

## Novel Oxazines with Potential Antiparasitic Activity obtained by Hetero Diels-Alder Reactions

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**SUMMARY.** Sixteen novel oxazines with potential antiparasitic activity were prepared using Diels-Alder reactions, starting from two structurally different types of dienes (purpurogallin and substituted cis-3,5-cyclohexadien-1,2-diol derivatives), and nitrosoarenes as dienophiles. The biological activity was evaluated by *in vitro* assays against *Plasmodium falciparum*, *Trypanosoma cruzi* and *T. brucei rhodesiense*. Some of these oxazines had activities in the range of 8-50  $\mu\text{M}$ , and could be lead compounds in the development of novel drugs.

**RESUMEN.** "Nuevas Oxazinas con Posible Actividad Antiparasitaria Obtenidos por Reacciones de Hetero Diels-Alder". Se prepararon 16 nuevas oxazinas con potencial actividad antiparasitaria por la reacción de Diels-Alder partiendo de dos series de dienos con estructuras diferentes (purpurogalina y derivados de cis-3,5-ciclohexadien-1,2-dioles), y nitrosoarenos como dienófilos. La actividad biológica fué evaluada por ensayos *in vitro* frente a *Plasmodium falciparum*, *Trypanosoma cruzi* y *T. brucei rhodesiense*. Algunas de las oxazinas mostraron actividad en el rango de 8-50  $\mu\text{M}$ , y pudieran ser compuestos de cabeza para desarrollar nuevos medicamentos.

### INTRODUCTION

Infectious diseases due to parasitic protozoa cause 300-500 million clinical cases and over one million deaths each year. These diseases are commonly associated with poverty, but are also a cause of poverty and a major hindrance to economic development. The most important protozoan infections are malaria, trypanosomiasis and leishmaniasis <sup>1</sup>. The most malignant forms of malaria are caused by *Plasmodium falciparum*, a particularly resistant parasite known to have high adaptability by mutation. This infectious disease is widespread in tropical and subtropical regions, including parts of the Americas, Asia and Africa. The extensive use of drugs to treat the infection have favoured the "selection" of resistant forms. This has hindered the eradication by chemotherapy of the disease, and increases the interest in the development of new lead compounds <sup>2</sup>.

Our group has already reported the design,

synthesis and biological evaluation of 12 oxazines obtained by Diels-Alder cycloaddition using dienes with the benzotropolone (tri-O-methylpurpurogallin) structure, and nitrosobenzene as dienophile <sup>3</sup>. More recently, we described the synthesis and biological activity of a further 12 oxazines obtained using as eucarvone and derivatives as dienes for reaction with nitrosoarenes as dienophiles <sup>4</sup>.

### MATERIALS AND METHODS

Melting points were determined using a Galenkamp apparatus and are uncorrected. IR spectra were recorded on a FT-IR Shimadzu model DR-8100 spectrometer and are reported as  $\lambda_{\text{max}}$  ( $\text{cm}^{-1}$ ). <sup>1</sup>H RMN and <sup>13</sup>C RMN spectra were recorded on a Bruker Avance DPX 400 MHz using CDCl<sub>3</sub> as solvent and TMS as internal standard and are reported in  $\delta$  values. Mass spectra were measured on a Shimadzu GC-MS QP 1100 EX, and on a Hewlett Packard 5980II

**KEY WORDS:** Oxazines; Diels-Alder reaction; antiparasitic activity

**PALABRAS CLAVE:** Oxazinas; Reacción de Diels-Alder; Actividad antiparasitaria.

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gas chromatograph coupled to a Hewlett Packard 5971 mass spectrometer at an ionizing potential of 70 eV. Elemental analyses were carried out on a Fisons EA 1108 CHNS-O analyzer. Column chromatography was performed with Silica gel (1.063-02 mm; J. T. Baker). All solvents were purified and dried before use, using standard methods. Aryl nitroso compounds were prepared according to literature procedures <sup>5</sup>.

### General procedures

#### Diels-Alder reaction

The corresponding diene (0.67 mmol) and the nitroso compound (0.74 mmol) were dissolved in the minimum amount of dried THF. The reaction was stirred at room temperature and in the absence of light until total consumption of diene. The solvent was evaporated under vacuum and the residue purified by column chromatography on silica-gel.

#### Protection of diols with 1,2-dimethoxypropane

(1 eq.) of the corresponding diol, 4 eq. of DMP, and catalytic amounts of p-TsOH acid were dissolved in acetone and stirred at room temperature until the reaction was complete. The residue was diluted with ether, washed with an aqueous solution of NaHCO<sub>3</sub> (1 x 2 mL), and with brine (1 x 2 mL). The organic phase was dried with anhydrous MgSO<sub>4</sub>, filtered, the solvent was evaporated under vacuum. The product was purified by column chromatography on Silica gel. Yield 96-98 %.

## RESULTS

### Synthetic results

(±)-4,5,6,9-tetramethoxy-11-phenyl-10-oxa-11-aza-tricyclo [7.2.2.0<sub>2</sub>,7] trideca-2,4,6,12-tetraen-8-one (3).

5,6 Tetra-O-methypurpurogallin (1.1 g, 4 mmol) and nitrosobenzene (0.52 g, 4.8 mmol) reacted in hexane as described in the general procedure. The reaction was complete in 40 h. The precipitate was filtered, washed with cold hexane, and recrystallized from methanol. Yield 94 %. mp: 105-107 °C. (Dec.). IR, λ: 3044, 2982, 2944, 1694, 1590, 1491, 1456, 1402, 1356, 1325, 1283, 1258, 1215, 1198, 1134, 1107, 1088, 1049, 1032, 988, 955, 918, 899, 878, 841, 806, 764, 695, 656. <sup>1</sup>H RMN CDCl<sub>3</sub>, δ: 7.21 (2H, dd; *J*<sub>1</sub> = 7.8 Hz, *J*<sub>2</sub> = 7.3 Hz), 7.09 (2H, d; *J* = 7.8 Hz), 6.93 (1H, d; *J* = 7.3 Hz), 6.77 (1H, s), 6.64 (1H, dd; *J*<sub>1</sub> = 9.3 Hz, *J*<sub>2</sub> = 6.7 Hz), 6.17 (1H, dd; *J*<sub>1</sub> = 9.3 Hz, *J*<sub>2</sub> = 0.8 Hz), 5.35 (1H, d; *J*<sub>1</sub> = 6.7 Hz, *J*<sub>2</sub> = 0.8 Hz), 3.93 (3H, s), 3.91 (3H, s), 3.86 (3H, s), 3.69 (3H, s). <sup>13</sup>C RMN CDCl<sub>3</sub>, δ: 189.62, 156.09,

155.83, 150.18, 143.44, 136.23, 135.22, 128.63, 126.89, 122.70, 120.59, 117.64, 108.71, 102.10, 68.72, 62.48, 61.46, 55.92, 52.73. MS EI, *m/z* (relative intensity): 276 (83), 218 (65), 107 (62), 77 (100), 51 (52). Anal. Calc. for C<sub>21</sub>H<sub>21</sub>NO<sub>6</sub>: C, 65.79; H, 5.52; N, 3.65; Found: C, 65.82; H, 5.80; N, 3.75.

(±)-4-(4,5,6,9-tetramethoxy-8-oxo-10-oxa-11-azatricyclo [7.2.2.0<sub>2</sub>,7]trideca-2,4,6,12-tetraen-11-yl)-benzaldehyde (4).

Tetra-O-methypurpurogallin (0.1 g, 0.36 mmol) and 4-nitrosobenzaldehyde (0.054 g, 0.39 mmol) reacted in THF as described in the general procedure. The reaction was complete in 12 h. The residue was purified by column chromatography on Silica gel using Hexane:EtOAc 8:2. Yield 96 %. mp: 154-155 °C. IR, λ: 2980, 2810, 2800, 2726, 1820, 1780, 1597, 1566, 1499, 1460, 1420, 1387, 1306, 1316, 1177, 1161, 1115, 1096, 1080, 1044, 1019, 967, 949, 893, 855, 833, 803, 718, 695, 650. <sup>1</sup>H RMN CDCl<sub>3</sub>, δ: 9.90 (1H, s), 7.82 (2H, d; *J* = 8.5 Hz), 7.29 (2H, d; *J* = 8.5 Hz), 6.75 (1H, s), 6.69 (1H, dd; *J*<sub>1</sub> = 9.2 Hz, *J*<sub>2</sub> = 6.7 Hz), 6.19 (1H, d; *J* = 9.2 Hz), 5.43 (1H, d; *J* = 6.7 Hz), 3.97 (3H, s), 3.93 (3H, s), 3.88 (3H, s), 3.74 (3H, s). <sup>13</sup>C RMN CDCl<sub>3</sub>, δ: 191.14, 189.46, 158.02, 156.85, 156.11, 144.48, 136.75, 134.71, 131.74, 131.60, 127.86, 120.45, 117.69, 109.23, 103.34, 68.64, 62.21, 61.47, 56.66, 53.58. MS EI, *m/z* (relative intensity): 276 (100), 218 (70), 135 (82), 77 (71), 51 (75). Anal. Calc. for C<sub>22</sub>H<sub>21</sub>NO<sub>7</sub>: C, 64.23; H, 5.14; N, 40; Found: C, 63.88; H, 5.22; N, 3.41.

(±)-11-(4-chlorophenyl)-4,5,6,9-tetramethoxy-10-oxa-11-aza-tricyclo [7.2.2.0<sub>2</sub>,7]trideca-2,4,6,12-tetraen-8-one (5).

Tetra-O-methypurpurogallin (0.1 g, 0.36 mmol) and 4-chloronitrosobenzene (0.056 g, 0.39 mmol) react in 10 mL of hexane. The reaction was complete in 15 h. 50 % of the solvent was evaporated under vacuum, and the precipitate was washed with cold hexane and recrystallized from methanol. Yield 93 %. mp: 131-132 °C. IR, λ: 3000, 2942, 1694, 1590, 1487, 1402, 1358, 1327, 1258, 1204, 1136, 1082, 1055, 1003, 961, 949, 901, 885, 843, 816, 795, 750, 710. <sup>1</sup>H RMN CDCl<sub>3</sub>, δ: 7.25 (2H, d; *J* = 8.8 Hz), 7.09 (2H, d; *J* = 8.8 Hz), 6.71 (1H, s), 6.62 (1H, dd; *J*<sub>1</sub> = 9.4 Hz, *J*<sub>2</sub> = 6.7 Hz), 6.20 (1H, d; *J* = 9.4 Hz), 5.21 (1H, d; *J* = 6.7 Hz), 3.95 (3H, s), 3.92 (3H, s), 3.87 (3H, s), 3.69 (3H, s). <sup>13</sup>C RMN CDCl<sub>3</sub>, δ: 189.04, 157.60, 156.43, 149.15, 144.42, 135.91, 134.99, 129.71, 128.95, 127.56, 123.34, 119.33, 108.86, 102.45, 69.37, 61.09, 56.25, 55.99, 53.05.

MS EI,  $m/z$  (relative intensity): 276 (100), 218 (56), 143 (20), 141 (52), 111 (77), 76 (23), 75 (56), 74 (17). Anal. Calc. for  $C_{21}H_{20}ClNO_6$ : C, 60.36; H, 4.82; N, 3.35; Found: C, 59.97; H, 5.20; N, 3.21.

(±)-4,5,6,9-tetramethoxy-11-*p*-toluyl-10-oxa-11-aza-tricyclo[7.2.2.0<sub>2</sub>,7]trideca-2,4,6,12-tetraen-8-one (6)

Tetra-*O*-methypurpurogallin (0.1 g, 0.36 mmol) and 4-nitrosotoluene (0.048 g, 0.39 mmol) reacted in 10 mL of hexane. The reaction was complete in 48 h. The precipitate was filtered, washed with cold hexane, and recrystallized from methanol. Yield 92 %. mp: 122-123 °C. IR,  $\lambda$ : 3000, 2938, 1680, 1590, 1505, 1489, 1402, 1356, 1327, 1258, 1136, 1103, 1082, 1055, 1003, 950, 901, 885, 849, 841, 818, 803, 783, 756, 725. <sup>1</sup>H RMN  $CDCl_3$ ,  $\delta$ : 7.10-7.04 (4H, m), 6.71 (1H, s), 6.61 (1H, dd;  $J_1 = 9.2$  Hz,  $J_2 = 6.7$  Hz), 6.20 (1H, d;  $J = 9.2$  Hz), 5.22 (1H, d;  $J = 6.7$  Hz), 3.95 (3H, s), 3.91 (3H, s), 3.86 (3H, s), 3.69 (3H, s), 2.29 (3H, s). <sup>13</sup>C RMN  $CDCl_3$ ,  $\delta$ : 190.21, 157.92, 156.69, 148.48, 144.17, 136.47, 135.93, 132.95, 129.84, 127.85, 123.70, 120.50, 118.37, 109.25, 102.61, 69.73, 62.16, 61.45, 56.59, 53.31, 21.01. MS EI,  $m/z$  (relative intensity): 276 (100), 218 (60), 121 (57), 91 (68), 65 (52). Anal. Calc. for  $C_{22}H_{23}NO_6$ : C, 66.49; H, 5.83; N, 3.52; Found: C, 66.81; H, 5.69; N, 3.58.

(3*S*,5*R*)-4,4,7-Trimethyl-9-phenyl-3,5,8-trioxa-9-aza-tricyclo[5.2.2.0<sub>2</sub>,6]undec-10-ene (13).

Compound 10 (0.26 g, 1.58 mmol) and nitrosobenzene (0.19 g, 1.74 mmol) reacted in THF as described in the general procedure. The reaction was complete in 20 h. The solvent was evaporated under vacuum and the residue purified by column chromatography on Silica gel using Hexane:EtOAc 8:2. Yield 78 %. mp: 108-109 °C. IR,  $\lambda$ : 2980, 2932, 1595, 1489, 1453, 1379, 1258, 1202, 1165, 1130, 1086, 1063, 1030, 999, 976, 924, 884, 855, 810, 762, 710, 696, 654. <sup>1</sup>H RMN  $CDCl_3$ ,  $\delta$ : 7.25 (2H, dd;  $J_1 = 7.8$  Hz,  $J_2 = 7.3$  Hz), 7.03 (2H, d;  $J = 7.8$  Hz), 6.97 (1H, t;  $J = 7.3$  Hz), 6.22 (1H, ddd;  $J_1 = 8.2$  Hz,  $J_2 = 1.3$  Hz,  $J_3 = 0.8$  Hz), 6.06 (1H, ddd;  $J_1 = 8.2$  Hz,  $J_2 = 5.7$  Hz,  $J_3 = 0.7$  Hz), 4.78 (1H, ddd;  $J_1 = 7.2$  Hz,  $J_2 = 4.1$  Hz,  $J_3 = 0.7$  Hz), 4.70 (1H, ddd;  $J_1 = 5.7$  Hz,  $J_2 = 4.1$  Hz,  $J_3 = 1.3$  Hz), 4.28 (1H, dd;  $J_1 = 7.2$  Hz,  $J_2 = 0.8$  Hz), 1.66 (3H, s), 1.37 (3H, s), 1.35 (3H, s). <sup>13</sup>C RMN  $CDCl_3$ ,  $\delta$ : 151.41, 133.85, 128.93, 128.60, 122.96, 118.00, 110.80, 79.33, 75.46, 75.22, 60.53, 26.14, 25.84, 20.84. MS EI,  $m/z$  (relative intensity): 109 (100), 108 (76), 107 (50), 80 (76), 79 (80), 77 (81). Anal. Calc. for

$C_{16}H_{19}NO_3$ : C, 70.31; H, 7.01; N, 5.12; Found: C, 70.17; H, 6.96; N, 5.15.

(3*S*,5*R*)-4-(4,4,7-trimethyl-3,5,8-trioxa-9-azatricyclo[5.2.2.0<sub>2</sub>,6]undec-10-en-9-yl)-benzaldehyde (14)

Compound 10 (0.13 g, 0.79 mmol) and 4-nitrosobenzaldehyde (0.12 g, 0.87 mmol) reacted in THF as described in the general procedure. The reaction was complete in 15 h. The solvent was evaporated under vacuum and the residue purified by column chromatography on Silica gel using Hexane:EtOAc 7:3. Yield 80 %. mp: 155-156 °C. IR,  $\lambda$ : 2992, 2977, 2832, 2745, 1698, 1595, 1499, 1458, 1420, 1385, 1374, 1267, 1237, 1213, 1183, 1167, 1084, 1059, 990, 924, 872, 857, 838, 735, 675, 633. <sup>1</sup>H RMN  $CDCl_3$ ,  $\delta$ : 9.85 (1H, s), 7.75 (2H, d;  $J = 8.6$  Hz), 7.09 (2H, d;  $J = 8.6$  Hz), 6.19 (1H, dd;  $J = 8.2$  Hz), 6.11 (1H, dd;  $J_1 = 8.2$  Hz,  $J_2 = 5.7$  Hz), 4.82-4.85 (1H, m), 4.72 (1H, dd;  $J_1 = 6.7$  Hz,  $J_2 = 4.1$  Hz), 4.27 (1H, d;  $J = 6.7$  Hz), 1.66 (3H, s), 1.33 (6H, s). <sup>13</sup>C RMN  $CDCl_3$ ,  $\delta$ : 190.83, 156.43, 133.51, 130.95, 130.71, 128.43, 116.53, 110.63, 78.72, 75.82, 74.28, 58.85, 25.73, 25.47, 20.41. MS EI,  $m/z$  (relative intensity): 301 (M<sup>+</sup>, 37), 109 (100), 108 (92), 100 (32), 80 (52), 77 (40), 43 (62). Anal. Calc. for  $C_{17}H_{19}NO_4$ : C, 67.76; H, 6.36; N, 4.65; Found: C, 67.48; H, 6.59; N, 4.67.

(3*S*,5*R*)-9-(4-chlorophenyl)-4,4,7-trimethyl-3,5,8-trioxa-9-aza-tricyclo[5.2.2.0<sub>2</sub>,6]undec-10-ene (15).

Compound 10 (0.13 g, 0.79 mmol) and 4-chloronitrosobenzene (0.12 g, 0.87 mmol) reacted in THF as described in the general procedure. The reaction was complete in 24 h. The residue was purified by column chromatography on Silica gel using Hexane:EtOAc 8:2. Yield 75 %. mp: 91-92 °C. IR,  $\lambda$ : 3058, 2979, 2932, 1736, 1592, 1487, 1456, 1404, 1264, 1210, 1179, 1163, 1684, 1063, 1007, 972, 918, 876, 853, 828, 812, 729, 714. <sup>1</sup>H RMN  $CDCl_3$ ,  $\delta$ : 7.17 (2H, d;  $J = 8.9$  Hz), 6.92 (2H, d;  $J = 8.9$  Hz), 6.19 (1H, ddd;  $J_1 = 8.3$  Hz,  $J_2 = 1.6$  Hz,  $J_3 = 0.8$  Hz), 6.00 (1H, ddd;  $J_1 = 8.3$  Hz,  $J_2 = 5.7$  Hz,  $J_3 = 0.7$  Hz), 4.72 (1H, ddd;  $J_1 = 7.2$  Hz,  $J_2 = 4.1$  Hz,  $J_3 = 0.7$  Hz), 4.61 (1H, ddd;  $J_1 = 5.7$  Hz,  $J_2 = 4.1$  Hz,  $J_3 = 1.6$  Hz), 4.24 (1H, dd;  $J_1 = 7.2$  Hz,  $J_2 = 0.8$  Hz), 1.61 (3H, s), 1.32 (3H, s), 1.31 (3H, s). <sup>13</sup>C RMN  $CDCl_3$ ,  $\delta$ : 149.72, 133.55, 128.59, 128.01, 127.59, 118.87, 110.41, 78.82, 75.02, 74.61, 60.24, 25.73, 25.45, 20.43. MS EI,  $m/z$  (relative intensity): 309 (M<sup>+</sup> +2; 11), 307 (M<sup>+</sup>; 31), 111 (48), 109 (85), 108 (100), 80 (46), 43 (51). Anal. Calc. for  $C_{16}H_{18}ClNO_3$ : C, 62.44; H, 5.89; N, 4.55; Found: C, 62.79; H, 5.96; N, 4.87.

*(3S,5R)-4,4,7-trimethyl-9-p-toluyyl-3,5,8-trioxa-9-aza-tricyclo[5.2.2.0<sub>2</sub>,6]undec-10-ene (16)*

Compound 10 (0.13 g, 0.79 mmol) and 4-nitrosotoluene (0.11 g, 0.87 mmol) reacted in THF as described in the general procedure. The reaction was complete in 48 h. The residue was purified by column chromatography on Silica gel using Hexane:EtOAc 8:2. Yield 70 %. mp: 75-76 °C. IR,  $\lambda$ : 2980, 2932, 1736, 1611, 1578, 1507, 1456, 1375, 1242, 1198, 1163, 1130, 1084, 1001, 976, 943, 922, 884, 855, 820, 758, 708, 646. <sup>1</sup>H RMN CDCl<sub>3</sub>,  $\delta$ : 7.02 (2H, d;  $J$  = 8.3 Hz), 6.89 (2H, d;  $J$  = 8.3 Hz), 6.19 (1H, d;  $J$  = 8.2 Hz), 6.02 (1H, dd;  $J_1$  = 8.2 Hz,  $J_2$  = 5.8 Hz), 4.74 (1H, dd;  $J_1$  = 6.9 Hz,  $J_2$  = 4.0 Hz), 4.63-4.60 (1H, m), 4.24 (1H, d;  $J$  = 6.9 Hz), 2.26 (3H, s), 1.61 (3H, s), 1.33 (3H, s), 1.32 (3H, s). <sup>13</sup>C RMN CDCl<sub>3</sub>,  $\delta$ : 148.64, 133.49, 132.03, 129.11, 128.22, 117.69, 110.23, 78.79, 74.77, 74.65, 60.28, 25.75, 25.46, 20.63, 20.49. MS EI,  $m/z$  (relative intensity): 288 (M+ +1, 10), 287 (M+, 51), 187 (51), 186 (17), 121 (100), 109 (45), 108 (42), 91 (68), 43 (49). Anal. Calc. for C<sub>17</sub>H<sub>21</sub>NO<sub>3</sub>: C, 71.06; H, 7.37; N, 4.87; Found: C, 71.31; H, 7.44; N, 4.79.

*(3S,5S)-7-Chloro-4,4-dimethyl-9-phenyl-3,5,8-trioxa-9-aza-tricyclo[5.2.2.0<sub>2</sub>,6]undec-10-ene (17)*

Compound 11 (0.2 g, 1.07 mmol) and nitrosobenzene (0.126 g, 1.18 mmol) reacted in THF in a similar way as described in the general procedure. The reaction was complete in 24 h. The residue was purified by column chromatography on Silica gel using Hexane:EtOAc 8:2. Yield 80 %. Colorless oil. IR,  $\lambda$ : 2990, 2940, 1692, 1595, 1489, 1375, 1266, 1211, 1161, 1117, 1084, 1051, 974, 949, 858, 766, 696, 511. <sup>1</sup>H RMN CDCl<sub>3</sub>,  $\delta$ : 7.28 (2H, dd;  $J_1$  = 8.7 Hz,  $J_2$  = 7.3 Hz, 7.05-7.02 (3H, m), 6.45 (1H, dd;  $J_1$  = 8.3 Hz,  $J_2$  = 1.5 Hz), 6.10 (1H, ddd;  $J_1$  = 8.3 Hz,  $J_2$  = 5.6 Hz,  $J_3$  = 1.0 Hz), 4.89 (1H, ddd;  $J_1$  = 7.1 Hz,  $J_2$  = 4.0 Hz,  $J_3$  = 1.0 Hz), 4.75 (1H, ddd;  $J_1$  = 5.6 Hz,  $J_2$  = 4.0 Hz,  $J_3$  = 1.5 Hz), 4.62 (1H, dd;  $J_1$  = 7.1 Hz,  $J_2$  = 1.1 Hz), 1.40 (6H, s). <sup>13</sup>C RMN CDCl<sub>3</sub>,  $\delta$ : 150.02, 132.71, 129.96, 128.75, 123.42, 117.65, 111.45, 94.65, 80.92, 75.47, 60.40, 25.47, 25.31. MS EI,  $m/z$  (relative intensity): 293 (44), 235 (55), 208 (39), 206 (100), 193 (55), 166 (95), 164 (100), 158 (40), 130 (68), 127 (45), 81 (89).

*(3S,5S)-4-(7-Chloro-4,4-dimethyl-3,5,8-trioxa-9-aza-tricyclo[5.2.2.0<sub>2</sub>,6]undec-10-en-9-yl)-benzaldehyde (18)*

Compound 11 (0.2 g, 1.18 mmol) and 4-nitrosobenzaldehyde (0.16 g, 0.74 mmol) react in THF as described in the general procedure. The

reaction was complete in 22 h. The residue was purified by column chromatography on Silica gel using Hexane:EtOAc 7:3. Yield 85 %. mp: 174-175 °C. IR,  $\lambda$ : 3093, 2992, 2977, 2937, 2832, 2803, 2745, 1697, 1597, 1578, 1501, 1458, 1426, 1383, 1375, 1343, 1311, 1306, 1267, 1213, 1163, 1119, 1086, 1051, 1005, 974, 949, 911, 862, 833, 808, 750, 739, 718, 691, 677, 658, 633, 615. <sup>1</sup>H RMN CDCl<sub>3</sub>,  $\delta$ : 9.91 (1H, s), 7.80 (2H, d;  $J$  = 8.7 Hz), 7.15 (2H, d;  $J$  = 8.7 Hz), 6.43 (1H, ddd;  $J_1$  = 8.6 Hz,  $J_2$  = 1.5 Hz,  $J_3$  = 1.2 Hz), 6.16 (1H, ddd;  $J_1$  = 8.6 Hz,  $J_2$  = 5.6 Hz,  $J_3$  = 1.2 Hz), 4.91 (1H, ddd;  $J_1$  = 5.6 Hz,  $J_2$  = 4.0 Hz,  $J_3$  = 1.5 Hz), 4.87 (1H, ddd;  $J_1$  = 7.0 Hz,  $J_2$  = 4.0 Hz,  $J_3$  = 1.2 Hz), 4.63 (1H, dd;  $J_1$  = 7.0 Hz,  $J_2$  = 1.2 Hz), 1.40 (6H, s). <sup>13</sup>C RMN CDCl<sub>3</sub>,  $\delta$ : 190.77, 155.39, 132.71, 131.67, 130.97, 130.08, 116.97, 111.78, 94.80, 80.77, 75.10, 59.42, 25.66, 25.48. MS EI,  $m/z$  (relative intensity): 135 (49), 129 (100), 128 (40), 100 (71), 77 (97), 65 (88). Anal. Calc. for C<sub>16</sub>H<sub>16</sub>ClNO<sub>4</sub>: C, 59.73; H, 5.01; N, 4.35; Found: C, 59.88; H, 5.21; N, 4.40.

*(3S,5S)-7-Chloro-9-(4-chlorophenyl)-4,4-dimethyl-3,5,8-trioxa-9-aza-tricyclo[5.2.2.0<sub>2</sub>,6]undec-10-ene (19)*

Compound 11 (0.3 g, 1.61 mmol) and 4-chloronitrosobenzene (0.25 g, 1.77 mmol) react in THF as described in the general procedure. The reaction was completed in 24 h. The residue was purified by column chromatography on Silica gel using Hexane:EtOAc 8:2. Yield 75 %. mp: 99-100 °C. IR,  $\lambda$ : 2992, 2936, 1589, 1487, 1404, 1375, 1339, 1265, 1211, 1161, 1117, 1086, 1051, 1009, 974, 951, 907, 858, 829, 810, 712, 559. <sup>1</sup>H RMN CDCl<sub>3</sub>,  $\delta$ : 7.23 (2H, d;  $J$  = 8.7 Hz), 6.97 (2H, d;  $J$  = 8.7 Hz), 6.43 (1H, d;  $J$  = 8.6 Hz), 6.08 (1H, dd;  $J_1$  = 8.6 Hz,  $J_2$  = 5.6 Hz), 4.86 (1H, dd;  $J_1$  = 7.0 Hz,  $J_2$  = 4.0 Hz), 4.69 (1H, ddd;  $J_1$  = 5.6 Hz,  $J_2$  = 4.0 Hz), 4.59 (1H, dd;  $J$  = 7.0 Hz), 1.39 (6H, s). <sup>13</sup>C RMN CDCl<sub>3</sub>,  $\delta$ : 148.66, 132.79, 129.79, 128.62, 128.54, 118.87, 111.55, 94.63, 80.76, 75.66, 60.19, 25.45, 25.30. MS EI,  $m/z$  (relative intensity): 235 (100), 200 (61), 199 (39), 95 (17 %). Anal. Calc. for C<sub>15</sub>H<sub>15</sub>Cl<sub>2</sub>NO<sub>3</sub>: C, 54.90; H, 4.61; N, 4.27; Found: C, 54.52; H, 5.02; N, 4.22.

*(3S,5S)-7-Chloro-4,4-dimethyl-9-p-toluyyl-3,5,8-trioxa-9-aza-tricyclo[5.2.2.0<sub>2</sub>,6]undec-10-ene (20)*

Compound 11 (0.3 g, 1.61 mmol) and 4-nitrosotoluene (0.22 g, 1.77 mmol) react in THF as described in the general procedure. The reaction was completed in 72 h. The residue was purified by column chromatography on Silica

gel using Hexane:EtOAc 8:2. Yield 70%. mp: 95-96 °C. IR,  $\lambda$ : 2915, 1574, 1558, 1377, 1337, 1260, 1200, 1165, 1086, 1036, 988, 930, 880, 790, 774, 677. <sup>1</sup>H RMN CDCl<sub>3</sub>,  $\delta$ : 7.08 (2H, d;  $J$  = 8.4 Hz), 6.89 (2H, d;  $J$  = 8.4 Hz), 6.40 (1H, dd;  $J_1$  = 8.5 Hz,  $J_2$  = 1.5 Hz), 6.08 (1H, dd;  $J_1$  = 8.5 Hz,  $J_2$  = 5.6 Hz), 4.51 (1H, dd;  $J_1$  = 7.0 Hz,  $J_2$  = 4.0 Hz), 4.30 (1H, ddd;  $J_1$  = 5.6 Hz,  $J_2$  = 4.0 Hz,  $J_3$  = 1.5 Hz), 4.24 (1H, d br;  $J$  = 7.0 Hz), 2.30 (3H, s), 1.42 (6H, s). <sup>13</sup>C RMN CDCl<sub>3</sub>,  $\delta$ : 150.20, 132.36, 133.20, 130.01, 129.19, 117.15, 111.30, 95.02, 81.10, 75.60, 60.30, 25.48, 25.21, 20.10.

*(3S,5S)-7-Bromo-4,4-dimethyl-9-phenyl-3,5,8-trioxo-9-aza-tricyclo[5.2.2.0<sub>2,6</sub>]undec-10-ene (21).*

Compound 12 (0.3 g, 1.30 mmol) and 4-nitrosobenzene (0.16 g, 1.43 mmol) reacted in THF as described in the general procedure. The reaction was complete in 24 h. The residue was purified by column chromatography on Silica gel using Hexane:EtOAc 8:2. Yield 90 %. mp: 89-90 °C. IR,  $\lambda$ : 3083, 3017, 2996, 2926, 1594, 1489, 1453, 1387, 1874, 1335, 1298, 1252, 1219, 1161, 1127, 1093, 1036, 974, 947, 897, 851, 812, 764, 747, 710, 696. <sup>1</sup>H RMN CDCl<sub>3</sub>,  $\delta$ : 7.27 (2H, dd;  $J_1$  = 8.2 Hz,  $J_2$  = 7.4 Hz), 7.03 (2H, d;  $J$  = 8.02 Hz), 6.90 (1H, t;  $J$  = 7.4 Hz), 6.57 (1H, dd;  $J_1$  = 8.4 Hz,  $J_2$  = 1.5 Hz), 6.02 (1H, dd;  $J_1$  = 8.4 Hz,  $J_2$  = 5.5 Hz), 4.85 (1H, ddd;  $J_1$  = 5.5 Hz,  $J_2$  = 4.1 Hz,  $J_3$  = 1.5 Hz), 4.76 (1H, dd;  $J_1$  = 7.0 Hz,  $J_2$  = 4.1 Hz), 4.75 (1H, d;  $J$  = 7.0 Hz), 1.42 (6H, s). <sup>13</sup>C RMN CDCl<sub>3</sub>,  $\delta$ : 150.40, 134.29, 130.24, 129.75, 123.83, 118.07, 110.95, 88.31, 82.21, 75.86, 60.42, 26.07, 25.84. MS EI,  $m/z$  (relative intensity): 175 (44), 173 (44), 94 (71), 65 (100). Anal. Calc. for C<sub>15</sub>H<sub>16</sub>BrNO<sub>3</sub>: C, 53.27; H, 4.77; N, 4.14; Found: C, 53.20; H, 5.12; N, 4.09.

*(3S,5S)-4-(7-Bromo-4,4-dimethyl-3,5,8-trioxo-9-aza-tricyclo[5.2.2.0<sub>2,6</sub>]undec-10-en-9-yl-benzaldehyde (22).*

Compound 12 (0.3 g, 1.30 mmol) and 4-nitrosobenzaldehyde (0.19 g, 1.43 mmol) reacted in THF as described in the general procedure. The reaction was complete in 20 h. The residue was purified by column chromatography on Silica gel using Hexane:EtOAc 7:3. Yield 90 %. mp: 140 °C (Dec.). IR,  $\lambda$ : 3013, 1705, 1590, 1499, 1487, 1404, 1374, 1320, 1267, 1213, 1161, 1080, 1036, 1011, 972, 943, 899, 849, 833, 799, 706, 639, 550, 515. <sup>1</sup>H RMN CDCl<sub>3</sub>,  $\delta$ : 9.91 (1H, s), 7.80 (2H, d;  $J$  = 8.7 Hz), 7.15 (2H, d;  $J$  = 8.7 Hz), 6.57 (1H, dd;  $J_1$  = 8.6 Hz,  $J_2$  = 1.5 Hz), 6.07 (1H, dd;  $J_1$  = 8.6 Hz,  $J_2$  = 5.6 Hz), 4.91 (1H, ddd;  $J_1$

= 5.6 Hz,  $J_2$  = 4.0 Hz,  $J_3$  = 1.5 Hz), 4.84 (1H, dd;  $J_1$  = 7.0 Hz,  $J_2$  = 4.0 Hz), 4.76 (1H, d;  $J$  = 7.0 Hz), 1.40 (6H, s). <sup>13</sup>C RMN CDCl<sub>3</sub>,  $\delta$ : 190.78, 155.40, 133.92, 131.69, 130.99, 129.97, 117.03, 111.59, 87.71, 81.69, 75.11, 59.07, 25.67, 25.47. MS EI,  $m/z$  (relative intensity): 186 (87), 129 (63), 128 (87), 127 (34), 115 (55), 100 (59), 94 (85), 85 (56), 77 (57), 65 (100). Anal. Calc. for C<sub>16</sub>H<sub>16</sub>BrNO<sub>4</sub>: C, 52.48; H, 4.40; N, 3.82; Found: C, 52.82; H, 4.53; N, 3.63.

*(3S,5S)-7-Bromo-9-(4-chlorophenyl)-4,4-dimethyl-3,5,8-trioxo-9-aza-tricyclo[5.2.2.0<sub>2,6</sub>]undec-10-ene (22).*

Compound 12 (0.07 g, 0.37 mmol) and 4-chloronitrosobenzene (0.057 g, 0.40 mmol) reacted in THF as described in the general procedure. The reaction was complete in 24 h. The residue was purified by column chromatography on Silica gel using Hexane:EtOAc 8:2. Yield 70 %. mp: 80 °C (Dec.). IR,  $\lambda$ : 3089, 2938, 2800, 1696, 1597, 1499, 1456, 1378, 1343, 1267, 1213, 1171, 1119, 1082, 1040, 972, 948, 903, 857, 831, 806, 746, 716, 633, 561, 513. <sup>1</sup>H RMN CDCl<sub>3</sub>,  $\delta$ : 7.26 (2H, d;  $J$  = 8.9 Hz), 6.97 (2H, d;  $J$  = 8.9 Hz), 6.58 (1H, dd;  $J_1$  = 8.6 Hz,  $J_2$  = 1.5 Hz), 6.01 (1H, ddd;  $J_1$  = 8.6 Hz,  $J_2$  = 5.6 Hz,  $J_3$  = 1.0 Hz), 4.84 (1H, ddd;  $J_1$  = 7.1 Hz,  $J_2$  = 4.1 Hz,  $J_3$  = 1.0 Hz), 4.72 (1H, dd;  $J_1$  = 7.1 Hz,  $J_2$  = 1.0 Hz), 4.70 (1H, ddd;  $J_1$  = 5.6 Hz,  $J_2$  = 4.1 Hz,  $J_3$  = 1.5 Hz), 1.41 (3H, s), 1.40 (3H, s). <sup>13</sup>C RMN CDCl<sub>3</sub>,  $\delta$ : 148.67, 134.04, 129.70, 128.79, 128.69, 119.04, 111.39, 87.76, 81.72, 75.36, 60.14, 25.68, 25.45. MS EI,  $m/z$  (relative intensity): 373 (M<sup>+</sup>; 8), 371 (25), 286 (20), 284 (20), 271 (10), 269 (10), 244 (18), 242 (13), 207 (10), 205 (9), 164 (28), 125 (13), 109 (23), 85 (22), 81 (100), 77 (18), 63 (23).

*(3S,5S)-7-Bromo-4,4-dimethyl-9-p-toluy-3,5,8-trioxo-9-aza-tricyclo[5.2.2.0<sub>2,6</sub>]undec-10-ene (24).*

Compound 12 (0.3 g, 1.30 mmol) and 4-nitrosotoluene (0.18 g, 1.43 mmol) reacted in THF as described in the general procedure. The reaction was complete in 30 h. The residue was purified by column chromatography on Silica gel using Hexane:EtOAc 8:2. Yield 70 %. Colorless oil. IR,  $\lambda$ : 3033, 2988, 2934, 2926, 1689, 1610, 1514, 1452, 1398, 1388, 1375, 1312, 1271, 1242, 1229, 1159, 1075, 1047, 1036, 999, 967, 911, 858, 814, 733, 672. <sup>1</sup>H RMN CDCl<sub>3</sub>,  $\delta$ : 7.05 (2H, d;  $J$  = 8.3 Hz), 6.90 (2H, d;  $J$  = 8.3 Hz), 6.73 (1H, dd;  $J_1$  = 8.3 Hz,  $J_2$  = 1.5 Hz), 6.21 (1H, dd;  $J_1$  = 8.3 Hz,  $J_2$  = 5.7 Hz), 4.56 (1H, ddd;  $J_1$  = 5.7 Hz,  $J_2$

Compound	<i>P. falciparum</i> (IC <sub>50</sub> μM)	<i>T. cruzi</i> (IC <sub>50</sub> μM)	<i>T. b. rhodesiense</i> (IC <sub>50</sub> μM)	Cytotoxicity (μM)
3	8.9	11.2	10.9	29.2
4	69.5	30.9	n.a.	240
5	n.a.	n.a.	n.a.	160
6	9.56	8.1	9.5	76.5
13	n.a.	108.9	35.2	180
14	85.0	n.a.	n.a.	280
15	42.3	52.3	26.3	530
16	8.24	9.2	8.7	260

**Table 1.** *In vitro* antiprotozoal activity of oxazines 3–6, 13–16 against *P. falciparum*, *T. cruzi*, and *T. brucei rhodesiense*.

= 4.0 Hz,  $J_3 = 1.5$  Hz), 4.46 (1H, dd;  $J_1 = 7.0$  Hz,  $J_2 = 4.0$  Hz), 4.24 (1H, d;  $J = 7.0$  Hz), 2.26 (3H, s), 1.41 (6H, s). <sup>13</sup>C RMN CDCl<sub>3</sub>, δ: 153.20, 138.02, 133.70, 129.74, 129.45, 117.03, 112.38, 87.74, 81.70, 75.22, 60.15, 27.43, 25.89, 20.04. MS EI, *m/z* (relative intensity): 353 (M<sup>+</sup>, 12), 351 (M<sup>+</sup>, 11), 295 (31), 295 (31), 266 (38), 264 (38), 224 (36), 222 (28), 199 (53), 144 (100), 81 (86), 77 (37).

### Biological results

The new compounds were assayed for their *in vitro* biological activity against *P. falciparum*, *T. cruzi*, and *T. brucei rhodesiense*, and their toxicity evaluated against KB cells (Table 1) <sup>6-8</sup>.

Chloroquine (IC<sub>50</sub> 3.0 nM), benznidazol, (IC<sub>50</sub> 4.7 μM), pentamidine (IC<sub>50</sub> 1.7 μM) were used as positives for *P. falciparum*, *T. cruzi*, and *T. brucei rhodesiense* respectively. Cytotoxicity was assayed against KB cells, using Podophyllotoxin (LD50 0.2 nM) as a standard. All determinations were performed in triplicate.

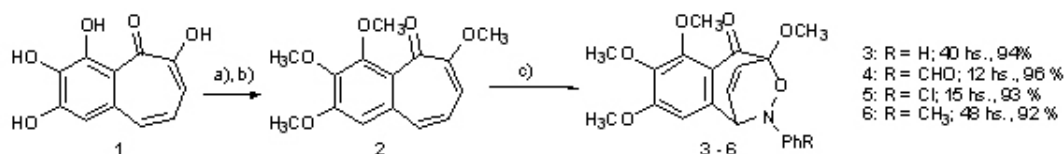
### DISCUSSION

In the present work we describe the synthesis of 16 new oxazines using the same methodology as in previous reports <sup>4,9</sup>. As dienes for the Diels-Alder reaction we used two structurally different groups of compounds, the benzotropone derivative tetra-*O*-methylpurpurogallin and 6-substituted *cis*-cyclohexadien-1,2-diols (methyl, bromo, chloro). As dienophiles we used nitrosobenzene and *p*-substituted derivatives (aldehyde, chloro and methyl groups).

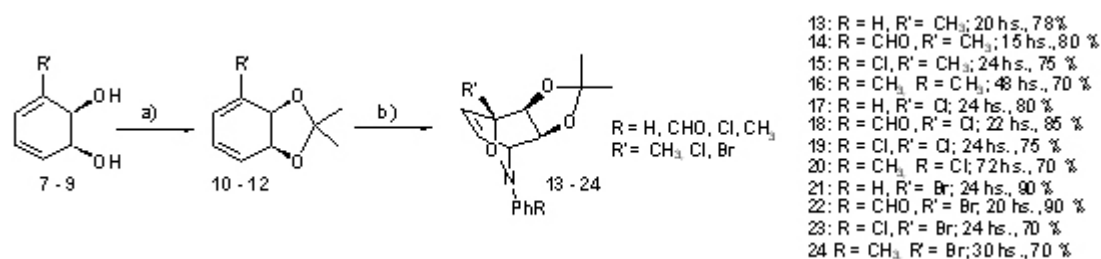
Tetra-*O*-methylpurpurogallin was synthesized from purpurogallin in two steps, using Bartrop's original technique, with dimethylsulfate as methylating agent <sup>10</sup>. The (1*S*,2*R*)-*cis*-cyclohexadiene-1,2-diol derivatives used as dienes, (1*S*,2*R*)-1,2-dihydroxy-3-methyl-3,5-cyclohexadiene, (1*S*,2*S*)-3-chloro-1,2-dihydroxy-3,5-cyclohexadiene and (1*S*,2*S*)-3-bromo-1,2-dihydroxy-3,5-cyclohexadiene, were obtained through microbial dihydroxylation of aromatic compounds with the F/39D strain of *Pseudomonas putida* <sup>11</sup>. The resulting diols were protected as acetonides with dimethoxypropane, using *p*-toluenesulfonic acid as catalyst <sup>12</sup>.

Oxazines **3** - **6** were synthesized from tetra-*O*-methylpurpurogallin (diene **2**), through Diels-Alder reactions using nitrosobenzene, 4-nitrosobenzaldehyde, 4-nitrosotoluene and 4-chloronitrosobenzene as dienophiles (Fig 1). Compound **3** had been previously synthesized and characterized by our group <sup>5,13</sup>. Compounds **4-6** were characterized by IR, MS, <sup>1</sup>H and <sup>13</sup>C NMR. In the reaction, the dienes were treated with a slight excess of the substituted nitrosoarenes at room temperature, using hexane as solvent. The cycloaddition reaction was clean and resulted in excellent yields (higher than 90%). In most cases the reactions were completed in less than 48 h. The regiochemistry of the reaction was assigned through <sup>1</sup>H and <sup>13</sup>C spectroscopy and later confirmed by X-Ray diffraction studies performed on oxazine **3**.

In the synthesis of adducts **13** to **24**, the re-



**Figure 1.** Route to oxazines **3** - **6**; Reagents and conditions: (a) (CH<sub>3</sub>)<sub>2</sub>SO<sub>4</sub>, NaOH, rt, 90%; (b) (CH<sub>3</sub>)<sub>2</sub>SO<sub>4</sub>, KOH, reflux, 95%; (c) *p*-NOPhR, THF, rt. Yields.



**Figure 2.** Route to oxazines **13** - **24**; Reagents and conditions: (a) DMP, *p*-TsOH, acetone, rt., 96 - 98 %; (b) *p*-NOPhR, THF, rt. Yields.

actions were performed in THF as solvent. In less than 48 h the products were obtained in high yields (70-90%). In all cases, only the *anti* stereoisomer was obtained. The regiochemistry of these hetero Diels-Alder reactions is the one shown in Fig. 2, which was established from the <sup>1</sup>H and <sup>13</sup>C NMR spectra of the products.

We include the biological evaluation of only 8 compounds, as some of them were unstable and could not be evaluated. As can be observed in Table 1, few of the assayed compounds show activity against *P. falciparum*, and those that are active are less active than the standard drug by a factor of 103. The results against *T. cruzi* and *T. brucei rhodesiense* are more promising, as the activity in some cases is only ten times lower than the standards, and could be improved by further chemical modifications. Compounds **6** and **16** are quite active on both these parasites, and compound **3** shows relatively good results on *T. cruzi*, although it is cytotoxic. As we previously observed <sup>4</sup>, the compounds that show activity in both series are those with less polar *p*-substituents in the aromatic ring. These results are being taken into consideration in experiments on other adducts which are being prepared.

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