

## SYNTHESIS OF 1H-BENZOXAZINE-2,4-DIONES FROM HETEROCYCLIC ANHYDRIDES: EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES

Juan I. Sarmiento-Sánchez<sup>a,\*</sup>, Julio Montes-Avila<sup>b</sup>, Adrián Ochoa-Terán<sup>c</sup>, Francisco Delgado-Vargas<sup>b</sup>, Víctor Wilson-Corral<sup>a</sup>, Sylvia P. Díaz-Camacho<sup>b</sup>, Fernando García-Páez<sup>a</sup> and Pedro Bastidas-Bastidas<sup>d</sup><sup>a</sup>Faculty of Engineering, Autonomous University of Sinaloa, Blvd. of the Americas S/N, 80040, Campus Culiacán, Culiacán, Sinaloa, México<sup>b</sup>Faculty of Chemical-Biological Sciences, Autonomous University of Sinaloa, Blvd. of the Americas S/N, 80040, Campus Culiacán, Culiacán, Sinaloa, México<sup>c</sup>Centro de Graduados e Investigación en Química, Instituto Tecnológico de Tijuana, Blvd. Alberto Limón Padilla S/N, 22500, Tijuana, B.C., México<sup>d</sup>Laboratorio de Análisis de Residuos de Plaguicidas, Centro de Investigación en Alimentación y Desarrollo, A.C., Unidad Culiacán, México

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A facile one-step synthesis of 1H-benzoxazine-2,4-diones from heterocyclic anhydrides and TMSA was described. This paper determines their antimicrobial activity against nine human bacterial pathogens by the broth microdilution method; antioxidant activity by DPPH<sup>•</sup> inactivation and a ferric-reducing power assay; and toxicity by a brine shrimp, *Artemia salina*, assay. The 1H-benzoxazine-2,4-dione yields were in the range of 57 to 98%. The novel compound 1H-pyrazino[2,3-][1,3]oxazine-2,4-dione **4c** showed the highest antioxidant capacity (DPPH 35.4% and FRAP 0.063 μmol TE<sub>s</sub>/μmol).

Keywords: 1H-benzoxazine-2,4-dione; antimicrobial activity; antioxidant activity; toxicity.

## INTRODUCTION

Currently, microbial resistance to first-line antibiotic agents is a public health concern, and new drugs are required to treat infected patients. Several heterocyclic structures have shown antibacterial activity, including benzoxazine-2,4-diones.<sup>1</sup> Benzoxazine-2,4-diones are potential pharmacophores because of their registered activities: potent inhibitor of hepatitis C virus,<sup>2</sup> butyrylcholinesterase inhibitor,<sup>3</sup> antiallergic,<sup>4</sup> antitumor,<sup>5</sup> antipsychotic,<sup>6</sup> antileishmanial,<sup>7</sup> and antimycobacterial,<sup>8</sup> among others. The syntheses of benzoxazine-2,4-diones from anthranilic acid derivatives,<sup>9</sup> isatin,<sup>10</sup> allenamides,<sup>11</sup> and carbamoylbenzoic acid<sup>12</sup> have been reported. Rao *et al.*<sup>13</sup> reported the synthesis of isatoic anhydride from phthalimide and sodium hypochlorite in a basic one-pot solution. This is the only methodology used to produce isatoic anhydride on a large scale. However, if the temperature is not controlled during the course of the reaction, the yield is significantly reduced. In addition, large effluents of wastewater are produced. In general, these processes involve several reaction steps and employ expensive, noncommercial, or dangerous reagents; thus, a better process must be designed.

Viewing the process from a different perspective, there is an increasing interest in compounds with high antioxidant activities, which could counteract the oxidative stress associated with diseases.<sup>14</sup> Antioxidant activity screening is commonly done by *in vitro* assays, as in the ABTS<sup>•+</sup>,<sup>15</sup> ferric-reducing,<sup>16,17</sup> and DPPH<sup>18,19</sup> methods.

Recently, we have reported the synthesis of heterocyclic compounds<sup>20-24</sup> with antiparasitary<sup>25</sup> and antioxidant<sup>26,27</sup> activities. In the following sections, an easy one-step synthesis of 1H-benzoxazine-2,4-diones from heterocyclic anhydride with TMSA is presented, and their toxicity, antimicrobial activities, and antioxidant activities are reported.

## EXPERIMENTAL

## Chemistry

All reagents were purchased in the highest quality available and were used without further purification. The solvents used in column chromatography were obtained from commercial suppliers and used without distillation. Nuclear Magnetic Resonance of <sup>1</sup>H (200 MHz) and <sup>13</sup>C (50 MHz) spectra were recorded on a Varian Mercury 200 MHz Spectrometer in DMSO-*d*<sub>6</sub> with TMS as an internal standard. Chemical ionization mass spectra were obtained with a Varian Titan 4000 ion trap GC-MS, and the intensities were reported as a percentage relative to the base peak after the corresponding *m/z* value. HR-MS was recorded on an ESI/APCI-TOF Bruker model MicroTOF-II-Focus<sup>TM</sup> at the Universidad Autónoma Metropolitana, Campus Iztapalapa. Infrared spectra were recorded on a Cary 660 series FTIR-ATR spectrophotometer. Melting points were obtained on a Stuart apparatus model SMP30; the reported value is the average of three separate experiments.

## General procedure for the synthesis of benzoxazine-2,4-diones

The heterocyclic anhydride in THF (1.0 mM) and trimethylsilylazide (TMSA, 1.05 equiv.) were stirred in a Schlenk bulb for 17 h at 53 °C, then temperature was increased to reflux and maintained for 2 h. The resulting solution was concentrated under vacuum pressure until a solid was formed. The solid was washed with diethyl ether (5 x 4 mL) to obtain 1H-benzoxazine-2,4-diones in high purity.

1H-3,1-benzoxazine-2,4-quinone (**4a**). (For full characterization see lit.);<sup>28,29</sup> CAS number: 118-48-9; yield 85%; white solid; *R*<sub>f</sub> 0.58 (petroleum ether/EtOAc 1:1 v/v); <sup>1</sup>H-NMR (200 MHz, DMSO-*d*<sub>6</sub>): δ 11.74 (br s, 1H), 7.91 (dd, *J*<sub>1</sub>=8.07, *J*<sub>2</sub>=1.1 Hz, 1H), 7.74 (td, *J*<sub>1</sub>=7.79, *J*<sub>2</sub>=1.28 Hz, 1H), 7.25 (m, 2H); <sup>13</sup>C-NMR (50 MHz, DMSO-*d*<sub>6</sub>): δ 159.9, 147.1, 141.4, 136.9, 128.9, 123.5, 115.3; CG-MS *m/z*: 164 [M+H]<sup>+</sup>

\*e-mail: jsarmiento@uas.edu.mx

1*H*-pyrido[2,3-*d*][1,3]oxazine-2,4-dione (**4b**). (For full characterization see lit.);<sup>30-32</sup> CAS number: 21038-63-1; yield 98%; pale brown solid;  $R_f$  0.13 (acetonitrile);  $^1\text{H-NMR}$  (200 MHz, DMSO- $d_6$ ):  $\delta$  12.30 (br s, 2H), 8.65 (dd,  $J=4.77$ , 1.87 Hz, 1H), 8.54 (dt,  $J=4.67$ , 2.25 Hz, 1H) 8.43-8.28 (m, 2H), 7.34-7.18 (m, 2H);  $^{13}\text{C-NMR}$  (50 MHz, DMSO- $d_6$ ):  $\delta$  166.9, 159.5, 155.9, 153.0, 147.0, 136.8, 135.5, 129.6, 127.3, 123.7, 119.8, 106.7; GC-MS  $m/z$ : 165 [M+H]<sup>+</sup>, 153 [M-CO<sub>2</sub>+MeOH]<sup>+</sup>

1*H*-pyrazino[2,3-*d*][1,3]oxazine-2,4-dione (**4c**). Yield 95%; mp 190-191 °C; pale brown solid;  $R_f$  0.25 (methanol);  $^1\text{H-NMR}$  (200 MHz, DMSO- $d_6$ ):  $\delta$  11.74 (br s, 1H), 8.32 (d,  $J=4.03$  Hz, 1H), 8.95 (d,  $J=4.03$  Hz, 1H);  $^{13}\text{C-NMR}$  (50 MHz, DMSO- $d_6$ ):  $\delta$  163.5, 153.8, 140.1, 134.3, 132.2, 123.5; FT-IR (ATR): 3725, 3068, 1749, 1710, 1606, 1558, 1502, 1452, 1413, 1239, 1186 cm<sup>-1</sup>; GC-MS  $m/z$ : 166 [M+H]<sup>+</sup>, 154 [M-CO<sub>2</sub>+MeOH]<sup>+</sup>; HR-MS calculated for C<sub>6</sub>H<sub>2</sub>N<sub>3</sub>O<sub>3</sub> (MH) 164.0102; found: 164.0124

6,7-dichloro-1*H*-benzo[*d*][1,3]oxazine-2,4-dione (**4d**). (For full characterization see lit.);<sup>33</sup> yield 98%; pale pink solid;  $R_f$  0.42 (petroleum ether/EtOAc 1:1 v/v);  $^1\text{H-NMR}$  (200 MHz, DMSO- $d_6$ ):  $\delta$  8.08 (s, 1H), 7.31 (s, 1H);  $^{13}\text{C-NMR}$  (50 MHz, DMSO- $d_6$ ):  $\delta$  158.2, 146.5, 140.8, 138.9, 129.8, 125.4, 116.8, 111.1; FT-IR (ATR): 3714, 3606, 3172, 3087, 1760, 1708, 1614, 1594, 1614, 1488, 1396, 1307, 1265, 1118, 1035 cm<sup>-1</sup>; GC-MS  $m/z$ : 233 [M+H]<sup>+</sup>, 220 [M-CO<sub>2</sub>+MeOH]<sup>+</sup>

Regioisomeric mixture of 6- and 7-methyl-1*H*-benzo[*d*][1,3]oxazine-2,4-dione (**4e**). (For full characterization see lit.);<sup>34,35</sup> CAS number: 63480-11-5, CAS number: 4692-99-3; yield 61%; white solid;  $R_f$  0.13 (petroleum ether/EtOAc 1:1 v/v);  $^1\text{H-NMR}$  (200 MHz, DMSO- $d_6$ ):  $\delta$  11.67 (d,  $J=6.23$  Hz, 2H), 7.78 (d,  $J=8.07$  Hz, 1H) 7.70 (s, 1H), 7.56 (dd,  $J=8.43$ , 1.83 Hz, 1H), 7.09-7.02 (m, 2H), 6.92 (s, 1H), 2.37 (s, 3H), 2.32 (s, 3H);  $^{13}\text{C-NMR}$  (50 MHz, DMSO- $d_6$ ):  $\delta$  159.9, 159.7, 147.3, 147.1, 141.4, 139.2, 137.9, 132.9, 128.9, 128.3, 124.8, 115.2, 115.0, 110.0, 107.7, 21.6, 20.0; FT-IR (ATR): 3714, 3606, 3085, 2956, 1779, 1710, 1625, 1596, 1511, 1477, 1413, 1367, 1276, 1151, 1012 cm<sup>-1</sup>; GC-MS  $m/z$ : 178 [M+H]<sup>+</sup>, 166 [M-CO<sub>2</sub>+MeOH]<sup>+</sup>

Regioisomeric mixture of 6- and 7-fluoro-1*H*-benzo[*d*][1,3]oxazine-2,4-dione (**4f**). (For full characterization see lit.);<sup>36,37</sup> CAS number: 321-50-6; yield 56%; white solid;  $R_f$  0.32 (acetonitrile);  $^1\text{H-NMR}$  (200 MHz, DMSO- $d_6$ ):  $\delta$  11.83 (br s, 2H), 7.99 (dd,  $J=8.8$ , 5.87 Hz, 1H), 7.72-7.60 (m, 2H), 7.21-7.06 (m, 2H), 6.88 (dd,  $J=9.72$ , 2.38 Hz, 1H);  $^{13}\text{C-NMR}$  (50 MHz, DMSO- $d_6$ ):  $\delta$  167.4 (d,  $J=254.8$  Hz, 1C), 159.9, 159.5, 158.2 (d,  $J=241.2$  Hz, 1C), 147.6, 147.4, 138.8, 138.7, 133.1, 125.2, 118.3, 118.1, 114.4, 112.2, 112.0, 102.2; FT-IR (ATR): 3714, 3611, 3185, 3102, 1758, 1697, 1625, 1614, 1515, 1488, 1425, 1336, 1257, 1162, 1037 cm<sup>-1</sup>; CG-MS  $m/z$ : 182 [M+H]<sup>+</sup>, 170 [M-CO<sub>2</sub>+MeOH]<sup>+</sup>

5,6,7,8-tetrahydro-1*H*-benzo[*d*][1,3]oxazine-2,4-dione (**4g**). (For full characterization see lit.);<sup>38</sup> yield 66%, white solid;  $R_f$  0.50 (petroleum ether/EtOAc 1:1 v/v);  $^1\text{H-NMR}$  (200 MHz, DMSO- $d_6$ ):  $\delta$  11.32 (br s, 1H), 2.32 (t,  $J=5.13$  Hz, 2H), 2.17 (t,  $J=4.95$  Hz, 2H), 1.69-1.56 (m, 4H);  $^{13}\text{C-NMR}$  (50 MHz, DMSO- $d_6$ ):  $\delta$  160.5, 153.6, 148.1, 100.9, 25.6, 21.0, 20.8, 20.5; GC-MS  $m/z$ : 124 [M-CO<sub>2</sub>]<sup>+</sup>, 156 [M-CO<sub>2</sub>+MeOH]<sup>+</sup>

### DPPH-scavenging activity

The DPPH<sup>•</sup> scavenging activity of the benzoxazine-2,4-diones was assessed as described by Sivakumar *et al.* with slight modifications.<sup>39</sup> This method is based on the reduction of DPPH in the presence of antioxidants. The antioxidant activity is detected as a change in the solution color from purple to yellow. A solution of DPPH (0.10 mM) in ethanol was prepared. Then, 50  $\mu\text{L}$  of the 1*H*-benzoxazine-2,4-dione (50  $\mu\text{g mL}^{-1}$  in ethanol) were mixed with 1.950 mL of the DPPH solution; the mixture was vigorously shaken, incubated for 20 min at 37 °C in darkness conditions, and the absorbance was measured

at 517 nm (Spectronic Genesys 20). The results were expressed as % inhibition using a calibration curve of Trolox (0–400  $\mu\text{g mL}^{-1}$ ). The DPPH<sup>•</sup> scavenging activity of the benzoxazine-2,4-dione was calculated as follows:

$$\text{DPPH scavenging effect (\%)} = [(A_0 - A_1)/A_0] \times 100,$$

where  $A_0$  is the absorbance of the control and  $A_1$  is the absorbance in the presence of the 1*H*-benzoxazine-2,4-diones (or the positive control, Trolox) at 100  $\mu\text{g mL}^{-1}$ . Calculated values correspond to the mean  $\pm$  one standard deviation of one experiment in quadruplicate and were determined by SPSS Statistics software v19 (IBM company).

### Ferric-reducing antioxidant power (FRAP) assay

The ferric-reducing power assay was performed as described by Zhang *et al.* with slight modifications.<sup>40</sup> The FRAP reagent was prepared by mixing 2.5 mL of 10 mM 2,4,6-tripyridyl-*s*-triazine (TPTZ), 2.5 mL of 40 mM HCl, 2.5 mL of 20 mM FeCl<sub>3</sub> and 25 mL of 0.3 M acetate buffer (pH 3.6). For the blank or compound assay, 1.95 mL of the freshly prepared FRAP reagent were mixed with ethanol (0.05 mL) or with the benzoxazine-2,4-dione (0.05 mL, 50  $\mu\text{g mL}^{-1}$ ). The mixture was incubated in darkness conditions (37 °C, 5 min), and the absorbance was measured at 593 nm. In the FRAP assay, the blue-colored Fe(II)-tripyridyltriazine compound was oxidized to the colorless Fe(III) form. Calculated values were the mean of four replicates and were reported as  $\mu\text{mol}$  of Trolox equivalents ( $\mu\text{mol TEs}/\mu\text{mol}$  of compound) in accordance with the equation of the Trolox calibration curve:

$$\text{Absorbance} = 19.57 \text{ Trolox } (\mu\text{mol}) + 0.1051 \quad (r^2=0.9928)$$

### Bacteria and the antibacterial assay

Activity against nine human bacterial pathogens was evaluated; two strains were ATCC (DIFCO Laboratories, MI, U.S.A.) (*i.e.*, *Staphylococcus aureus* 29213 and *Escherichia coli* 25922), and seven clinical isolates were provided by the bacteriology laboratory of the Instituto Nacional de Pediatría, Secretaría de Salud in Mexico, D.F. (*i.e.*, *Streptococcus group A-4*, *Staphylococcus aureus* 3, *E. coli* AO11, *E. coli* AO19, *E. coli* AO55, *Salmonella typhi*, and *Shigella dysenteriae*).

For the antibacterial activity evaluation, the benzoxazine-2,4-diones were dissolved in aqueous DMSO (5% v/v) and evaluated at the following concentrations: 3.125, 6.25, 12.5, 25, 50, 100, 200, 225, 250, 275, 300, 350, and 400  $\mu\text{g mL}^{-1}$ . The antibacterial activity was determined by a microdilution assay in 96-well plates.<sup>41,42</sup> Strains were cultured in Petri dishes (TSA, trypticase soy agar) for 18–20 h at 37 °C; two to five colonies were suspended in 1.0 mL of 0.85% NaCl (w/v) and density adjusted to 10<sup>8</sup> CFU mL<sup>-1</sup> (0.5 McFarland value). Bacterial cultures (50  $\mu\text{L}$ , 10<sup>6</sup> CFU mL<sup>-1</sup>) and 50  $\mu\text{L}$  of each compound/concentration were mixed in the wells. Gentamicin (0.5, 1, 2, 4, 8, 16, and 32  $\mu\text{g mL}^{-1}$ ) was used as a positive control, microorganisms without additives as a negative control, and dissolving solvents without compounds as a toxicity control. The 96-well plates were incubated for 18–20 h at 37 °C.

### Brine shrimp lethality bioassay

The toxicities of the benzoxazine-2,4-diones were evaluated by the brine shrimp larvae assay.<sup>43</sup> Dried brine shrimp eggs were incubated in a saline medium under light conditions for 48 h. One-day-old larvae (10–12 per vial in 100  $\mu\text{L}$  of saline solution) were transferred into 96-well plates and exposed to 100  $\mu\text{L}$  of the benzoxazine-2,4-diones at 100, 300, 500, 700, and 1000  $\mu\text{g mL}^{-1}$ . Four replicates of

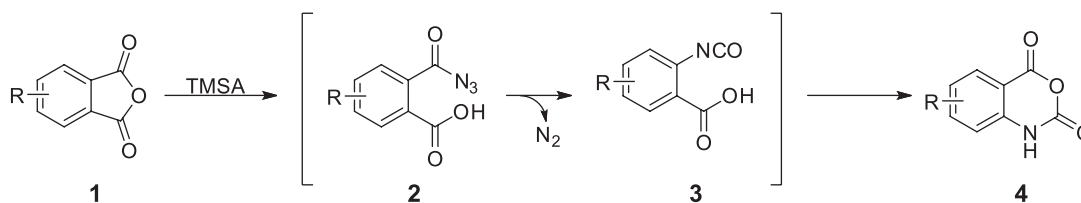


Figure 1. Synthesis of benzoxazine-2,4-diones

each concentration were done. The dead larvae were counted after 24 h of incubation, and the Median Lethal Concentration ( $LC_{50}$ ) and 95% confidence intervals were determined by probit analysis with SPSS Statistics software v19 (IBM company). Evaluated compounds were classified by the  $LC_{50}$  values as follows:  $LC_{50} \geq 1000 \mu\text{g mL}^{-1}$ , classified as non-toxic;  $100 < LC_{50} < 1000$ , classified as moderately toxic; and  $10 < LC_{50} < 100$ , classified as very toxic.<sup>44,45</sup>

## RESULTS AND DISCUSSION

### Chemistry

As depicted in Figure 1, the synthesis of the 1*H*-benzoxazine-2,4-diones involved an acylazide intermediate **2**, followed by a subsequent Curtius rearrangement to generate an isocyanate **3**, and an intramolecular cyclization to provide the benzoxazine-2,4-diones **4**. At first, the reaction conditions were optimized following the synthesis of the benzoxazine-2,4-dione **4a**: the initial conditions were those recommended by Washburne,<sup>46</sup> the starting materials were refluxed in

benzene for 19 h, and the reaction product was detected by GC-MS in positive chemical ionization. Later, the reaction conditions were changed: temperatures were 53 °C for the first 17 h, followed by 85 °C for 2 h, resulting in isolation of the compound **4a** with 54% yield and high purity. The same conditions were used for the synthesis of **4a** employing acetonitrile, benzene-acetonitrile (4:1 v/v), DMSO, and THF as solvents. Some unidentified byproducts were detected with acetonitrile and DMSO as solvents. In contrast, yields were high for the reactions with THF (85%) and benzene-acetonitrile 4:1 v/v (50%). The higher yield for THF could be associated with the solubility of reagents and intermediates in this solvent. Washburne<sup>46</sup> reported that oxazine-2,6-diones were obtained as silylated products. However, silylated benzoxazine-2,4-diones were not detected under our modified reaction conditions. The benzoxazine-2,4-diones synthesis using heterocyclic anhydrides and TMSA has the following advantages: i) reaction conditions are simple, ii) the products are obtained in good yields, and iii) less waste is produced in comparison with the phthalimides method.

The benzoxazine-2,4-dione yields were similar to those reported

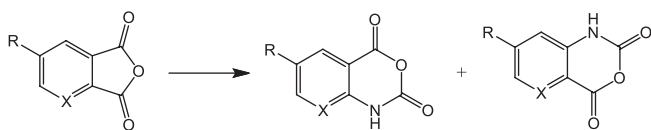
Table 1. Toxicity and antioxidant activities of synthetic benzoxazine-2,4-diones

Product	Structure	Yield (%)	% DPPH-scavenging activity <sup>a</sup>	FRAP assay ( $\mu\text{mol TEs}/\mu\text{mol compound}$ )	Toxicity $LC_{50}$ ( $\mu\text{g mL}^{-1}$ )
4a		85	17.2 ± 4.15	1.35x10 <sup>-2</sup>	264.44
4b		98	8.0 ± 0.95	1.03x10 <sup>-2</sup>	887.64
4c		95	35.4 ± 0.83	6.31x10 <sup>-2</sup>	459.00
4d		98	1.5 ± 0.67	1.02x10 <sup>-2</sup>	279.33
4e		61	2.9 ± 1.04	4.65x10 <sup>-3</sup>	617.04
4f		56	2.1 ± 0.15	1.66x10 <sup>-2</sup>	965.04
4g		66	3.55 ± 0.93	NE	NE

<sup>a</sup>Values represent mean ± standard deviation, n = 4, p ≤ 0.05; NE – compound not evaluated.

in the literature (56–98%, Table 1). Compounds **4b**, **4e**, and **4f** were obtained as a mixture of regioisomers in the 6- and 7-positions (Table 2).

**Table 2.** Isomers distribution in the synthesis of benzoxazine-2,4-diones



Product	R, X	6-isomer <sup>a</sup> %	7-isomer <sup>a</sup> %
<b>4e</b>	R = CH <sub>3</sub> , X = CH	53	47
<b>4b</b>	R = H, X = N	56	44
<b>4f</b>	R = F, X = CH	77	23

<sup>a</sup>Isomers ratio were obtained by <sup>1</sup>H-NMR of the isomers mixture.

Table 2 shows the regioisomeric distribution of benzoxazine-2,4-diones resulting from the formation of non-symmetric heterocyclic anhydrides. The 7-isomer was always detected, ranging from 23% (R = F) to 47% (R = CH<sub>3</sub>); thus, the regioselectivity in the benzoxazine-2,4-dione synthesis resulted from a combination of electronic and steric effects. A similar phenomenon was registered for the aryloxazine-2,6-dione synthesis.<sup>47</sup> The electronic effect is clear when contrasting **4e** and **4f**; the carbonyl in position 1 was more electropositive than that in position 2 by the effect of the fluorine atom attached at position 4 (**4f**).

#### Antioxidant, antibacterial, and toxicity activities

All of the synthetic benzoxazine-2,4-diones acted as reducing agents over the DPPH<sup>•</sup> radical, with **4a** and **4c** as the best antioxidants (Table 1). Based on the structure-activity analysis, antioxidant activity can be associated with substitutions of the aromatic ring; compounds with substituents at C-5 or C-6 of the aromatic ring showed lower DPPH<sup>•</sup> scavenging activities. Moreover, the activity of 1*H*-pyrazino[2,3-*d*][1,3]oxazine-2,4-dione (**4c**) was 4.4-fold and 2-fold higher than **4b** and **4a**, respectively. In the FRAP assay, **4a-b**, **4d**, and **4f** compounds showed similar activity; benzoxazine-2,4-diones **4f** and **4c** were the most active. Compound **4c** was a 3.8-fold better reducing agent than **4f** (Table 1). In the antimicrobial assay, the synthetic 1*H*-benzoxazine-2,4-diones assayed up to 400 μg mL<sup>-1</sup> were inactive against the tested human pathogenic bacteria. By contrast, several 3*H*-benzoxazine-2,4-diones have good antimycobacterial activities against *M. tuberculosis*, *M. avium*, and *M. kansasii*,<sup>8,48,49</sup> but reports about their evaluation against other bacteria are scarce. Moreover and more recently, the antimycobacterial activities of 1*H*-benzoxazine-2,4-diones have not been evaluated.

From the *Artemia salina* assay, substitutions in the aromatic ring of the benzoxazine-2,4-diones decreased the toxicity as compared with the non-substituted **4a**. Furthermore, in the heteroaromatic compounds, more nitrogen atoms in the aromatic ring correlated with a higher toxicity (**4b-c**). The toxicity of **4b**, **4e**, and **4f** are reported as regioisomer mixtures. The tested compounds **4a-g** were classified as moderately toxic.

#### CONCLUSION

The proposed one-step method produced 1*H*-benzoxazine-2,4-diones from heterocyclic anhydrides with TMSA in good yields and with high purity. This methodology is simple, and the starting materials are economic, accessible, and commercial. The 1*H*-pyrazino[2,3-*d*][1,3]oxazine-2,4-dione **4c** showed the highest

antioxidant activity for the two methods tested (DPPH radical scavenging and ferric-reducing power).

#### SUPPLEMENTARY MATERIAL

Available at <http://quimicanova.sbg.org.br> in the form of a PDF file, with free access: the <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra for compounds **4a-g** (Figure S1 to S7) and HRMS-ESI for compound **4c** (Figure S3).

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**SYNTHESIS OF 1H-BENZOXAZINE-2,4-DIONES FROM HETEROCYCLIC ANHYDRIDES: EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES**

Juan I. Sarmiento-Sánchez<sup>a,\*</sup>, Julio Montes-Avila<sup>b</sup>, Adrián Ochoa-Terán<sup>c</sup>, Francisco Delgado-Vargas<sup>b</sup>, Víctor Wilson-Corral<sup>a</sup>, Sylvia P. Díaz-Camacho<sup>b</sup>, Fernando García-Páez<sup>a</sup> and Pedro Bastidas-Bastidas<sup>d</sup>

<sup>a</sup>Faculty of Engineering, Autonomous University of Sinaloa, Blvd. of the Americas S/N, 80040, Campus Culiacán, Culiacán, Sinaloa, México

<sup>b</sup>Faculty of Chemical-Biological Sciences, Autonomous University of Sinaloa, Blvd. of the Americas S/N, 80040, Campus Culiacán, Culiacán, Sinaloa, México

<sup>c</sup>Centro de Graduados e Investigación en Química, Instituto Tecnológico de Tijuana, Blvd. Alberto Limón Padilla S/N, 22500, Tijuana, B.C., México

<sup>d</sup>Laboratorio de Análisis de Residuos de Plaguicidas, Centro de Investigación en Alimentación y Desarrollo, A.C., Unidad Culiacán, México

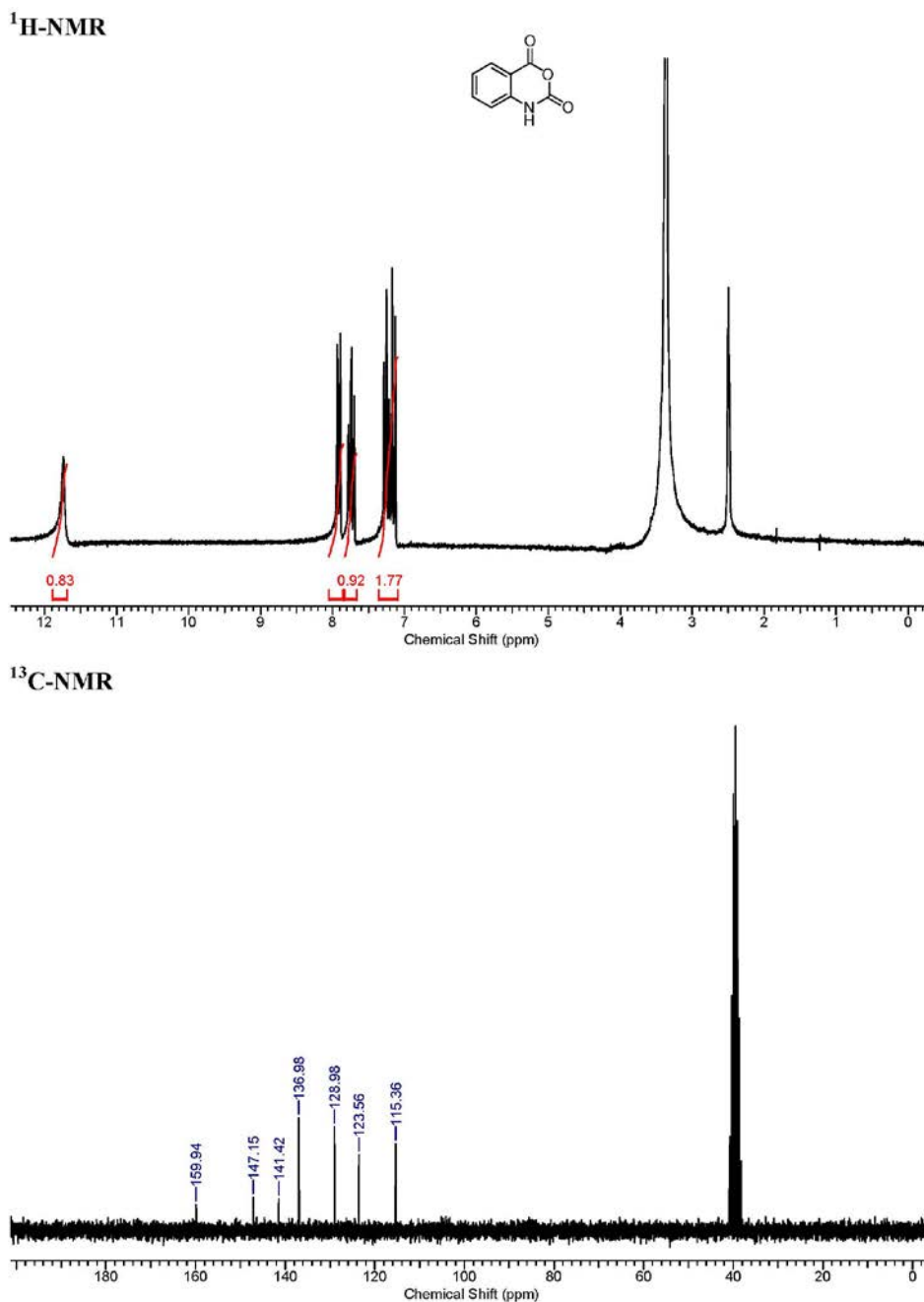


Figure 1S. 1H-benzo[d][1,3]oxazine-2,4-dione (4a)

\*e-mail: jsarmiento@uas.edu.mx

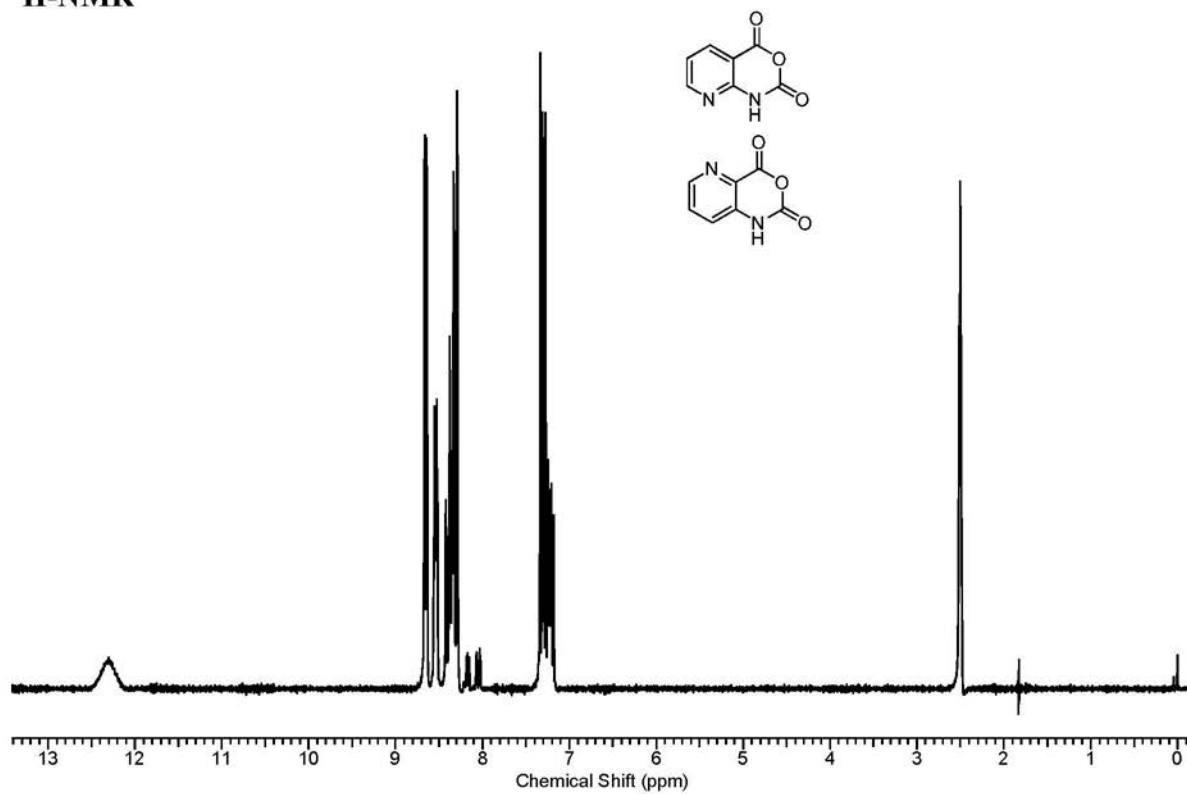
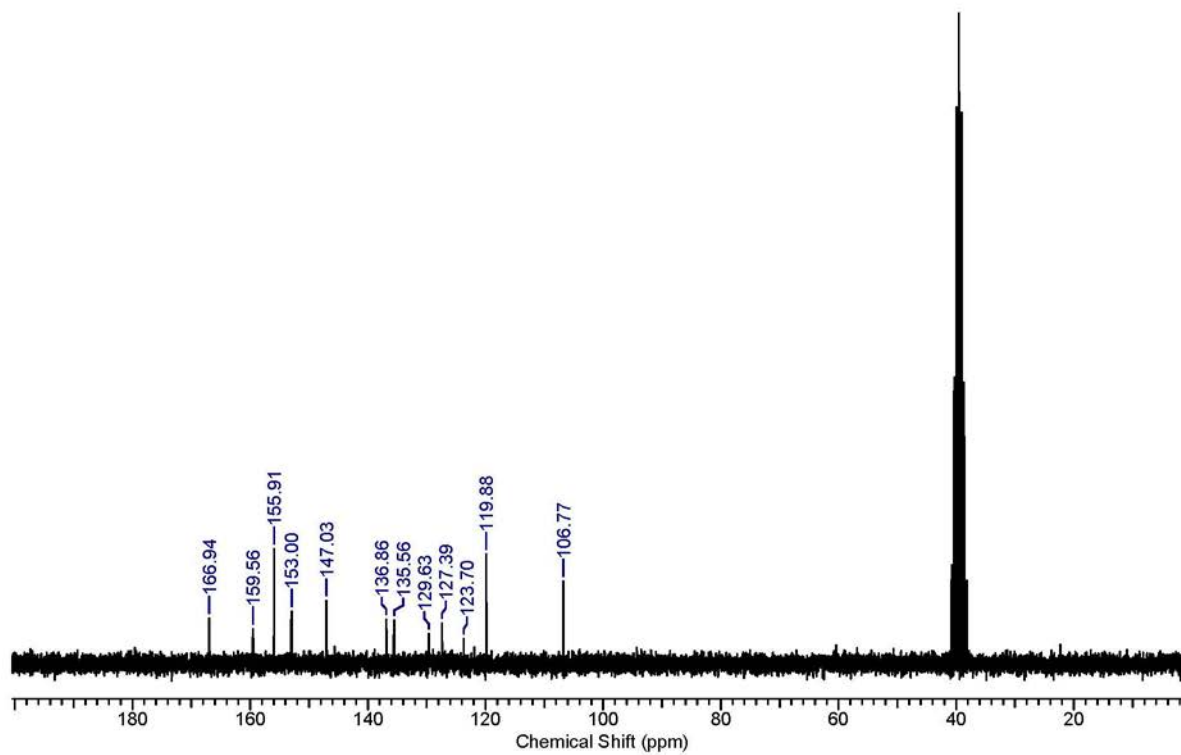
<sup>1</sup>H-NMR<sup>13</sup>C-NMR

Figure 2S. *1H*-pyrido[2,3-*d*][1,3]oxazine-2,4-dione (**4b**)

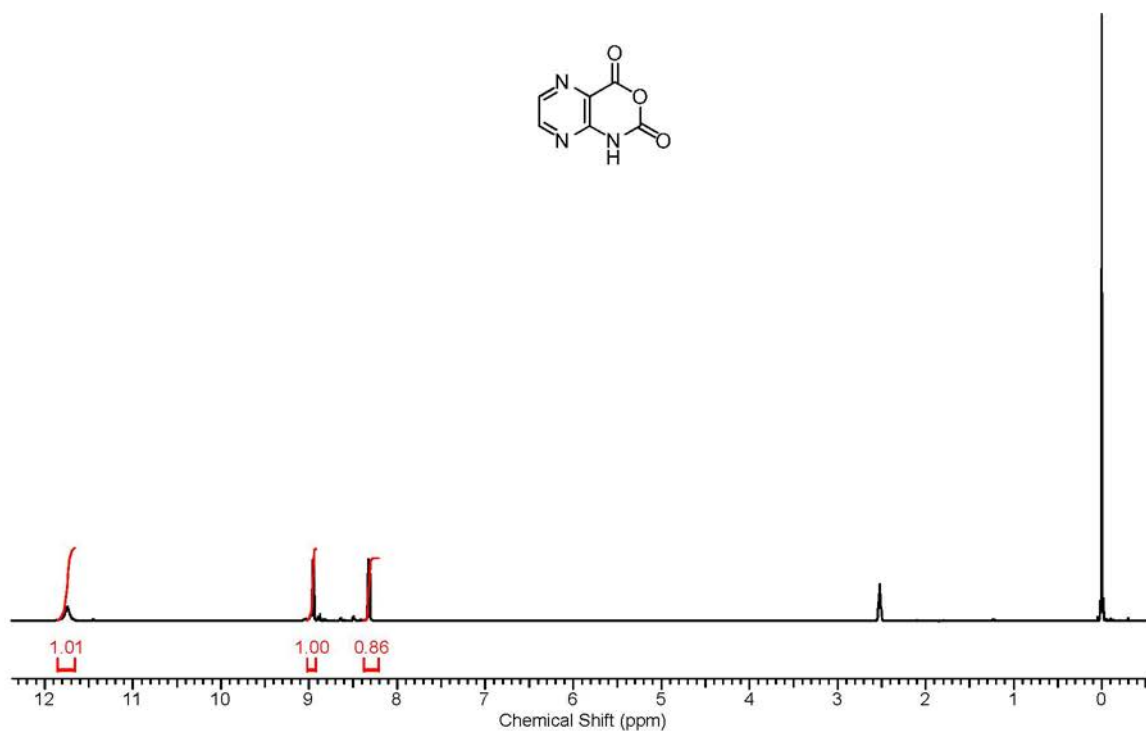
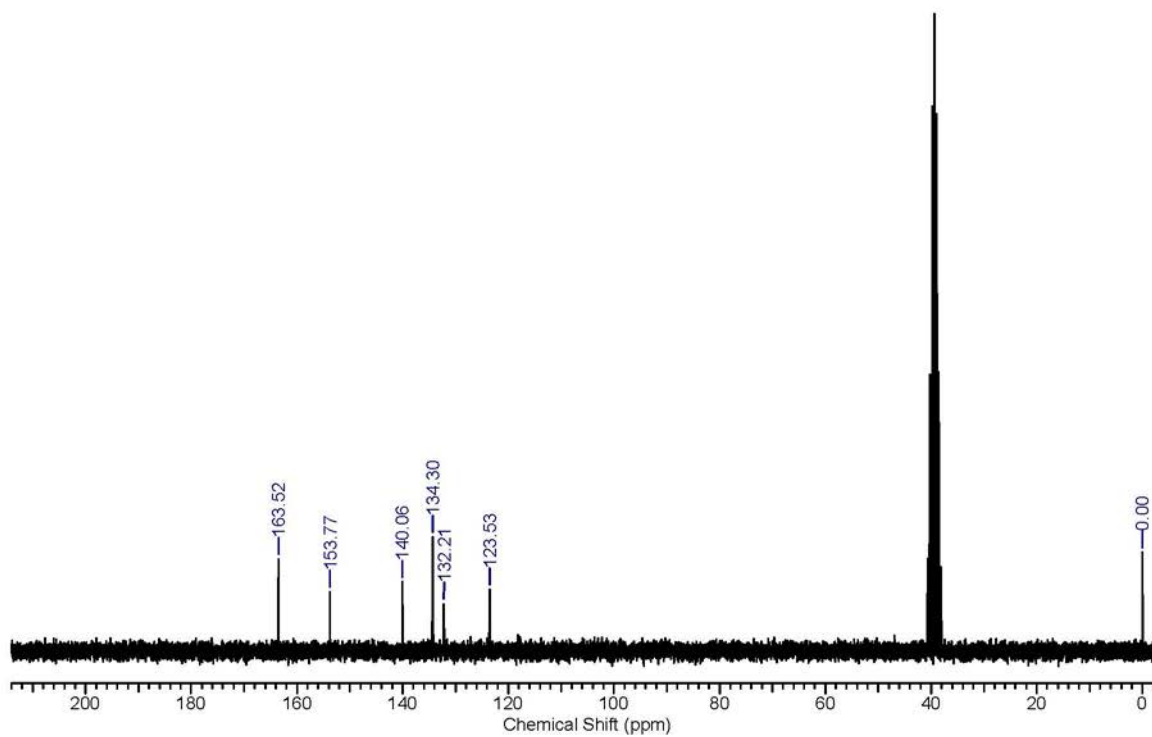
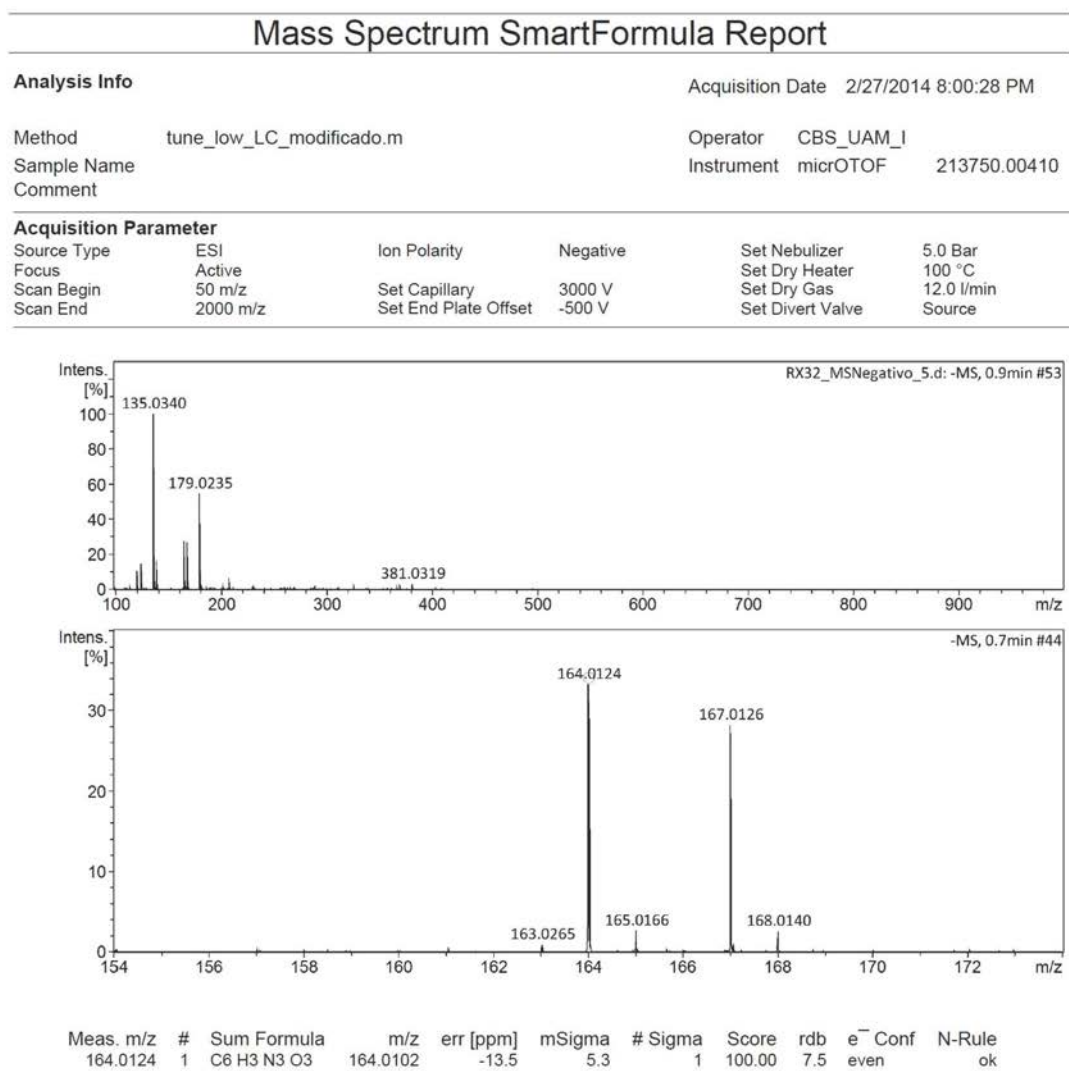
<sup>1</sup>H-NMR<sup>13</sup>C-NMR

Figure 3S. 1*H*-pyrazino[2,3-*d*][1,3]oxazine-2,4-dione (**4c**)



## HRMS-ESI Negative

Figure 3S. 1H-pyrazino[2,3-d][1,3]oxazine-2,4-dione (**4c**) (cont.)

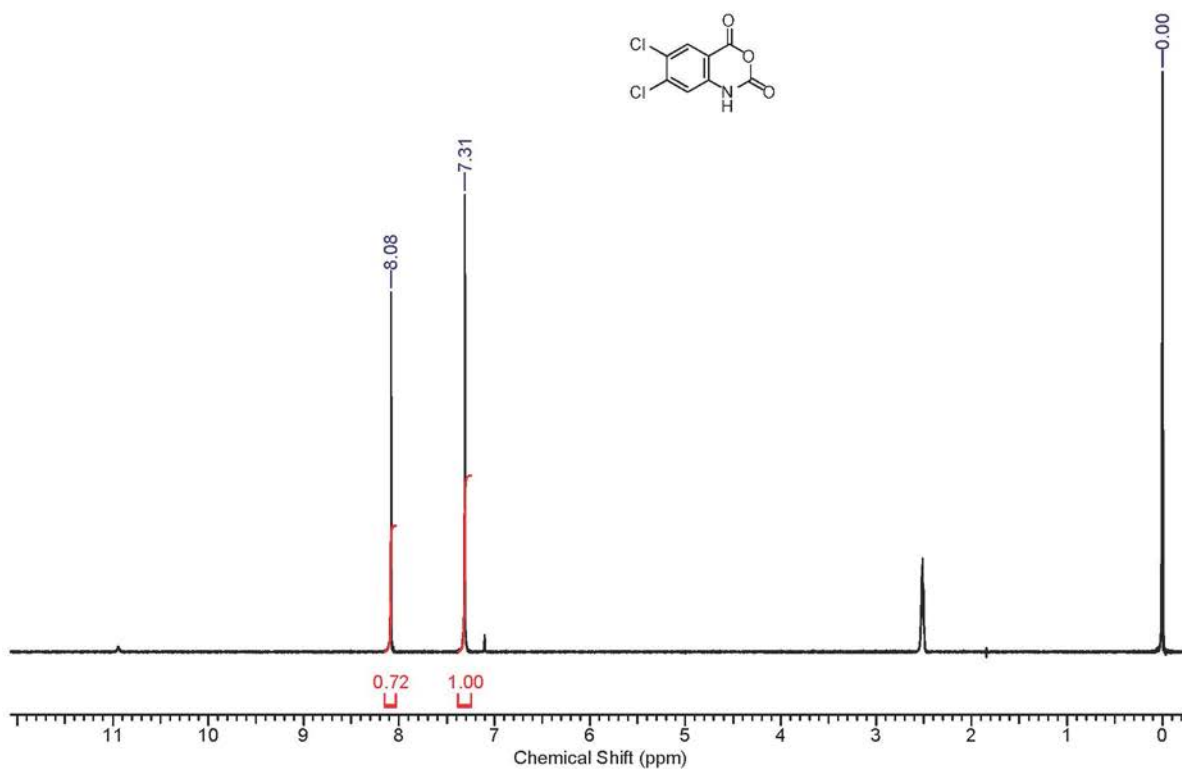
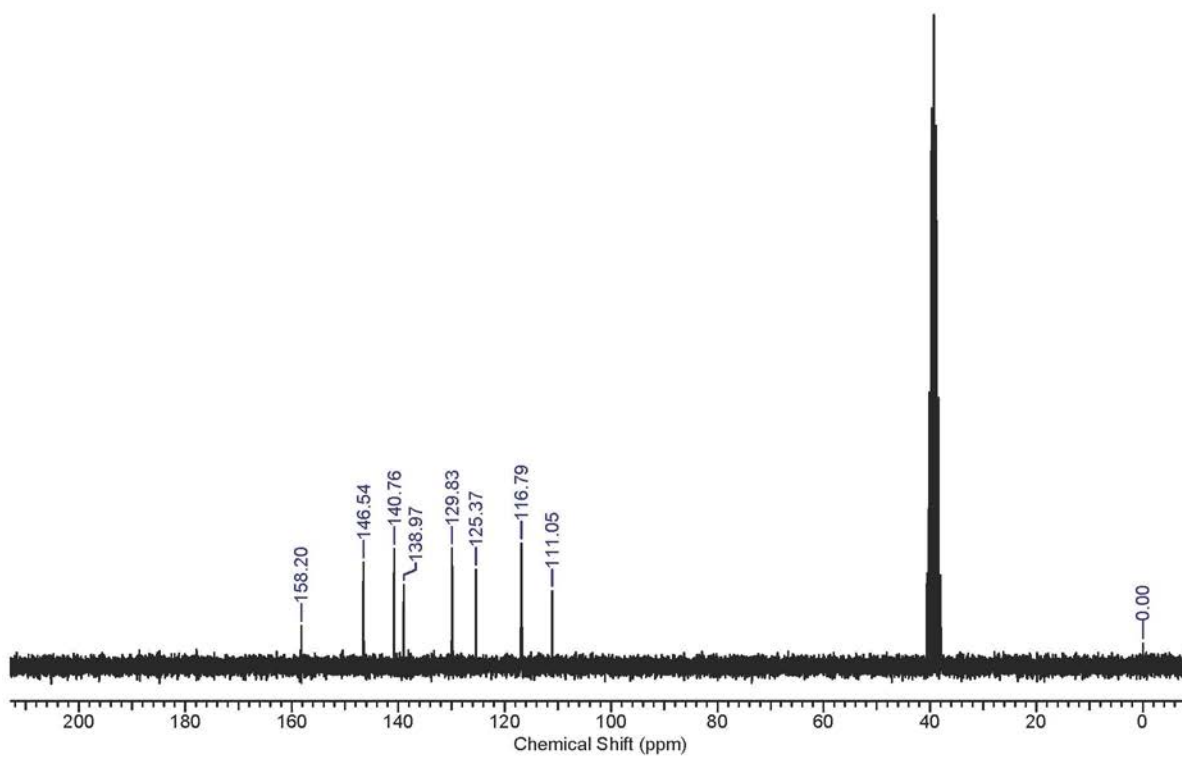
<sup>1</sup>H-NMR<sup>13</sup>C-NMR

Figure 4S. 6,7-dichloro-1*H*-benzo[*d*][1,3]oxazine-2,4-dione (4d)

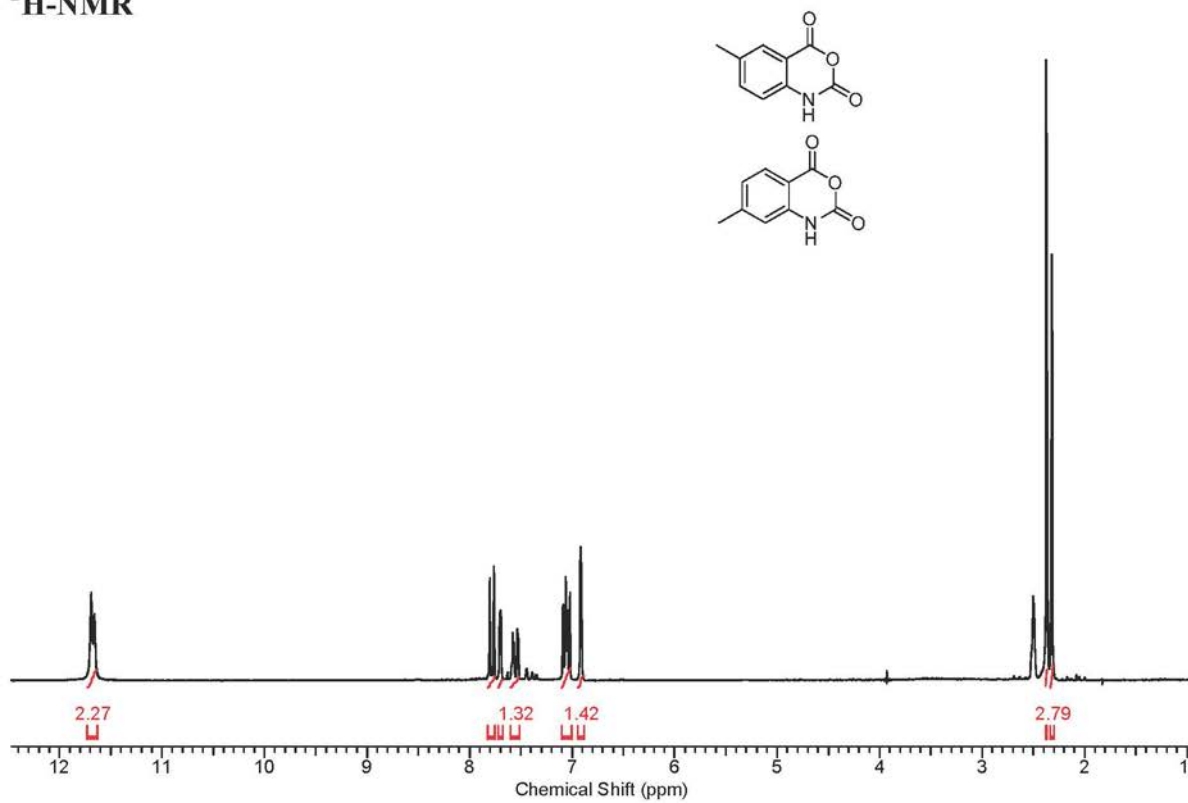
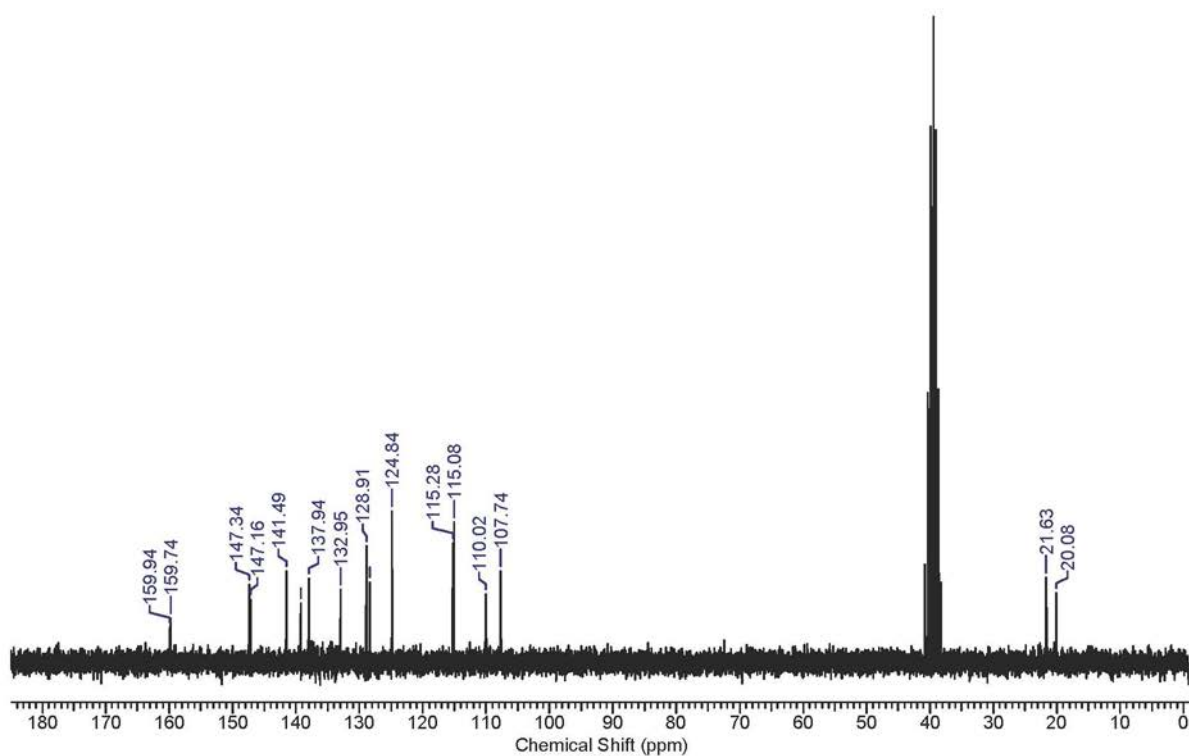
$^1\text{H-NMR}$  $^{13}\text{C-NMR}$ 

Figure 5S. 6-methyl and 7-methyl 1H-benzo[d][1,3]oxazine-2,4-dione (**4e**)

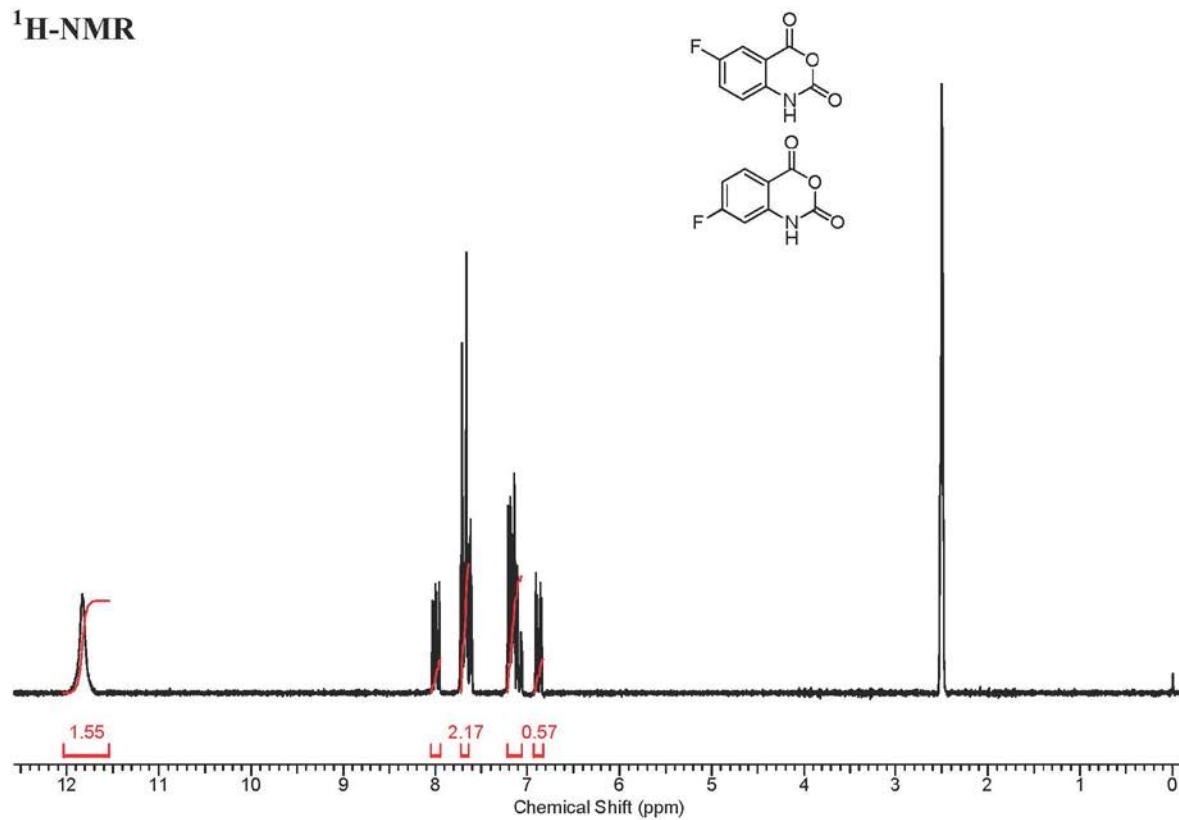
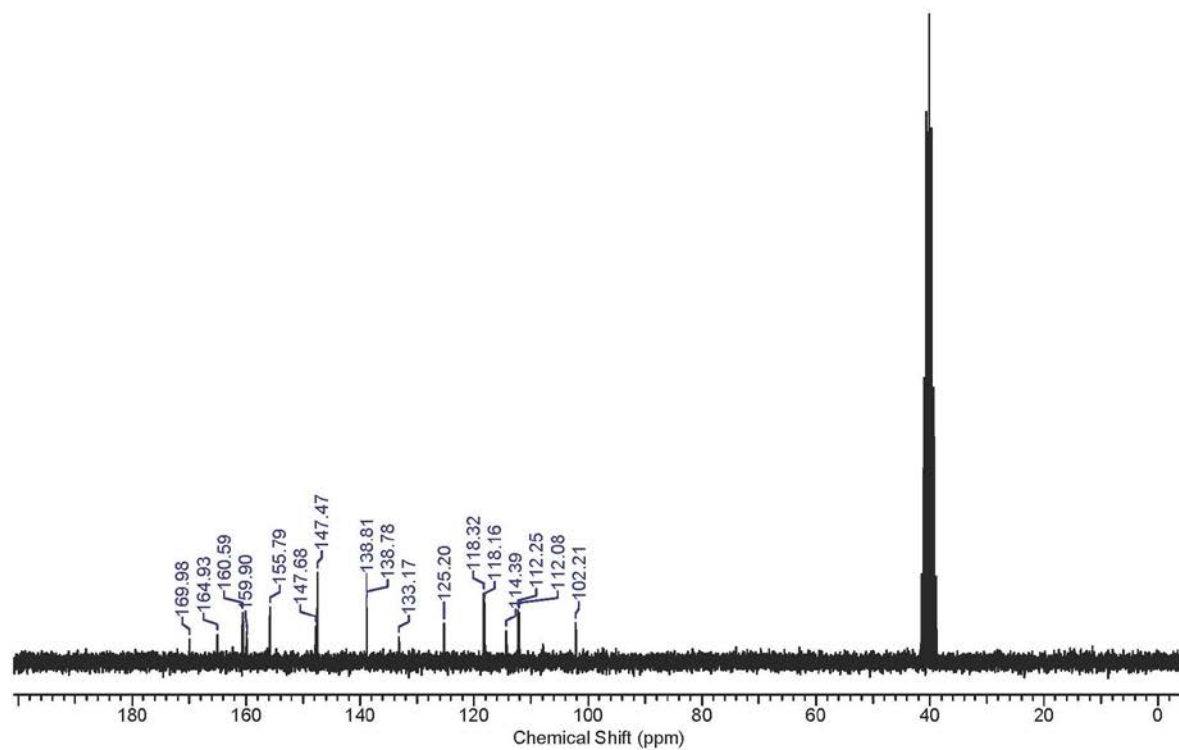
<sup>1</sup>H-NMR<sup>13</sup>C-NMR

Figure 6S. 6-fluoro and 7-fluoro-1H-benzo[d][1,3]oxazine-2,4-dione (4f)

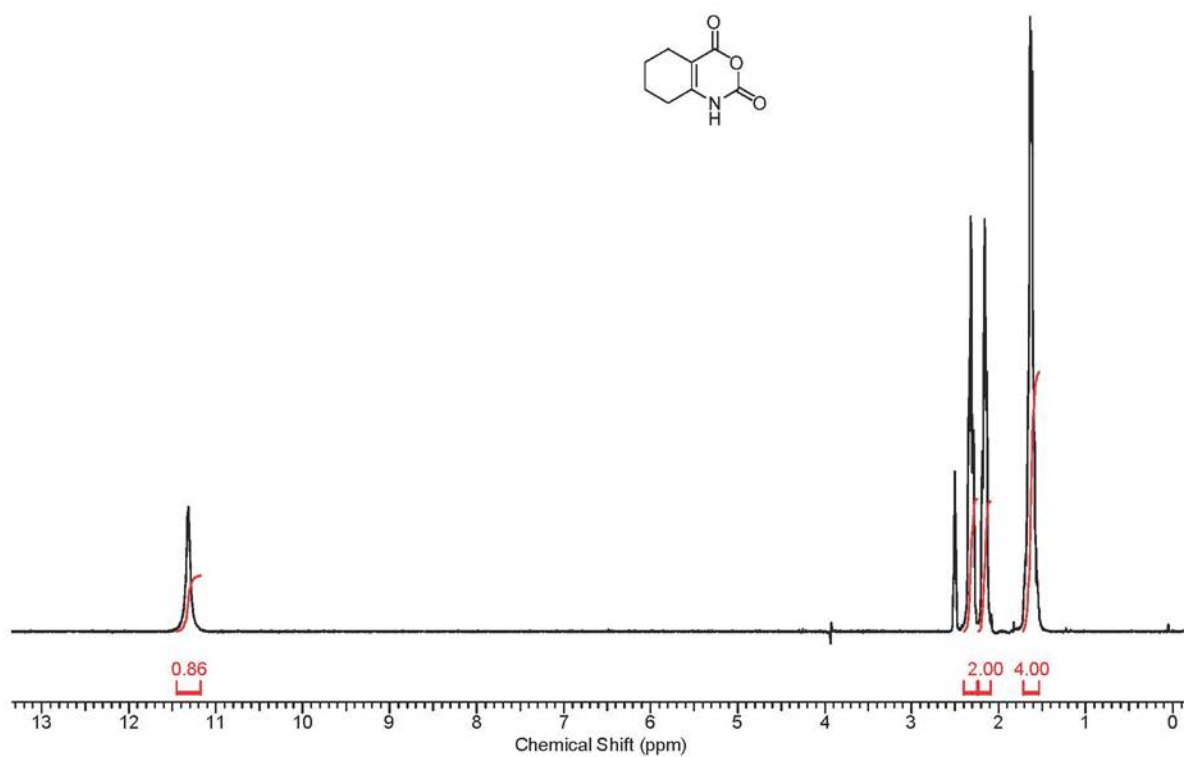
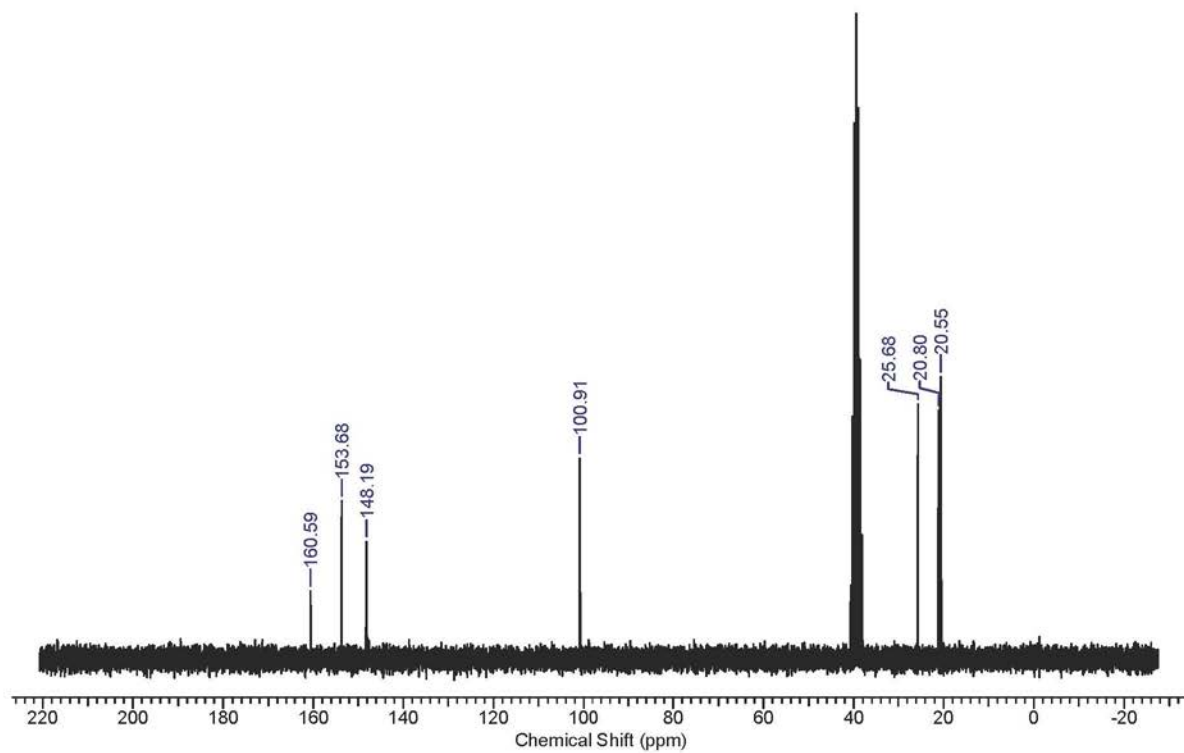
$^1\text{H-NMR}$  $^{13}\text{C-NMR}$ 

Figure 7S. 5,6,7,8-tetrahydro-1H-benzo[d][1,3]oxazine-2,4-dione (4g)