

Supporting Information

Fragment-Based Drug Design and Identification of HJC0123, A Novel Orally

Bioavailable STAT3 Inhibitor for Cancer Therapy

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States

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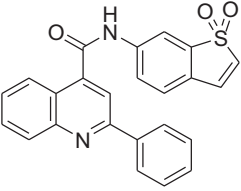
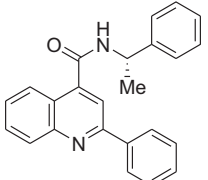
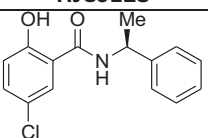
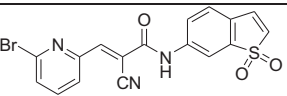
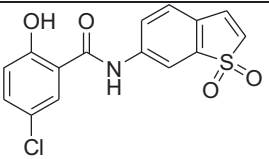
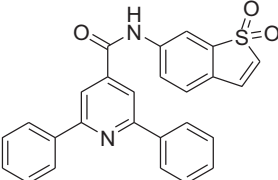
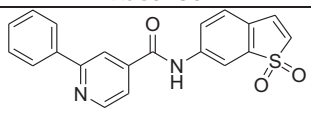
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Table of Contents

1. Table S1. Page 2
2. Figure S1. Page 3
3. Figure S2. Page 4
4. Figure S3. Page 5
5. Morphological Changes. Page 6
6. Cell Apoptosis Assay. Page 6
7. Cell Cycle Distribution Analysis. Page 6
8. References. Page 6
9. Copies of ¹H and ¹³C NMR spectra Page 7 to Page 30

Table S1. Physicochemical parameters¹⁻² of selected novel STAT3 inhibitors

| Compound | Chemical Structure | TPSA | cLogP | MW | HD (nOHNH) | HA (nON) |
|----------|---|------|-------|---------|---------------|-------------|
| 5 |  HJC0123 | 76.1 | 4.20 | 412.47 | 1 | 5 |
| 8 |  HJC0128 | 42.0 | 5.10 | 352.437 | 1 | 3 |
| 9 |  HJC0130 | 49.3 | 4.04 | 275.735 | 2 | 3 |
| 10 |  HJC0371 | 99.9 | 2.63 | 416.256 | 1 | 6 |
| 12 |  HJC0149 | 83.5 | 2.88 | 335.768 | 2 | 5 |
| 19 |  HJC0136 | 76.1 | 4.65 | 438.508 | 1 | 5 |
| 22 |  HJC0430 | 76.1 | 2.95 | 362.41 | 1 | 5 |

cLogP (Average LogP): <http://146.107.217.178/lab/alogps/start.html>

TPSA: <http://www.molinspiration.com/cgi-bin/properties>

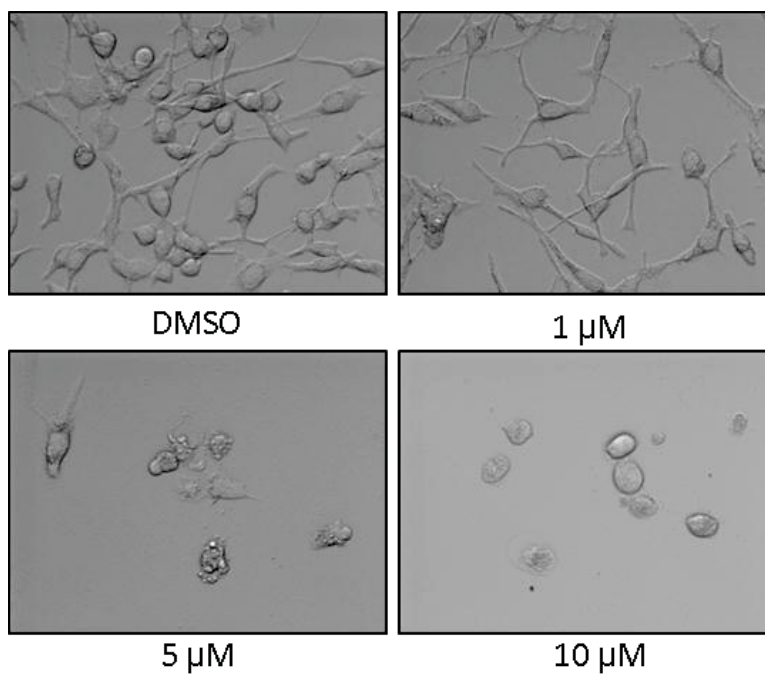


Figure S1. Effect of **5 (HJC0123)** on cell growth and cellular morphological change. Exponentially growing MDA-MB-231 breast cancer cells were incubated with **HJC0123** for 48 h. Cell morphology was evaluated under light microscopy.

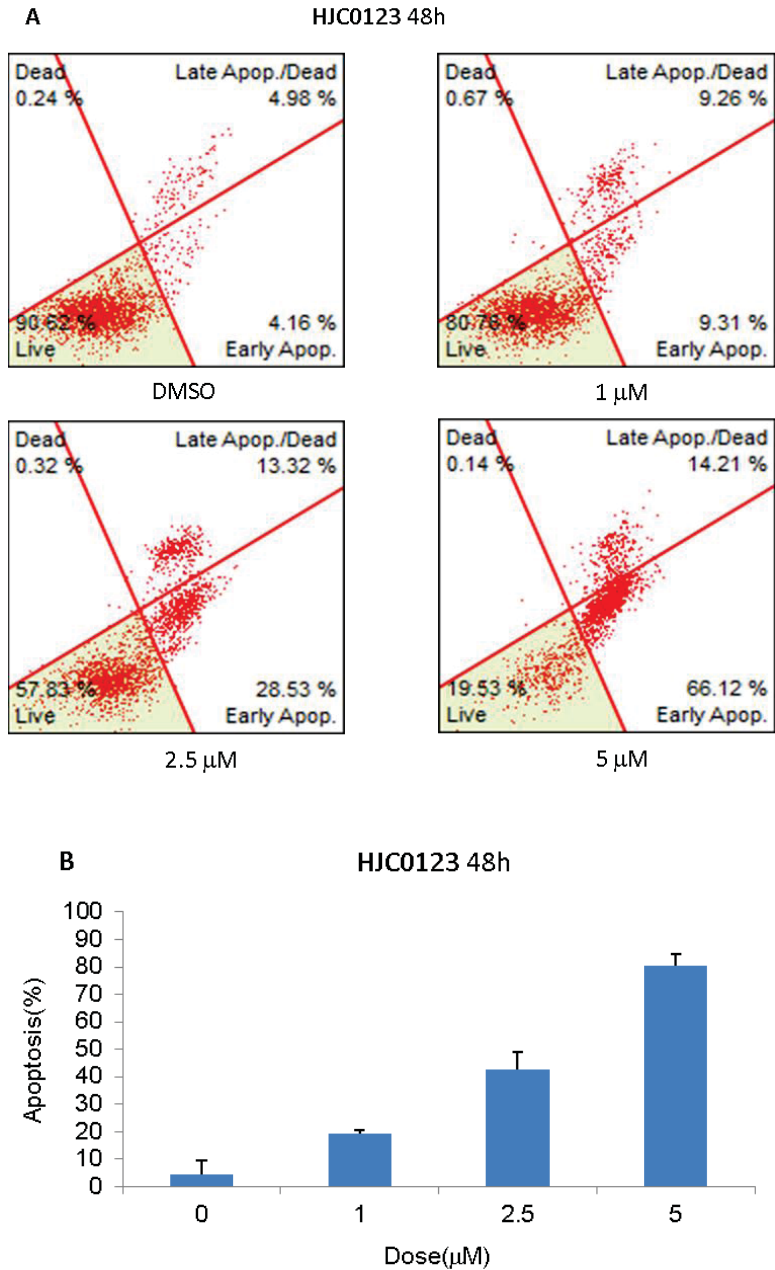


Figure S2. Induction of apoptosis on MDA-MB-231 cells by **HJC0123**. Cells were not treated or treated with 1 μ M, 2.5 μ M, and 5 μ M concentration of **HJC0123** for 48 h.

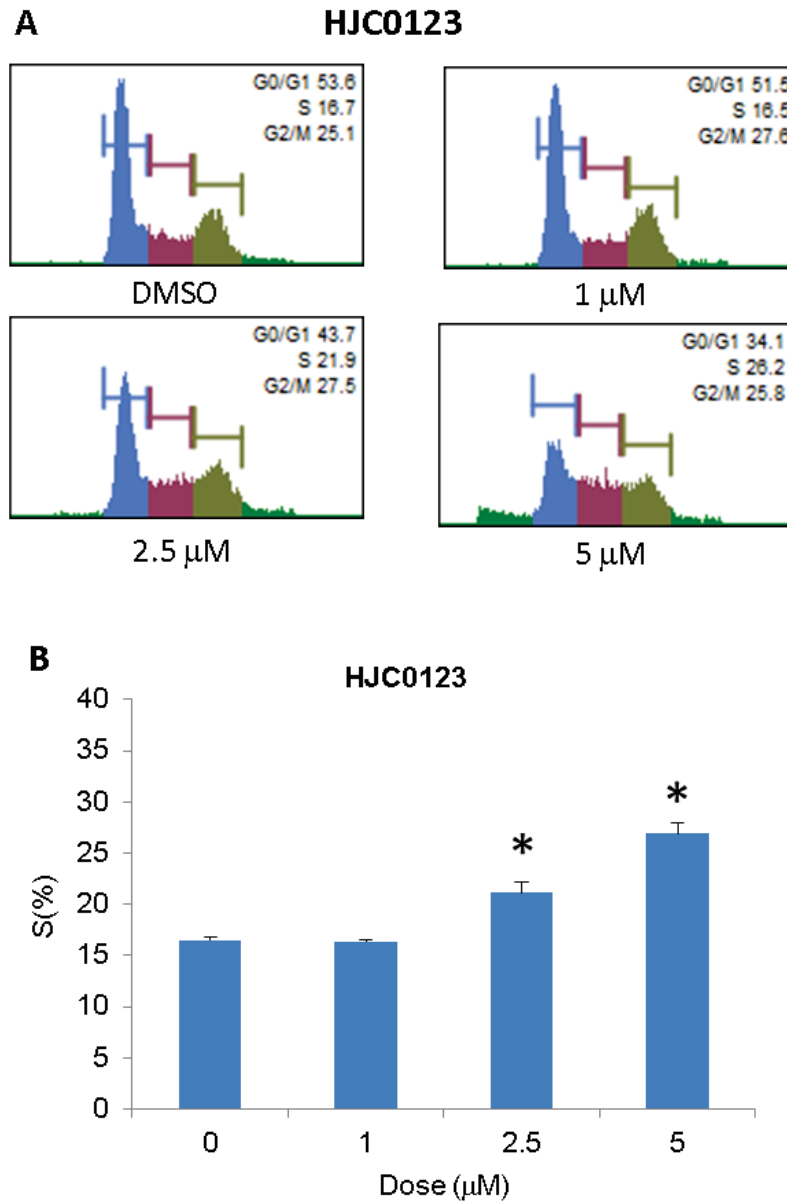


Figure S3. Changes of cell cycle distribution in MDA-MB-231 cells after treatment with **HJC0123**. (A) Cell cycle profiles of MDA-MB-231 cells after treated with **HJC0123** for 24 h. (B) The cell cycle distribution expressed in percentage. Cells were not treated or treated with 1 μM, 2.5 μM, and 5 μM concentration of **HJC0123**. Error bar represents standard deviations. * represents: $p \leq 0.05$.

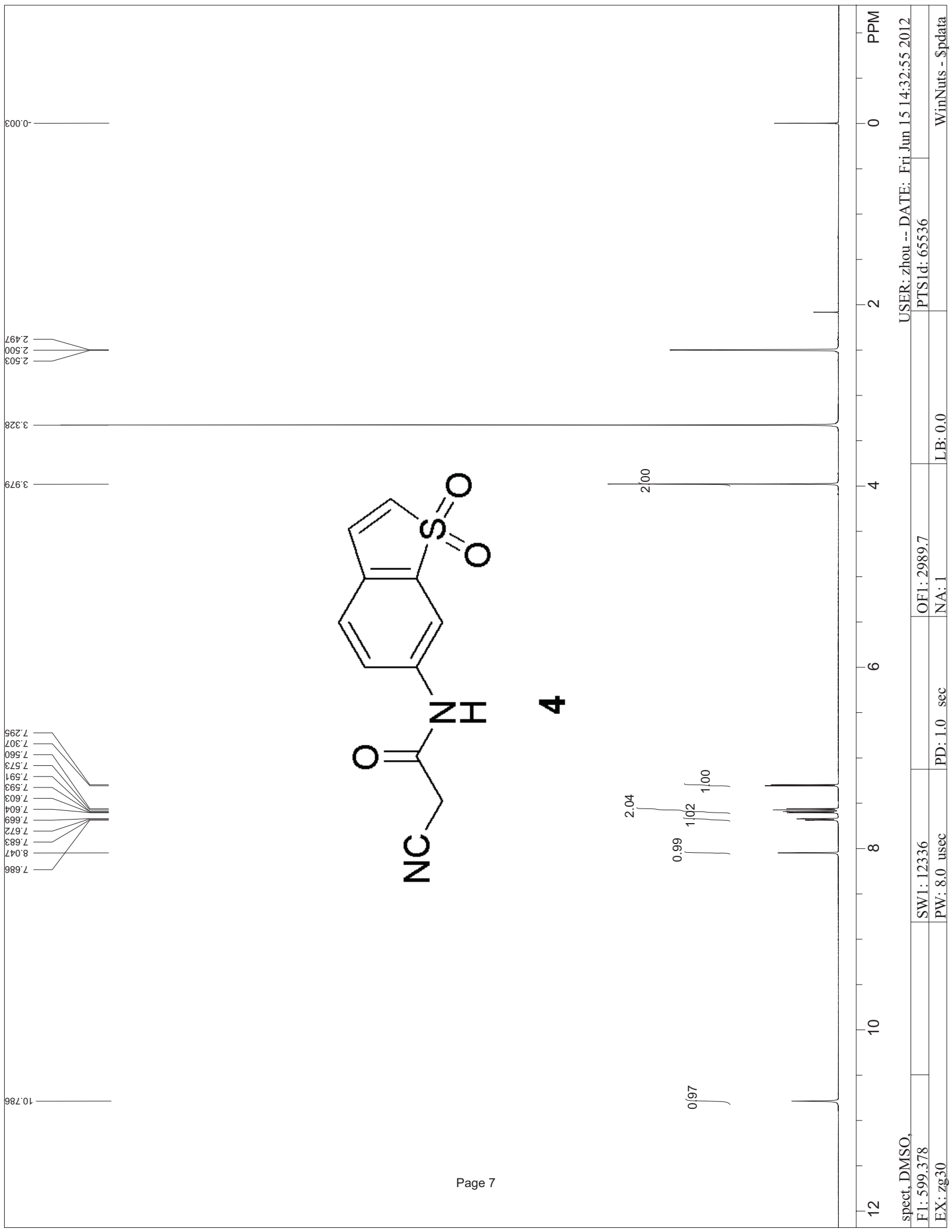
Morphological Changes. MDA-MB-231 cells were seeded in 6-well plates at a density of 2×10^5 cells/well and grown in RPMI 1640 (Cellgro, VA, USA) overnight at 37 °C in a humidified atmosphere of 95% air and 5% CO₂. The cells were then treated with vehicle and individual compounds. Growth media was removed 48 h after treatment and replaced with $1 \times$ PBS. Images were taken using Olympus BX41 microscope (Olympus, Melville, USA).

Cell Apoptosis Assay. Breast cancer MDA-MB-231 cells were incubated in 6-well plates (2.5×10^5 /well). Cells were then treated with DMSO, compound **5 (HJC0123)** at different concentrations for 48 h, and then both adherent and floating cells were collected, washed once with PBS. Resuspended cells were incubated with 100 μ L PBS containing 1% BSA and 100 μ L Annexin V and dead cell detection reagent at room temperature for 20 min. Apoptosis was measured immediately using the Muse Cell Analyzer with the Muse™ Apoptosis Kit (Catalog No. MCH100105).

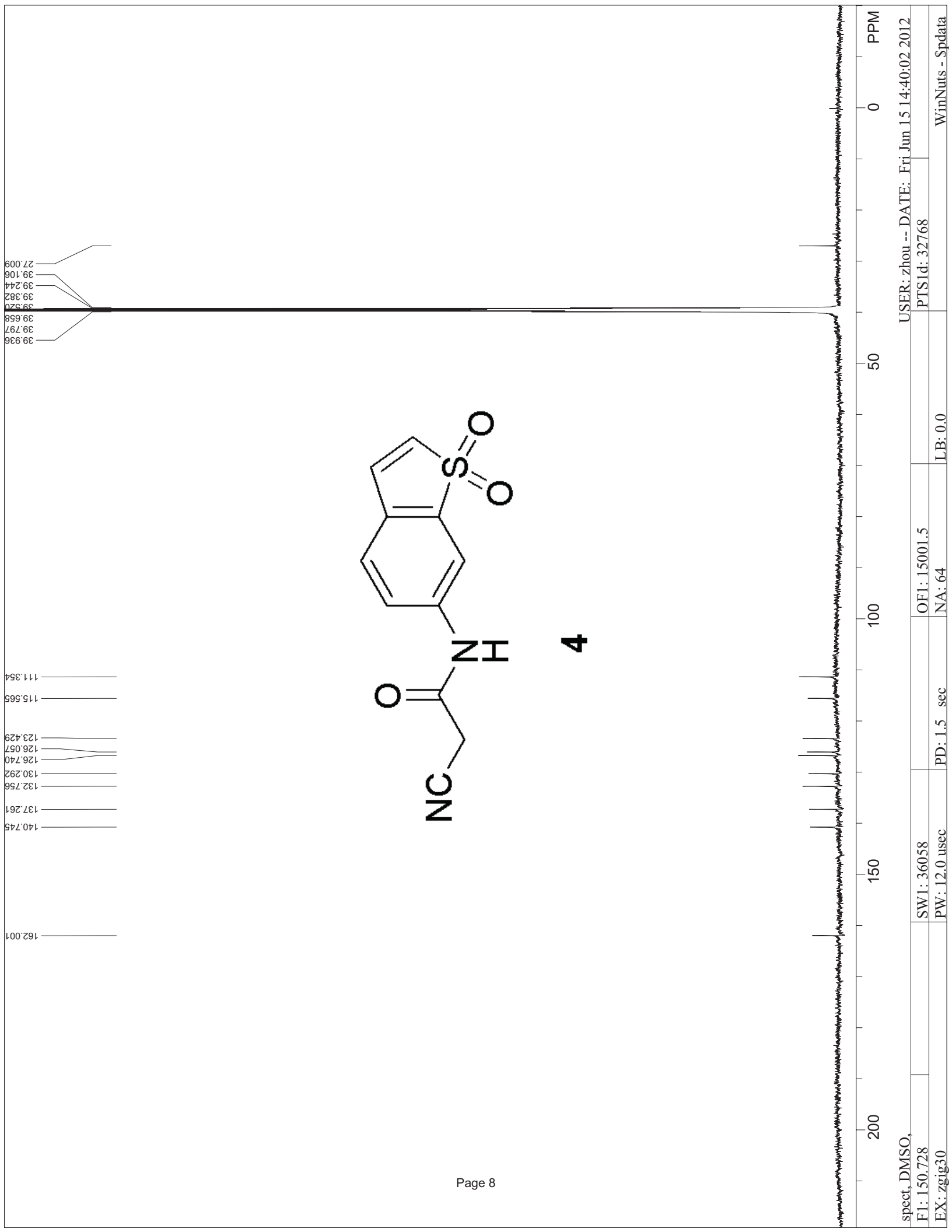
Cell Cycle Distribution Analysis. Breast cancer MDA-MB-231 cells were incubated in 6-well plates (2.5×10^5 /well). Cells were then treated with vehicle, compound **5 (HJC0123)** at different concentrations. After 24 h and 48 h of treatment, both adherent and floating cells were collected, washed once with PBS and fixed in cold ethanol for at least 4 h. The fixed cells were then washed twice with PBS and resuspended in 200 μ L cell cycle detection reagent, incubated for 30 min in dark at room temperature. Cell cycle distribution was measured by the Muse Cell Analyzer with Muse™ Cell Cycle Kit (Catalog No. MCH100106) purchased from EMD Millipore.

References:

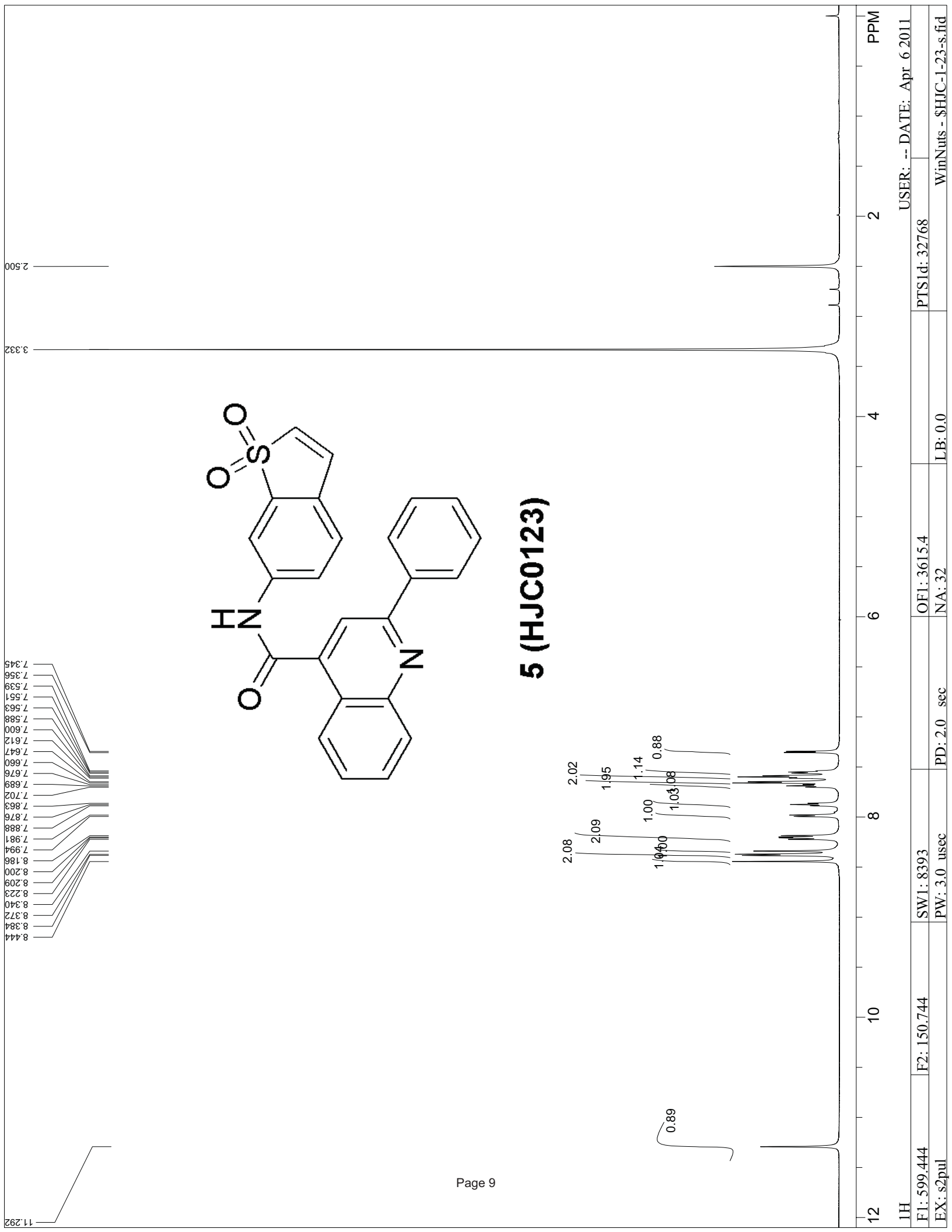
1. Tetko, I. V.; Gasteiger, J.; Todeschini, R.; Mauri, A.; Livingstone, D.; Ertl, P.; Palyulin, V. A.; Radchenko, E. V.; Zefirov, N. S.; Makarenko, A. S.; Tanchuk, V. Y.; Prokopenko, V. V. Virtual computational chemistry laboratory - design and description, *J. Comput. Aid. Mol. Des.* **2005**, *19*, 453-463.
2. Tetko, I. V. Computing chemistry on the web, *Drug Discov. Today* **2005**, *10*, 1497-500.



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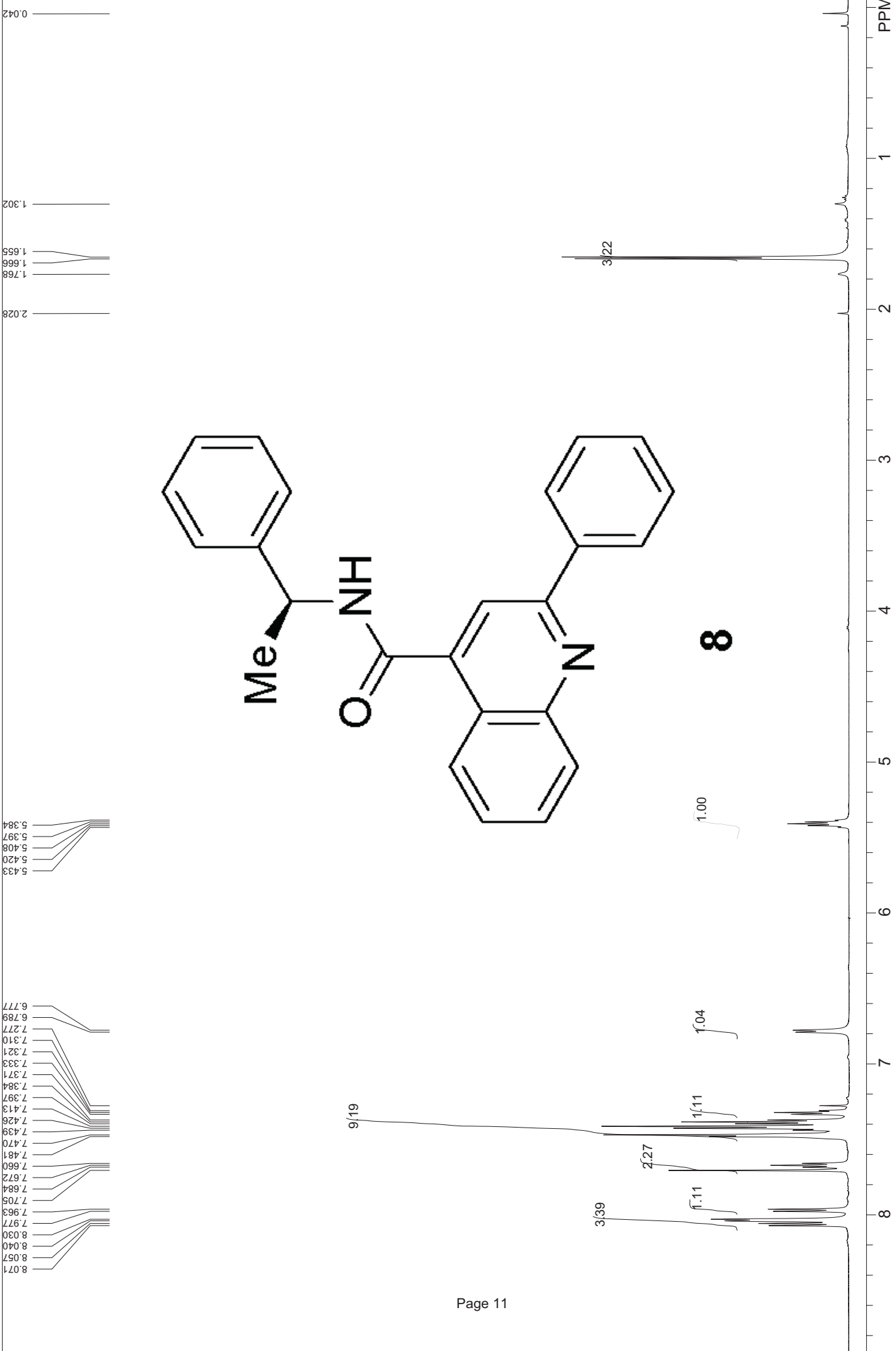
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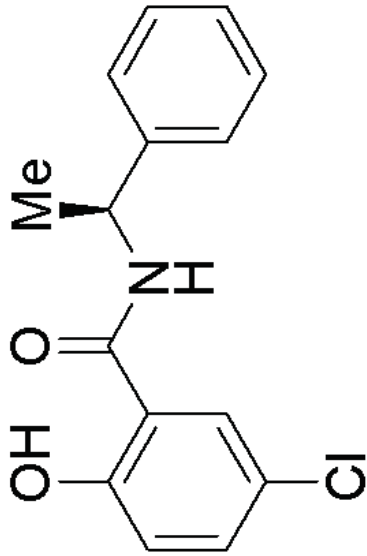
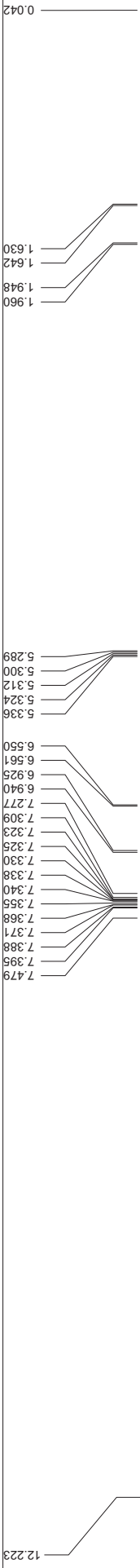
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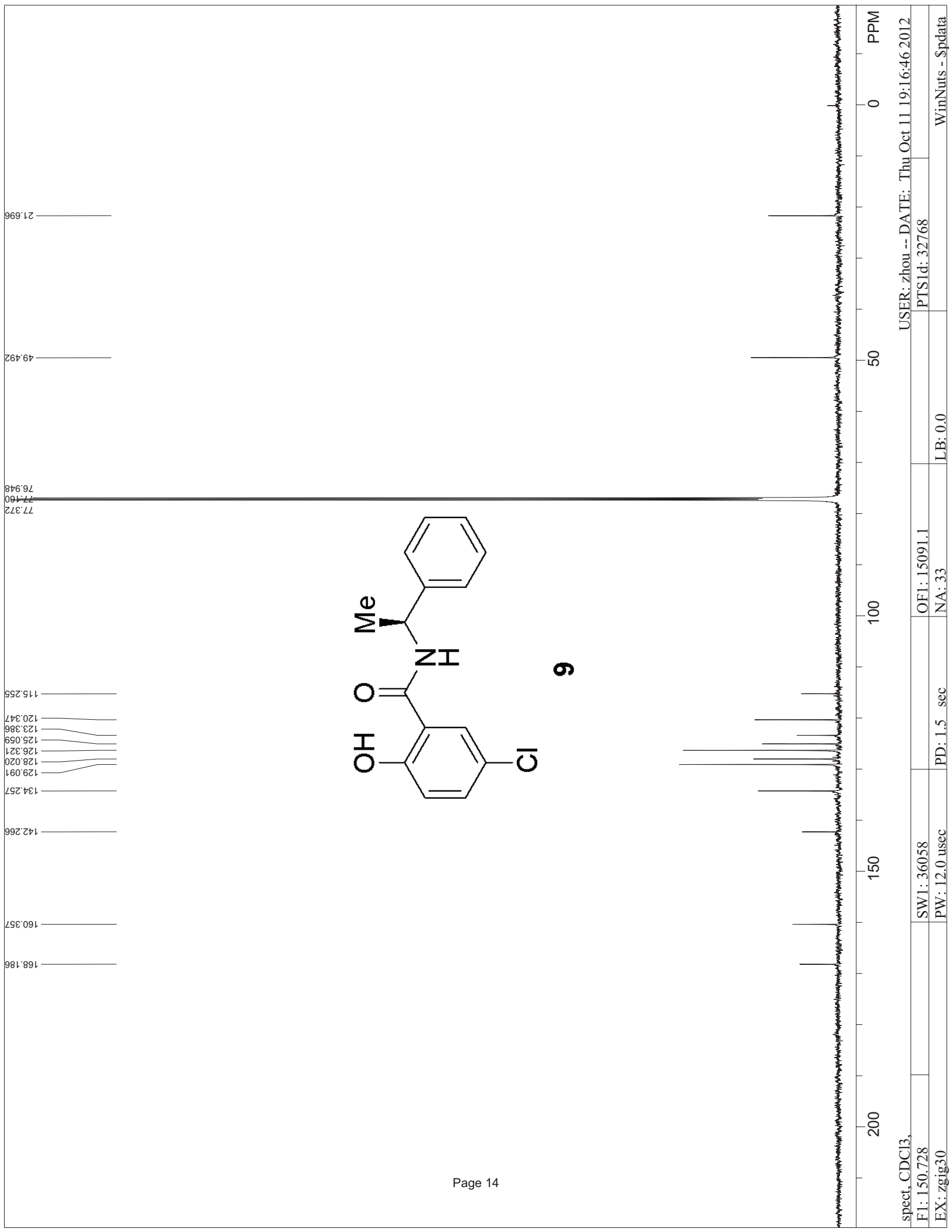


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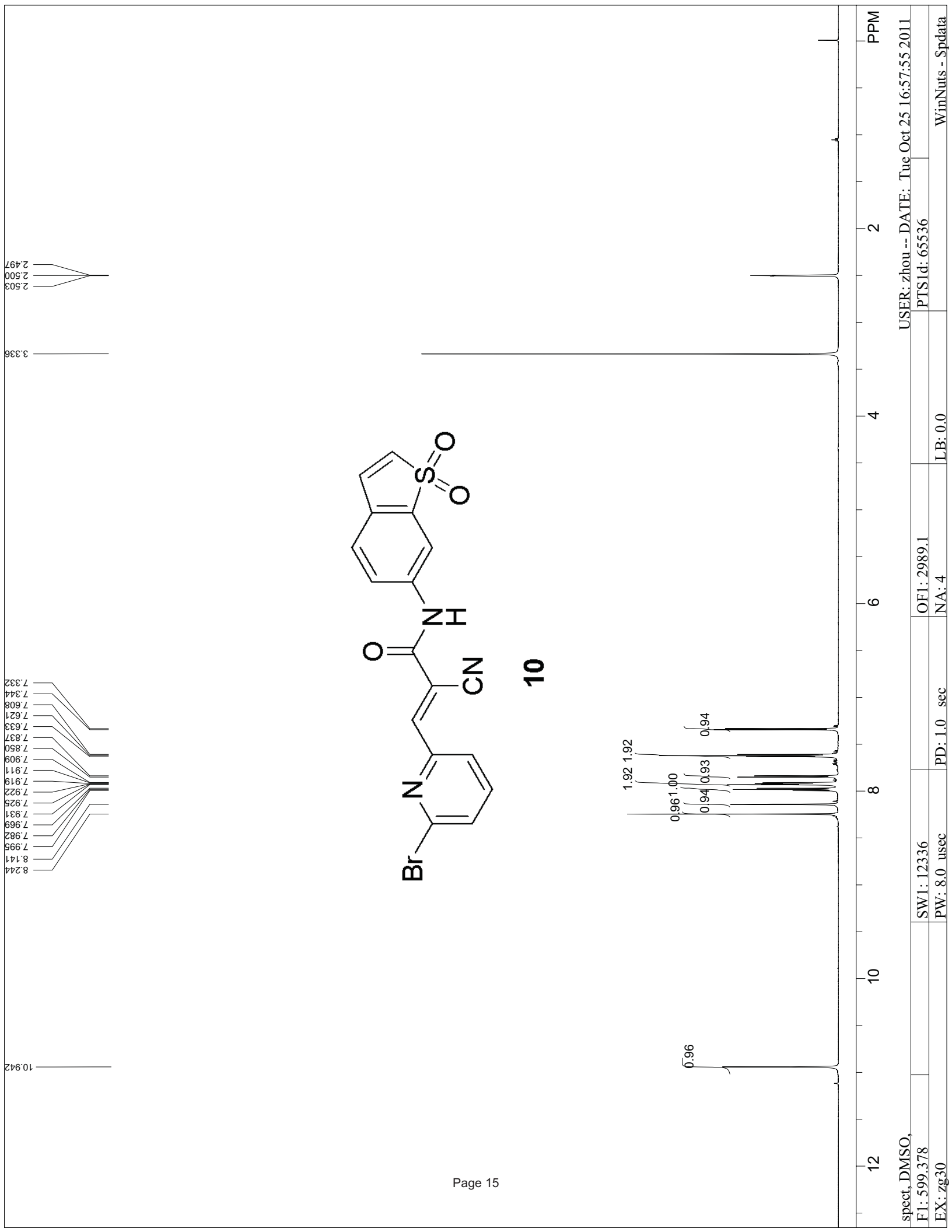


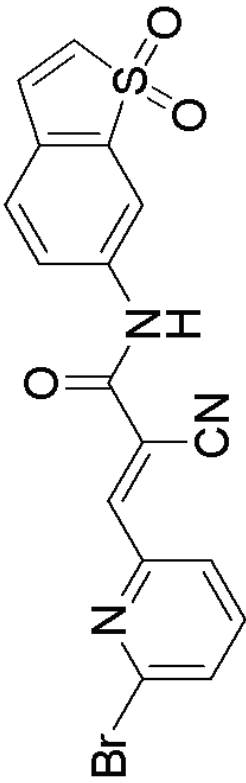
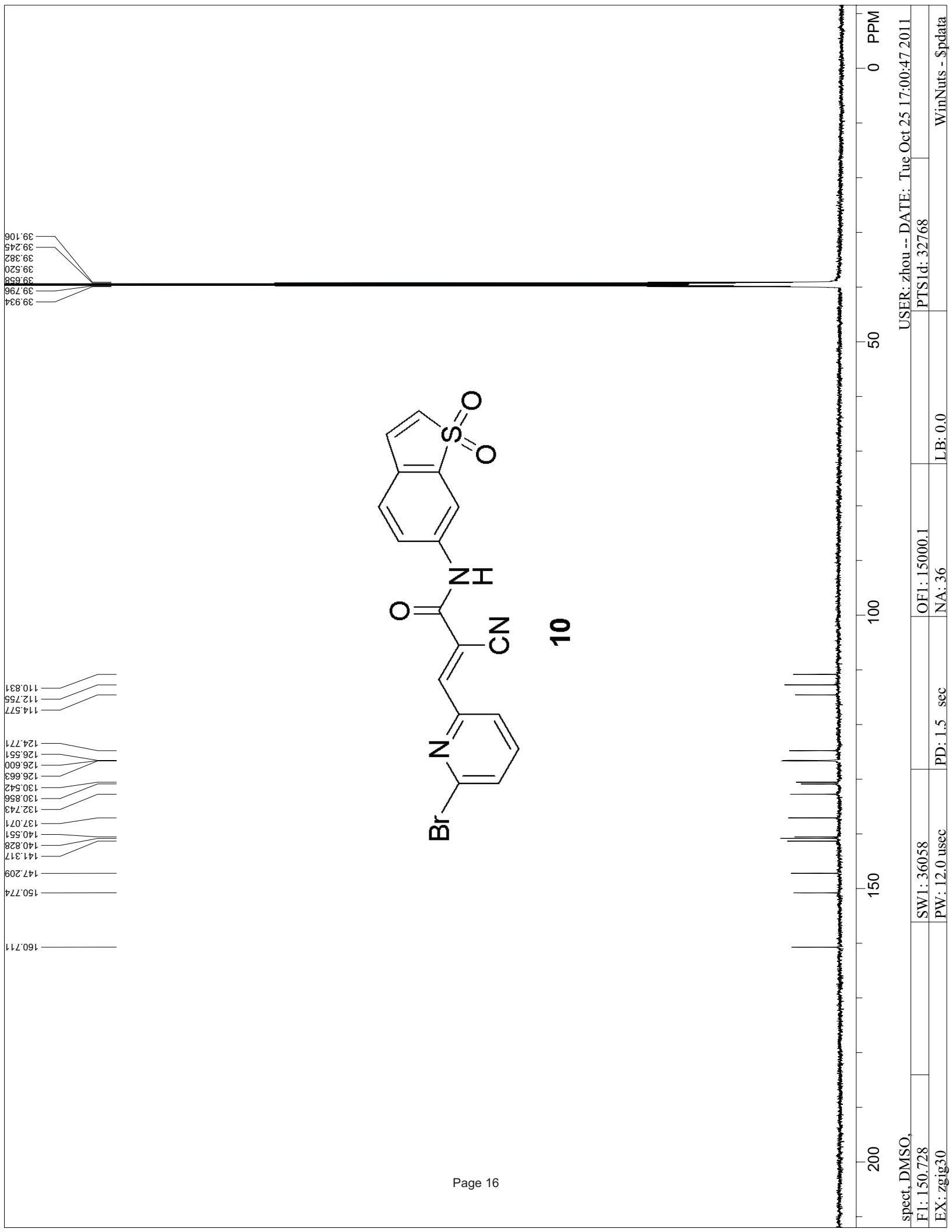
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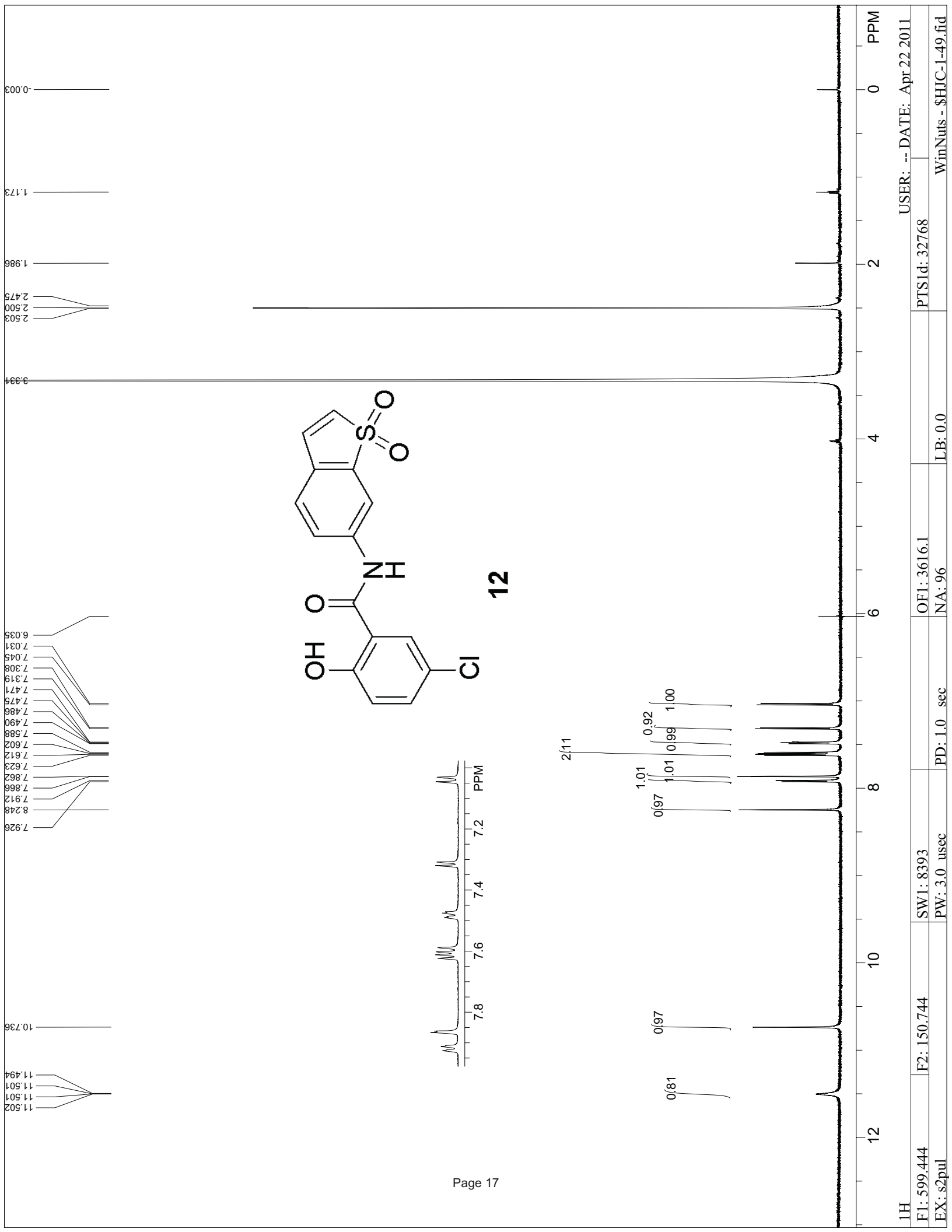




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1H

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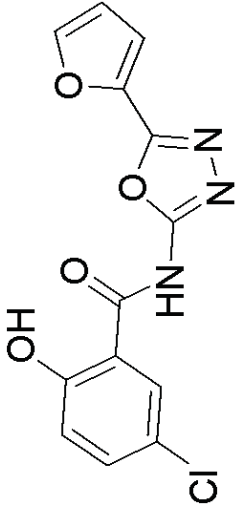
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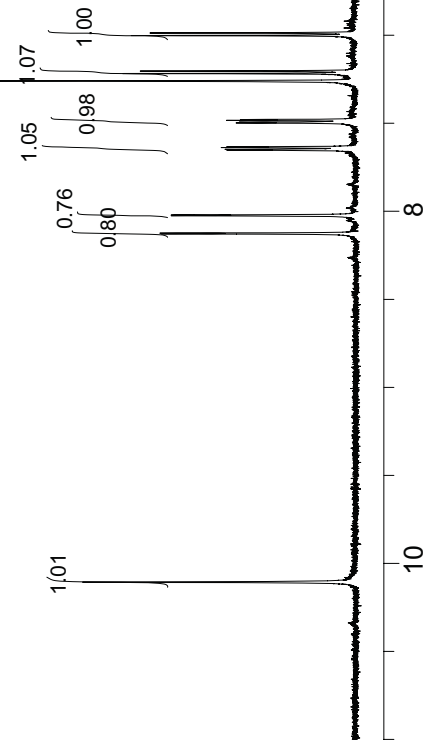
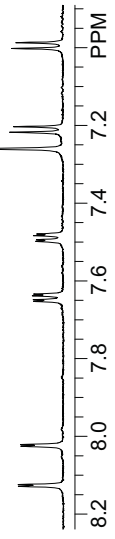
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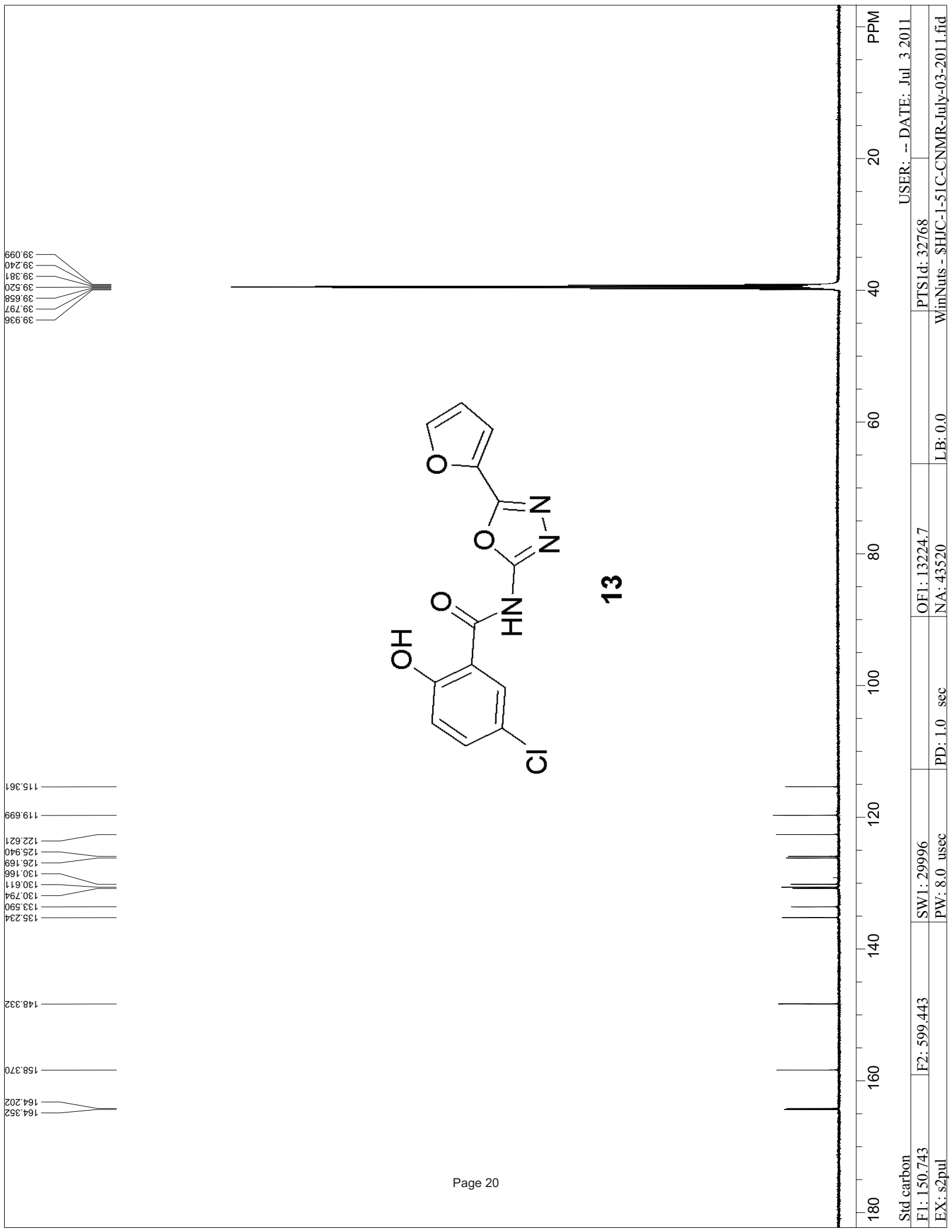
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13



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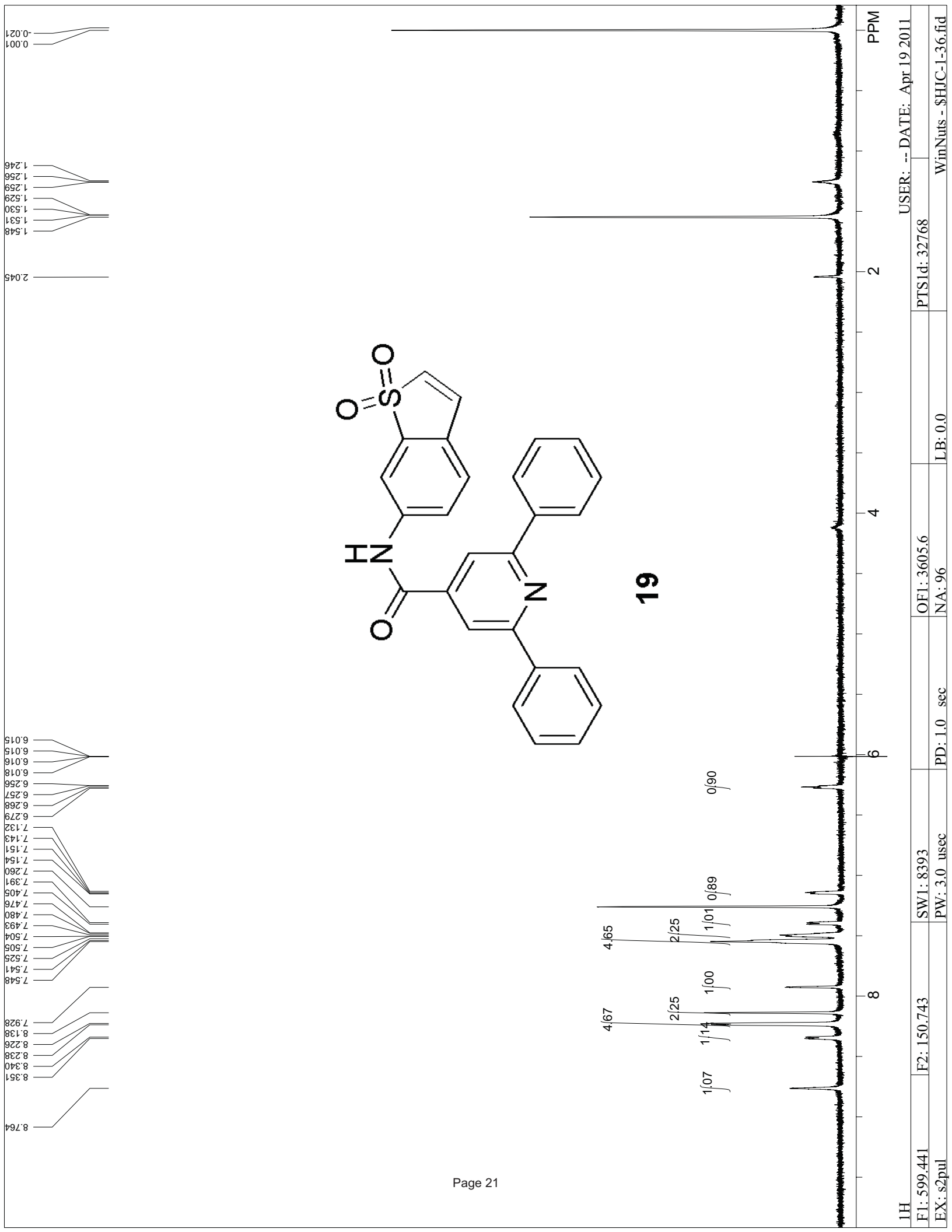
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EX: s2pul

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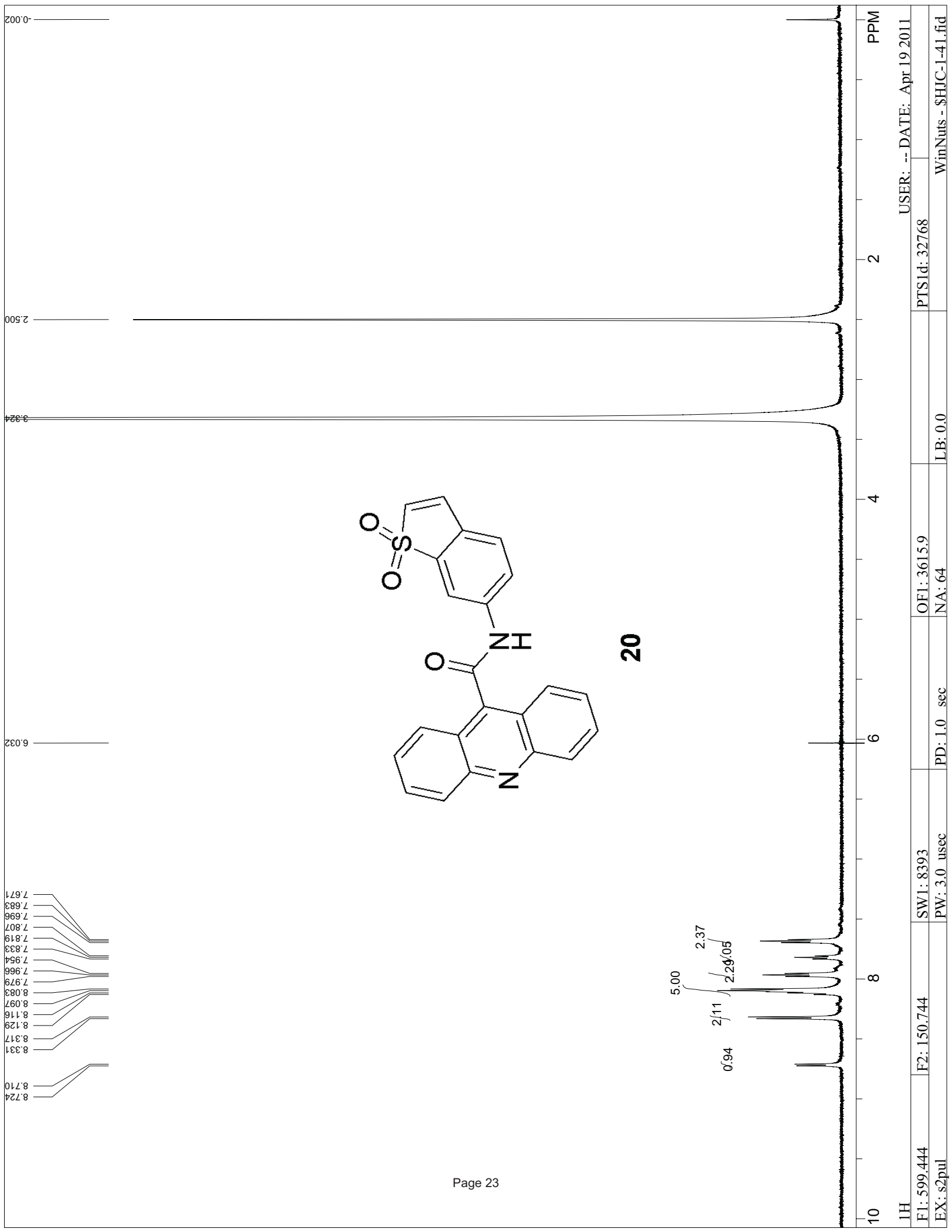
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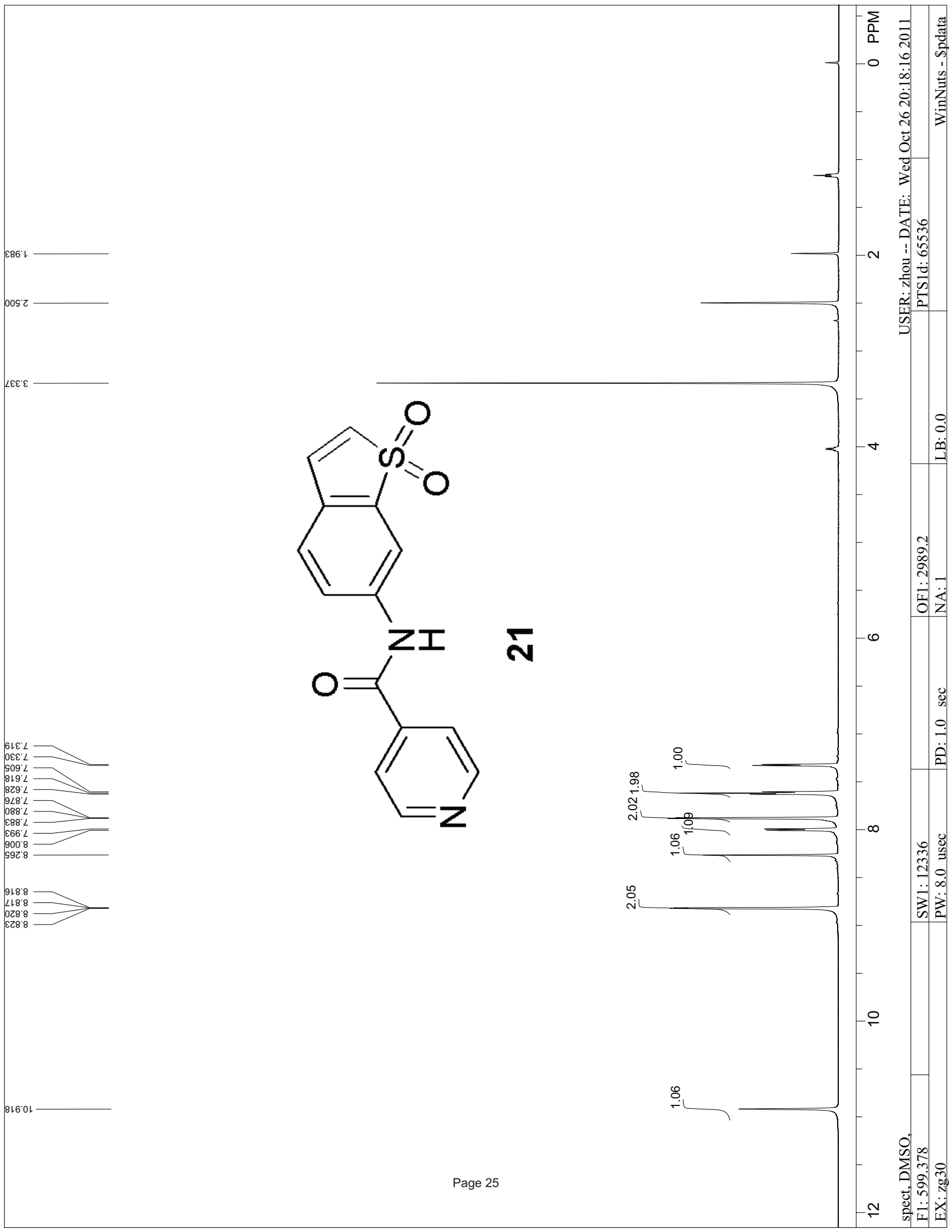
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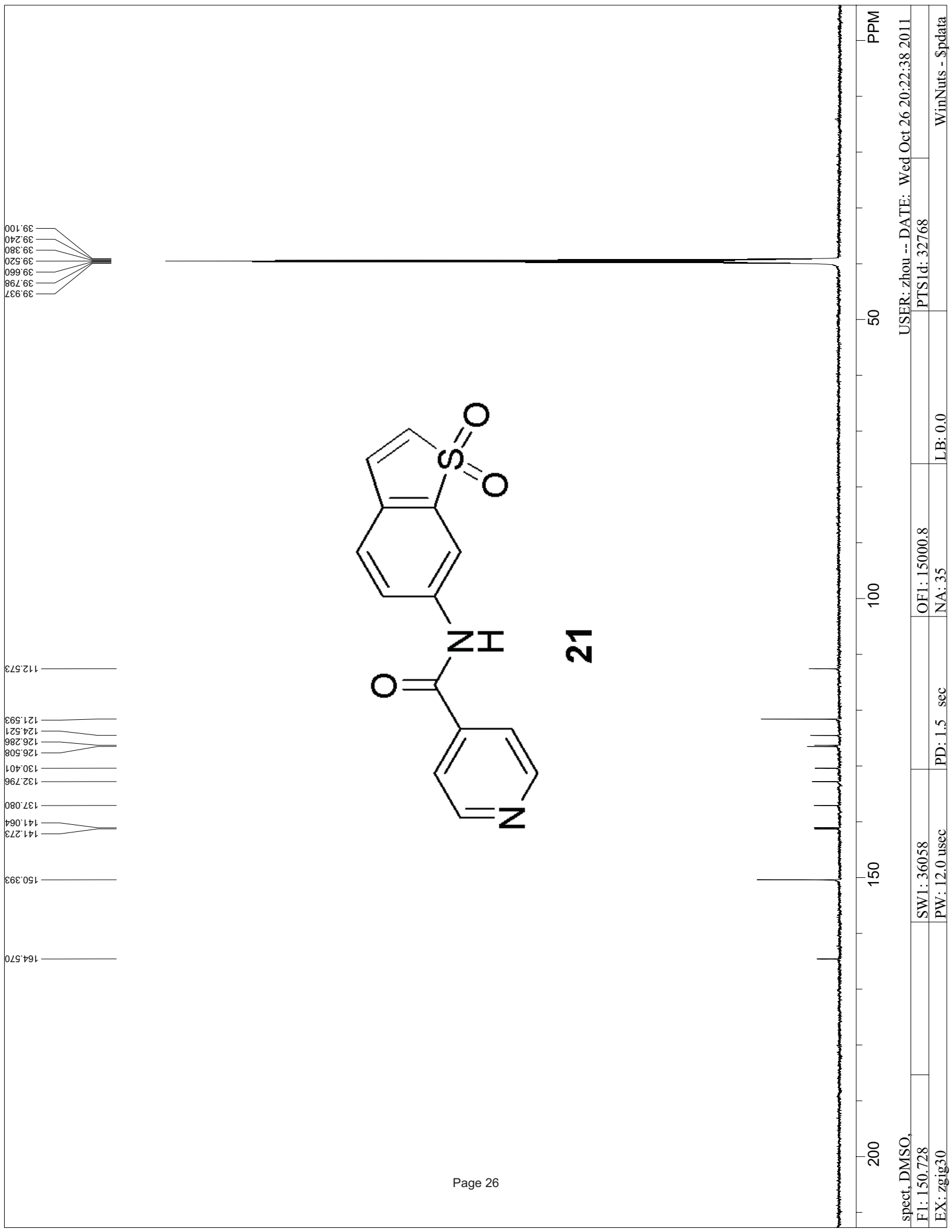


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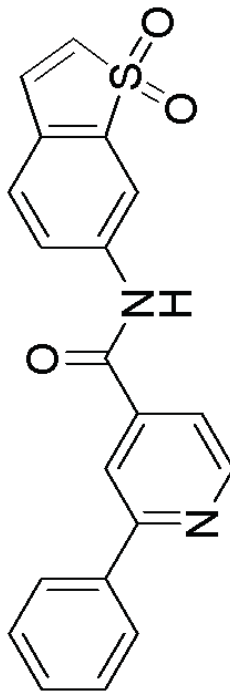
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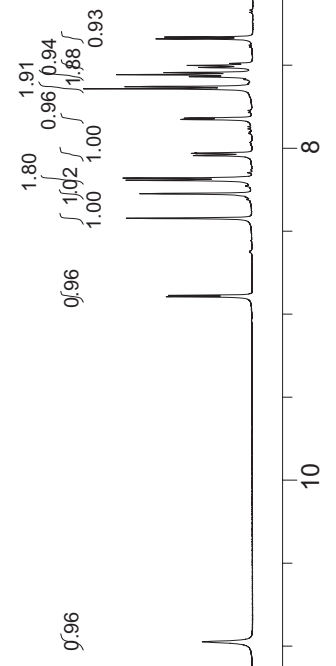
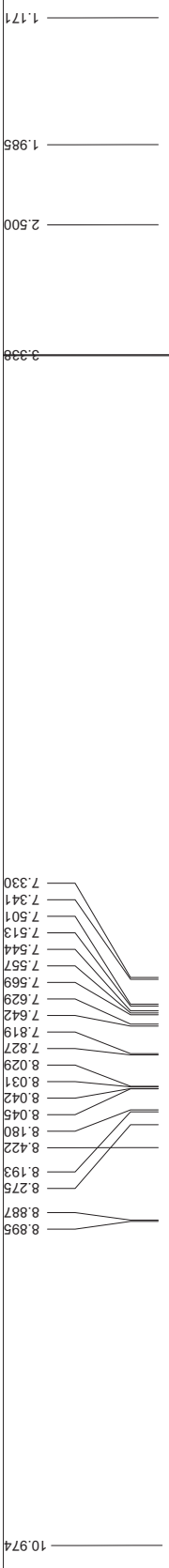




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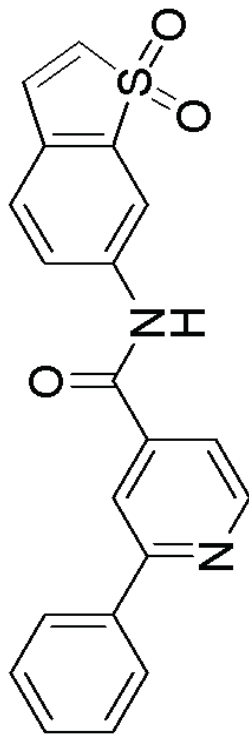


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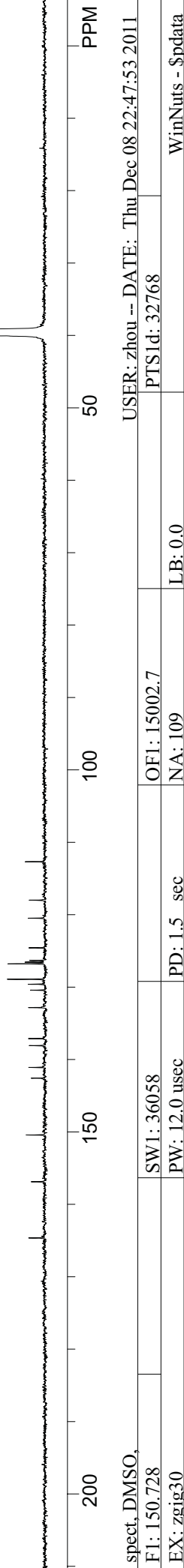


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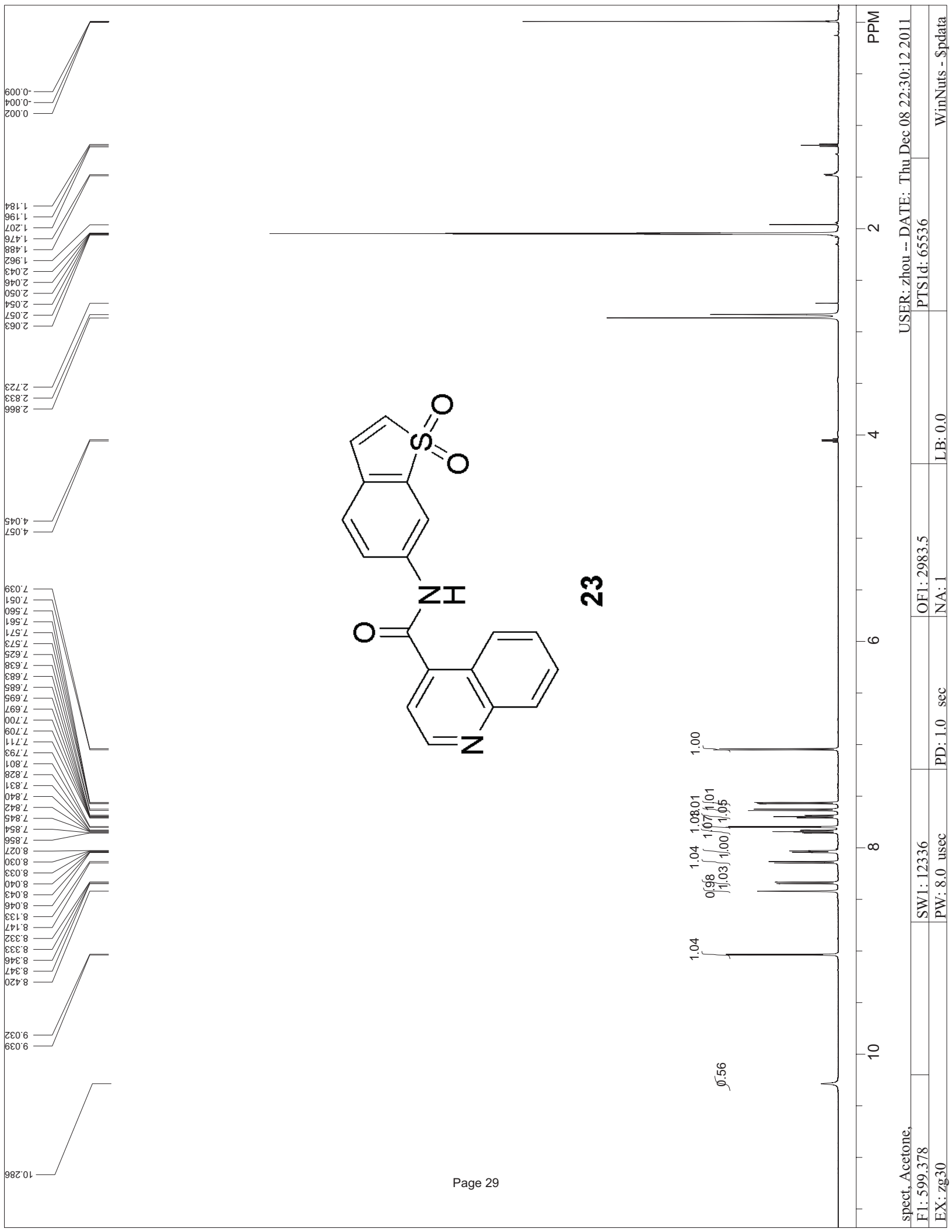


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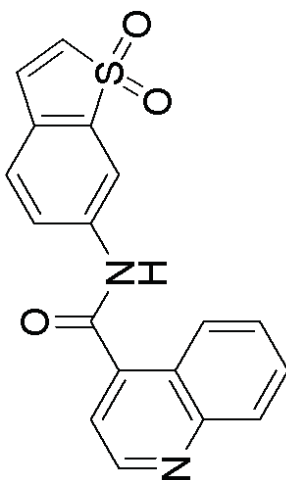
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