottomed flask (50 mL) containing 4-methoxybenzaldehyde (2.0 g, 14.7 mmol) in acetonitrile (30.0 mL) was added aluminium chloride hexahydrate ($AlCl_3.6H_2O$, 0.150 g, 1.13 mmol), followed by a dropwise addition of 30% aqueous H_2O_2 (5.2 mL, 37.5 mmol). The reaction mixture was stirred at room temperature for about 6 h. The mixture was quenched with water (30 mL) and extracted with EtOAc (3 × 30 mL). The combined organic layer was washed with sodium bicarbonate solution (2 × 30 mL), dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The obtained product **21** (1.96 g, 72% yield) was used in the further step without purification.[33]

The dihydroperoxides **14**, **21** and **22** were prepared employing a procedure similar to that described for compound **21**; the yields are presented in Table 1.

2.2.3 *3,3,6,6-Tetramethyl-1,2,4,5-tetraoxane (**23**)*

To a two-neck round-bottomed flask (50 mL) containing 2,2-dihydroperoxypropane (**10**) (0.58 g, 5.37 mmol) in CH_2Cl_2 (20.0 mL) at 4 °C was added propanone (0.31 g, 5.37 mmol), followed by dropwise addition of a cooled H_2SO_4/CH_3CN solution (3.3 mL, 1:10 v/v). After stirring for 4 h, the mixture was quenched by addition of sodium bicarbonate solution (25 mL) and CH_2Cl_2 (25 mL). The water layer was additionally extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and evaporated to dryness.[23] The residue was purified by column chromatography on silica gel eluted with hexane-diethyl ether (15:1 v/v), yielding 68.0 mg (11%) of **23**: IR (CsI, cm^{-1}) $\bar{\nu}_{max}$ 2921, 2854, 2362, 2163, 1462, 1367, 1268, 1197, 943, 930, 858, 814, 686, 522; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (s, 6H, H-8 and H-10), 1.79 (s, 6H, H-7 and H-9); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.8 (C-8 and C-10), 22.6 (C-7 and C-9), 107.8 (C-3 and C-6); HRMS *m/z* (M + Na$^+$): calcd for $C_6H_{11}NaO_4$, 170.0555; found, 170.0583.

Compounds **24** to **42** were prepared employing a procedure similar to that described for compound **23**; the yields are presented in Table 1. Structures of all tetraoxanes were confirmed on the basis of IR and NMR spectral analyses. The molecular formulas were validated by mass spectrometry analyses employing the following techniques: electron spray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI). Structures for the remaining compounds are supported by the following spectroscopic and spectrometric data.

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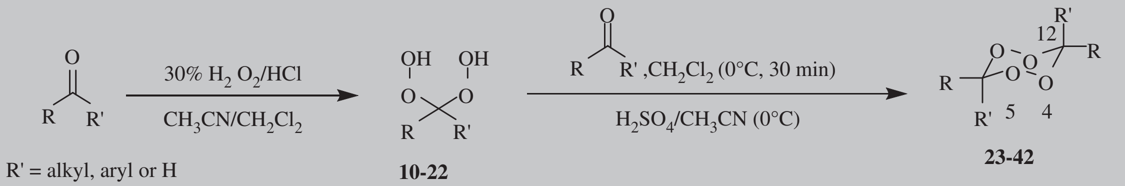
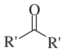
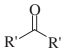
Table 1. Preparation of hydroperoxides **10** to **22** and tetraoxanes **23** to **42**



Ketone/aldehyde 	Hydroperoxide (yield %)	Ketone/aldehyde 	Tetraoxane (yield %)
Propanone	**10** (62)	Propanone	**23** (11)
Pentan-3-one	**11** (82)	Pentan-3-one	**24** (21)
Heptan-4-one	**12** (71)	Heptan-4-one	**25** (21)
Nonan-5-one	**13** (55)	Nonan-5-one	**26** (52)
4-methylpentan-2-one[a]	**14** (41)	4-methylpentan-2-one	**27** (30)
		Cyclopentanone	**28** (13)
Cyclopentanone	**15** (77)	Cyclohexanone	**29** (10)
		Cycloheptanone	**30** (28)
		Cyclooctanone	**31** (10)
Cyclohexanone	**16** (75)	Cyclohexanone	**32** (41)
Cycloheptanone	**17** (83)	Cycloheptanone	**33** (34)
Cyclooctanone	**18** (36)	Cyclooctanone	**34** (11)
		Cyclopentanone	**35** (10)
		Cyclohexanone	**36** (18)
Benzaldehyde	**19** (86)	Cycloheptanone	**37** (42)
		Cyclooctanone	**38** (16)
		Benzaldehyde	**39** (22)
4-ethylbenzaldehyde	**20** (91)	4-ethylbenzaldehyde	**40** (24)
4-methoxybenzaldehyde[a]	**21** (72)	4-methoxybenzaldehyde	**41** (16)
2,4,6-trimethoxybenzaldehyde[a]	**22** (50)	2,4,6-trimethoxybenzaldehyde	**42** (26)

[a] Hydroperoxide obtained using $AlCl_3$ as catalyst.

*2.2.4 Data for 3,3,6,6-tetraethyl-1,2,4,5-tetraoxane (**24**)*

Pale-yellow oil; purified by silica gel column chromatography, eluent hexane-diethyl ether (15:1 v/v); IR (CsI, cm^{-1}) $\bar{\nu}_{max}$ 2980, 2935, 2885, 1461, 1382, 1350, 1301, 1275, 1229, 1156, 1144, 1070, 1044, 957, 926, 805, 764, 670; ^1H NMR (300 MHz, $CDCl_3$) δ 0.94 (t, 12H, $J_{8,7} = J_{10,9} = J_{12,11} = J_{14,13} = 7.5$ Hz, H-8, H-10, H-12 and H-14), 1.57–1.65 (m, 4H, H-7b, H-9b, H-11b and H-13), 2.22–2.29 (m, 4H, H-7a, H-9a, H-11a and H-13a); ^{13}C NMR (75 MHz, $CDCl_3$) δ 6.6 (C-10 and C-14), 8.2 (C-8 and C-12), 23.7 (C-9 and C-13), 26.3 (C-7 and C-11), 111.2 (C-3 and C-6); HRMS *m/z* ($M + Na^+$): calcd for $C_{10}H_{19}NaO_4$, 226.1307; found, 226.1199.

*2.2.5 Data for 3,3,6,6-tetrapropyl-1,2,4,5-tetraoxane (**25**)*

Pale-yellow oil; purified by column chromatography, eluent hexane-diethyl ether (18:1 v/v); IR (CsI, cm^{-1}) $\bar{\nu}_{max}$ 2962, 2934, 2874, 2152, 1468, 1450, 1422, 1375, 1315, 1299, 1265, 1249, 1206, 1169, 1144, 1075, 1011, 953, 911, 849, 809, 743, 624, 492; ^1H NMR (300 MHz, $CDCl_3$) δ [signals related to the minor isomer are presented with a prime (′)] 0.88–1.17 (m, 18H, H-9, H-9′, H-12, H-12′, H-15, H-15′, H-18 and H-18′), 1.30–1.42 (m, 12H, H-8, H-8′, H-11, H-11′, H-14, H-14′, H-17 and H-17′), 1.53–1.59 (m, 4H, H-7b, H-10-b, H-13b and H-16b), 2.05–2.21 (m, 46H, H-7a, H-7′, H-10a, H-10′, H-13a, H-13′, H-16a and H-16′); ^{13}C NMR (75 MHz, $CDCl_3$) (major isomer) δ 14.6 (C-9, C-12, C-15 and C-18), 15.7 (C-11 and C-13), 17.3 (C-8 and C-14), 33.4 (C-10 and C-16), 36.3 (C-7 and C-13), 110.7 (C-3 and C-6); (minor isomer) δ 14.5 (C-11′ and C-17′), 14.6 (C-9′, C12′, C-15′ and C-18′), 33.4 (C-10′ and C-16′), 35.9 (C-7 and C-13′), 109.1 (C-3′ and C-6′); HRMS *m/z* ($M + Na^+$): calcd for $C_{14}H_{27}NaO_4$, 282.1807; found, 282.1771.

*2.2.6 Data for 3,3,6,6-tetrabutyl-1,2,4,5-tetraoxane (**26**)*

Pale-yellow oil; purified by silica gel column chromatography, eluent hexane-diethyl ether (20:1 v/v); IR (CsI, cm^{-1}) $\bar{\nu}_{max}$ 2952, 2928, 2869, 2160, 2003, 1468, 1455, 1377, 1343, 1260, 1221, 1159, 1135, 1027, 1003, 967, 899, 823, 728, 605, 505; ^1H NMR (300 MHz, $CDCl_3$) δ 0.92 (t, 12H, $J_{10,9} = J_{14,13} = J_{18,17} = J_{22,21} = 6.9$ Hz, H-10, H-14, H-18 and H-22), 1.19–1.53 (m, 20H, H-7b, H-8, H-9, H-11b, H-12, H-13, H-15b, H-16, H-17, H-19b, H-20 and H-21), 1.74–1.82 (t, 4H, $J_{7a,8} = J_{11a,12} = J_{15a,16} = J_{19a,20} = 9.9$ Hz, H-7a, H-11a, H-15a and H-19a); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.3 (C-10, C-14, C-18 and C-22), 23.1 (C-9, C-13, C-17 and C-21), 26.0 (C-8, C-12, C-16 and C-20), 30.0 (C-7, C-11, C-15 and C-19), 111.1 (C-3 and C-6); HRMS *m/z* ($M + Na^+$): calcd for $C_{18}H_{35}NaO_4$, 338.2433; found, 338.2401.

*2.2.7 Data for 3,6-diisobutyl-3,6-dimethyl-1,2,4,5-tetraoxane (**27**)*

Pale-yellow oil; purified by column chromatography, eluent hexane-diethyl ether (30:1 v/v); IR (CsI, cm^{-1}) $\bar{\nu}_{max}$ 2957, 2873, 2359, 2170, 1466, 1375, 1239, 1158, 1085, 1048, 921, 718, 535; ^1H NMR (300 MHz, $CDCl_3$) δ signals related to the minor isomer are marked with a prime) 0.90–1.02 (m, 18H, H-9, H-9′, H-12, H-12′,

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H-13, H-13′, H-14, H-14′, H-15, H-15′, H-16 and H-16′), 1.19–2.20 (m, 18H, H-7, H-7′, H-8, H-8′, H-10, H-10′, H-11, H-11′); ^{13}C NMR (75 MHz, CDCl$_3$) (major isomer) δ 19.7 (C-8, C-11, C-14 and C-16), 24.2 (C-9, C-12, C-13 and C-15), 45.5 (C-7 and C-10), 109.7 (C-3 and C-6); (minor isomer) δ 20.8 (C-8′, C-11′, C-14′ and C-16′), 23.6 (C-9′, C-12′, C-13′ and C-15′), 41.0 (C-7′ and C-10′), 110.1 (C-3′ and C-6′); HRMS *m/z* (M + Na$^+$): calcd for $C_{12}H_{23}NaO_4$, 254.1494; found, 254.1507.

2.2.8 *Data for 3,6-dicyclopentyl-1,2,4,5-tetraoxane (**28**)*

White solid; purified by column chromatography, eluent hexane-diethyl ether (12:1 v/v); mp 114.5–114.9 °C; IR (CsI, cm^{-1}) $\bar{\nu}_{max}$ 2997, 2876, 2366, 2029, 1430, 1326, 1311, 1199, 1175, 1069, 1013, 967, 849, 790, 709; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.67–1.79 (m, 12H, H-7b, H-8, H-9, H-10b, H-11b, H-12, H-13 and H-14b), 2.40 (t, 4H, $J_{7a,8} = J_{10a,9} = J_{11a,12} = J_{14a,13} = 6.9$ Hz, H-7a, H-10a, H-11a and H-14a); ^{13}C NMR (75 MHz, CDCl$_3$) δ 24.1 (C-9 and C-13), 25.3 (C-8 and C-12), 34.6 (C-10 and C-14), 35.5 (C-7 and C-11), 120.0 (C-3 and C-6); HRMS *m/z* (M$^+$): calcd for $C_{10}H_{16}O_4$, 200.1049; found, 200.0983.

2.2.9 *Data for 3-cyclohexyl-6-cyclopentyl-1,2,4,5-tetraoxane (**29**)*

White solid; purified by silica gel column chromatography, eluent hexane-diethyl ether (20:1 v/v); mp 122.3–123.5 °C; IR (CsI, cm^{-1}) $\bar{\nu}_{max}$ 2937, 2853, 2161, 1979, 1446, 1361, 1340, 1271, 1256, 1162, 1146, 1070, 1030, 945, 921, 851, 821, 778, 676, 559, 505; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.47 (m, 6H, H-12, H-13 and H-14), 1.57–1.59 (m, 8H, H-7b, H-8, H-9, H-10b, H-11b and H-15b), 2.28 (m, 4H, H-7a, H-10a, H-11a and H-15a); ^{13}C NMR (75 MHz, CDCl$_3$) δ 22.1 (C-9), 22.4 (C-8), 25.6 (C-12, C-13 and C-14), 29.8 (C-15), 32.0 (C-11), 34.6 (C-10), 35.5 (C-7), 108.4 (C-3), 119.9 (C-6); HRMS *m/z* (M + Na$^+$): calcd for $C_{11}H_{17}NaO_4$, 236.1025; found, 236.1035.

2.2.10 *Data for 3-cycloheptyl-6-cyclopentyl-1,2,4,5-tetraoxane (**30**)*

White solid; purified by silica gel column chromatography, eluent hexane-diethyl ether (40:1 v/v); mp 130.0–131.2 °C; IR (CsI, cm^{-1}) $\bar{\nu}_{max}$ 2930, 2861, 2362, 2032, 1977, 1444, 1361, 1340, 1274, 1202, 1179, 1110, 1073, 1032, 1015, 989, 961, 896, 861, 770, 692, 515, 470; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.57–1.59 (m, 10H, H-11b, H-12, H-13, H-14, H-15 and H-16b), 1.66–1.68 (m, 6H, H-7b, H-8, H-9 and H-10b), 2.41 (m, 4H, H-7a, H-10a, H-11a and H-16a); ^{13}C NMR (75 MHz, CDCl$_3$) δ 22.7 (C-14), 22.8 (C-13), 24.1 (C-9), 25.3 (C-8), 29.8 (C-15), 30.5 (C-12), 31.3 (C-16), 34.5 (C-10), 35.4 (C-7), 36.3 (C-11), 112.7 (C-3), 119.5 (C-6); HRMS *m/z* (M + Na$^+$): calcd for $C_{12}H_{19}NaO_4$, 250.1181; found, 250.1162.

2.2.11 *Data for 3-cyclooctyl-6-cyclopentyl-1,2,4,5-tetraoxane (**31**)*

White solid; purified by silica gel column chromatography, eluted with hexane-diethyl ether (80:1 v/v); mp 137.8–138.2 °C; IR (CsI, cm^{-1}) $\bar{\nu}_{max}$ 2960, 2925, 2871, 2359, 2156, 1975, 1470, 1445, 1328, 1244, 1180, 1068, 969, 911, 858, 782, 744, 504, 412; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.54–1.73 (m, 18H, H-7b, H-8, H-9, H-10b, H-11b, H-12, H-13, H-14, H-15, H-16 and H-17b), 2.37–2.44 (m, 4H, H-7a, H-10a, H-11a and H-17a); ^{13}C NMR (75 MHz, CDCl$_3$) δ 21.4 (C-15), 22.5 (C-13), 24.1 (C-9), 25.2 (C-14), 25.3 (C-8), 26.3 (C-16), 26.9 (C-12), 29.3 (C-17), 33.0 (C-11), 34.6 (C-10), 35.5 (C-7), 112.1 (C-3), 119.5 (C-6); HRMS *m/z* (M$^+$): calcd for $C_{13}H_{22}O_4$, 242.1318; found, 242.1289.

2.2.12 *Data for 3,6-dicyclohexyl-1,2,4,5-tetraoxane (**32**)*

White solid; purified by silica gel column chromatography, eluent hexane-diethyl ether (14:1 v/v); mp 129.2–130.2 °C; IR (CsI, cm^{-1}) $\bar{\nu}_{max}$ 2935, 2861, 2187, 1448, 1359, 1346, 1270, 1260, 1231, 1160, 1148, 1093, 1065, 1029, 954, 913, 847, 829; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.47 (m, 4H, H-9 and H-14), 1.58 (m, 12H, H-7b, H-8, H-9, H-10, H-11b, H-12b, H-13, H-14, H-15 and H-16b), 2.28 (m, 4H, H-7a, H-11a, H-12a and H-16a); ^{13}C NMR (75 MHz, CDCl$_3$) δ 22.0 (C-9 and C-14), 25.4 (C-8, C-10, C-13 and C-15), 29.7 (C-11 and C-16), 31.7 (C-7 and C-12), 108.1 (C-3 and C-6); HRMS *m/z* (M + Na$^+$): calcd for $C_{12}H_{19}NaO_4$, 250.1181; found, 250.1221.

2.2.13 *Data for 3,6-dicycloheptyl-1,2,4,5-tetraoxane (**33**)*

White solid; purified by silica gel column chromatography, eluent hexane-diethyl ether (15:1 v/v); mp 145.5–145.9 °C; IR (CsI, cm^{-1}) $\bar{\nu}_{max}$ 2984, 2931, 2861, 2360, 1982, 1455, 1443, 1360, 1350, 1229, 1200, 1178, 1090, 1032, 1017, 989, 896, 752; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.56–1.60 (m, 16H, H-8, H-9, H-10, H-11, H-14, H-15, H-16 and H-17), 1.65 (s, 4H, H-7b, H-12b, H-13b and H-18b), 2.41 (m, 4H, H-7a, H-12a, H-13a and H-18a); ^{13}C NMR (75 MHz, CDCl$_3$) δ 22.6 (C-10 and C-16), 22.8 (C-9 and C-15), 29.8 (C-11 and C-17), 30.5 (C-8 and C-14), 31.3 (C-12 and C-18), 36.3 (C-7 and C-13), 112.7 (C-3 and C-6); HRMS *m/z* (M + Na$^+$): calcd for $C_{14}H_{23}NaO_4$, 278.1594; found, 278.1627.

2.2.14 *Data for 3,6-dicyclooctyl-1,2,4,5-tetraoxane (**34**)*

White solid; purified by silica gel column chromatography, eluent hexane-diethyl ether (30:1 v/v); mp 161.3–162.6 °C; IR (CsI, cm^{-1}) $\bar{\nu}_{max}$ 2912, 2852, 2362, 2335, 1970, 1467, 1453, 1443, 1375, 1292, 1242, 1225, 1162, 1127, 1065, 986, 963, 904, 872, 734, 699; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.42–1.68 (m, 24H, H-7b, H-8, H-9, H-10, H-11, H-12, H-13b, H-14b, H-15, H-16, H-17, H-18, H-19 and H-20b), 2.42–2.47 (m, 4H, H-7a, H-13a, H-14a and H-20a); ^{13}C NMR (75 MHz, CDCl$_3$) δ 21.4 (C-11 and C-18), 22.4 (C-9 and C-16), 25.3 (C-10 and C-17), 26.3 (C-12 and C-19), 26.9 (C-8 and C-15), 29.3 (C-13 and C-20), 33.0 (C-7 and C-14), 111.7 (C-3 and C-6); HRMS *m/z* (M + Na$^+$): calcd for $C_{16}H_{27}NaO_4$, 306.1907; found, 306.2103.

2.2.15 *Data for 3-cyclopentyl-6-phenyl-1,2,4,5-tetraoxane (**35**)*

White solid; purified by silica gel column chromatography, eluent hexane-diethyl ether (16:1 v/v); mp 170.0–171.0 °C; IR (CsI, cm^{-1}) $\bar{\nu}_{max}$ 3070, 3039, 2963, 2877, 2159, 1980, 1602, 1495, 1455, 1430, 1360, 1329, 1312, 1196, 1118, 1075, 991, 908, 769, 739, 639; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.73–1.91 (m, 6H, H-13b, H-14, H-15 and H-16b), 2.56 (t, 2H, $J_{13a,14} = J_{16a,15} = 7.2$ Hz, H-13a and H-16a), 6.74 (s, 1H, H-6), 7.37–7.54 (m, 5H, H-8, H-9, H-10, H-11 and H-12); ^{13}C NMR (75 MHz, CDCl$_3$) δ 24.3 (C-15), 25.5 (C-14), 35.2 (C-16), 35.5 (C-13), 108.1 (C-3), 120.5 (C-6), 127.9 (C-9 and C-11), 128.9 (C-8 and C-12), 131.4 (C-10), 131.6 (C-7); HRMS *m/z* (M$^+$): calcd for $C_{12}H_{14}O_4$, 222.0892; found, 222.0869.

2.2.16 *Data for 3-cyclohexyl-6-phenyl-1,2,4,5-tetraoxane (**36**)*

White solid; purified by silica gel column chromatography, eluent hexane-diethyl ether (12:1 v/v); mp 175.5–176.0 °C; IR (CsI, cm^{-1}) $\bar{\nu}_{max}$ 3040, 2991, 2948, 2936, 2901, 2858, 2019, 1976, 1602, 1496, 1449, 1358, 1313, 1196, 1089, 1068, 1006, 987, 920, 751, 693, 635, 553; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.48–1.54 (m, 2H, H-15), 1.59–1.73 (m, 6H, H-13b, H-14, H-16 and H-17b), 2.45 (t, 2H, $J_{13a,14} = J_{17a,16} = 6.0$ Hz, H-13a and H-17a), 6.67 (s, 1H, H-6), 7.37–7.43 (m, 5H, H-8, H-9, H-10, H-11 and H-12); ^{13}C NMR (75 MHz,

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$CDCl_3$) δ 22.1 (C-15), 22.5 (C-16), 25.6 (C-14), 30.4 (C-17), 30.1 (C-13), 108.1 (C-3), 109.0 (C-6), 127.8 (C-9 and C-11), 128.9 (C-8 and C-12), 131.3 (C-10), 131.8 (C-7); HRMS *m/z* (M^+): calcd for $C_{13}H_{16}O_4$, 236.1049; found, 236.1007.

*2.2.17 Data for 3-cycloheptyl-6-phenyl-1,2,4,5-tetraoxane (**37**)*

White solid; purified by silica gel column chromatography, eluent hexane-diethyl ether (16:1 v/v); mp 182.3–183.0 °C; IR (CsI, cm^{-1}) $\bar{\nu}_{max}$ 3070, 3039, 2930, 2852, 2043, 2016, 1972, 1608, 1494, 1455, 1358, 1310, 1196, 1174, 1111, 1021, 1007, 980, 893, 749, 691, 672, 653; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.57–1.77 (m, 10H, H-13b, H-14, H-15, H-16, H-17 and H-18b), 2.59–2.63 (m, 2H, H-13a and H-18a), 6.64 (s, 1H, H-6), 7.40–7.49 (m, 5H, H-8, H-9, H-10, H-11 and H-12); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.7 (C-16), 23.0 (C-15), 29.9 (C-14), 30.5 (C-17), 32.0 (C-18), 36.3 (C-13), 107.8 (C-3), 113.8 (C-6), 127.8 (C-9 and C-11), 128.9 (C-8 and C-12), 131.3 (C-10), 131.7 (C-7); HRMS *m/z* (M^+): calcd for $C_{14}H_{18}O_4$, 250.1205; found, 250.1187.

*2.2.18 Data for 3-cyclooctyl-6-phenyl-1,2,4,5-tetraoxane (**38**)*

White solid; purified by silica gel column chromatography, using as eluent hexane-diethyl ether (20:1 v/v); mp 190.1–190.8 °C; IR (CsI, cm^{-1}) $\bar{\nu}_{max}$ 3066, 2937, 2862, 2362, 1984, 1602, 1493, 1469, 1454, 1358, 1314, 1266, 1220, 1194, 1179, 1120, 1065, 999, 898, 735, 697, 635; ^{1}H NMR (300 MHz, $CDCl_3$) δ (signals related to the minor isomer are presented within brackets) 1.60–1.80 (m, 12H, H-13b, H-14, H-15, H-16, H-17, H-18 and H-19b), 2.60–2.64 (m, 2H, H-13a and H-19a), 6.63 (s, 1H, H-6), [6.94 (s, 1H, H-6′)], 7.38–7.54 (m, 5H, H-8, H-9, H-10, H-11 and H-12), [7.38–7.54 (m, 5H, H-8′, H-9′, H-10′, H-11′ and H-12′)]; ^{13}C NMR (75 MHz, $CDCl_3$) (major isomer) δ 21.6 (C-17), 22.5 (C-15), 25.3 (C-16), 26.9 (C-18), 27.1 (C-14), 29.3 (C-19), 33.1 (C-13), 107.8 (C-6), 112.80 (C-3), 127.8 (C-9 and C-11), 128.9 (C-8 and C-12), 131.3 (C-10), 131.6 (C-7); (minor isomer) δ 108.5 (C-6′), 128.1 (C-9′ and C-11′), 129.1 (C-8′ and C-12′), 131.1 (C-10′), 131.8 (C-7′); HRMS *m/z* (M^+): calcd for $C_{15}H_{20}O_4$, 264.1362; found, 264.1322.

*2.2.19 Data for 3,6-diphenyl-1,2,4,5-tetraoxane (**39**)*

White solid; purified by silica gel column chromatography, eluent hexane-diethyl ether (12:1 v/v); mp 201.0–202.0 °C; IR (CsI, cm^{-1}) $\bar{\nu}_{max}$ 3069, 3037, 2941, 2028, 2003, 1994, 1602, 1493, 1453, 1364, 1314, 1195, 1173, 1015, 998, 921, 907, 743, 709, 691, 645, 509; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.94 (s, 2H, H-3 and H-6), 7.41–7.54 (m, 10H, H-8, H-9, H-10, H-11, H-12, H-14, H-15, H-16, H-17 and H-18); ^{13}C NMR (75 MHz, $CDCl_3$) δ 108.5 (C-3 and C-6), 128.1 (C-9, C-11, C-15 and C-17), 129.1 (C-8, C-12, C-14 and C-18), 131.1 (C-10 and C-16), 131.6 (C-7 and C-13); HRMS *m/z* (M^+): calcd for $C_{14}H_{12}O_4$, 244.0736; found, 244.0623.

*2.2.20 Data for 3,6-bis(4-ethylphenyl)-1,2,4,5-tetraoxane (**40**)*

White solid; purified by silica gel column chromatography, eluent hexane-diethyl ether (18:1 v/v); mp 230.7–231.2 °C; IR (CsI, cm^{-1}) $\bar{\nu}_{max}$ 2970, 2933, 2877, 2363, 2042, 2008, 1610, 1516, 1456, 1420, 1360, 1308, 1198, 1180, 1119, 1018, 1002, 954, 909, 837, 801, 772; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24 (t, 6H, $J_{20,19} = J_{22,21} = 7.6$ Hz, H-20 and H-22), 2.68 (q, 4H, $J_{19,20} = J_{21,22} = 7.6$ Hz, H-19 and H-21), 6.89 (s, 2H, H-3 and H-6), 7.27 (d, 4H, $J_{9,8} = J_{11,12} = J_{15,14} = J_{17,18} = 8.1$ Hz, H-9, H-11, H-15 and H-17), 7.43 (dd, 4H, $J_{8,12} = J_{12,8} = J_{14,18} = J_{18,14} = 1.5$ Hz, $J_{12,11} = J_{8,9} = J_{14,15} = J_{18,17} = 6.6$ Hz, H-8, H-12, H-14 and H-18); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.6 (C-20 and C-22), 29.1 (C-19 and C-21), 108.4 (C-3 and C-6), 128.1 (C-9, C-11, C-15 and C-17), 128.5 (C-8, C-12, C-14 and C-18), 128.6 (C-10 and C-16), 148.1 (C-7 and C-13); HRMS *m/z* ($M + Na^+$): calcd for $C_{18}H_{19}NaO_4$, 322.1181; found, 322.1164.

*2.2.21 Data for 3,6-bis(4-methoxyphenyl)-1,2,4,5-tetraoxane (**41**)*

Pale-yellow oil; purified by silica gel column chromatography, eluent hexane-diethyl ether (8:1 v/v); IR (CsI, cm^{-1}) $\bar{\nu}_{max}$ 3004, 2946, 2918, 2840, 2165, 2039, 1549, 1501, 1465, 1442, 1298, 1249, 1181, 1097, 1028, 855, 831, 791, 733, 695, 607; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.80 (s, 6H, 19 and 20-$OC\underline{H}_3$), 6.89 (t, 2H, $J_{8,9} = J_{8,12} = J_{14,15} = J_{14,18} = 2.4$ Hz, H-8 and H-14), 6.92 (t, 2H, $J_{12,11} = J_{18,8} = J_{18,17} = J_{18,14} = 2.4$ Hz, H-12 and H-18), 7.04 (t, 2H, $J_{11,12} = J_{11,9} = J_{17,18} = J_{17,15} = 2.4$ Hz, H-11 and H-17), 7.07 (t, 2H, $J_{9,8} = J_{9,11} = J_{14,15} = J_{14,17} = 2.4$ Hz, H-9 and H-14), 8.28 (s, 2H, H-3 and H-6); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.8 (19 and 20-OCH_3), 114.9 (C-8, C-12, C-14 and C-18), 122.2 (C-9, C-11, C-15 and C-17), 143.6 (C-3 and C-6), 157.9 (C-7 and C-13), 160.0 (C-10 and C-16); HRMS *m/z* ($M + Na^+$): calcd for $C_{16}H_{16}NaO_6$, 327.0839; found, 327.0832.

*2.2.22 Data for 3,6-bis(2,4,6-trimethoxyphenyl)-1,2,4,5-tetraoxane (**42**)*

White solid; purified by silica gel column chromatography, eluent hexane-diethyl ether (3:1 v/v); mp 302.0–303.0 °C; IR (CsI, cm^{-1}) $\bar{\nu}_{max}$ 2961, 2940, 2847, 2360, 2160, 2008, 1346, 1700, 1613, 1593, 1505, 1467, 1439, 1421, 1409, 1357, 1233, 1206, 1191, 1101, 1051, 1029, 948, 920, 806, 749, 716, 528; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.80 (s, 6H, 20 and 23-$OC\underline{H}_3$), 3.81 (s, 12H, 19, 21, 22 and 24-$OC\underline{H}_3$), 6.18 (s, 4H, H-9, H-11, H-15 and H-17), 8.22 (s, 2H, H-3 and H-6); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.8 (20 and 23-$O\underline{C}H_3$), 55.3 (19, 21, 22 and 24-$OC\underline{H}_3$), 91.6 (C-9, C-11, C-15 and C-17), 93.3 (C-8, C-12, C-14 and C-18), 121.8 (C-3 and C-6), 152.7 (C-7 and C-13), 159.7 (C-10 and C-16); HRMS *m/z* ($M + Na^+$): calcd for $C_{20}H_{24}NaO_{10}$, 447.1189; found, 447.1238.

2.3 Preliminary phytotoxic tests

Two target species were selected: *Cucumis sativus* and *Sorghum bicolor*. Stock solutions at 1.0 mM of each tested compound were prepared as follows. Each compound was dissolved in a mixture of pentan-3-one (10 µL), the surfactant Tween 80® (50 µL) and distilled water (10 mL).[19] The resulting suspension was shaken for 1 min and then transferred to a volumetric flask. The volume was completed with water to 50 mL and sonicated for 10 min. These solutions were diluted with distilled water, so that test concentrations for each compound (0.5, 0.25 and 0.125 mM) were reached.

The post-emergent commercial herbicides glyphosate and imazethapyr were used as positive at the same concentrations and conditions as the compounds studied. A solution with the same composition described above, but without the active ingredient under evaluation, was also used as control in the biological assays.

2.3.1 Radicle and shoot elongation assay

For each experiment, a group of 20 pregerminated seeds of *C. sativus* or *S. bicolor* were placed in petri dishes (9 cm) lined with one sheet of Whatman No. 1 filter paper. Aliquots of 5 mL of each solution were applied. The petri dishes were sealed with Parafilm and incubated at 25 ± 1 °C in the absence of light in a controlled environment growth chamber.[34,35] The bioassays took 7 days in the

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case of *S. bicolor* and 10 days for *C. sativus*. After growth, the plants were frozen at −10 °C for 24 h to avoid subsequent growth during the measurement process. This helped the handling of the plants and allowed a more accurate measurement of root and shoot lengths. All treatments were replicated 3 times in a completely randomised design.[19,34,35]

2.3.2 Statistical analysis

Seeds were considered to have germinated if a radical protruded as least 1 mm beyond the seed body. The percentage of germination was calculated according to the number of seeds that germinated in relation to the total number of seeds sown. The percentages of radicle and aerial part inhibition, or stimulation, were calculated in relation to the radicle and aerial part length of the control.[35]

2.3.3 Greenhouse assays with weeds

The most active compounds from the preliminary phytotoxic assay (**29, 30, 31, 32, 33, 35** and **36**) and also the commercial herbicides glyphosate and imazethapyr were subsequently evaluated against *Bidens pilosa*, *Brachiaria brizantha*, *Euphorbia heterophylla* and *Sorghum arundinaceum*. Plastic pots (0.05 L) were filled with acid washed sand (70 g), which was saturated with 5.0 mL of stock solution (0.25 and 1.0 mM, the preparation of which was similar to that previously described). Four seeds of each target species were placed in each pot. Seedlings were grown in a greenhouse and watered as required with tap water or every 2 days with half-strength Hoagland solution,[36] to maintain the humidity at 13.3% w/w, in the presence of light. Forty days after sowing, plants were harvested, and the roots and shoots were separated and dried at 70 °C until constant weight.[19] The percentage of root and shoot growth inhibition was calculated with respect to the mass of the control. The data were analysed using Scott–Knott's test at a 0.05 probability level.[37]

3 RESULTS AND DISCUSSION

3.1 Synthesis of hydroperoxides

The conversion of carbonyl compounds into the corresponding dihydroperoxides can be achieved by a number of methodologies, all of which involve the use of some type of acid. Some common procedures involve hydrochloric acid[23] and aluminium chloride, usually resulting in the hydroperoxides in high yields.[33] Tetrafluoroboric acid,[38] iodine[39] and boron trifluoride[40] have also been used successfully. In this work the preparation of the dihydroperoxides was carried out via two different sets of methodologies.

In the first set of experiments, the carbonyl compound was treated with concentrated HCl in 30% H_2O_2 in CH_3CN/CH_2Cl_2.[23] This procedure afforded the hydroperoxides **10** to **13** and **15** to **20** in yields ranging from 55 to 91% in all cases except in the case of cyclooctanone (36%) (Table 1). Although this reaction was repeated twice, no improvement in the yields could be achieved. This could be due to hysteric hindrance caused by the very flexible large ring. In the case of 4-methylpentan-2-one, the dihydroperoxide was not formed when this methodology was applied because the carbonyl was more hindered than in pentan-3-one. Most electron-rich aldehydes, such as 4-methoxybenzaldehyde and 2,4,6-trimethoxybenzaldehyde, did not yield peroxides. In such cases we employed $AlCl_3$ in the presence of 30% H_2O_2 and the carbonyl compound dissolved in CH_3CN.[33] This procedure afforded the required hydroperoxides **14**, **21** and **22** in good yields (Table 1).

The dihydroperoxides were characterised by IR spectroscopy and were not purified prior to further reactions. In the IR spectra of the crude products, the major feature observed was the absence of a strong absorption band around 1690–1720 cm^{-1} due to the (C–O) bonds. A strong absorption around 1030–1150 cm^{-1}, due to the stretching of peroxide (O–O) bonds, and another band at 3550 cm^{-1}, associated with the hydroxy (OH) bonds, were observed in all spectra.

3.2 Synthesis of tetraoxanes

For the preparation of tetraoxanes **23** to **42**, we applied the methodology described by Opsenica *et al.*,[23] in which dihydroperoxide was dissolved in dichloromethane (DCM) and cooled to 0 °C. Then, the carbonyl compound was added slowly, followed by the addition of a cooled solution of H_2SO_4/CH_3CN.

The dihydroperoxides previously prepared were reacted with acyclic and cyclic ketones and aromatic aldehydes. The choice of acyclic and cyclic ketones for the synthesis of tetraoxanes is based on the proven antimalarial action already reported for this chemistry, and also the fact that previous studies showed that 1,2,4-trioxolanes prepared from ketones were phytotoxic.[18,19] The introduction of an aromatic unit in the products is a strategy for subsequent functionalization of the molecules, allowing further studies of the quantitative relationship between chemical structure and biological activity (SAR) to be carried out.

Following the procedure mentioned above, four series of tetraoxanes were obtained, as depicted in Fig. 2. Features of the four types of tetraoxane are: (a) derived only from acyclic ketones (**23** to **27**); (b) obtained from cyclic ketones (**28** to **34**); (c) obtained from cyclic ketones and aromatic aldehydes (**35** to **38**); (d) obtained from two aromatic aldehydes (**39** to **42**).

Analyses of the reaction mixture by TLC indicated that the hydroperoxides were not fully consumed, in spite of variation in the conditions and time. Thus, yields obtained for the products are low, ranging 10 to 42% (Table 1). These low yields can in part be ascribed to the formation of byproducts, as revealed by TLC analyses. These byproducts can be derived from cleavage around the oxygenated ring, leading to the formation of ketones and esters,[41,42] or they can be constituted of hexaoxane derivatives.[23]

Attempts to improve the methodology for the synthesis of tetraoxanes were made by varying the concentration of the peroxide, the amount and nature of the acid catalyst and the order of addition of the reagents, without great success. The yields for the dihydroperoxides were high; however, their conversion into tetraoxanes was low yielding (Table 1). In spite of such difficulties, enough material was obtained for biological evaluation.

3.3 Biological activity

In a preliminary screening, phytotoxic activity of tetraoxanes **23** to **42** on growth of *Sorghum bicolor* and *Cucumis sativus* was evaluated.[43] None of the compounds, or the commercial herbicides used as positive control, affected seed germination. At all concentrations tested the germination rate was above 80%.

Regarding the growth of root and shoot, compounds **29**, **33**, **35**, **36**, **39** and **40** at 1.0 mM caused a higher percentage of inhibition of *S. bicolor* than the commercial herbicides (Fig. 3). At this same dose, all tetraoxanes except **27** cause inhibition of root growth above 80%. At concentrations of 0.5–0.25 mM, some compounds were more active while others were less active than the commercial herbicides. However, compound **29** caused root and shoot inhibition (73 and 76% respectively), statistically equivalent to those caused by the herbicides.

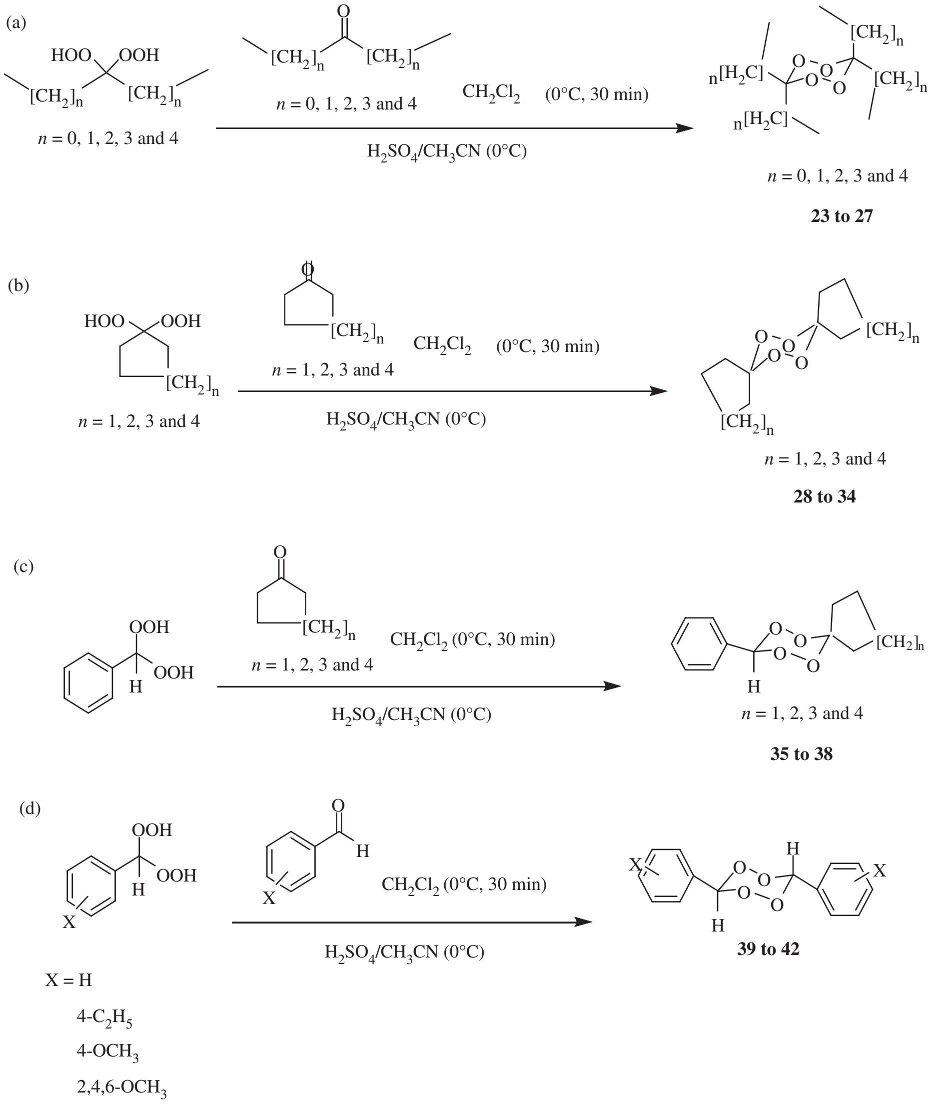

Figure 2. Synthetic scheme for the preparation of four series of tetraoxanes.

The inhibitory effect of all tetraoxanes tested on *C. sativus* was lower compared with the effect on *S. bicolor*. At the highest concentration (1.0 mM), compounds **29**, **30**, **32**, **33**, **35**, **36**, **37**, **41** and **42** were more active than the herbicides (Fig. 4). In this case it can be pointed out that, at the lower concentration (0.125 mM), the majority of compounds were more active than the commercial herbicides.

There was a correlation between dose and effect for all the compounds (Figs 3 and 4). Such results can indicate that the observed inhibition is a direct consequence of the interaction of the compounds with some key enzymes involved in the plant development.

The effects of the most active compounds (**29**, **30**, **31**, **32**, **33**, **35** and **36**), as selected on the basis of analysis of a large amount of data (Figs 3 and 4), were further evaluated on four important weed species, *Sorghum arundinaceum*, *Euphorbia heterophylla*, *Brachiaria brizantha* and *Bidens pilosa*. For this study, the compounds and the commercial herbicides were tested at 0.25 and 1.0 mM (Table 2).

No important inhibitory effect was observed for the compounds at 0.25 mM for *S. arundinaceum* (Table 2). At this concentration, glyphosate (post-emergent herbicide) stimulated root and shoot growth at low concentration, as did some compounds such as 29 and 35 (82 and 36.2% shoot growth induction respectively). These apparently unexpected results have a precedent in the literature,[44] where glyphosate, when applied at low doses, stimulated root and shoot growth up to 6 weeks after application. This phenomenon is known as hormesis, which is characterised by low-dose stimulation and high-dose inhibition.[45,46]

At higher concentration (1.0 mM), most compounds caused some inhibition of plant development. The most pronounced effect was caused by **36**, which inhibited the growth of both root and shoot by 95.5 and 92.9% respectively. This effect is comparable with the inhibition caused by the post-emergent herbicide imazethapyr (90.9 and 93.1% respectively). Although less active, compounds **30** and **31** caused root and shoot growth inhibition ranging from 45.2 to 66.6%, comparable with the effects caused by glyphosate (around 52–55%).

Compounds **32**, **33** and **35** caused necrosis in the roots, and compound **35** caused reddening of the leaves, besides inhibiting the growth of secondary roots (Fig. 5). These symptoms are

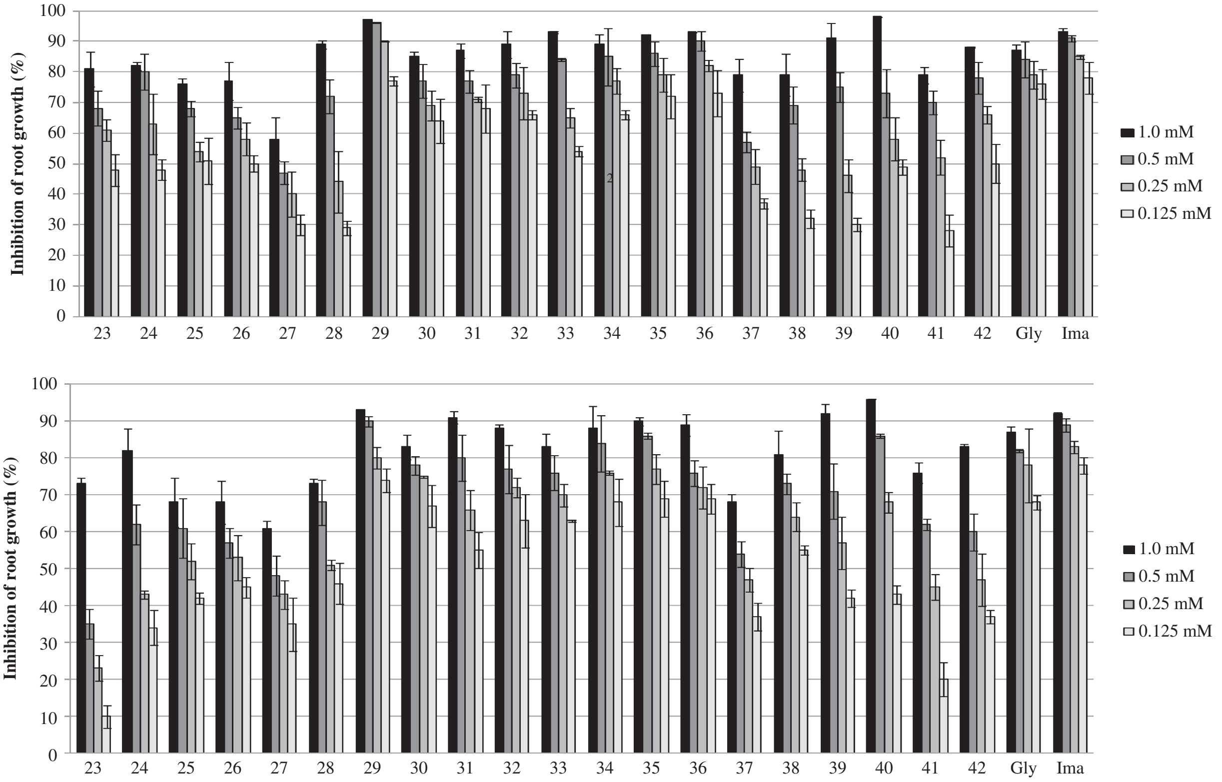

Figure 3. Growth inhibition of roots and shoots in *S. bicolor*, caused by tetraoxanes and commercial herbicides. Gly = glyphosate; Ima = imazethapyr.

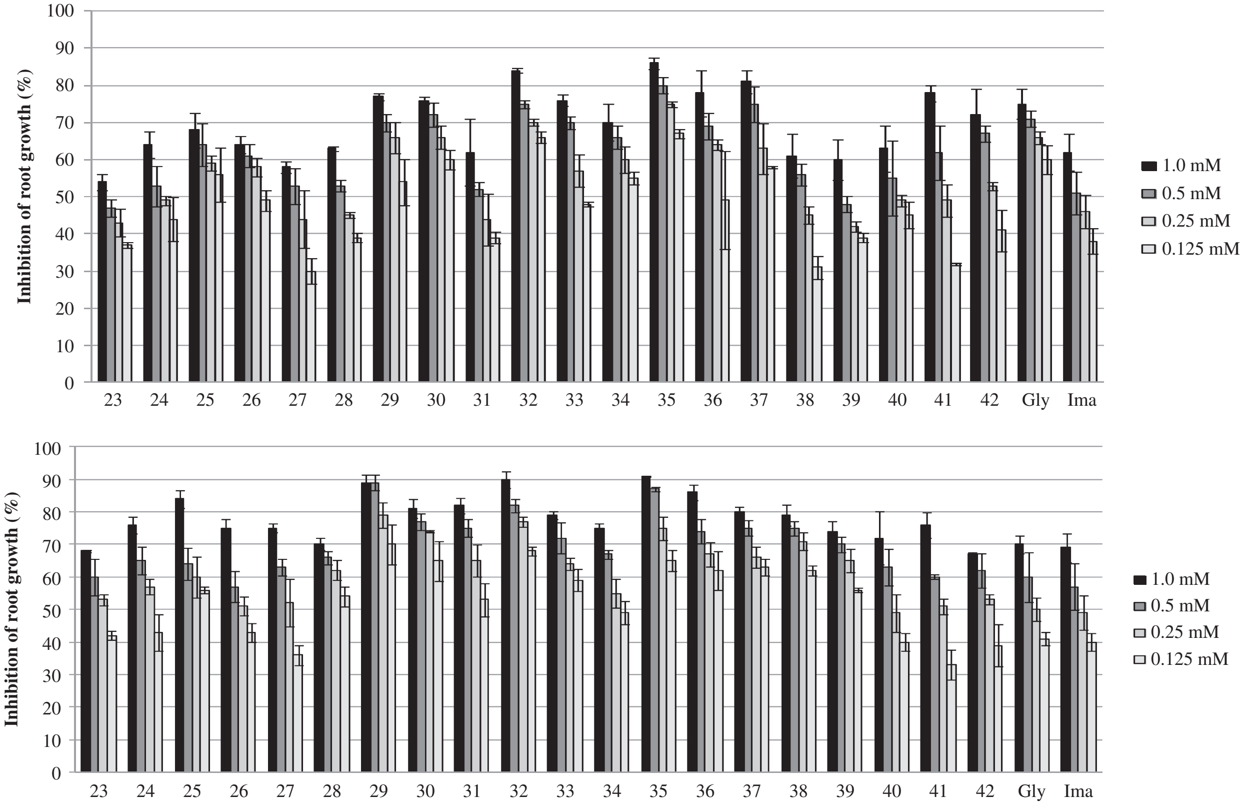

Figure 4. Growth inhibition of roots and shoots in *C. sativus*, caused by tetraoxanes and commercial herbicides. Gly = glyphosate; Ima = imazethapyr.

Table 2. Effect of herbicide compounds **29** to **33, 35** and **36** on the dry weight (roots and shoots) under greenhouse conditions after 40 days of growth

Sorghum arundinaceum[d]

Compound	0.25 mM				1.0 mM			
	Shoots[a]	Inhibition[b]	Roots[a]	Inhibition[b]	Shoots[a]	Inhibition[b]	Roots[a]	Inhibition[b]
29	245.1 aB	−19.0	655.2 bB	−82.0	157.3 aC	23.6	235.3 bC	34.6
30	160.1 bB	22.3	269.2 dB	25.2	87.3 bC	57.6	178.3 cC	50.5
31	142.9 bB	30.6	285.5 dB	20.7	112.9 bC	45.2	120.4 cC	66.6
32	158.4 bB	23.1	406.8 cB	−13.0	156.2 aC	19.6	386.4 aC	−7.3
33	173.9 bB	15.6	380.2 dB	−5.6	147.2 aC	28.5	281.6 bC	21.8
35	229.6 aB	−11.5	490.6 cB	−36.3	147.4 aC	28.5	232.8 bC	35.3
36	56.6 cB	72.5	108.9 eB	79.7	6.95 cC	95.5	23.9 dC	92.9
Glyphosate	287.1 aB	−39.4	760.9 aB	−111.4	93.1 bC	54.8	147.9 cC	52.2
Imazethapyr	43.9 cB	78.7	10.6 eB	97.0	18.8 cC	90.9	24.8 dC	93.1
Control	201.0 bA	0.0	353.0 dA	0.0	201.0 aA	0.0	353.0 bA	0.0

Euphorbia heterophylla[e]

Compound	0.25 mM				1.0 mM			
	Shoots[a]	Inhibition[b]	Root[a]	Inhibition[b]	Shoots[a]	Inhibition[b]	Roots[a]	Inhibition[b]
29	109.6 bB	20.6	54.4 aB	32.0	54.8 cC	80.1	16.8 dC	89.5
30	126.7 bB	8.2	50.2 aB	37.2	27.4 cC	85.1	12.9 dC	87.9
31	58.1 cB	68.4	47.2 bB	55.7	100.9 aC	26.9	44.5 bC	44.4
32	75.6 bB	44.9	19.1 bB	76.1	54.8 cC	80.1	16.8 dC	89.5
33	111.7 bB	39.3	41.2 bB	61.4	67.5 bC	51.1	20.4 cC	74.5
35	66.2 cB	76.0	36.2 bB	77.3	12.2 dC	97.8	8.5 dC	96.6
36	212.8 aB	−54.2	81.7 aB	−2.1	139.1 aC	2.0	54.8 bC	31.4
Glyphosate	43.9 cB	68.2	10.6 cB	86.7	18.8 cC	86.4	5.2 dC	93.7
Imazethapyr	–[c]	–	–	–	–	–	–	–
Control	141.0 bA	0.0	80.0 aA	0.0	141.0 aA	0.0	80.0 aC	0.0

Brachiaria brizantha[f]

Compound	0.25 mM				1.0 mM			
	Shoots[a]	Inhibition[b]	Roota	Inhibition[b]	Shoots[a]	Inhibition[b]	Roots[a]	Inhibition[b]
29	258.3 aB	21.2	437.6 aB	41.6	46.9 cC	92.8	58.7 cC	96.1
30	145.1 bB	55.8	394.3 aB	47.3	27.8 cC	91.5	35.8 cC	95.2
31	139.2 bB	57.6	308.1 aB	58.9	52.9 bC	83.9	89.2 bC	88.1
32	242.1 aB	25.7	464.1 aB	38.0	118.3 aC	63.7	174.9 aC	76.6
33	189.1 bB	42.3	385.5 aB	48.5	12.0 cC	99.1	23.9 cC	99.2
35	221.9 aB	32.3	424.8 aB	44.3	143.1 aC	56.4	189.9 aC	74.6
36	175.0 bB	41.4	347.3 aB	53.6	40.9 cC	97.5	37.4 cC	95.0
Glyphosate	141.9 bB	56.7	207.0 bB	72.4	13.6 cC	96.9	30.3 cC	97.0
Imazethapyr	–[c]	–	–	–	–	–	–	–
Control	329.0 aA	0.0	749.0 aA	0.0	329.0 aA	0.0	749.0 aA	0.0

Bidens pilosa[g]

Compound	0.25 mM				1.0 mM			
	Shoots[a]	Inhibition[b]	Roots[a]	Inhibition[b]	Shoots[a]	Inhibition[b]	Roots[a]	Inhibition[b]
29	193.2 aB	18.2	75.7 cB	64.6	41.0 cC	89.2	16.0 dC	90.0
30	189.5 aB	20.4	215.8 aB	−0.8	124.3 cC	47.8	232.9 bC	−8.8
31	164.9 bB	30.7	191.2 aB	10.7	102.2 cC	52.4	83.2 cC	61.1
32	148.8 bB	36.9	144.6 bB	32.4	158.8 bC	32.7	113.2 cC	47.1
33	153.8 bB	35.4	124.4 bB	36.7	150.9 bC	36.6	75.1 cC	64.9
35	217.9 aB	8.4	210.2 aB	−1.01	271.4 aC	−14.1	290.0 aC	−35.5
36	200.1 aB	15.9	146.2 bB	31.7	252.9 aC	−6.3	93.9 cC	56.1
Glyphosate	185.2 aB	22.2	56.0 cB	73.8	13.6 dC	94.3	2.6 dC	98.2
Imazethapyr	–[c]	–	–	–	–	–	–	–
Control	236.0 aA	0.0	208.0 aA	0.0	236.0 aC	0.0	208.0 bC	0.0

[a] The mass corresponds to the dry weight (mg).

[b] Inhibition (%).

[c] A dash (—) denotes no germination. Means followed by the same letter (columns – lower-case letters; lines – upper-case letters) do not differ statistically from each other at $P = 0.01\%$ by Scott–Knott's test.

[d] CV% (root) = 27.78, CV% (shoot) = 32.95.

[e] CV% (root) = 36.98, CV% (shoot) = 32.82.

[f] CV% (root) = 21.19, CV% (shoot) = 38.43.

[g] CV% (root) = 38.63, CV% (shoot) = 34.06.

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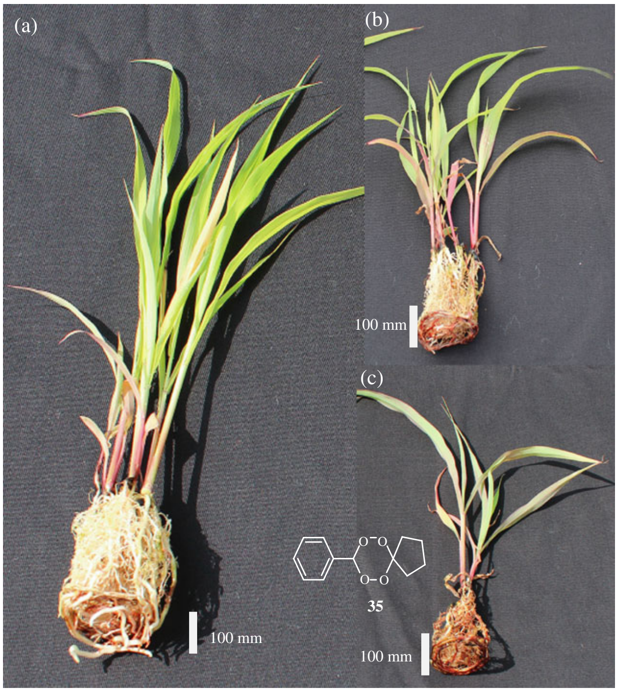

Figure 5. Effect of compound **35** on the development of *S. arundinaceum* under greenhouse conditions after 40 days of sowing: (a) control; (b) plant treated with glyphosate; (c) plant treated with tetraoxane **35**. Test concentration 1.0 mM.

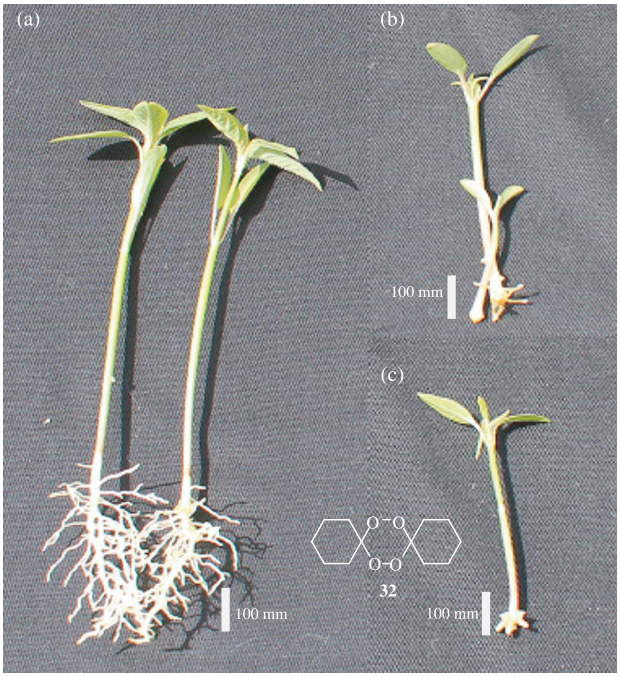

Figure 6. Effect of compound **32** on the growth of *E. heterophylla* under greenhouse conditions after 40 days of sowing: (a) control; (b) plant treated with glyphosate; (c) plant treated with tetraoxane **32**. Test concentration 1.0 mM.

characteristic of compounds causing stress on plants as well as on *S. arundinaceum*.[47]

For *E. heterophylla* (dicot), imazethapyr completely inhibited seed germination, and no comparison was possible. On the other hand, glyphosate caused inhibitions of root growth of 68.2 and 86.5% at 0.25 and 1.0 mM respectively. For the shoots, the inhibitions were 86.7% (0.25 mM) and 93.7% (1.0 mM).

At 1.0 mM, compounds **29**, **30**, **32** and **35** inhibited root and aerial part growth in a comparable manner to glyphosate (Table 2). At 0.25 mM, compound **35** inhibited the roots and shoots by approximately 76%, while **32** caused 76.1% inhibition of shoot growth.

As can be seen in Fig. 6, compound **32** completely inhibited root development of *E. heterophylla*. The tetraoxanes **33** and **35** caused a similar effect, including inhibition of the growth of secondary roots (Fig. 7). These symptoms are characteristic of compounds that inhibit microtubule formation during cell division in the prophase stage, with consequent inhibition of radicle growth and formation of secondary roots, while action on leaf development is less evident.[48–50]

For *B. brizantha*, imazethapyr completely inhibited germination, and glyphosate caused 97% inhibition (roots and shoot) at 1.0 mM but was less potent at 0.25 mM (56.7% shoot inhibition; 72.4% root inhibition). As observed in the previous cases, all compounds caused some growth inhibition at both concentrations tested, and the effect was dose dependent. At 0.25 mM, compounds **31** and **36** were the most active, causing more than 50% inhibition of root development (Table 2). At a higher concentration, compounds **29**, **30**, **33** and **36** caused shoot and root inhibition ranging from 91.5 to 99.1%, comparable with the effects observed for glyphosate.

The inhibition of root and shoot growth of *B. brizantha* by tetraoxane is comparable with that caused by glyphosate. In both cases the leaves turned yellow (Fig. 8).

Figure 7. Effect of the compounds **33** and **35** on the development of *E. heterophylla* under greenhouse conditions after 40 days of sowing: (a) control; (b) plant treated with tetraoxane **33**; (c) plant treated with tetraoxane **35**. Test concentration 1.0 mM.

For the dicotyledonous species *B. pilosa*, the effects of the tetraoxanes were compared with those of glyphosate, as

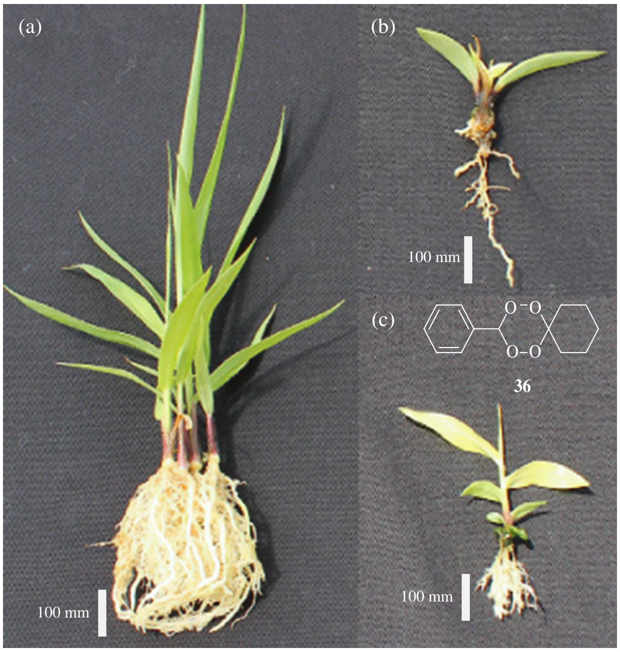

Figure 8. Effect of compound **36** on the development of *B. brizantha* under greenhouse conditions after 40 days of sowing: (a) control; (b) plant treated with glyphosate; (c) plant treated with tetraoxane **36**. Test concentration 1.0 mM.

imazethapyr caused 100% inhibition of seed germination. As expected, all compounds, including glyphosate, were more active at higher concentration (1.0 mM), with the commercial herbicide causing 94.3 and 98.2% shoot and root inhibition respectively.

The compounds were not too efficient in inhibiting the growth of roots and shoots of *B. pilosa*, as observed for the other species investigated (Table 2). In general, compounds **31**, **33** and **36** caused a shoot inhibition of above 50%. The most active compound **29** inhibited the shoots and roots by 89.2 and 90% respectively, an activity comparable with glyphosate.

The inhibitory activity profile displayed by the most active tetraoxanes tested (compounds **29, 30, 31, 32, 33, 35** and **36**) in some cases was comparable with the tested herbicides (glyphosate and imazethapyr), but there is a need to synthesise new compounds to show that this class is promising for the development of a potential new herbicide.

4 CONCLUSIONS

A new set of tetraoxanes were synthesised from simple commercial ketones and aldehydes and hydrogen peroxide catalysed under acidic conditions in only two steps. The herbicidal activity of this class of compounds was tested, and seven of the tetraoxanes presented a phytotoxic activity comparable with that of the commercial herbicides tested.

The activity of tetraoxane **36** was superior to the tested herbicides (glyphosate and imazethapyr), and furthermore this compound showed some selectivity towards monocot weed species.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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