SUPPLEMENTARY MATERIALS

Synthesis of aminophenoxazinones and evaluation of their phytotoxicity in the search for new natural herbicides

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File S1. Wheat coleoptile bioassay protocol.

Approximately 100 wheat caryopses (*Triticum aestivum* L. cv. Burgos) were sown in 15 cm diameter Petri dishes (11 cm × 11 cm × 3.5 cm) lined with one sheet of qualitative filter paper and moistened with 10 mL of distilled water. The dishes were placed in a germination chamber for 4 days at 25±1 °C in the dark (Hancock, Barlow and Lacey, 1964). Following this period, the etiolated seedling coleoptiles were cut into 4 mm pieces with the aid of a Van der Veij guillotine under safe green light for use in bioassays. The compounds were pre-solubilized in dimethylsulfoxide (DMSO) and diluted with a buffered solution (pH 5.6) containing citric acid monohydrate (1.05 g L^{-1}), potassium hydrogen phosphate trihydrate (2.9 g L^{-1}) and 2% sucrose (Nitsch and Nitsch, 1956). The solutions were tested at concentrations the compounds were tested at concentrations of 10 μM, 30 μM, 100 μM, 300 μM and 1000 μM. A constant DMSO concentration of 5 μL/mL was maintained for each solution evaluated . Each treatment was tested in triplicate by adding 2 mL of each solution and five fragments of wheat coleoptile to a glass test tube (16 × 100 mm, 10 mL). One control containing the buffer solution plus DMSO (5 μL/mL) was performed. The active ingredient of herbicide Stomp Aqua®, penditelamine, was used as an internal reference, at the same concentrations and under the same conditions as reported previously. The test tubes were capped and kept in a growth chamber at 25±1 °C in the dark and with constant rotation in a drum tube rotator (0.25 rpm). After 24 h, the coleoptiles were removed from the tubes, photographed and measured. Data were analyzed statistically using Welch's test and are presented as percentage difference with respect to the control. Positive values represent stimulation, and negative values represent inhibition.

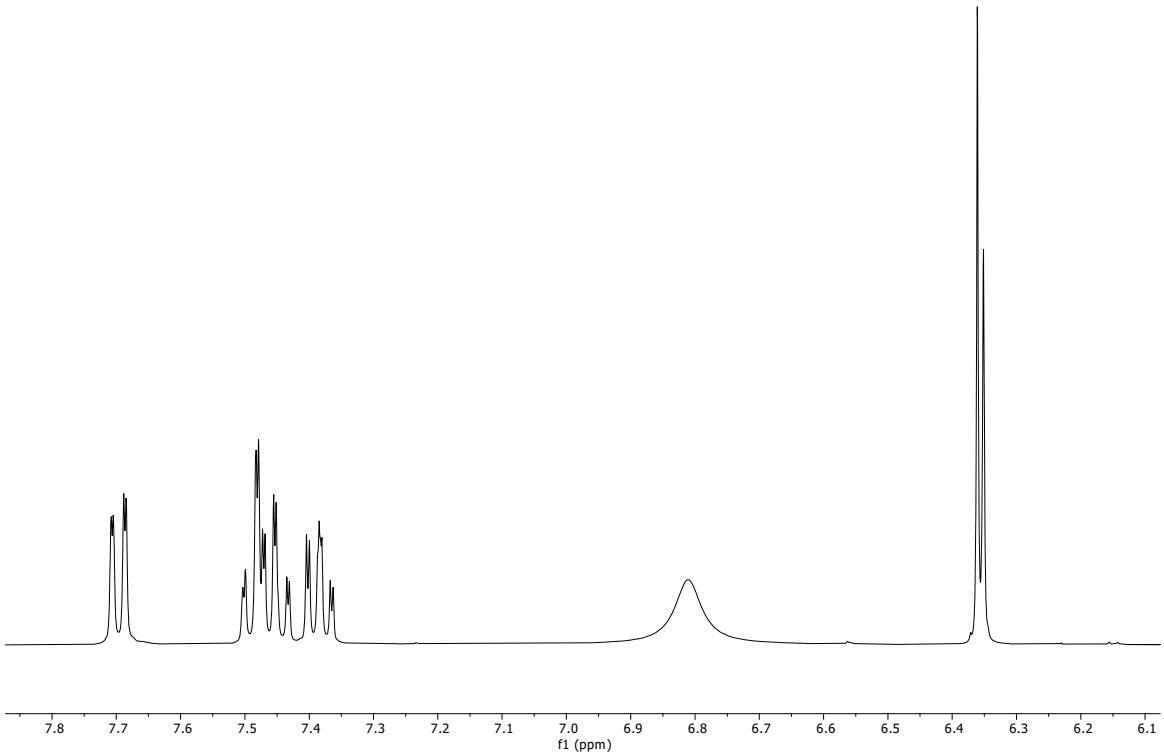
File S2. Phytotoxic bioassay protocol.

The compounds were assessed for phytotoxic activity in the following two species, which are agricultural weeds: *Lolium rigidum* and *Portulaca oleracea*. For the bioassay, seeds from the recipient species were distributed in Petri dishes (5 cm diameter) lined with one sheet of Whatman N° 1 qualitative filter paper moistened with 1 ml of the compounds or control solutions, separately.

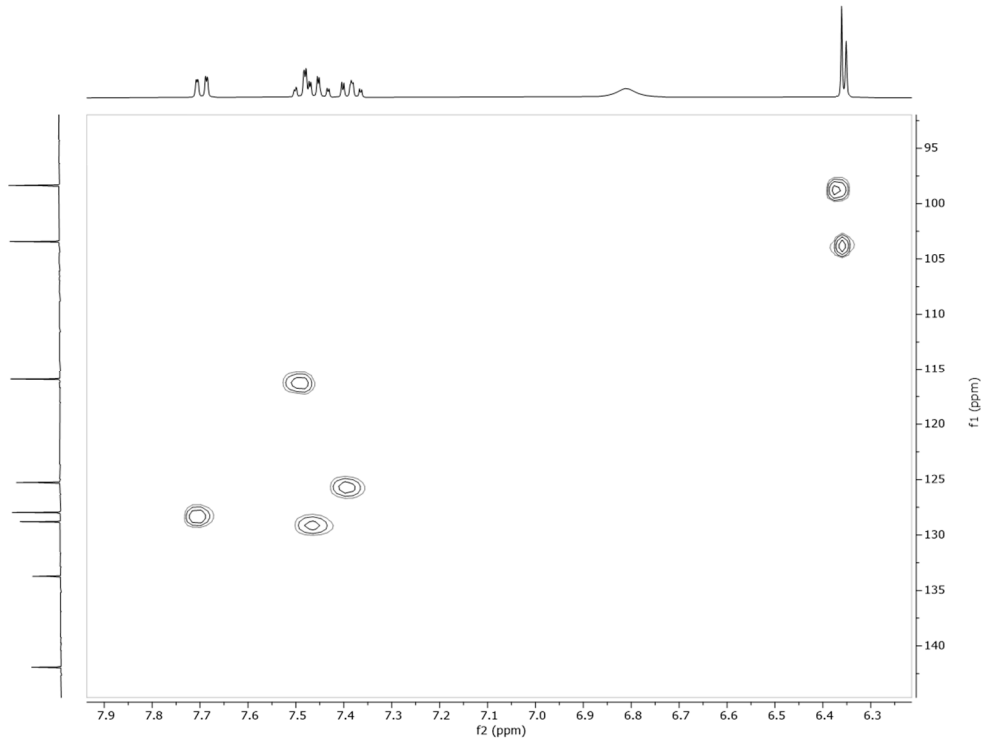
Germination and growth were conducted in aqueous solutions at controlled pH by using 10^{-2} M 2-[*N*-morpholino] ethanesulfonic acid and 1 M NaOH (pH 6.0). The compounds to be assayed were dissolved in DMSO, and these solutions were diluted with buffer (5 μL DMSO solution/mL buffer) to give test concentrations for each compound (10 μM, 30 μM, 100 μM, 300 μM and 1000 μM). Parallel controls were also run as described above for the coleoptile bioassay.

The experimental design was randomized, with four replicates of 20 seeds per treatment. The experiment was conducted in a germination chamber at 25 °C in the dark for 7 days. After growth, plants were frozen at –10°C for 24 h to avoid subsequent growth during the measurement process. The germination rate, root length, and shoot length were recorded using a Fitomed system. Data were analyzed statistically using Welch's test, with significance fixed at 0.01 and 0.05. Results are presented as percentage differences with respect to the control. Zero represents control, positive values represent stimulation, and negative values represent inhibition.

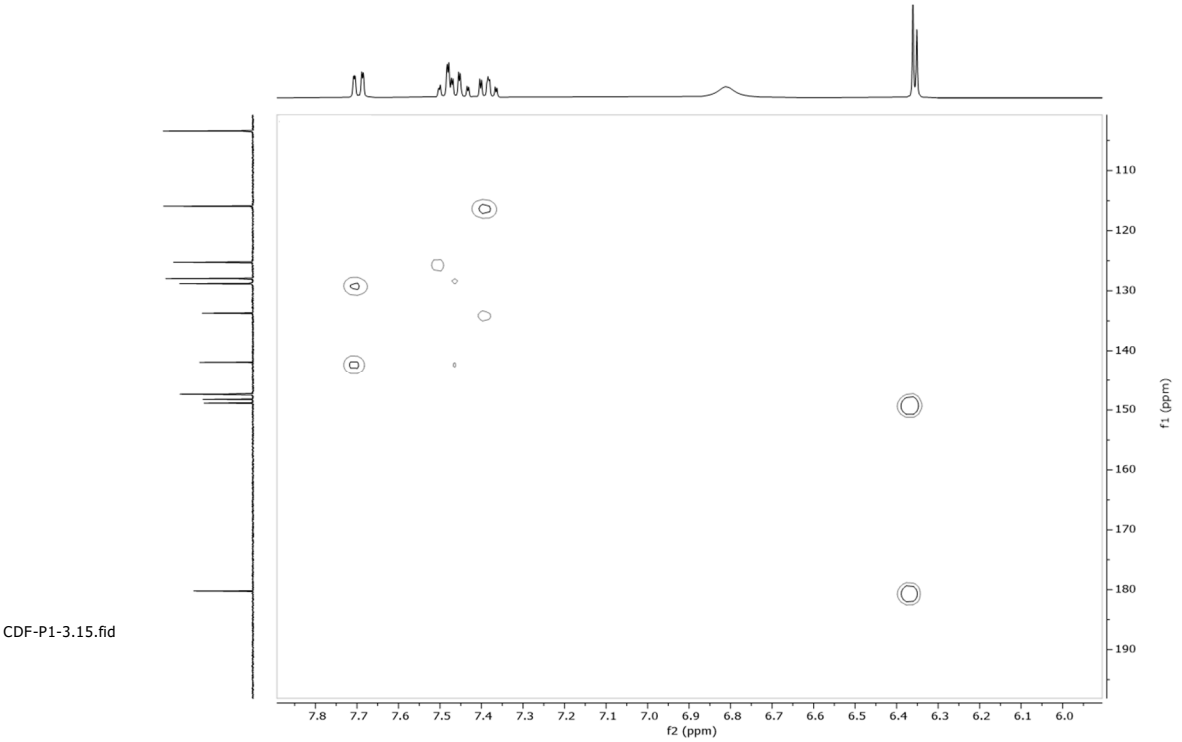
Figure S1. Structural characterization of 2-amino-3*H*-phenoxazin-3-one



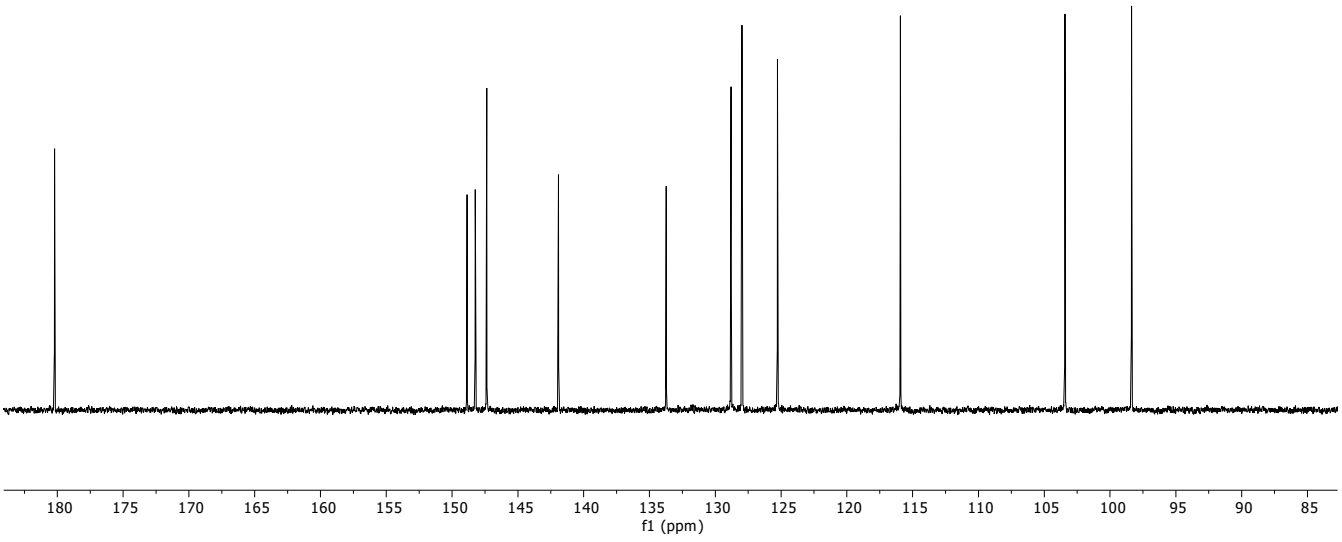
^{1}H-NMR Spectrum of 2-amino-3H-phenoxazin-3-one



HSQC Spectrum of 2-amino-3H-phenoxazin-3-one

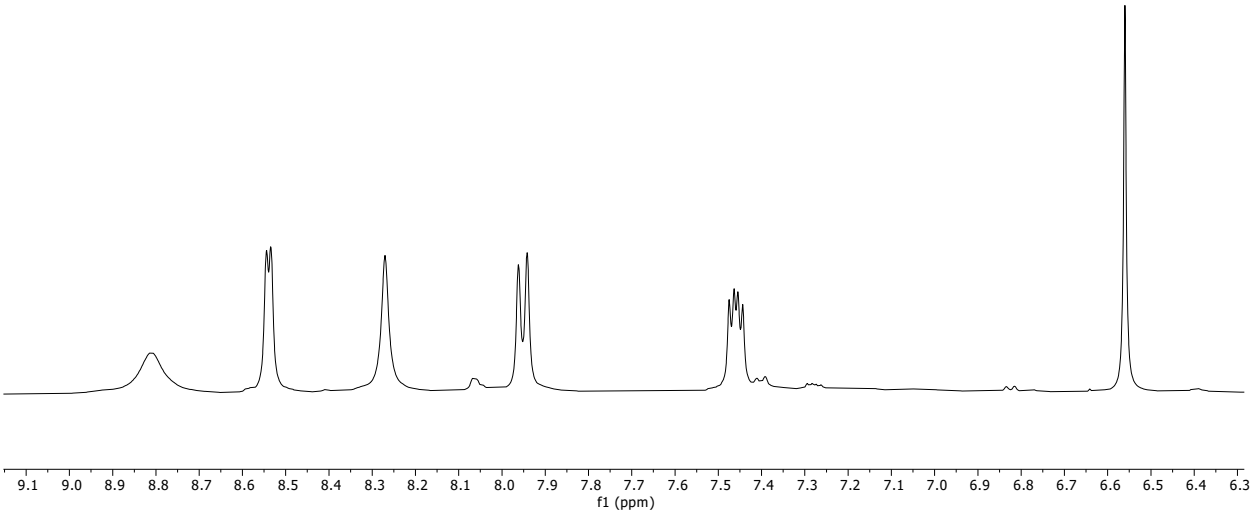


HMBC Spectrum of 2-amino-3H-phenoxazin-3-one



^{13}C-NMR Spectrum of 2-amino-3H-phenoxazin-3-one

Figure S2. Structural characterization of 2-amino-3*H*-dipyrido [3,2-b: 2 ', 3'-e] [1,4]-oxazin-3-one



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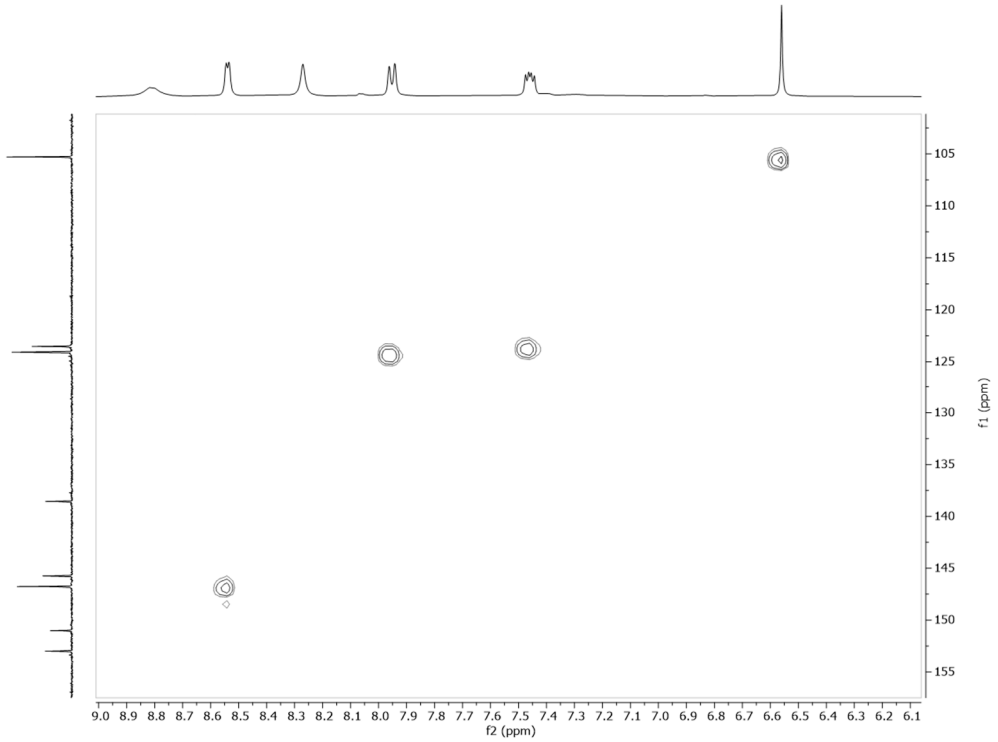

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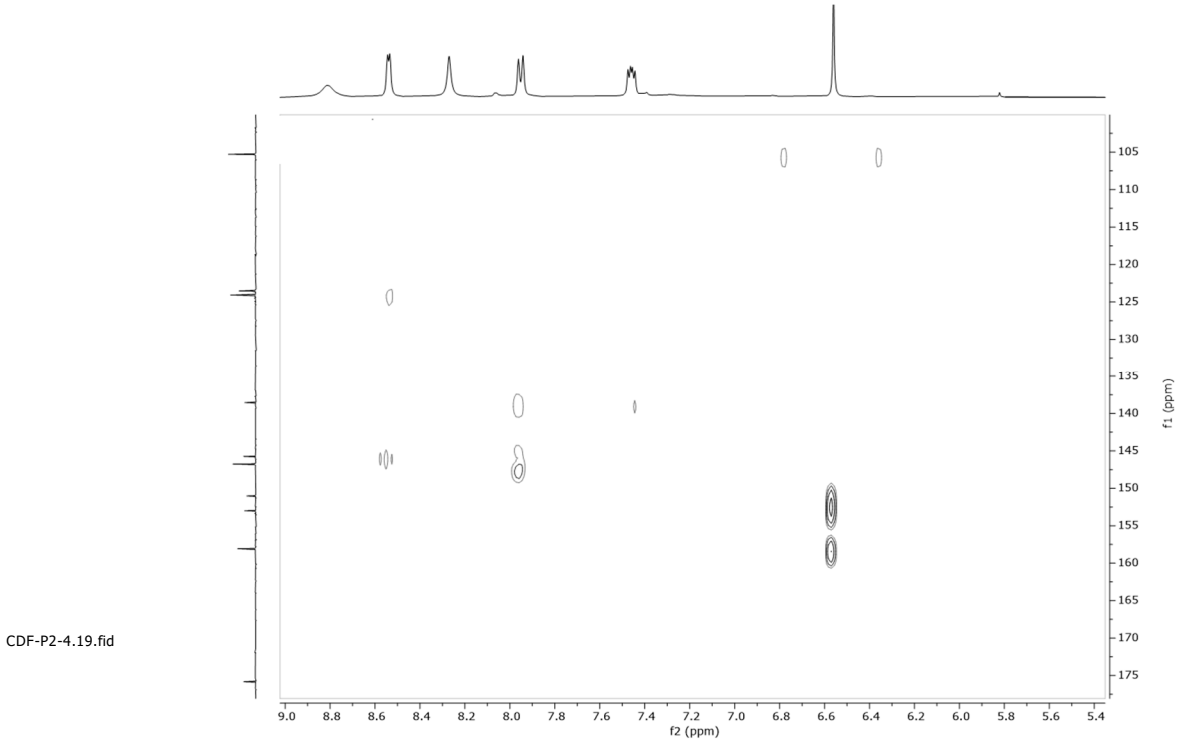

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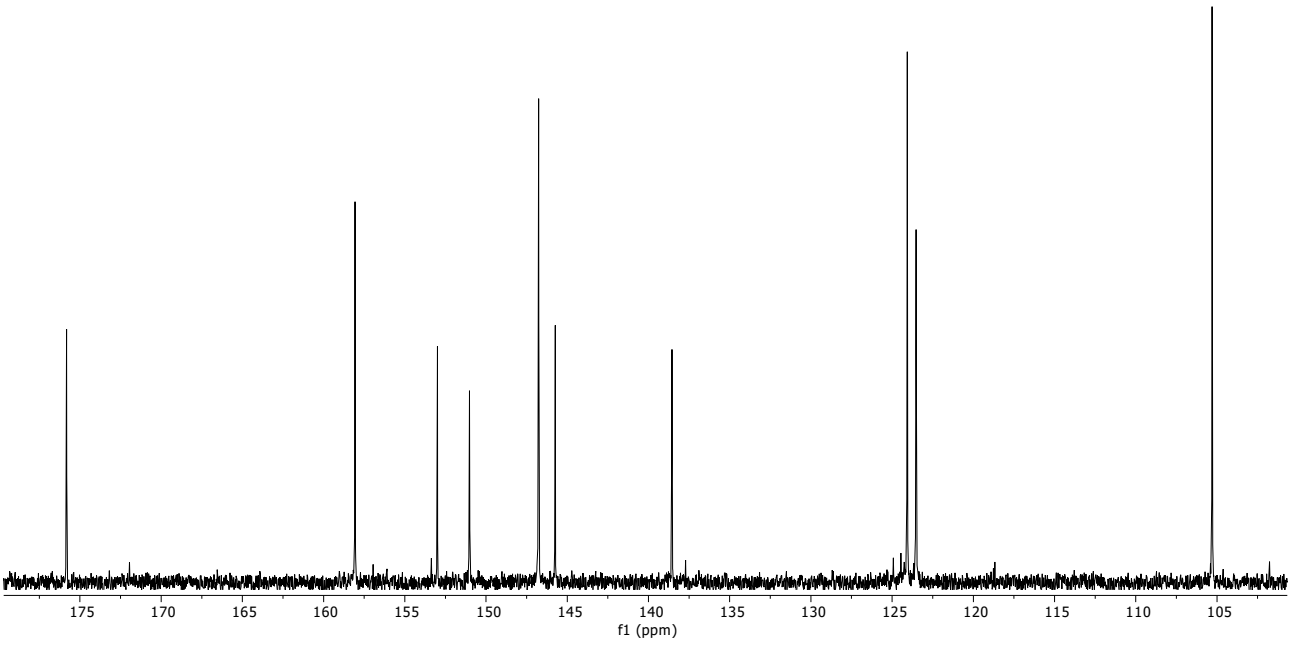
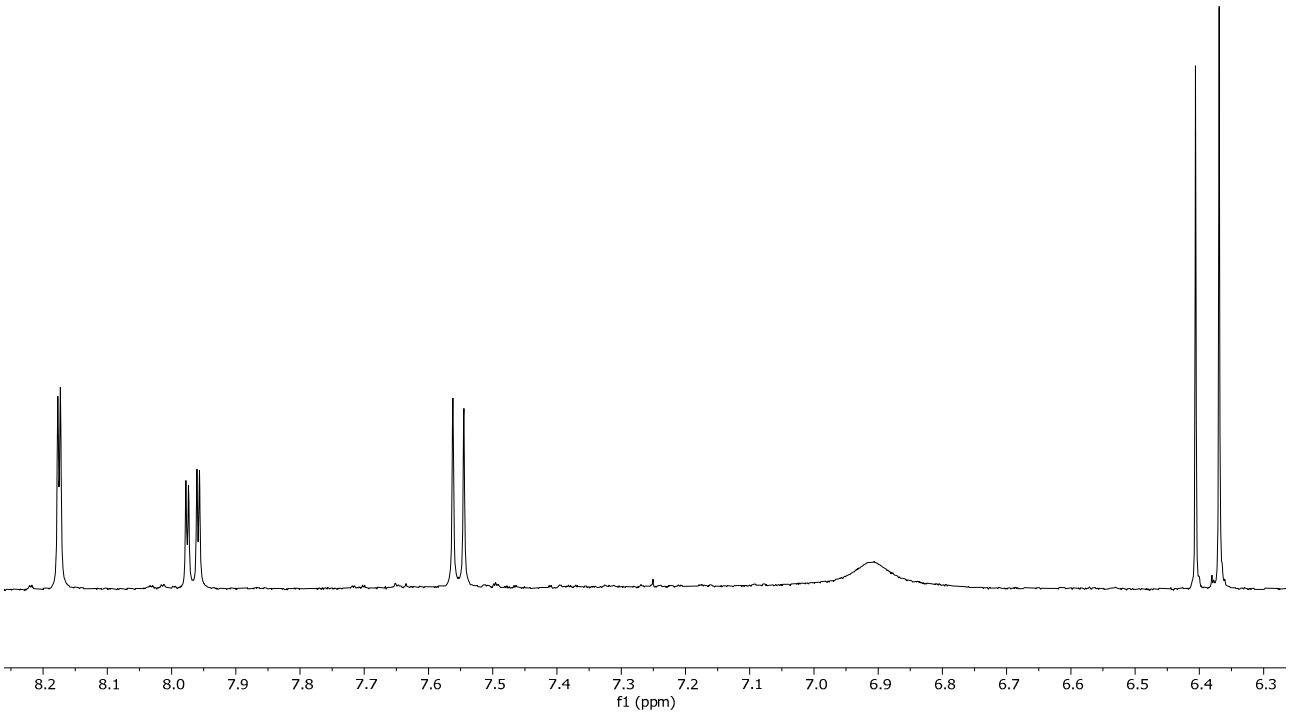
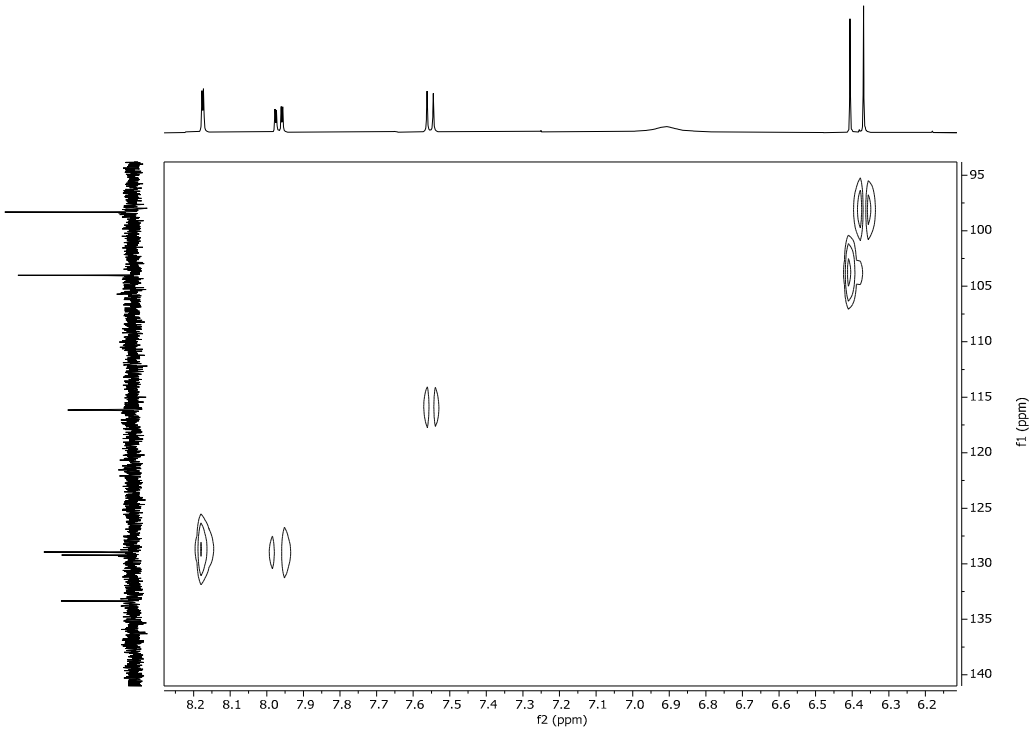


^{13}C-NMR Spectrum of 2-amino-3H-dipyrido [3,2-b: 2 ′, 3′-e] [1,4]-oxazin-3-one

Figure S3. Structural characterization of 2-amino-3-oxo-*3H*-phenoxazine-8-carboxylic acid



[1]H-NMR Spectrum of 2-amino-3-oxo-3H-phenoxazine-8-carboxylic acid



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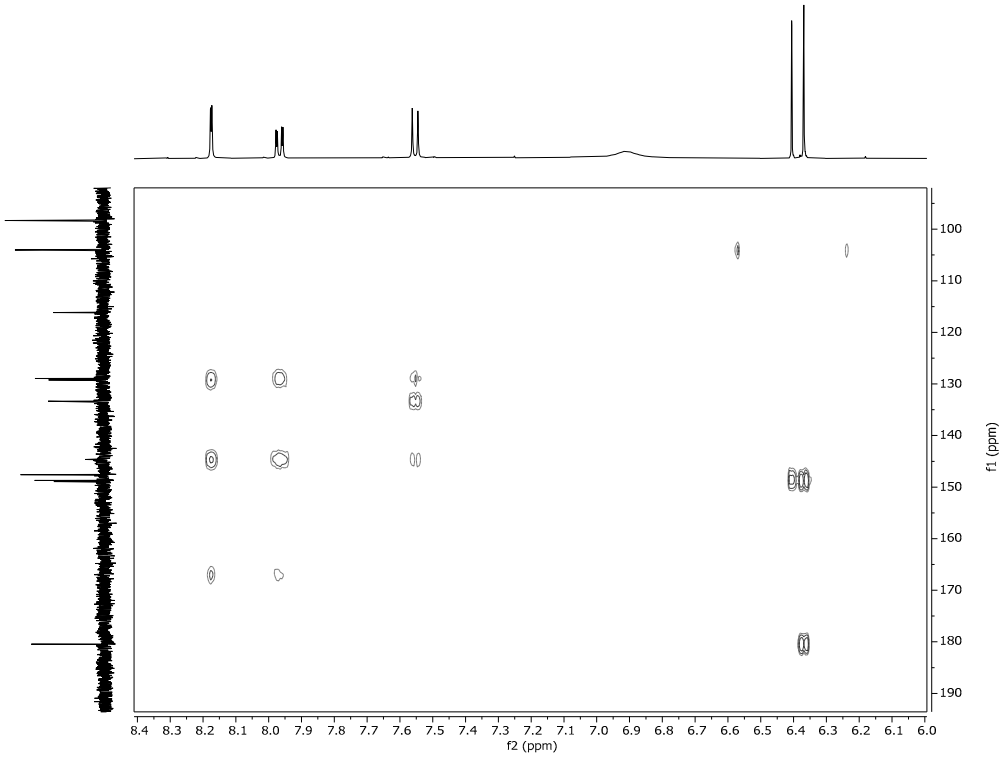

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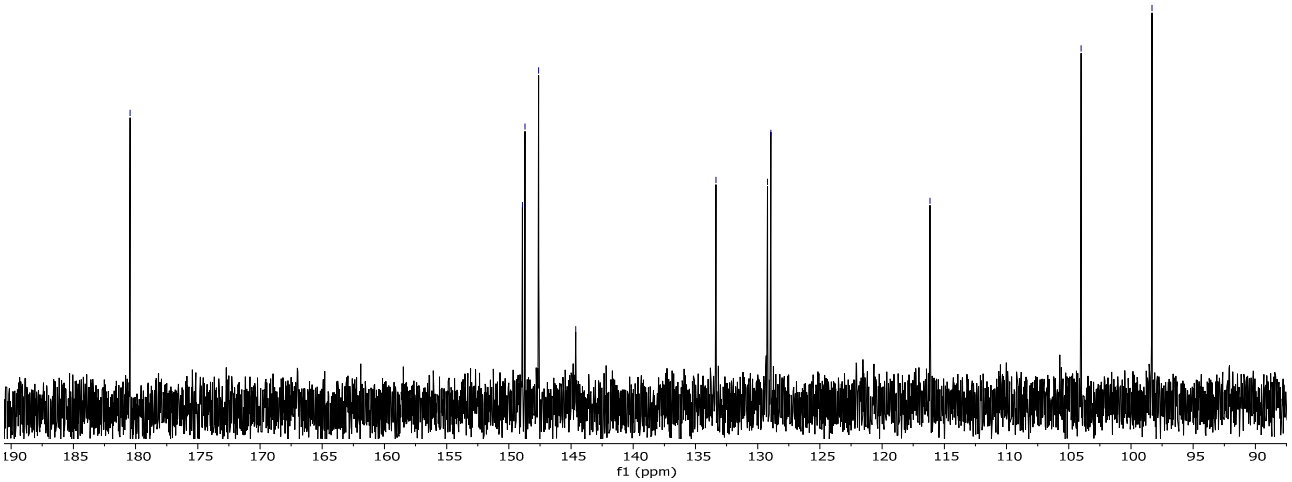
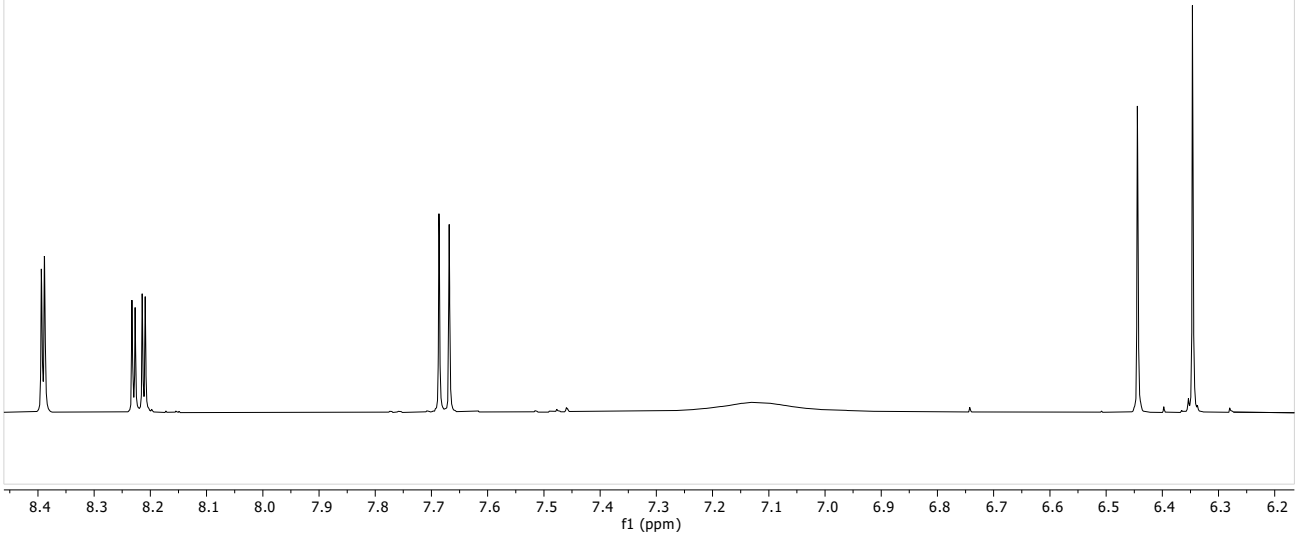


^{13}C-NMR Spectrum of 2-amino-3-oxo-3H-phenoxazine-8-carboxylic acid

Figure S4. Structural characterization of 2-amino-8-nitro-*3H*-phenoxazin-3-one



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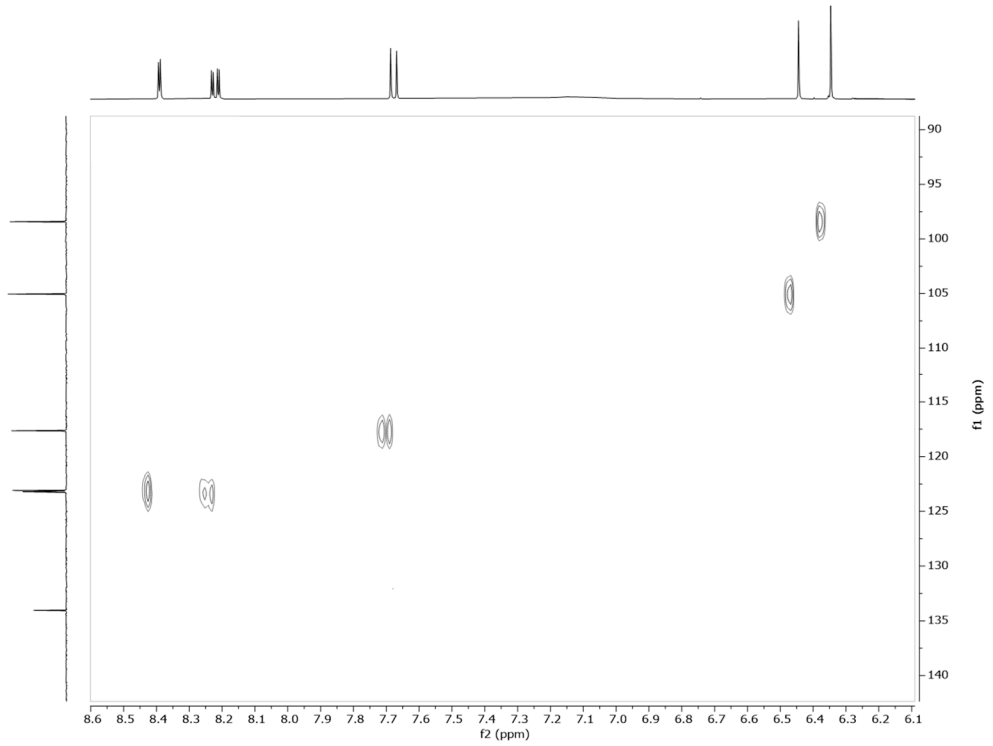
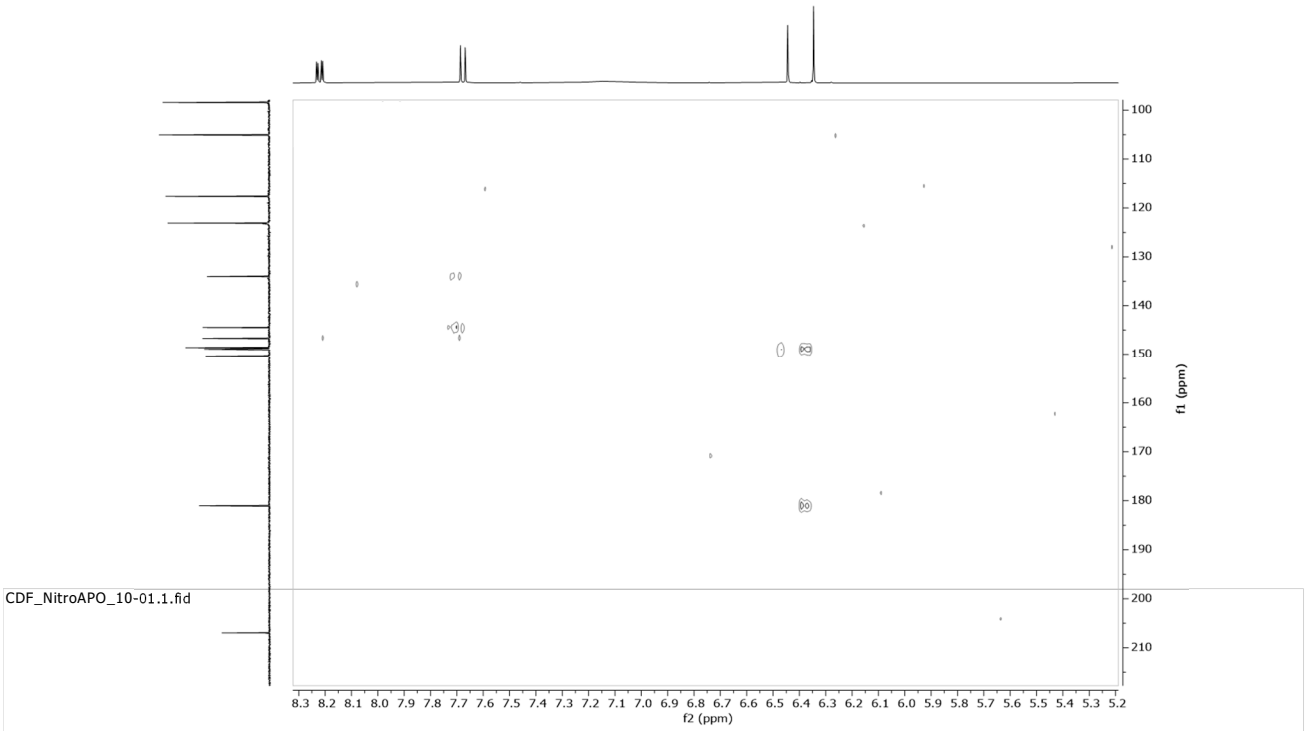


HSQC Spectrum of 2-amino-8-nitro-3H-phenoxazin-3-one



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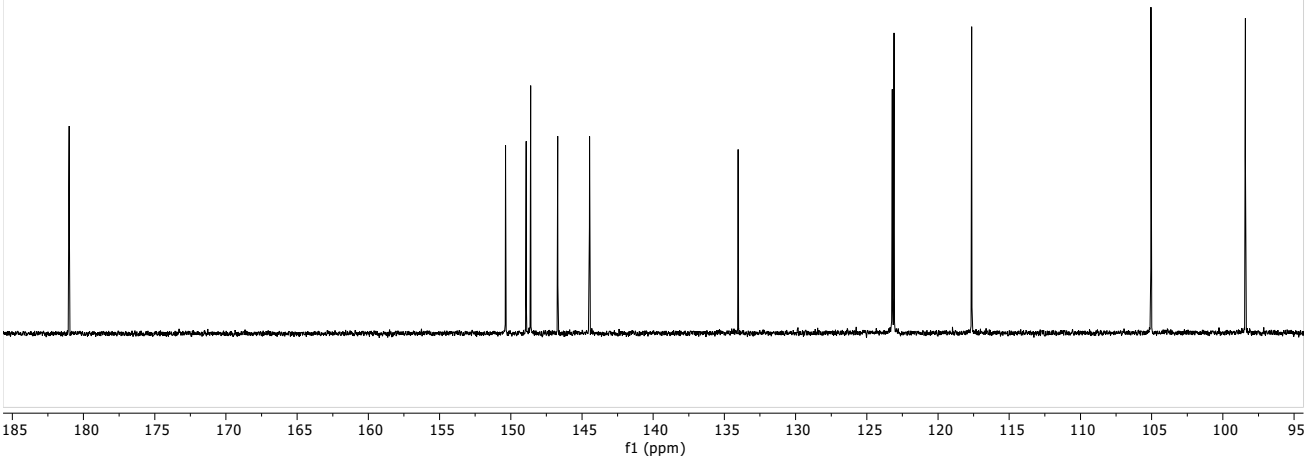
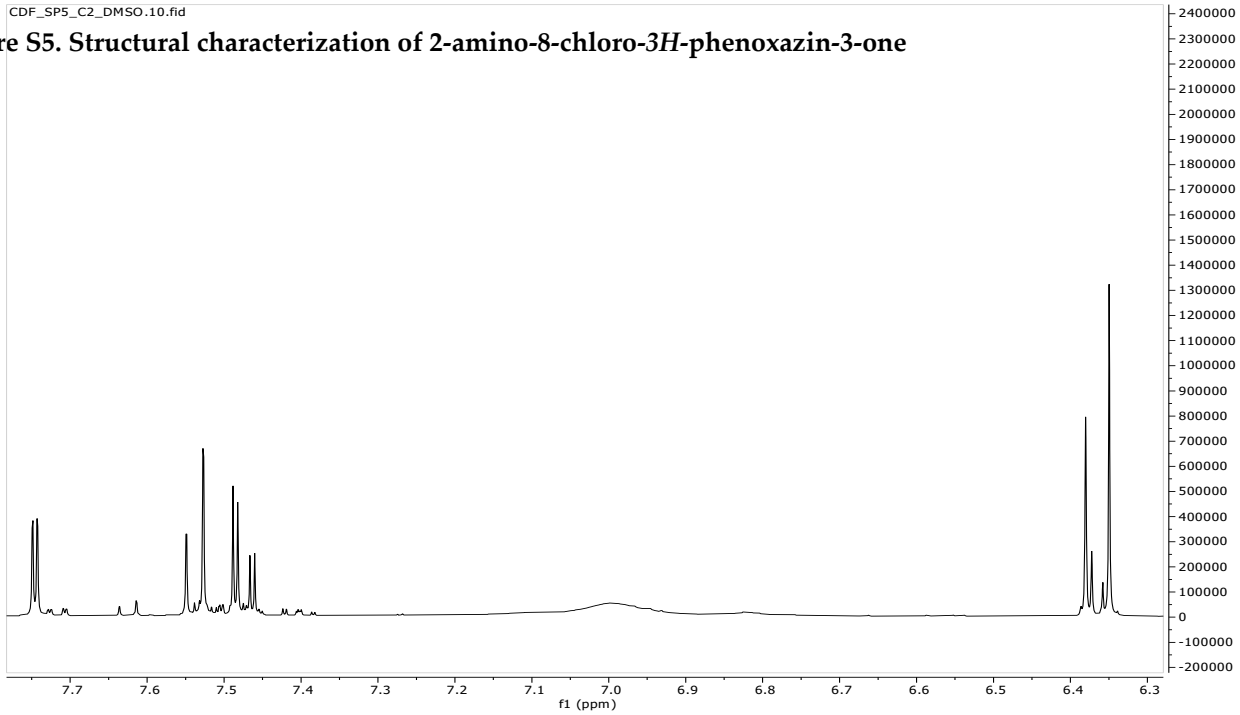


^{13}C-NMR Spectrum of 2-amino-8-nitro-3H-phenoxazin-3-one

Figure S5. Structural characterization of 2-amino-8-chloro-*3H*-phenoxazin-3-one



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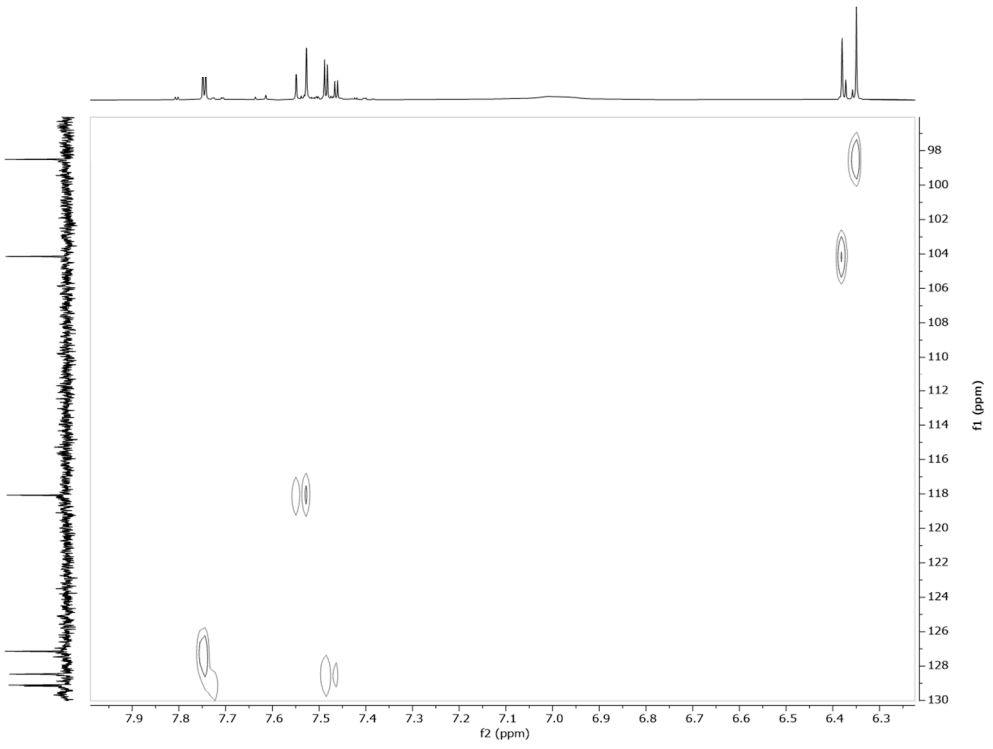

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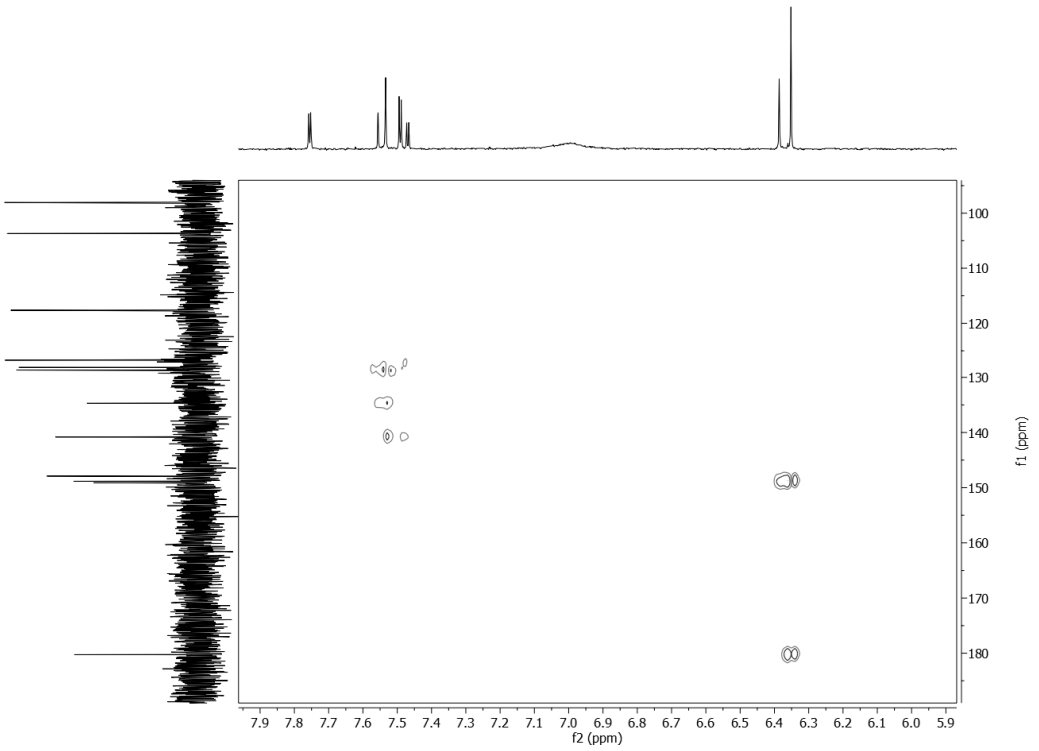
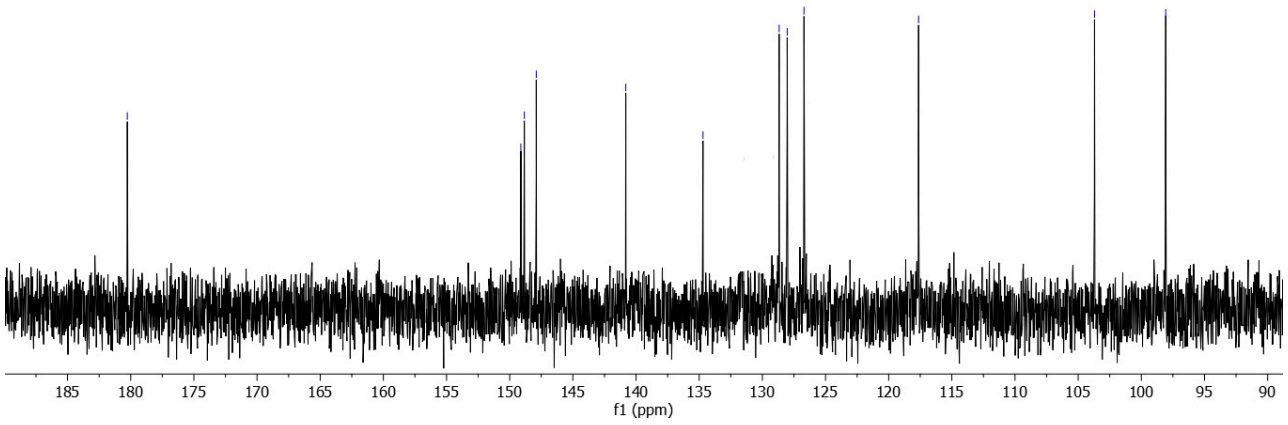
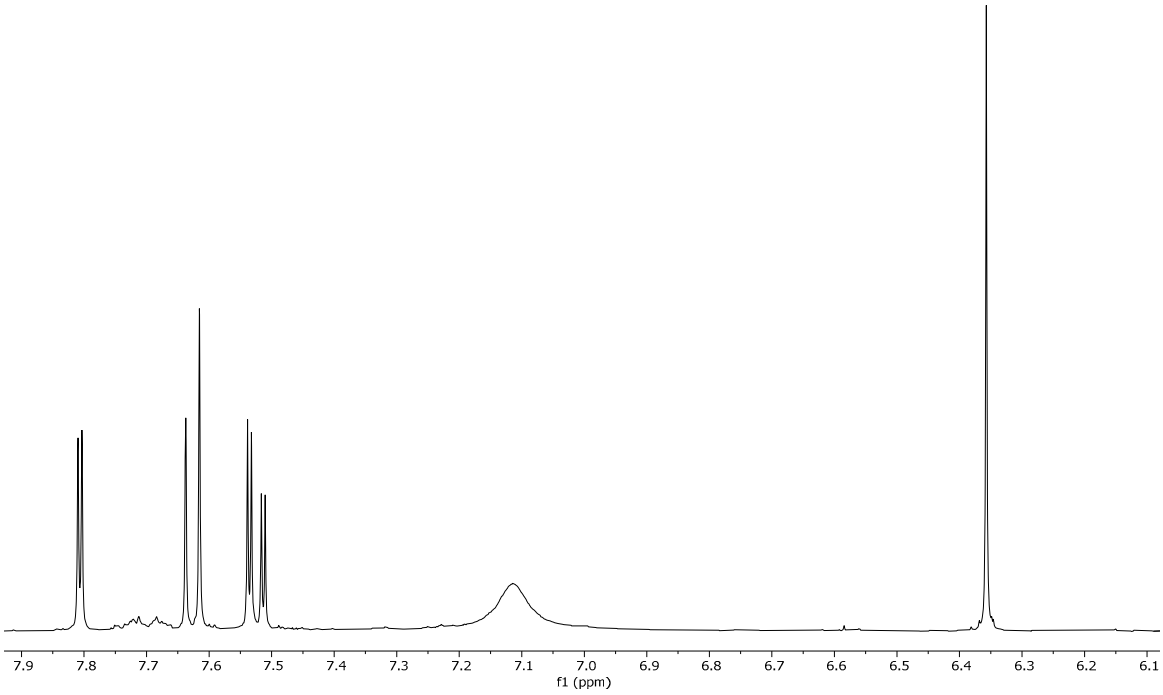


HMBC Spectrum of 2-amino-8-chloro-3H-phenoxazin-3-one



^{13}C-NMR Spectrum of 2-amino-8-chloro-3H-phenoxazin-3-one

Figure S6. Structural characterization of 2-amino-8-chloro-4-iodo-*3H*-phenoxazin-3-one



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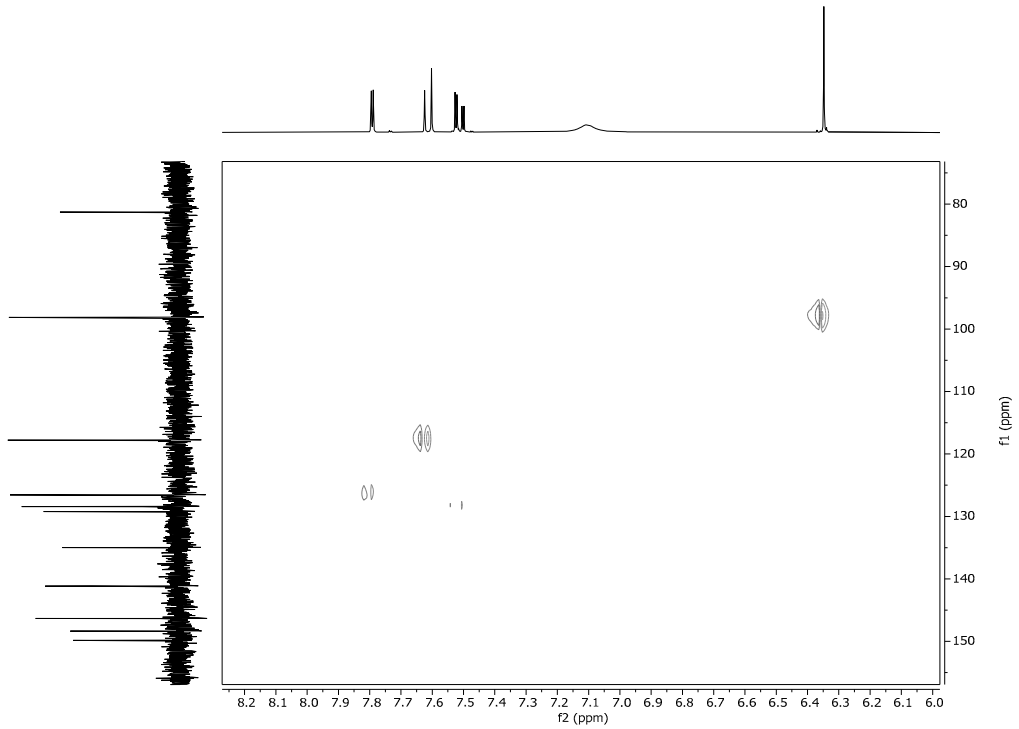
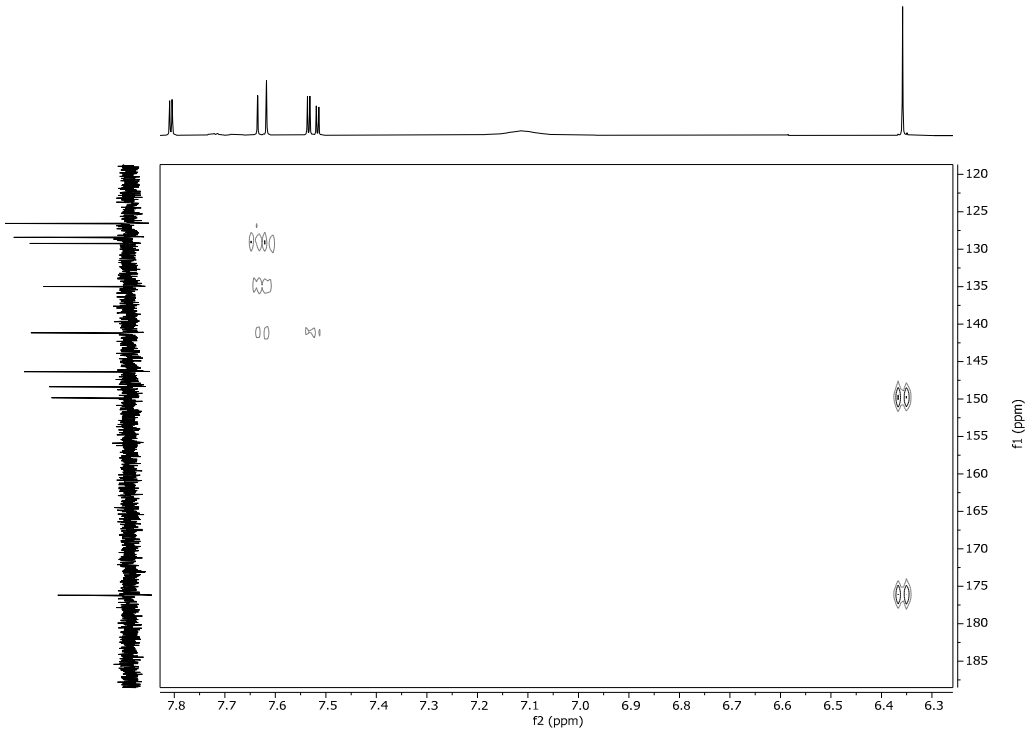
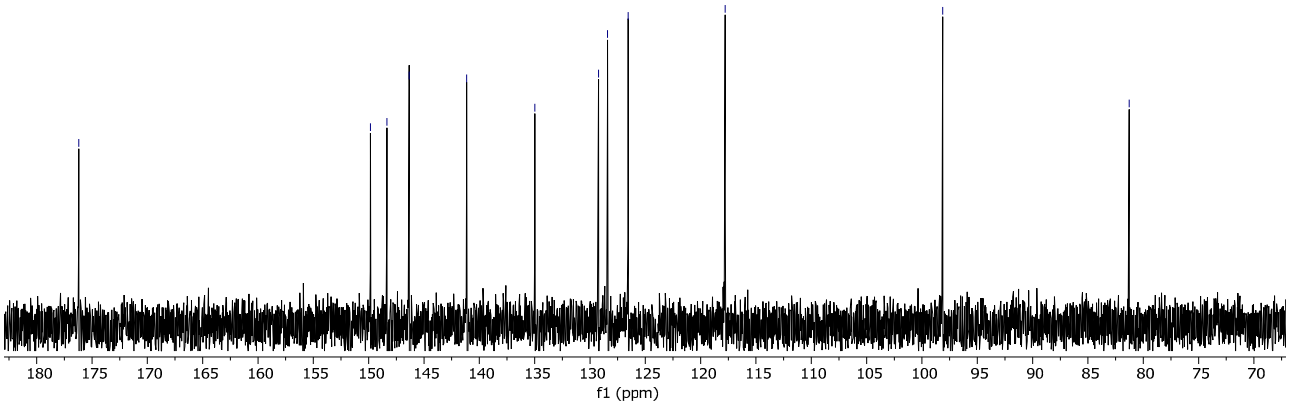


HSQC Spectrum of 2-amino-8-chloro-4-iodo-3H-phenoxazin-3-one

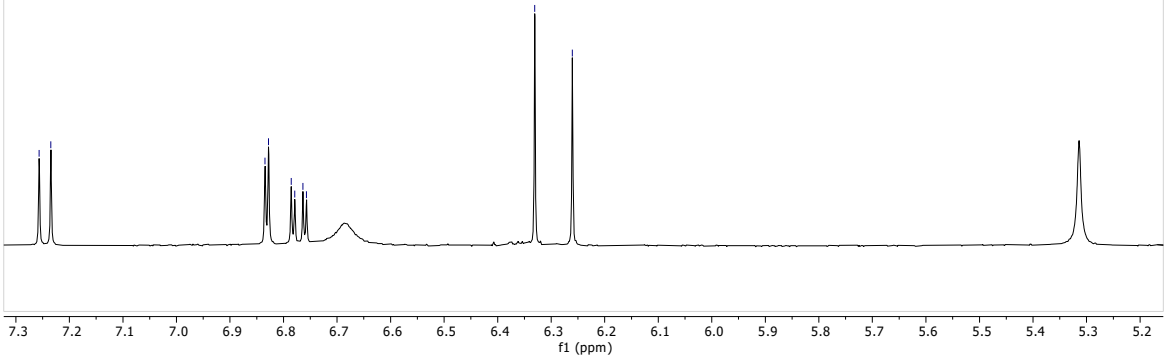


HMBC Spectrum of 2-amino-8-chloro-4-iodo-3H-phenoxazin-3-one



^{13}C-NMR Spectrum of 2-amino-8-chloro-4-iodo-3H-phenoxazin-3-one

Figure S7. Structural characterization of 2,8-diamino-*3H*-phenoxazin-3-one



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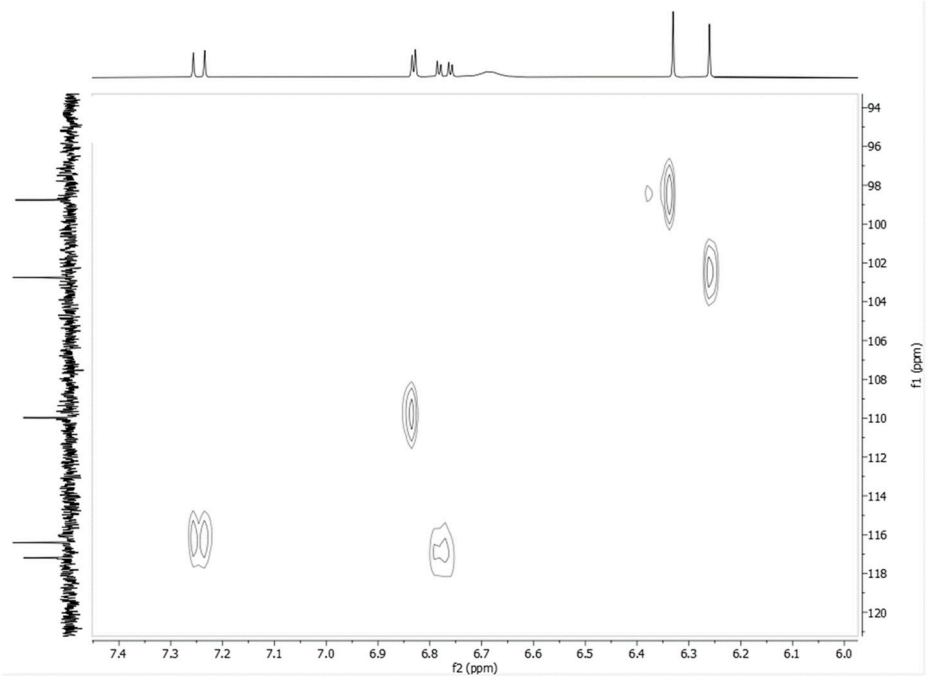
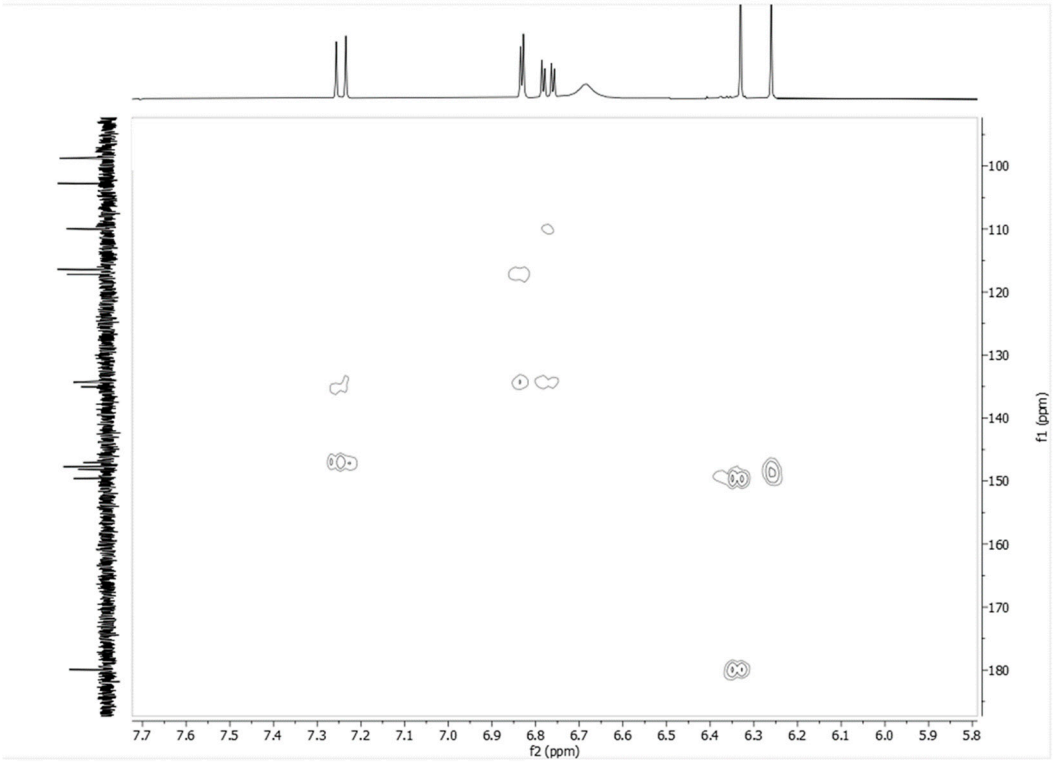


HSQC Spectrum of 2,8-diamino-3H-phenoxazin-3-one



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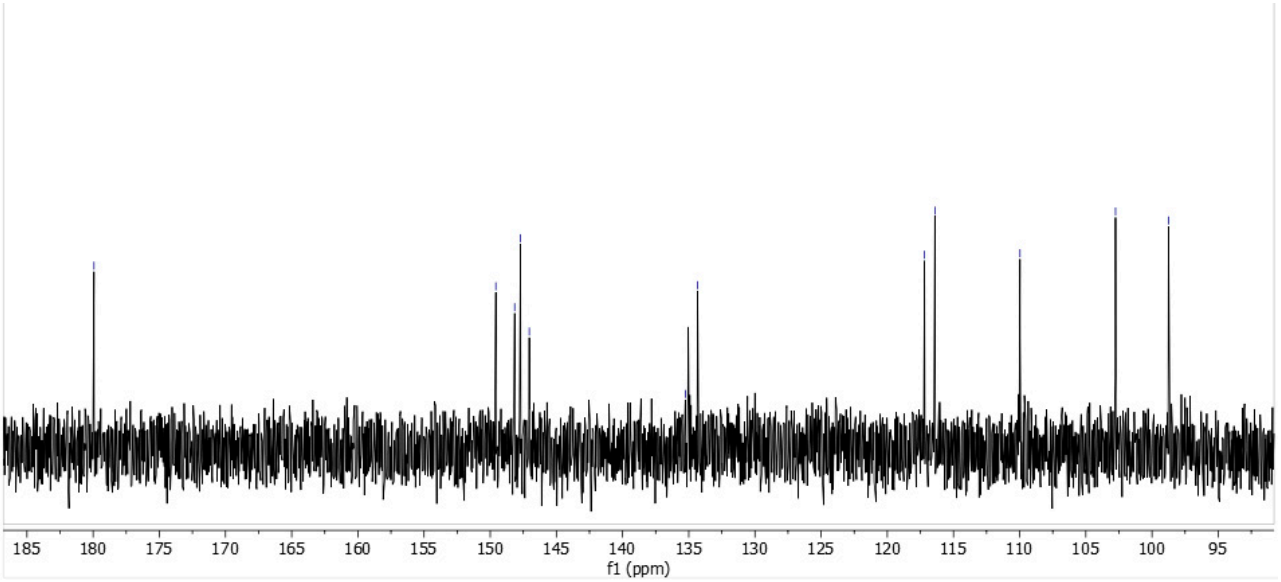

^{13}C-NMR Spectrum of 2,8-diamino-3H-phenoxazin-3-one