

Supporting Information

Hybrid Benzylidene Thiazolidine-2,4-diones as Potent Apoptosis-Inducing Anticancer Agents: Design-Driven Optimization, Cytotoxic Profiling, and Mechanistic Validation in Prostate Cancer

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Chemistry: Methods and Materials

All solvents and reagents were purchased from Sigma Aldrich and were used without any prior purifications. Melting points (MP) in °C were recorded on the Gallenkamp instrument. The IR spectra (KBr, ν cm⁻¹) were recorded at King Faisal University on a Mattson 5000 FTIR Spectrophotometer.

The structure characterization of the prepared materials is based on ¹H-NMR, ¹³C-NMR (Agilent Technologies 500 and 600 MHz VNMRS in DMSO-d₆ and pyridine-d₅ solution, with tetramethylsilane as internal standard. The high-resolution mass spectra were measured at Martin Luther University, Halle, Germany, on Thermo Scientific Q Exactive Plus mit HESI, APCI, sowie LIFDI-Quelle (Linden CMS).

Synthesis of 4-phenylthiazol-2-amine (3)¹

A mixture of phenacyl bromide (3 mmol) and thiourea (3.6 mmol) in EtOH (15 mL) was refluxed for 4 h. After cooling, the resulting precipitate was filtered, washed with water, and dried to give the compound 3 as a white solid in 99 % yield, mp 150-152 °C (Lit. 149-150 °C).

Synthesis of 2-chloro-N-(4-phenylthiazol-2-yl)acetamide (4)²

To a solution of 4-phenylthiazol-2-amine (3) (1 mmol) in dichloromethane (15 ml), triethylamine (50 μ l) was added while stirring at room temperature. The mixture was then cooled to 0-5 °C and then chloroacetyl chloride (1.5 mmol) was added dropwise, and the mixture was stirred for 1 h, and then for 3 h at room temperature. After completion, water (30 mL) was added, and the reaction was extracted 3 times with saturated brine. The organic layers were combined, dried over anhydrous sodium sulfate, evaporated to dryness to give a white solid, yield: 89%, m.p.141-142 °C (Lit. 143-144 °C).

Synthesis of Thiazolidine-2,4-dione³

A mixture of thiourea (100 mmol) and monochloroacetic acid (100 mmol) was dissolved in distilled water (250 mL) and then refluxed for 3 days. The mixture was then cooled, and the resulting white crystals were isolated and dried.

Synthesis of Potassium salt of 2,4-thiazolidinediones³

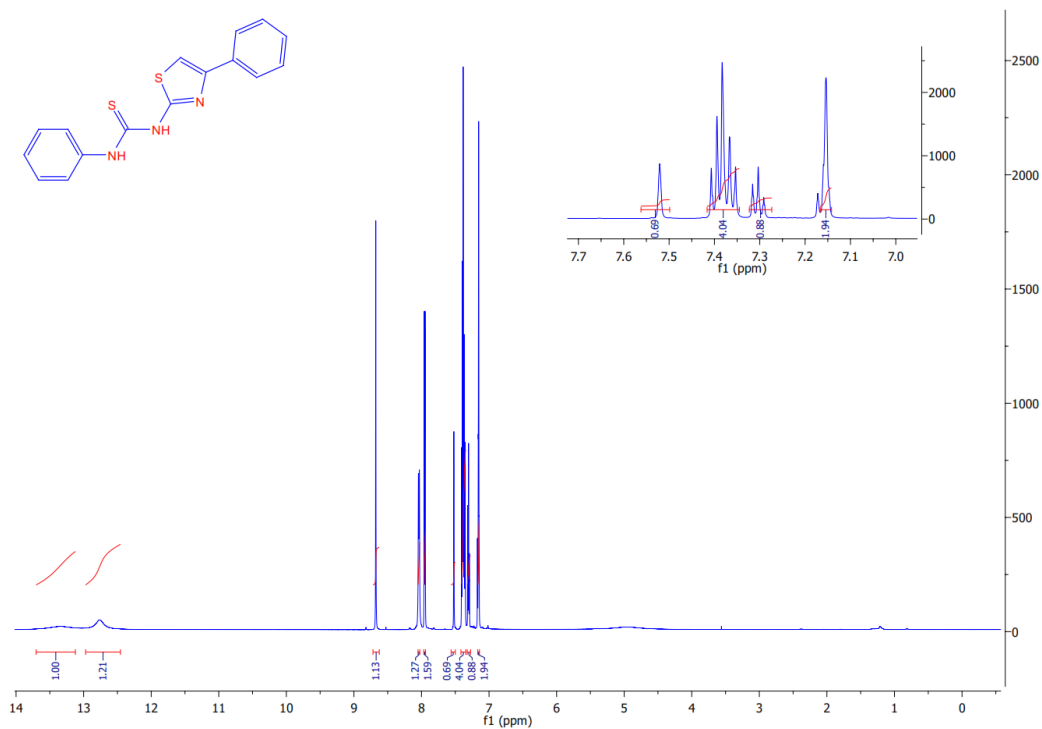
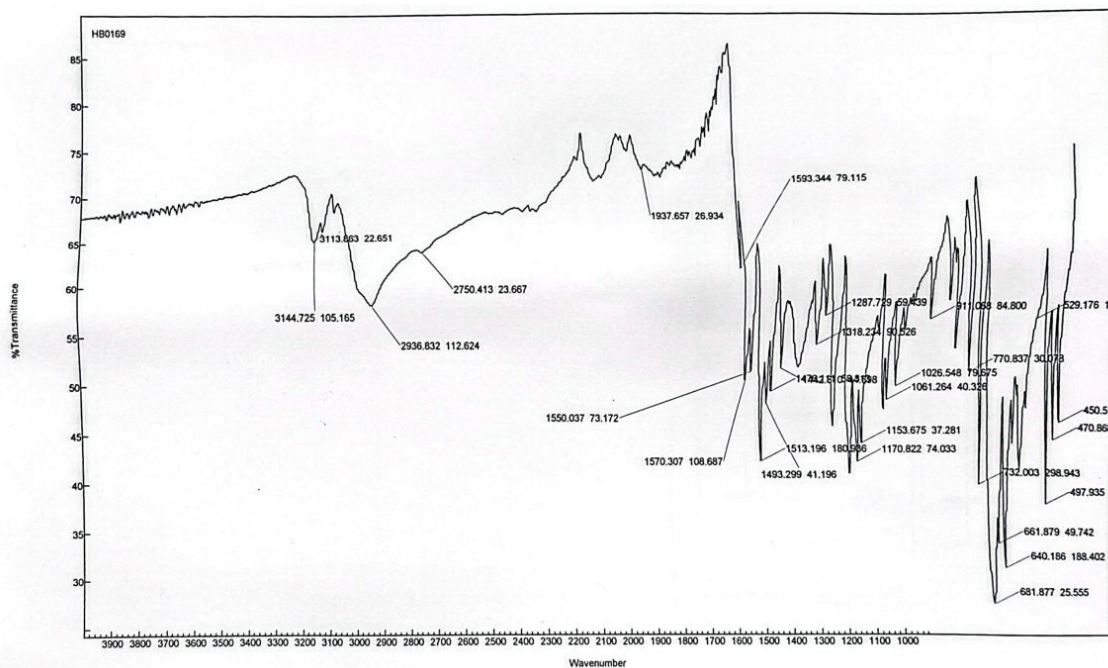
Thiazolidine-2,4-dione (8 g) was dissolved in 12.5 ml of ethanol. To this solution, KOH (4.2 g) in ethanol (10 mL) was added. The mixture was stirred for 2 h. The white crystalline solid

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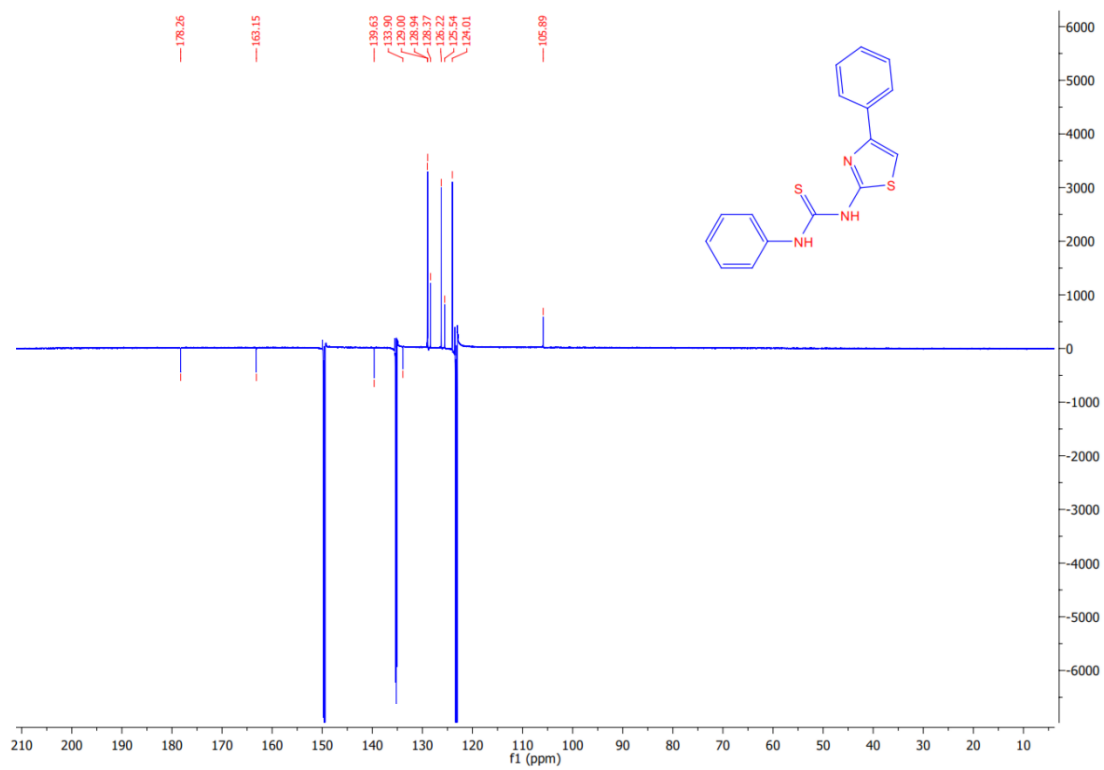
was collected after filtration, washed with ethanol, and dried under vacuum to get the potassium salt of thiazolidinedione.

Spectroscopic Data

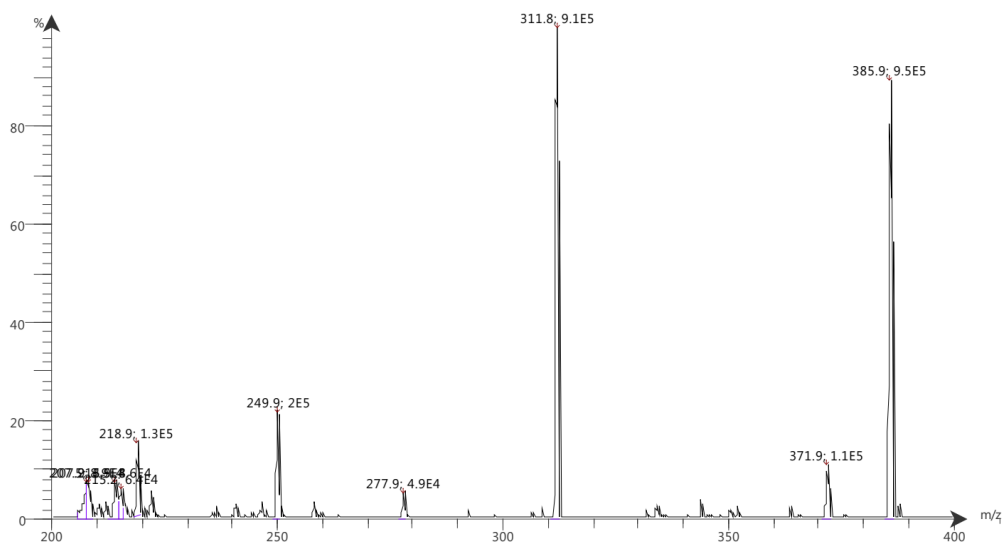
1-phenyl-3-(4-phenylthiazol-2-yl)thiourea (HB169)



Supporting Information

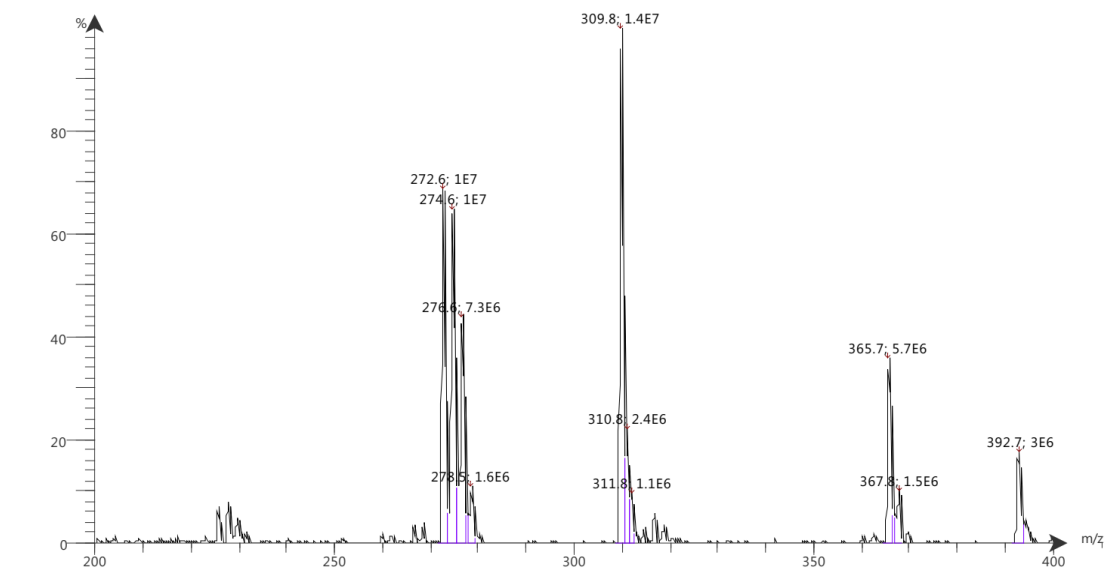


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ESI + Max: 1.2E6



Supporting Information

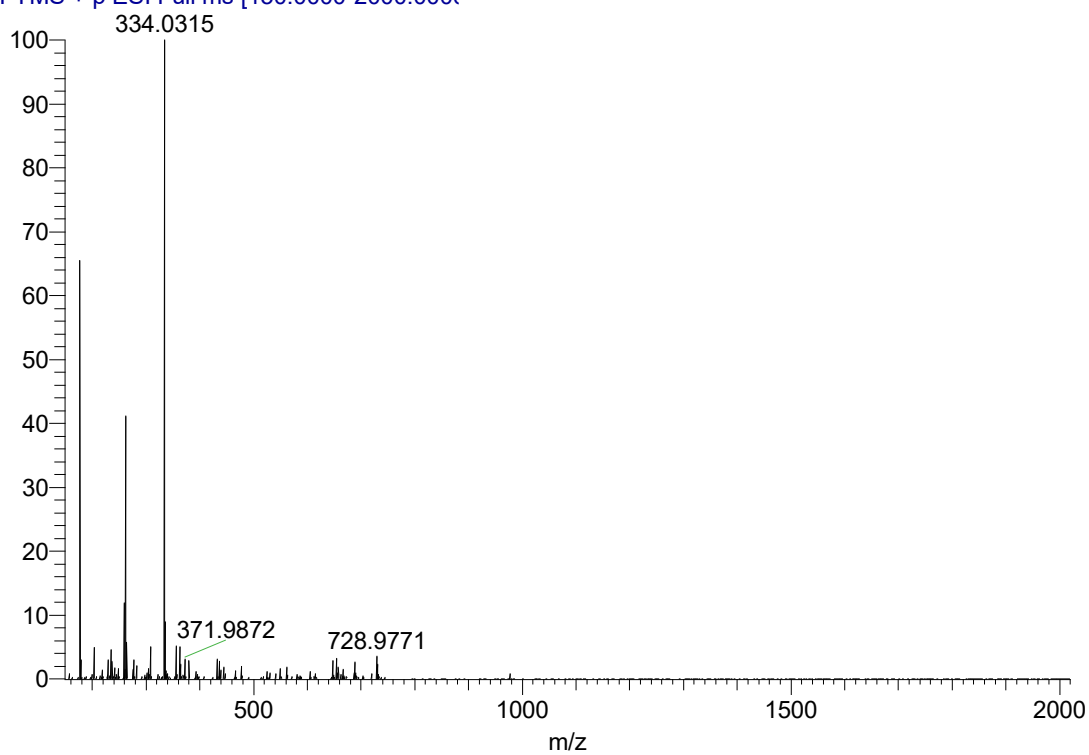
Spectrum RT 0:38 - 1:21 (83 scans) - Background Subtracted 0 - 0:38
Alaasar-HB169-2_Scan1_is1.datx 2025.01.15 09:43:53 ;
ESI - Max: 1.6E7



Supporting Information

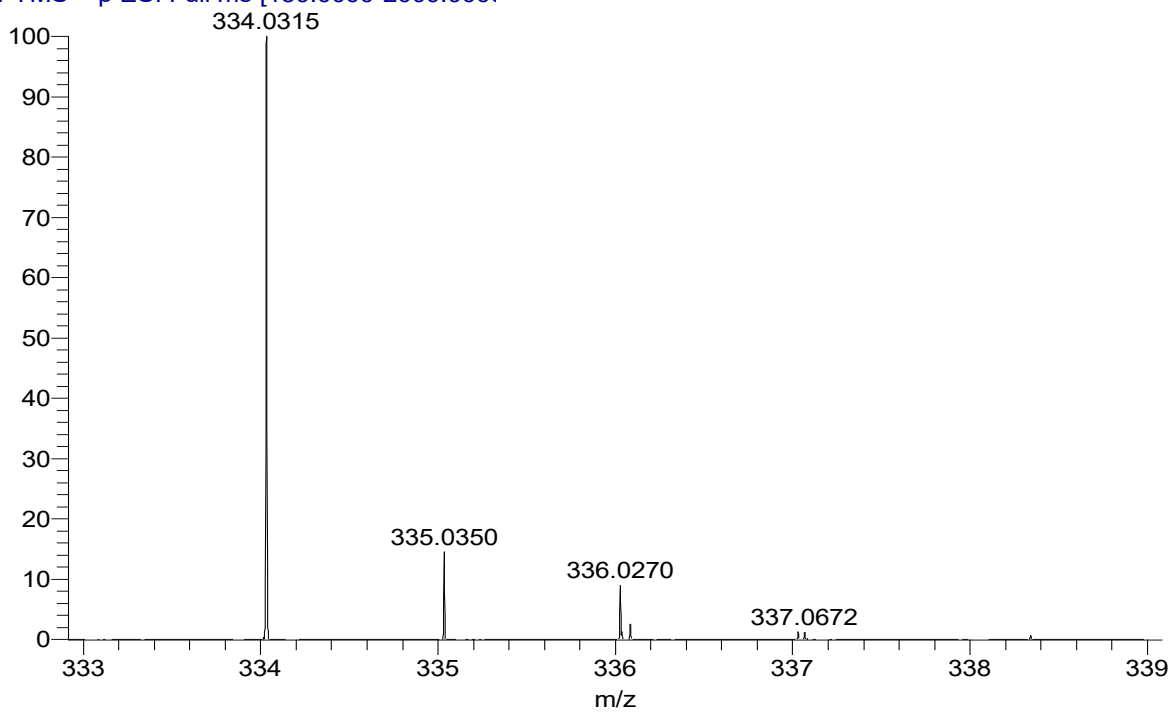
HB045 #1-100 RT: 0.00-0.45 AV: 100 NL: 2.97E8

T: FTMS + p ESI Full ms [150.0000-2000.0000]



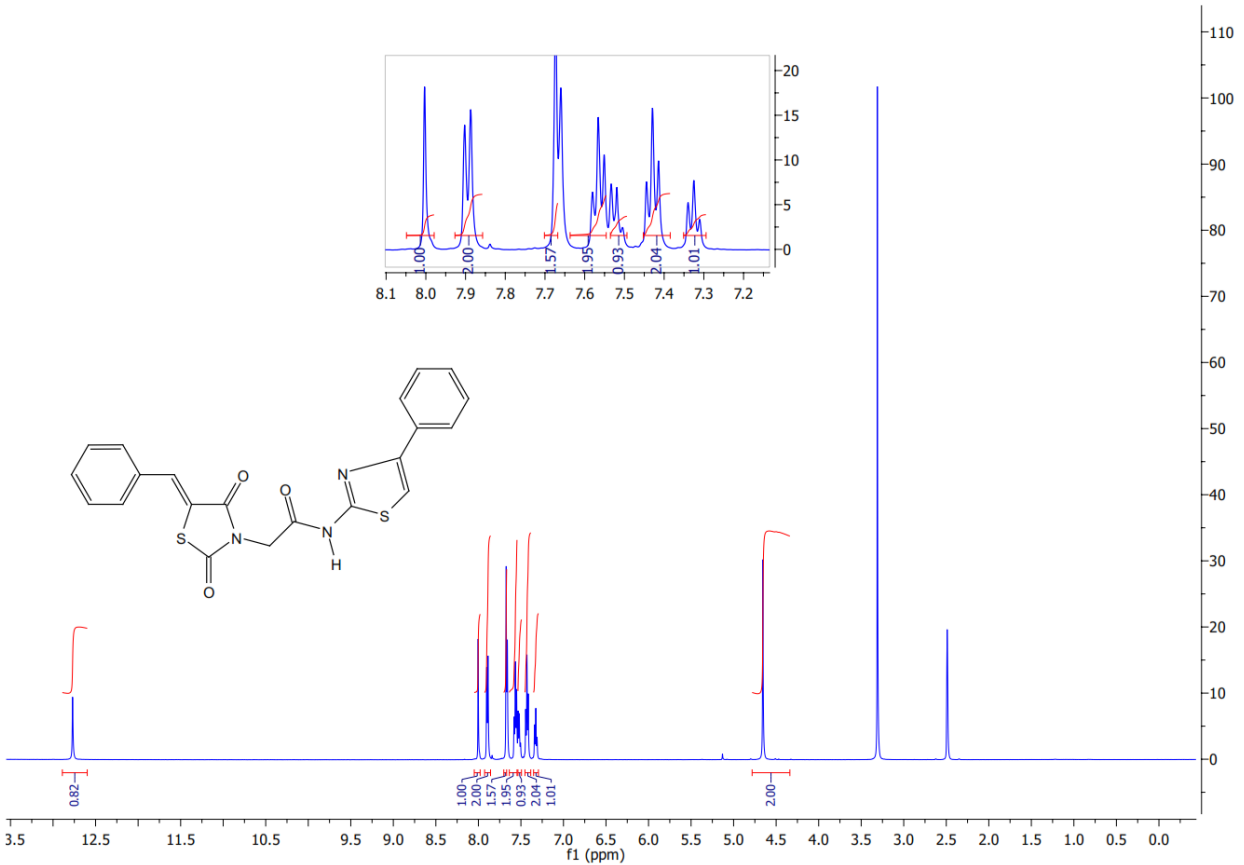
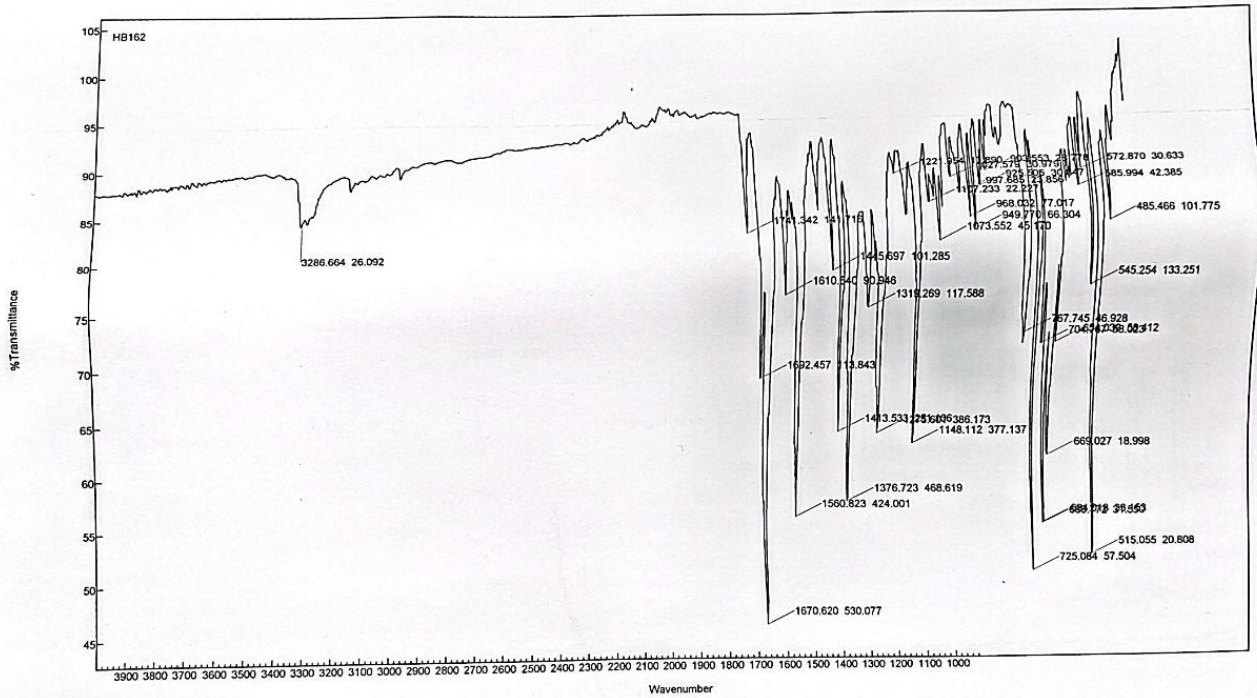
HB045 #1-100 RT: 0.00-0.45 AV: 100 NL: 2.97E8

T: FTMS + p ESI Full ms [150.0000-2000.0000]

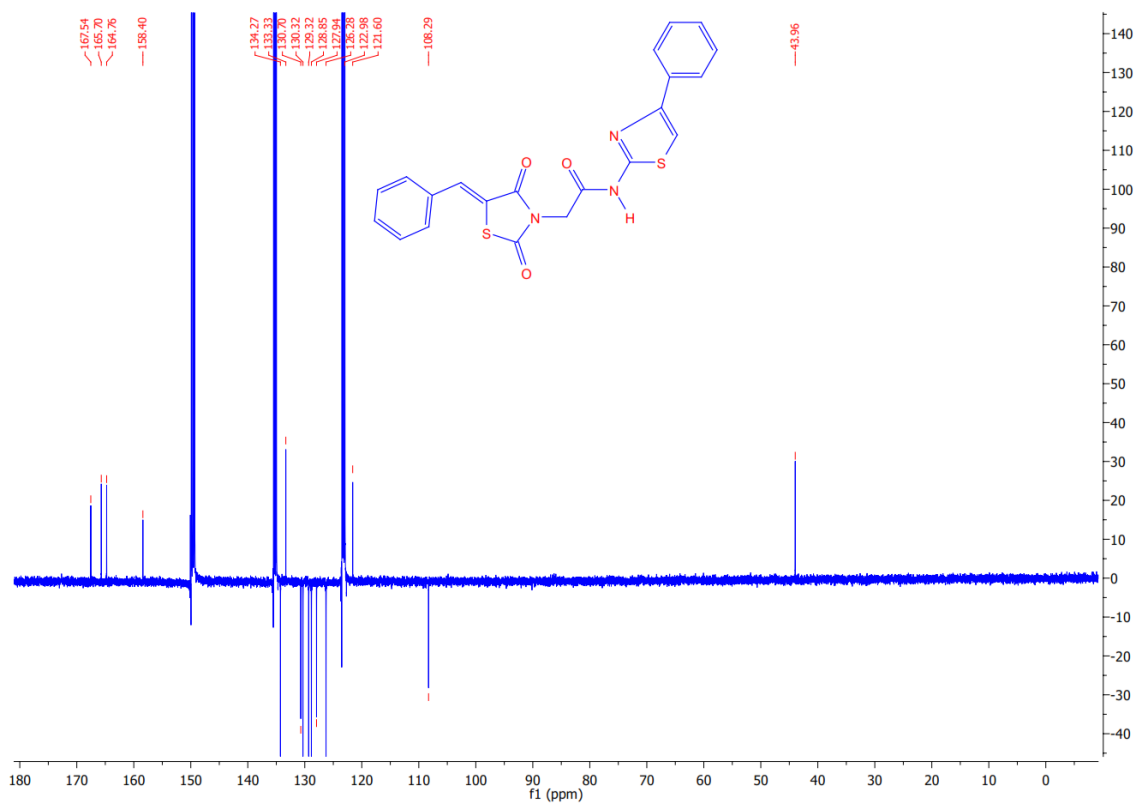


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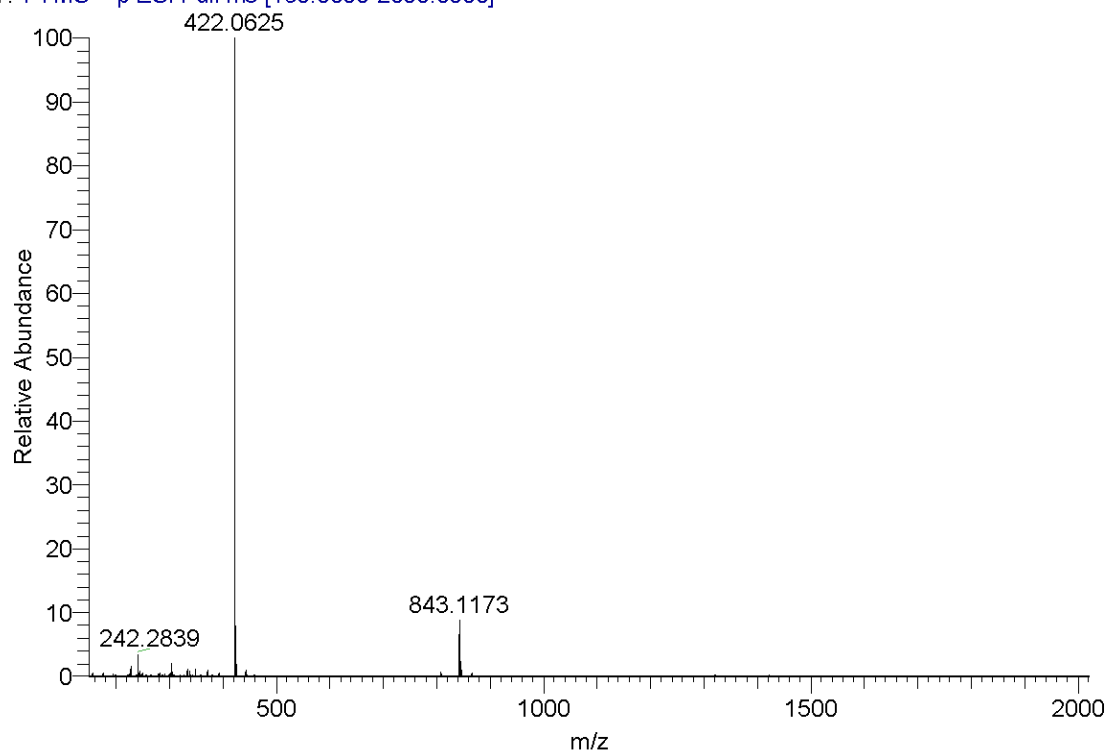
2-(5-benzylidene-2,4-dioxothiazolidin-3-yl)-N-(4-phenylthiazol-2-yl)acetamide (HB162)



Supporting Information

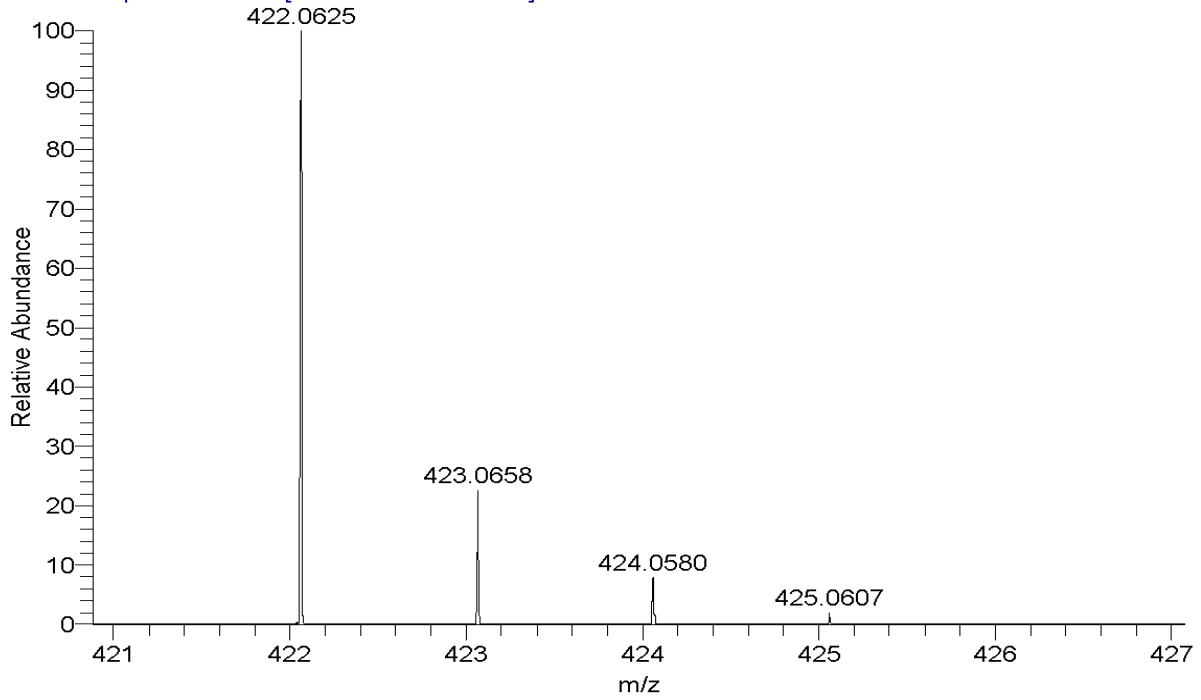


HB162 #1-100 RT: 0.00-0.45 AV: 100 NL: 1.79E7
T: FTMS + p ESI Full ms [150.0000-2000.0000]

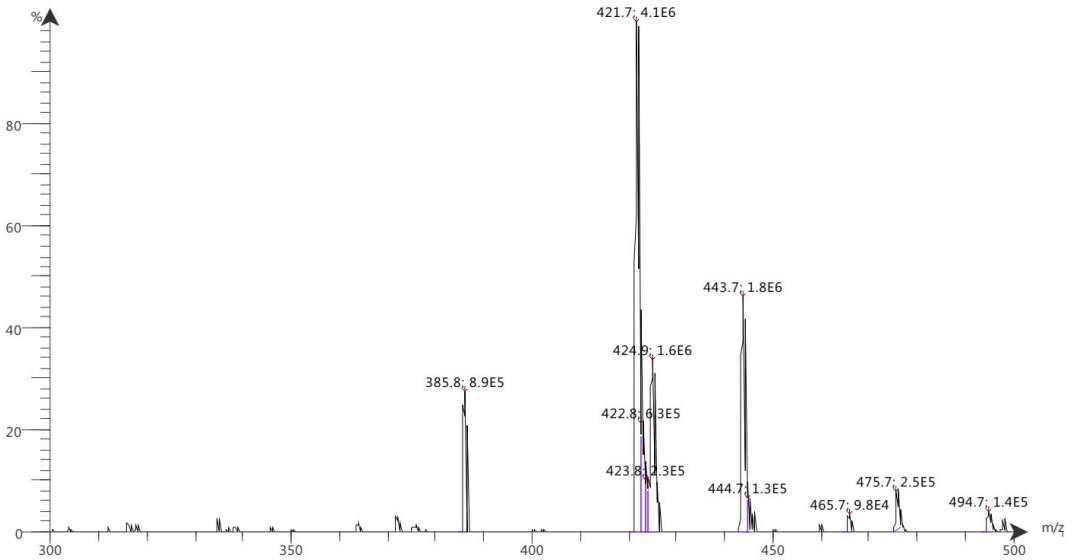


Supporting Information

HB162 #1-100 RT: 0.00-0.45 AV: 100 NL: 1.79E7
T: FTMS + p ESI Full ms [150.0000-2000.0000]

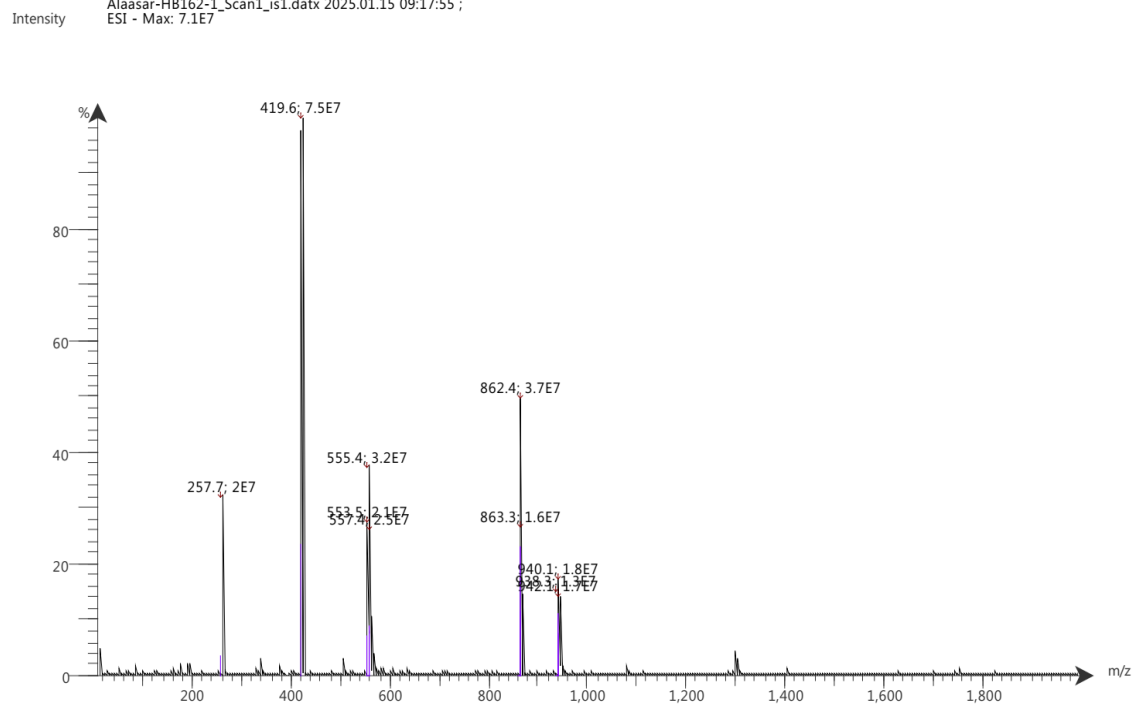


Spectrum RT 0:37 - 1:13 (69 scans) - Background Subtracted 0 - 0:37
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ESI + Max: 4.1E6

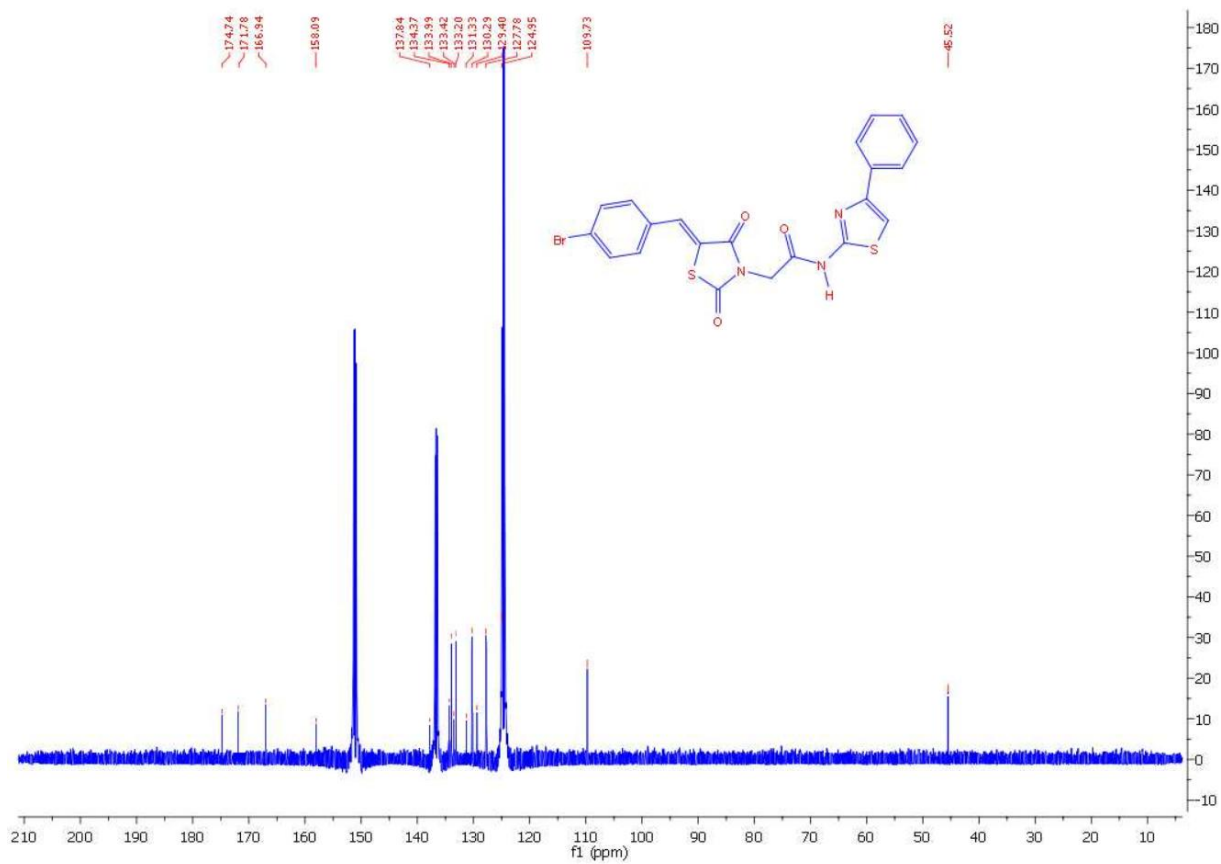


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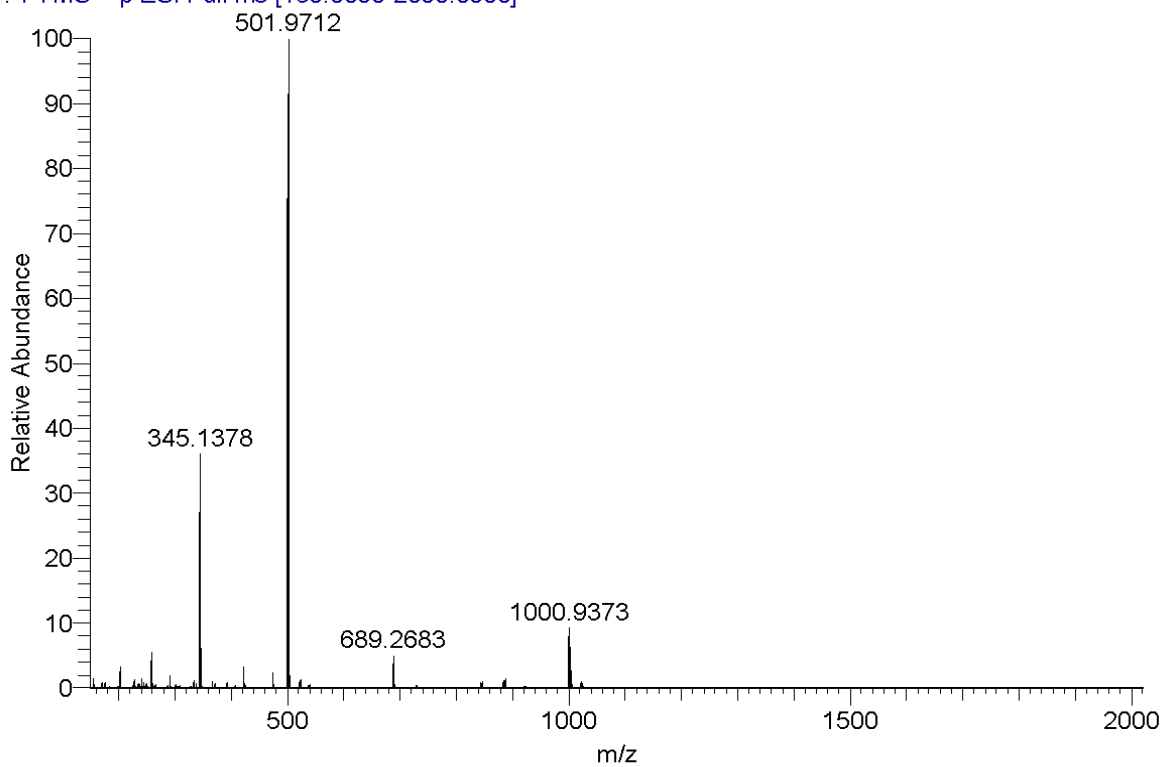
Spectrum RT 0:33 - 1:21 (11 scans) - Background Subtracted 0 - 0:29
Alaasar-HB162-1_Scan1_is1.datx 2025.01.15 09:17:55 ;
ESI - Max: 7.1E7



Supporting Information

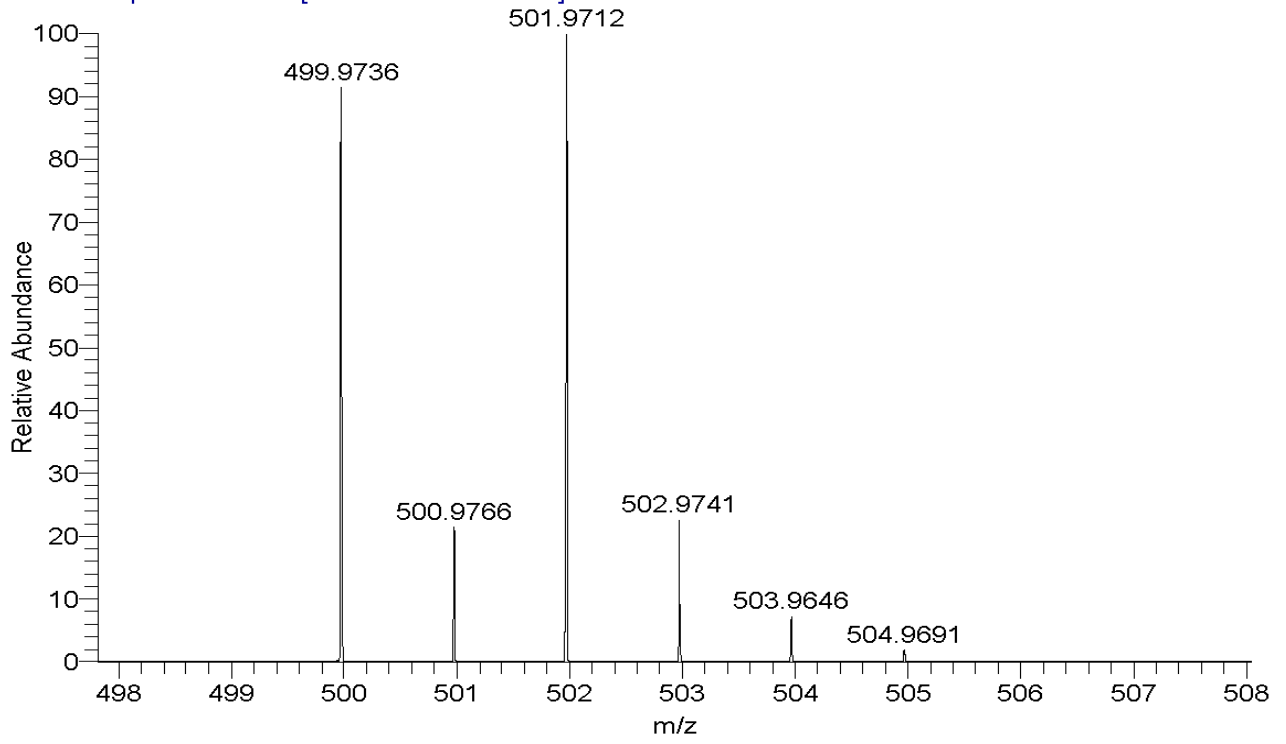


HB161 #1-100 RT: 0.00-0.45 AV: 100 NL: 1.08E8
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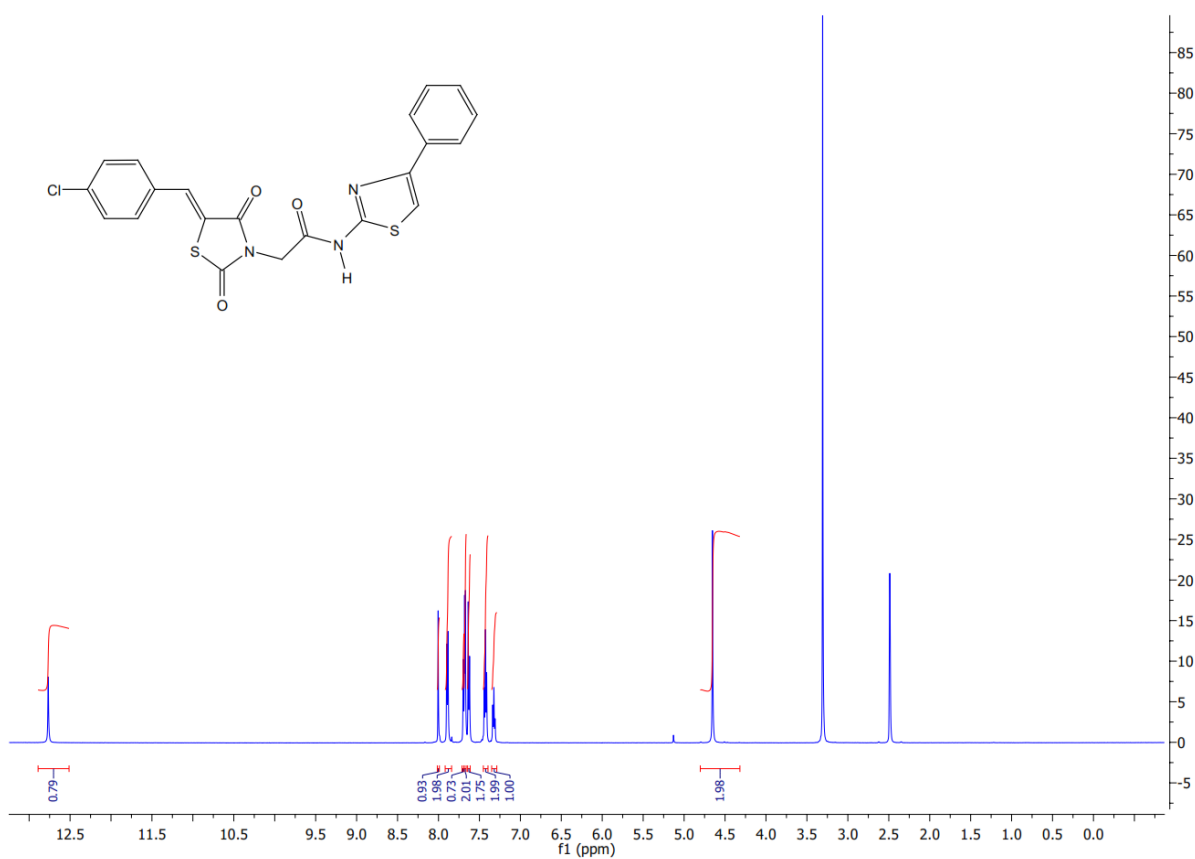
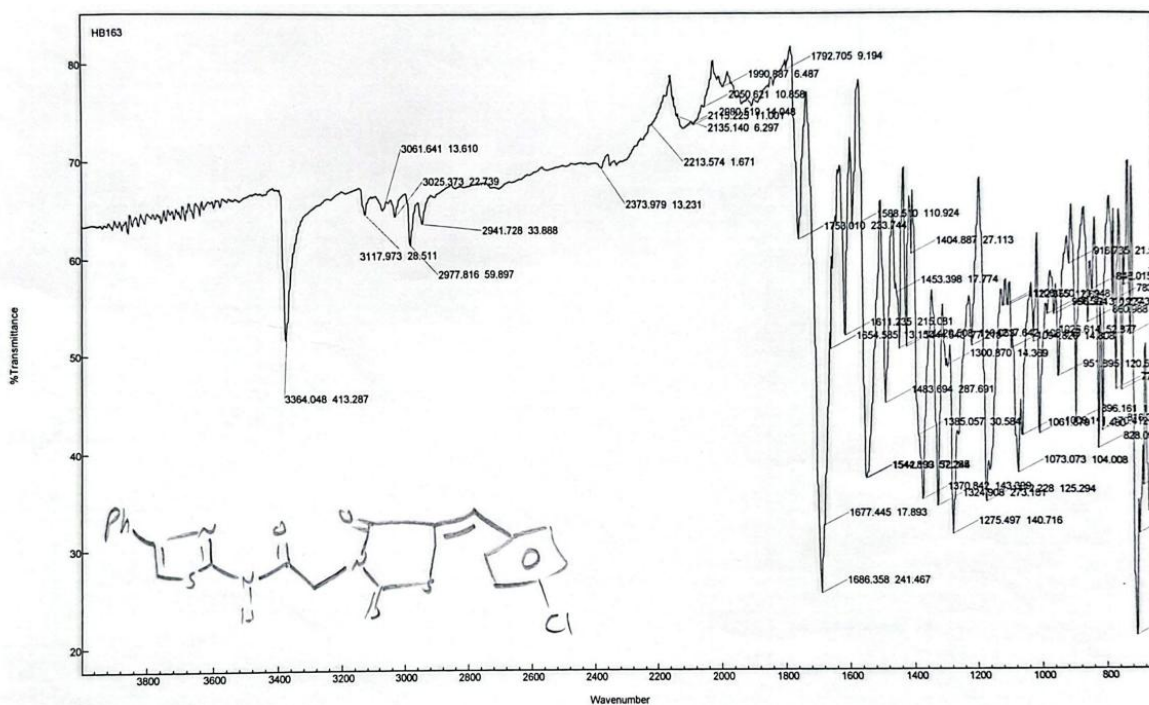
Supporting Information

HB161 #1-100 RT: 0.00-0.45 AV: 100 NL: 1.08E8
T: FTMS + p ESI Full ms [150.0000-2000.0000]

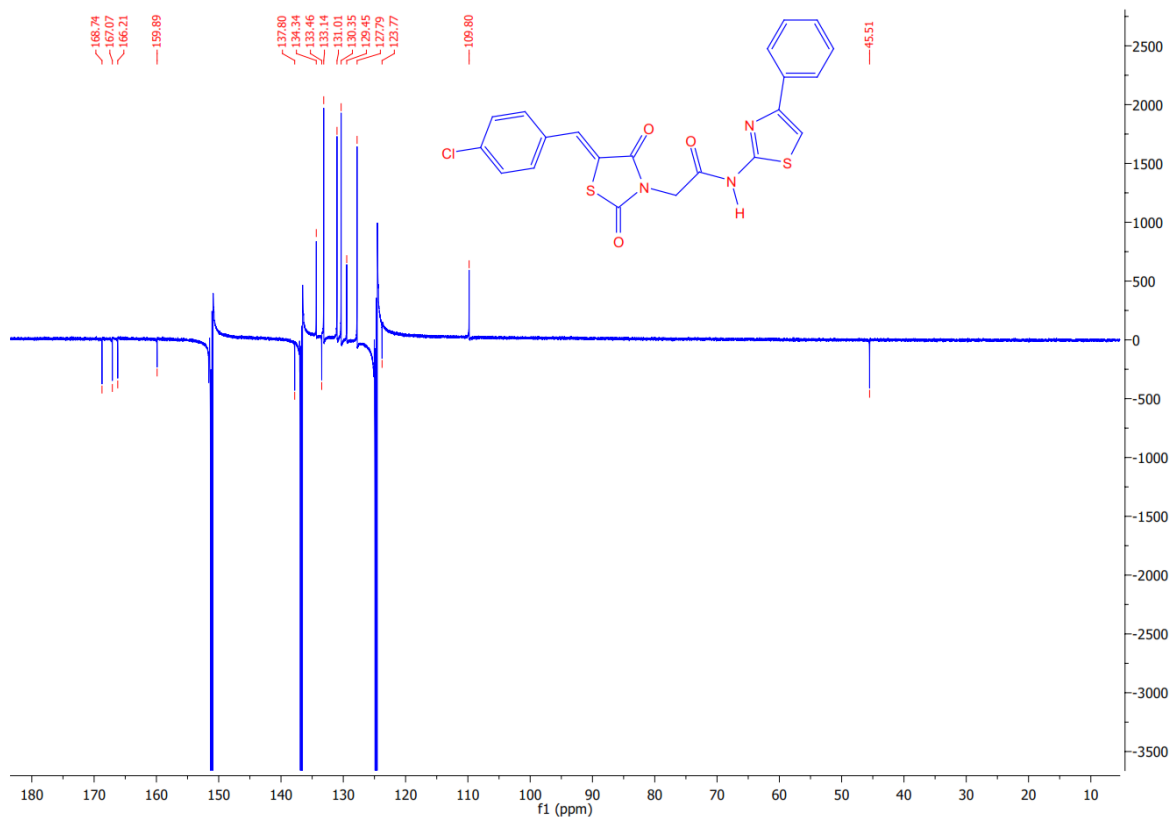


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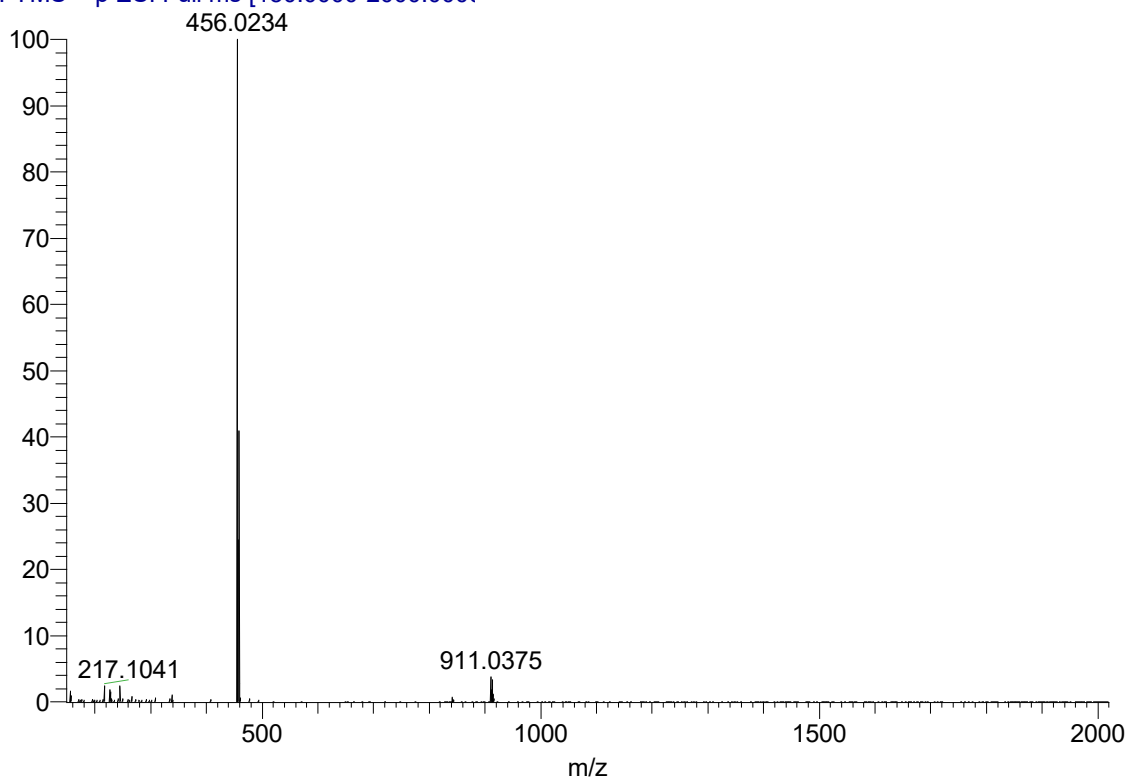
Synthesis of 2-(5-(4-chlorobenzylidene)-2,4-dioxothiazolidin-3-yl)-N-(4-phenylthiazol-2-yl)acetamide (HB163)



Supporting Information

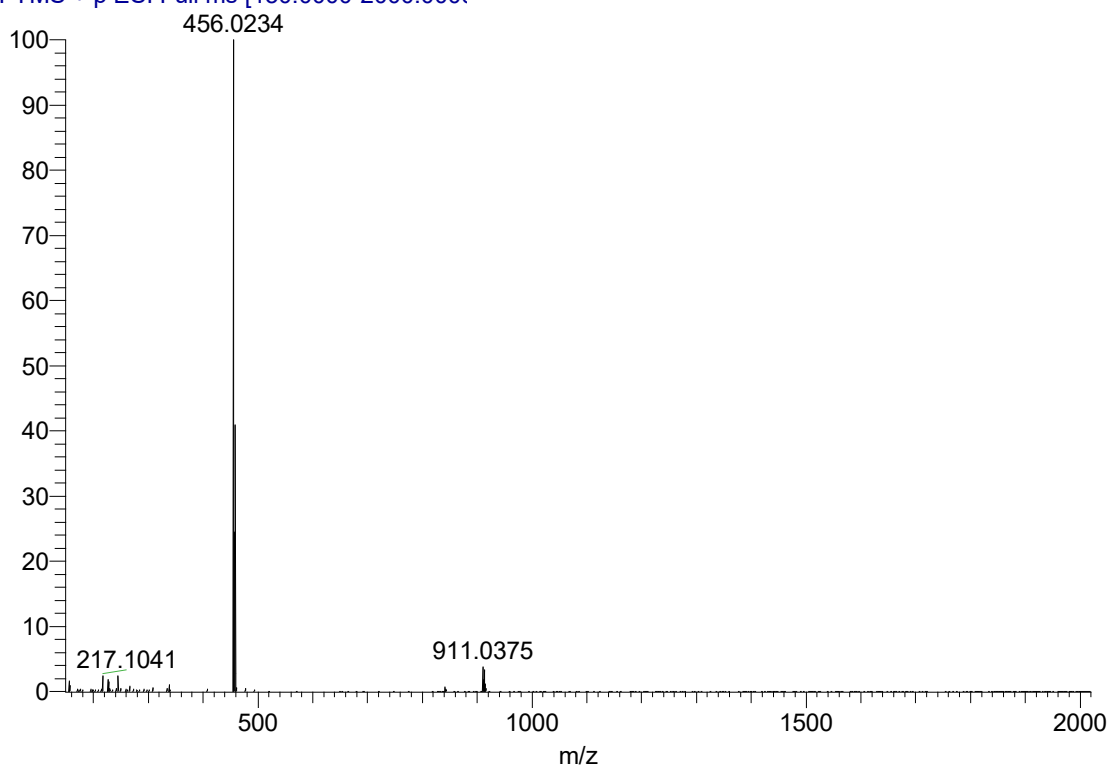


HB163_20240116093205 #1-100 RT: 0.00-0.45 AV: 100 NL: 4.37E6
T: FTMS + p ESI Full ms [150.0000-2000.0000]

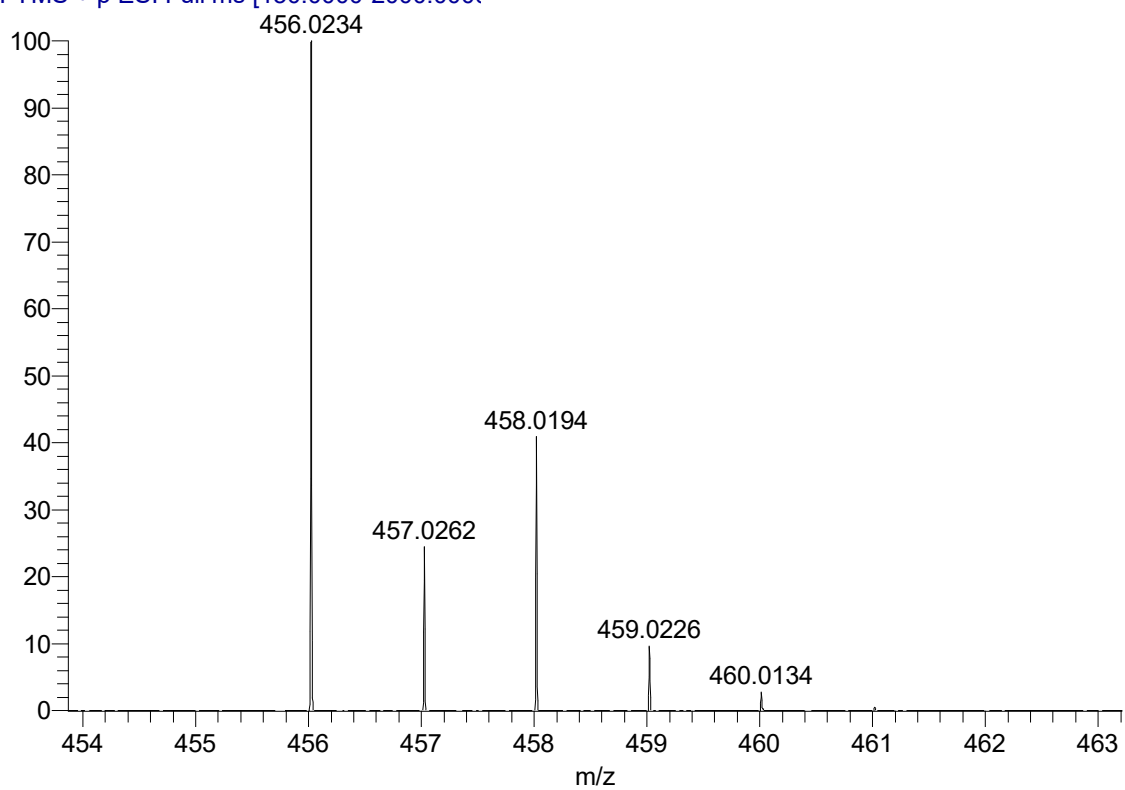


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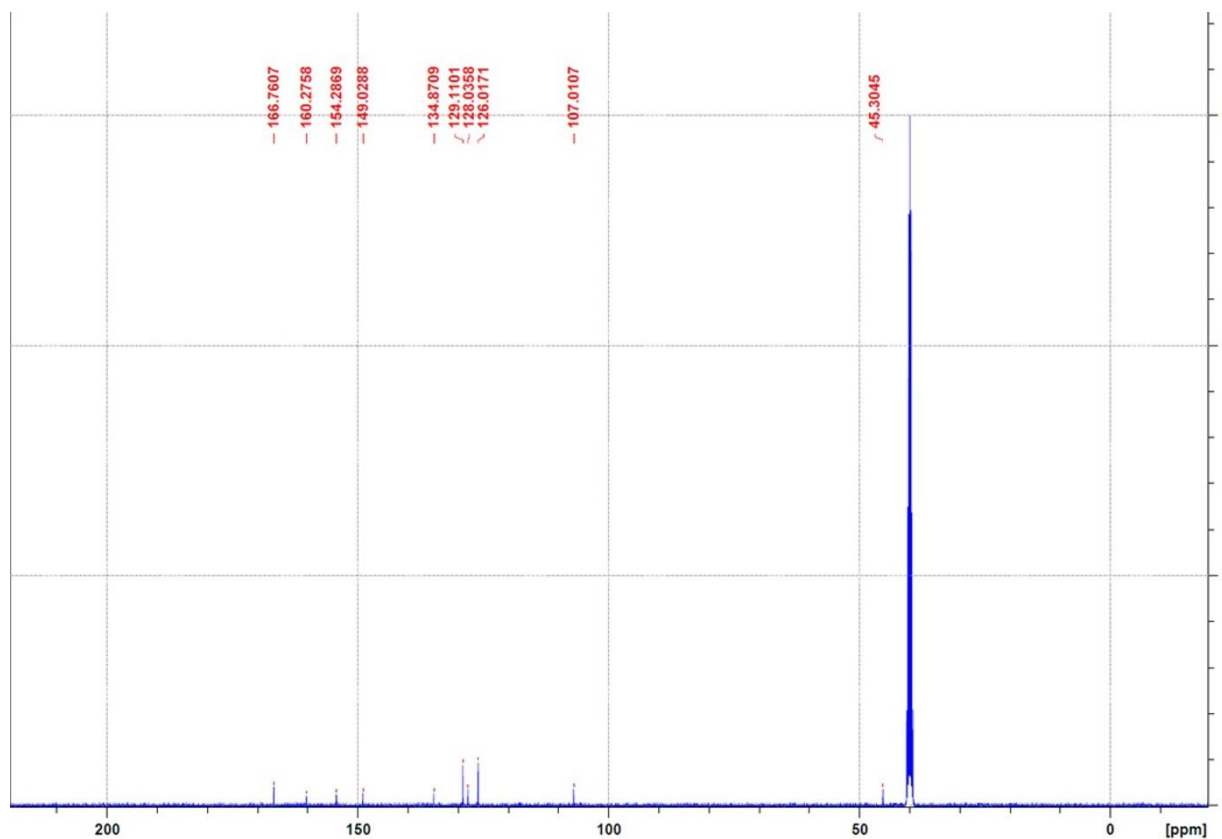
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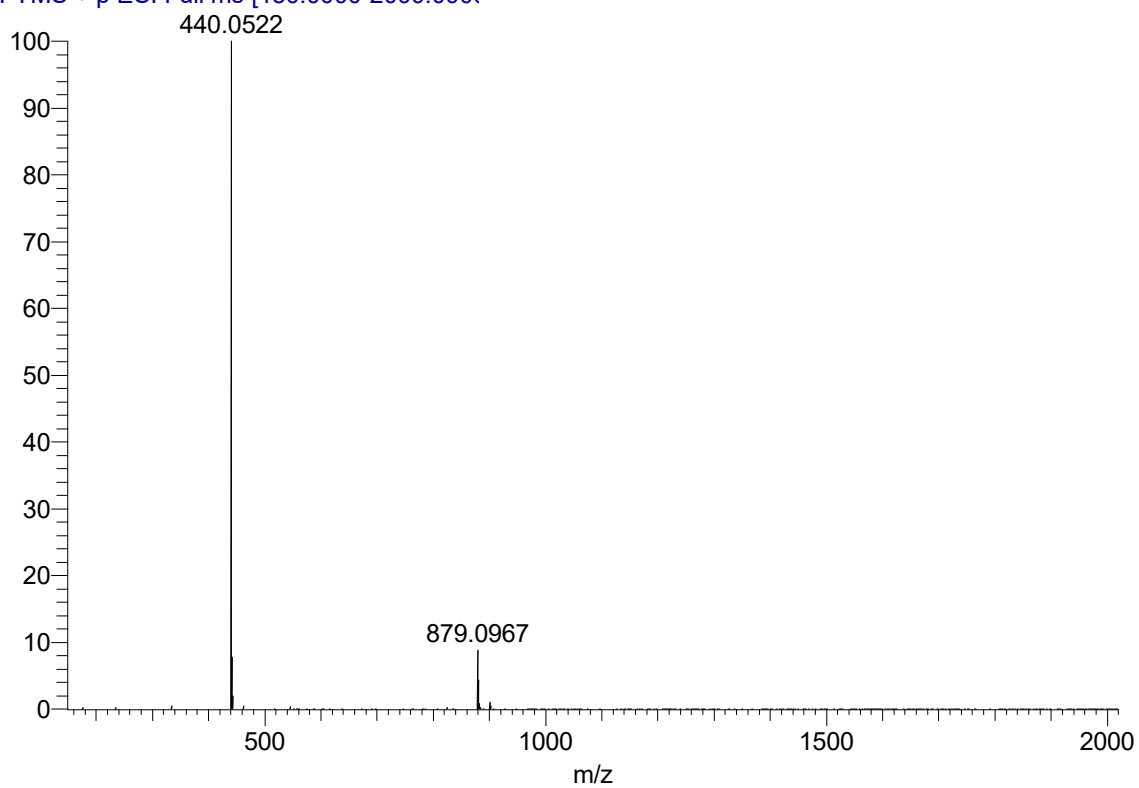
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T: FTMS + p ESI Full ms [150.0000-2000.0000]



Supporting Information

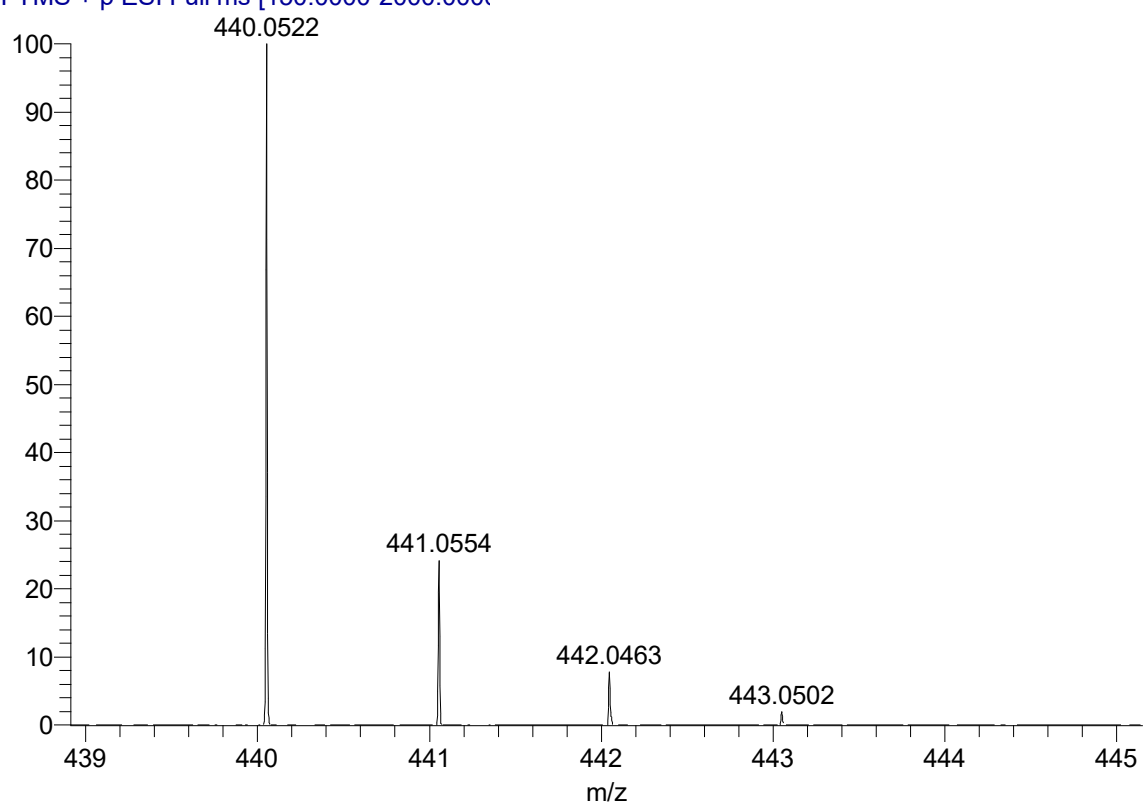


HB121 #1-100 RT: 0.00-0.45 AV: 100 NL: 1.48E8
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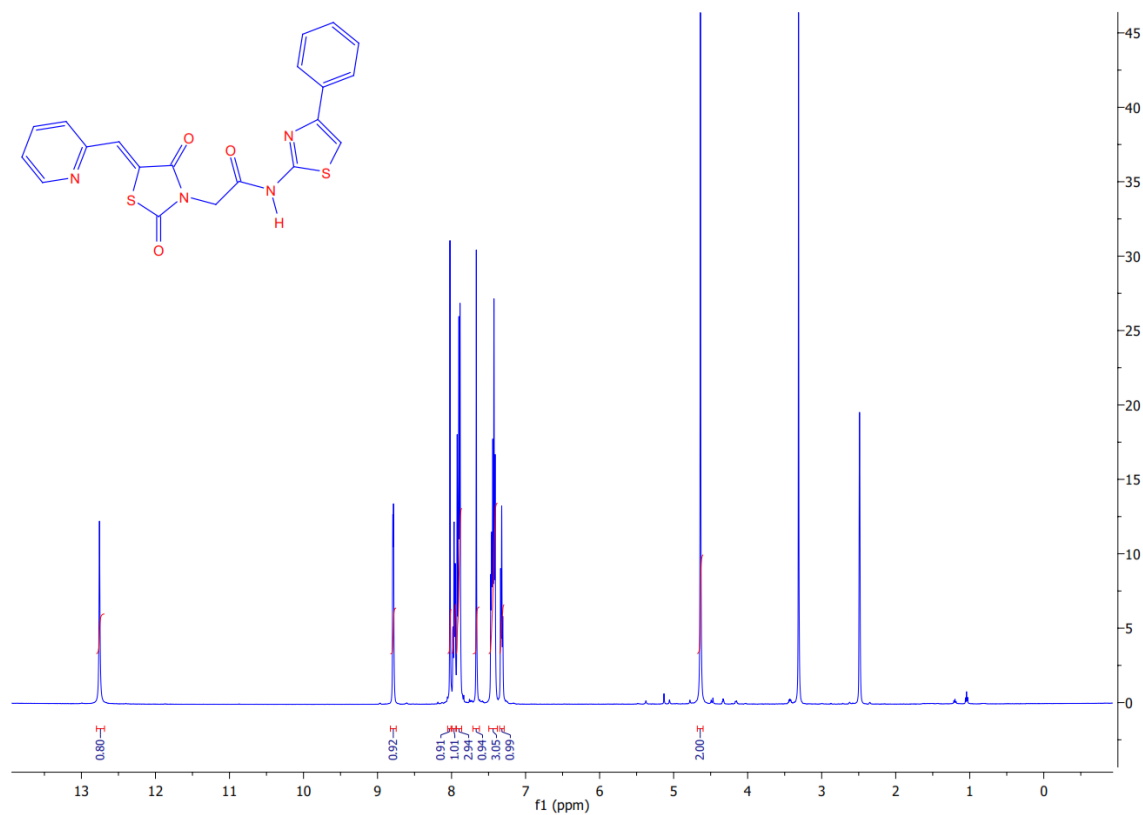
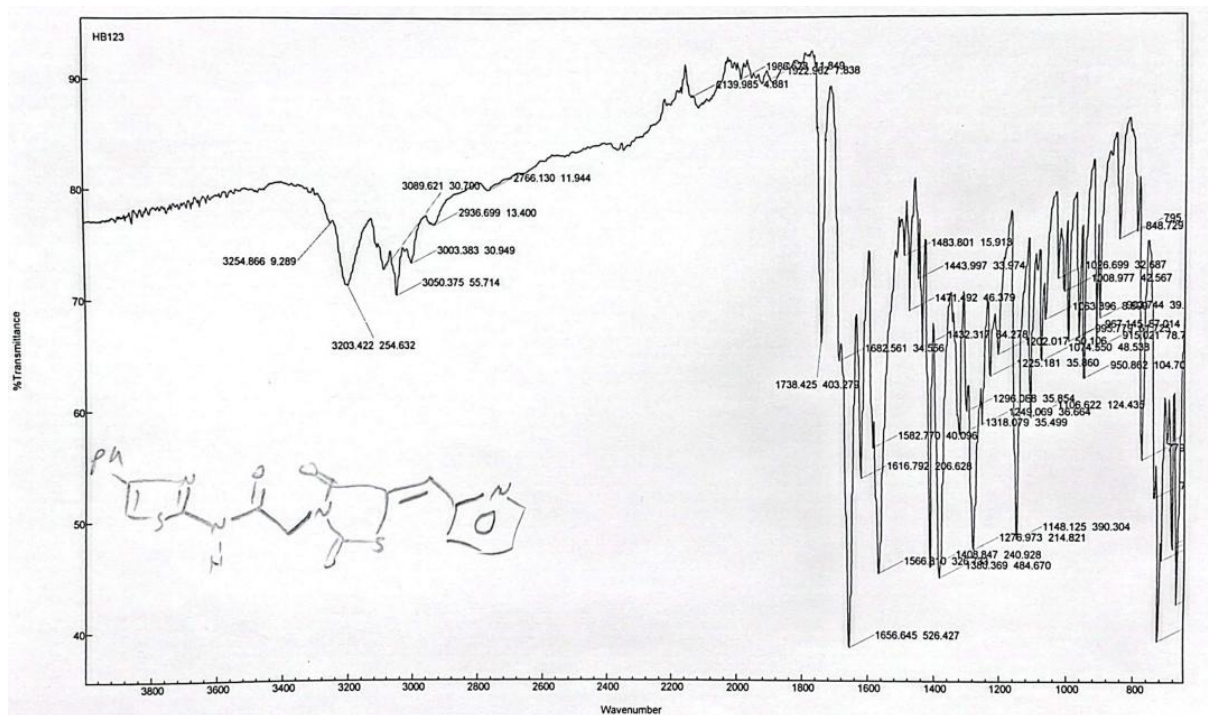
Supporting Information

HB121 #1-100 RT: 0.00-0.45 AV: 100 NL: 1.48E8
T: FTMS + p ESI Full ms [150.0000-2000.0000]

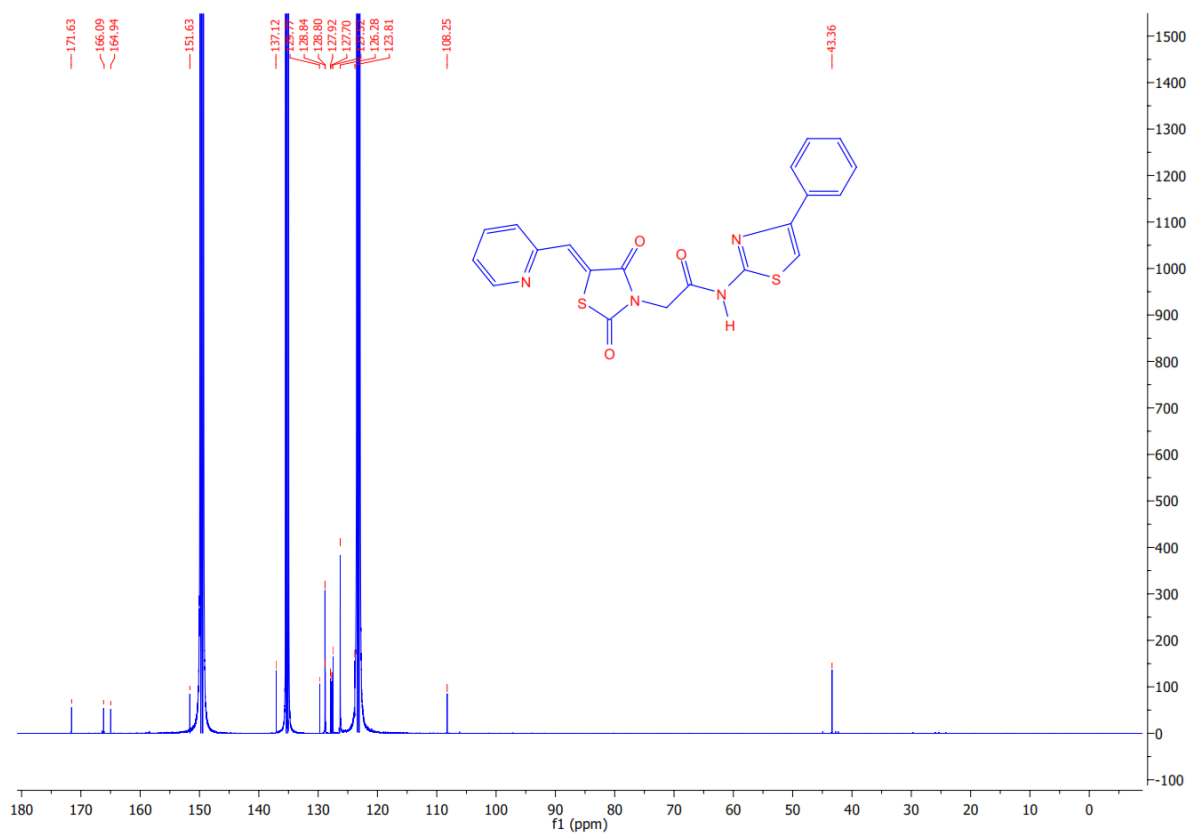


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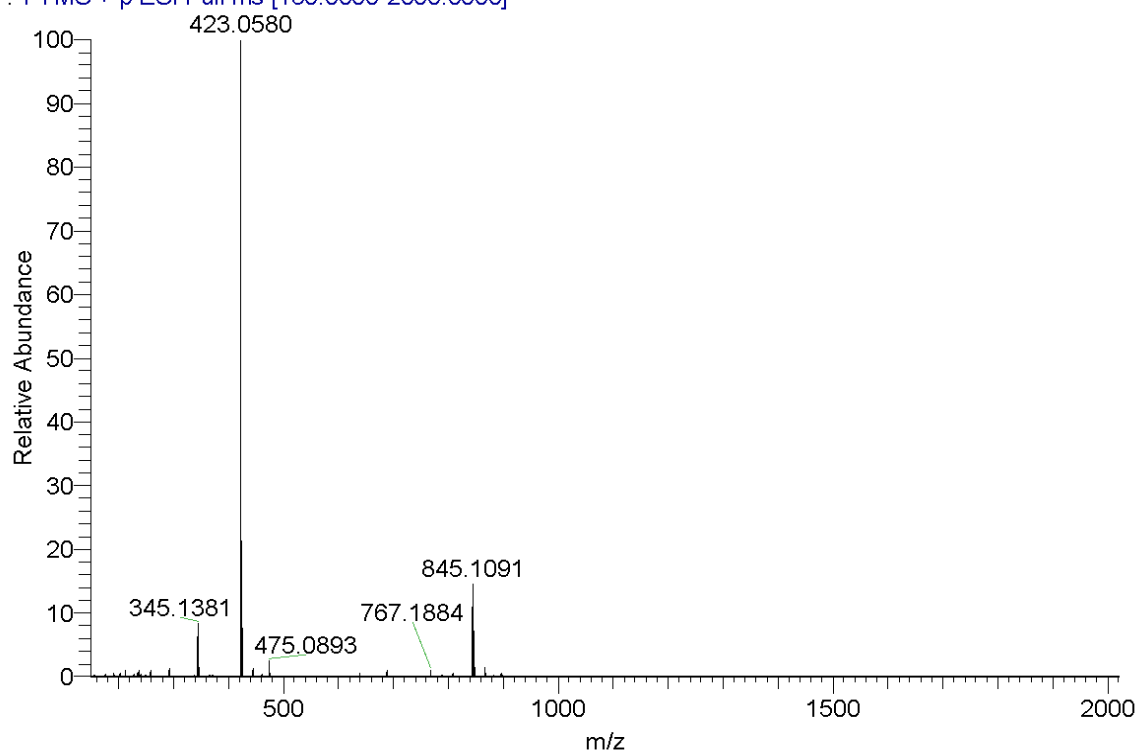
2-(2,4-dioxo-5-(pyridin-2-ylmethylene)thiazolidin-3-yl)-N-(4-phenylthiazol-2-yl)acetamide (HB123)



Supporting Information

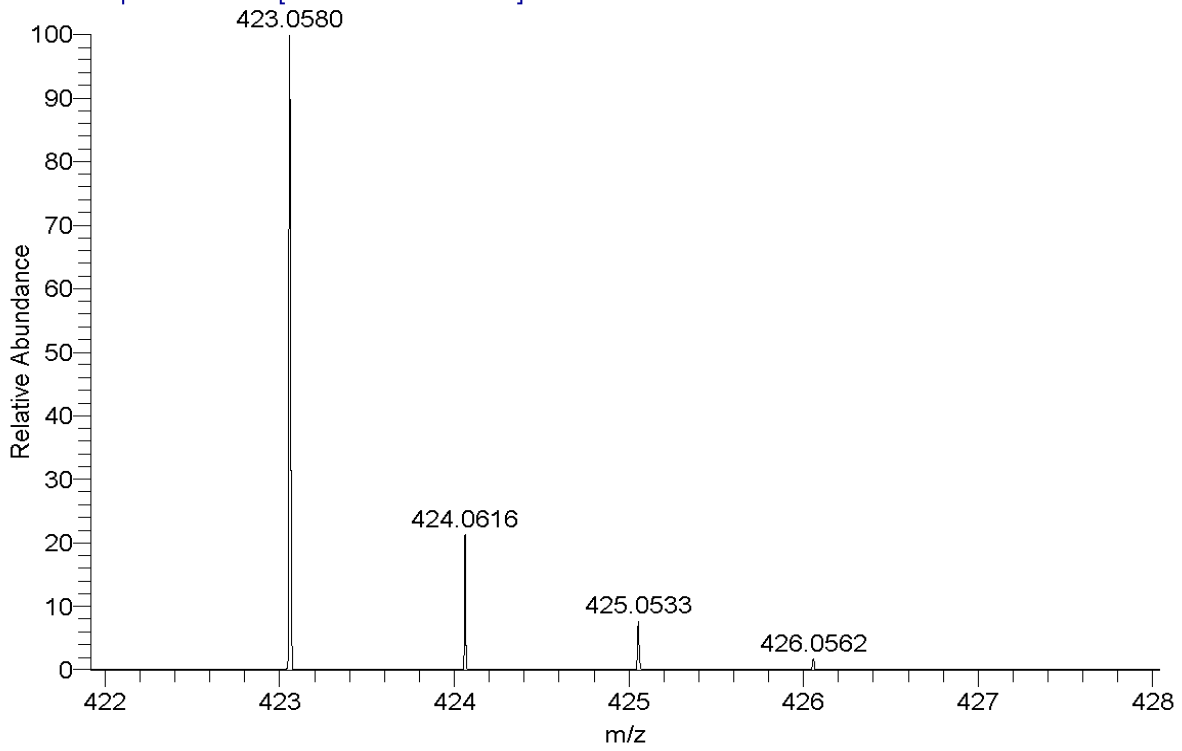


HB123 #1-100 RT: 0.00-0.45 AV: 100 NL: 2.35E8
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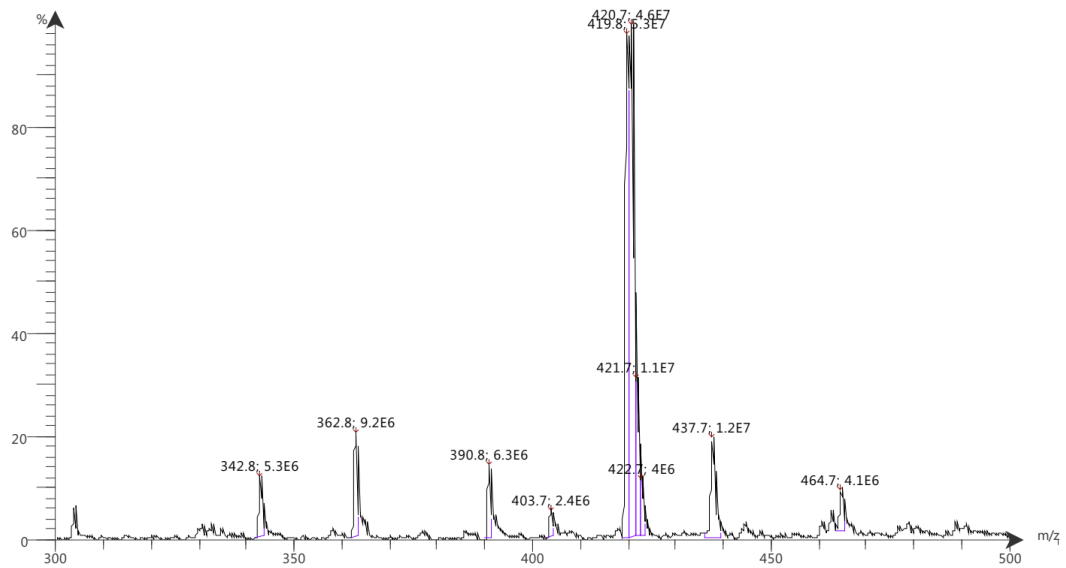


Supporting Information

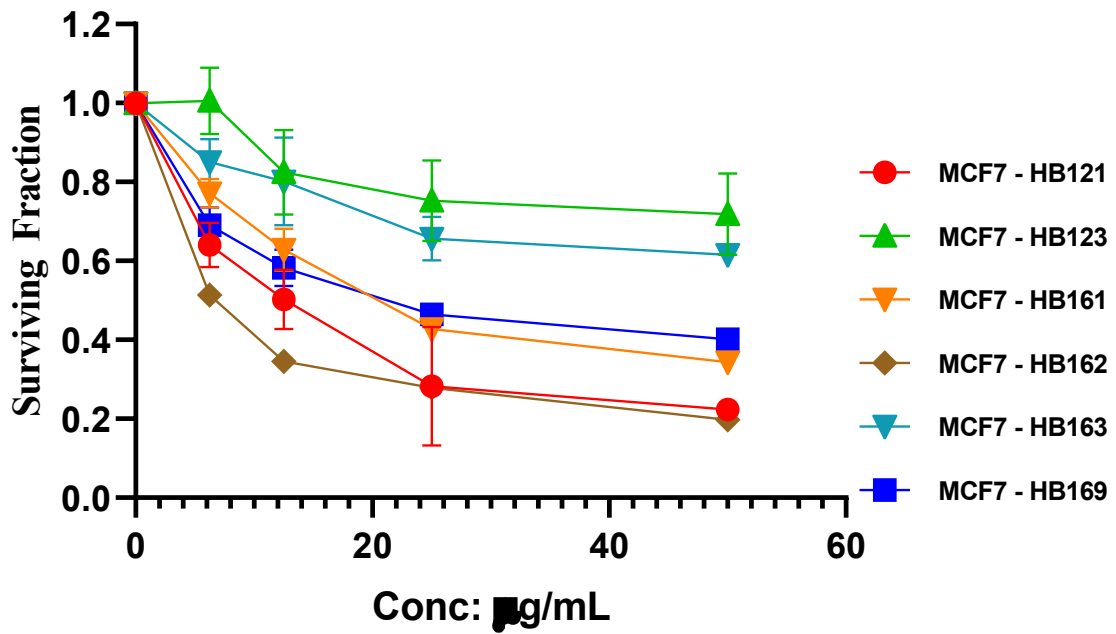
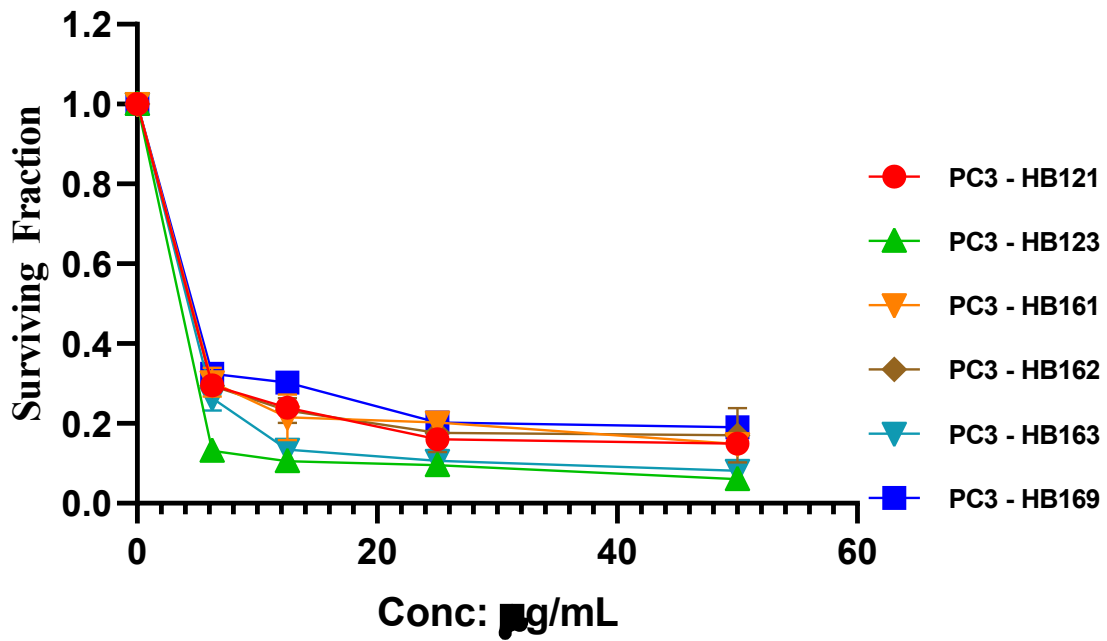
HB123 #1-100 RT: 0.00-0.45 AV: 100 NL: 2.35E8
T: FTMS + p ESI Full ms [150.0000-2000.0000]



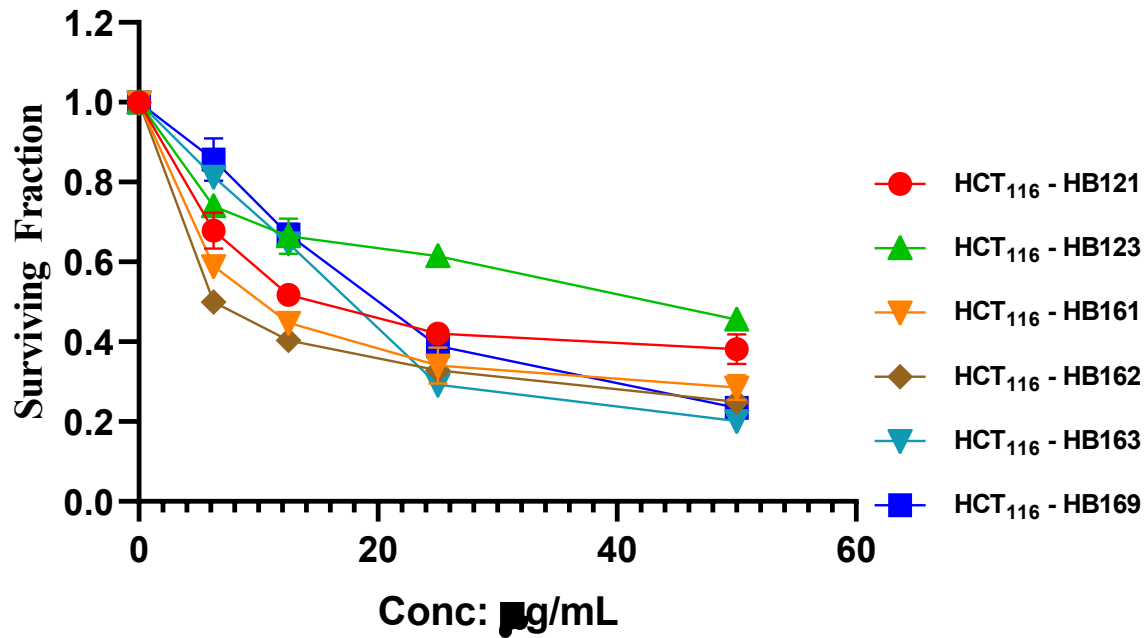
Spectrum RT 0:31 - 1:08 (71 scans) - Background Subtracted 0:05 - 0:28
Alaasar-HB123-2_Scan1_is1.datx 2025.01.15 09:12:14 ;
ESI - Max: 4.9E7



Biological Data



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Detailed representations of the IC₅₀ calculation curves of each tested cell line with the evaluated new analogues (HB121–HB169).

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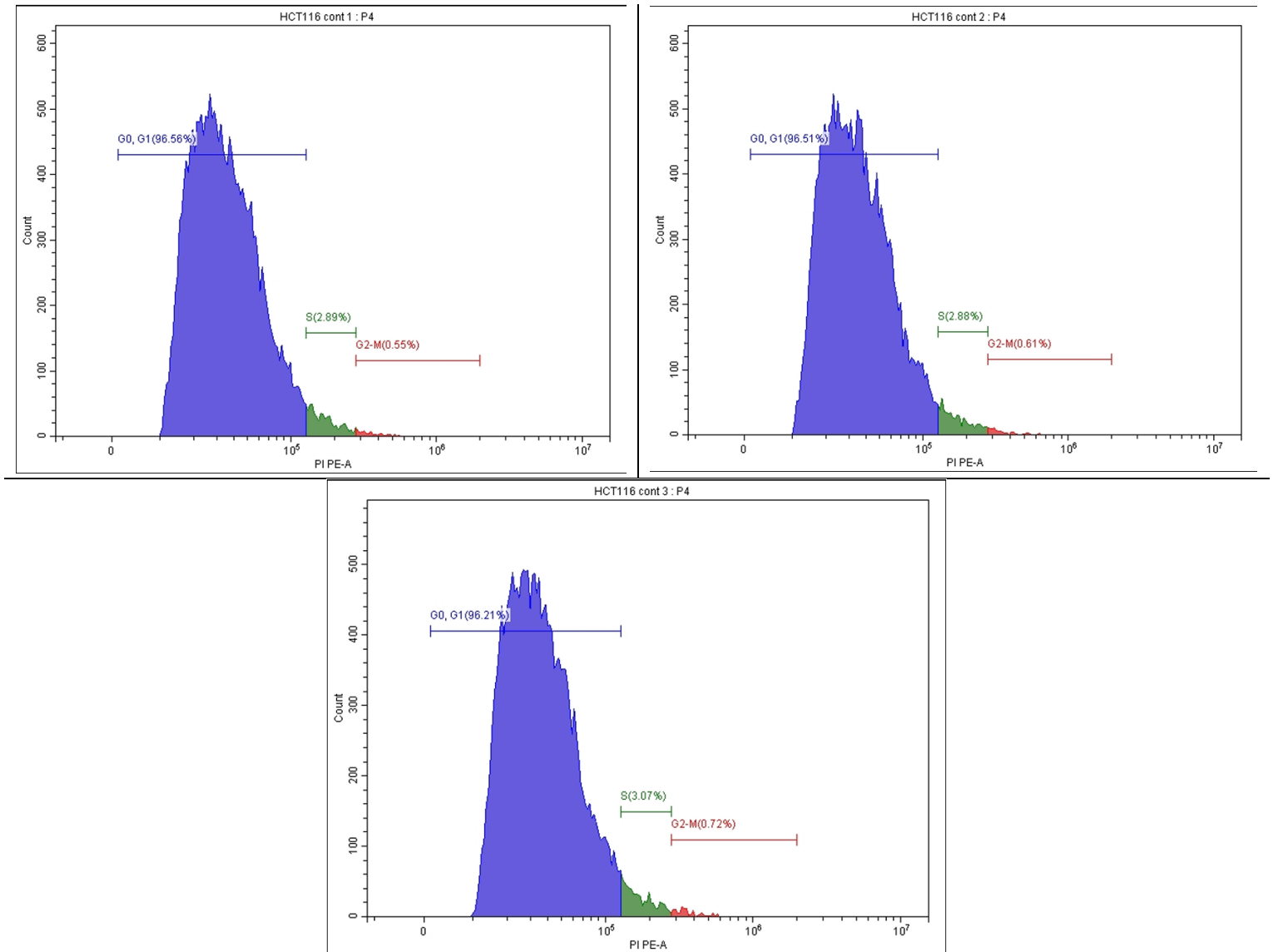


Figure S1. The triplicate experiments of the cell cycle analysis histogram of the untreated control HCT₁₁₆ cancer cell line.

Supporting Information

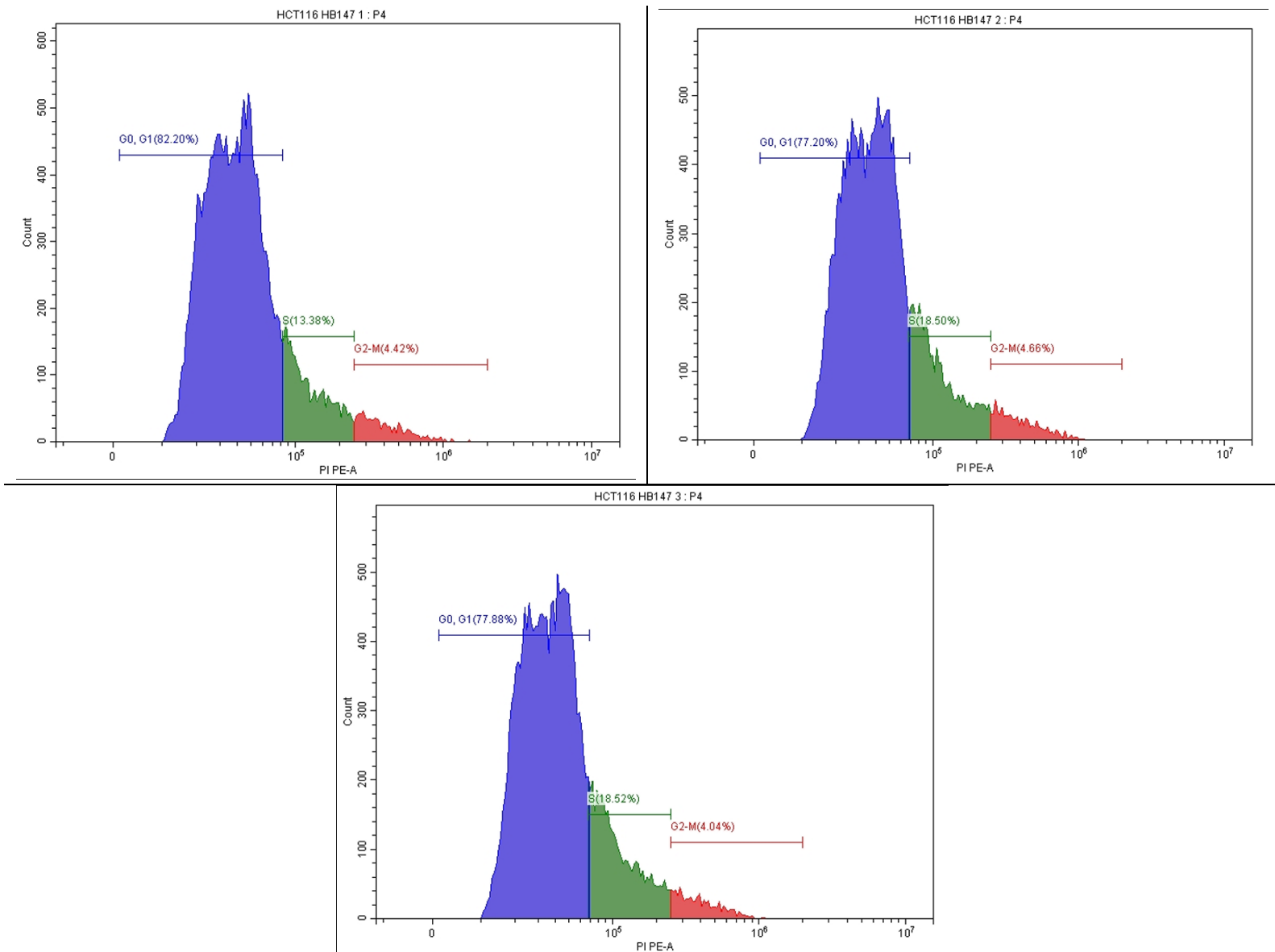


Figure S2. The triplicate experiments of the cell cycle analysis histogram of the **HB147**-treated HCT₁₁₆ cancer cell line.

ADMET and Physicochemical Studies

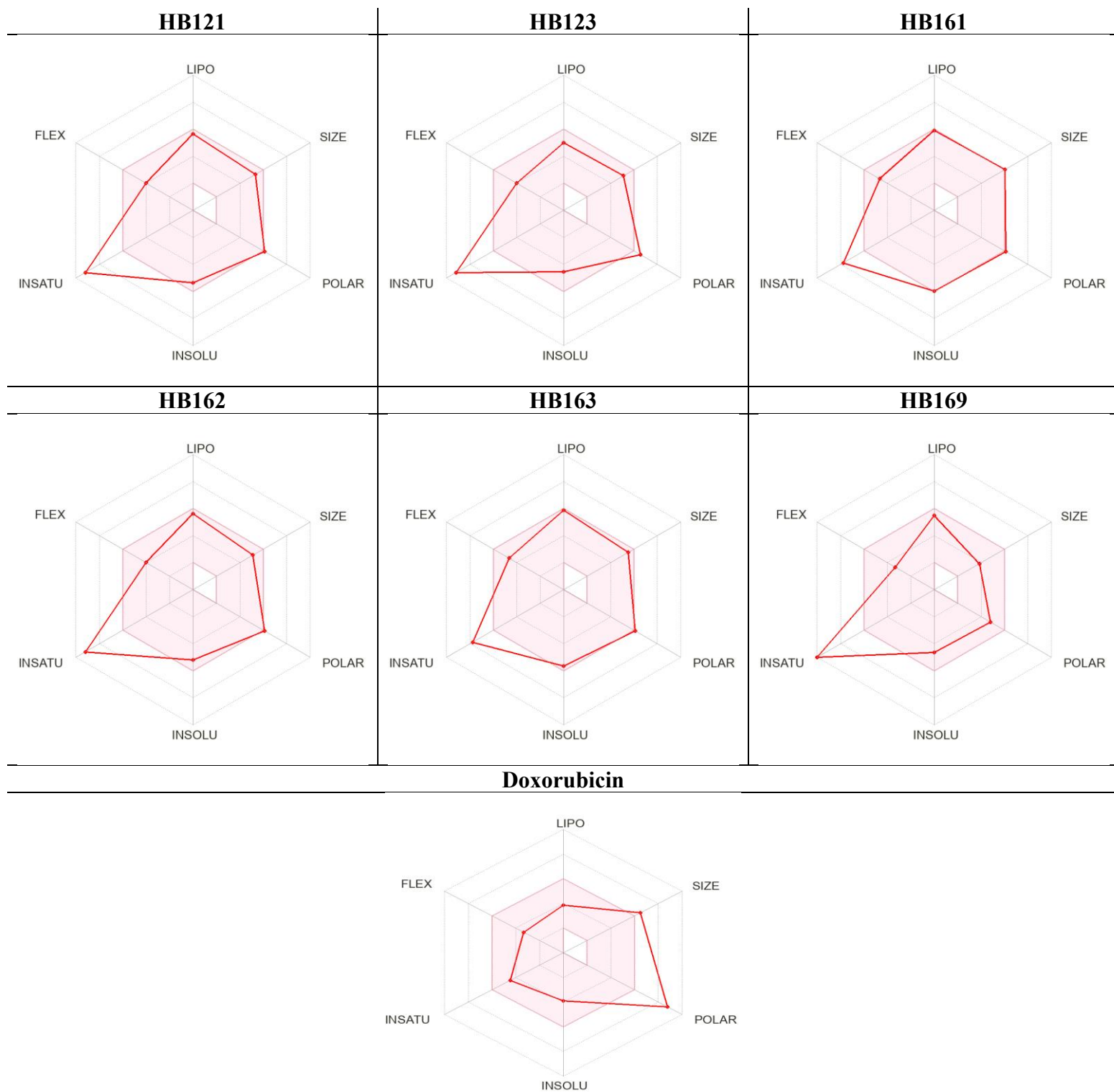


Figure S3. Radar bioavailability for the studied compounds (**5c** and **5f**), along with doxorubicin, in which the area in pink displays the specific property optimal range. (LIPO = lipophilicity

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expressed as XLOGP3 (range from -0.7 to 5.0). SIZE = size expressed as molecular weight (range from 150 g/mol to 500 g/mol). POLAR = polarity expressed as TPSA (topological polar surface area) (range from 20 Å² to 130 Å²). INSOLU = water insolubility by log S (ESOL) (range from -6 to 0). INSATU = insaturation expressed as for each carbon fraction in sp³ hybridization (range from 0.25 to 1). FLEX = flexibility, expressed as the number of rotatable bonds (range from 0 to 9).

References

1. E. O. Olawode, R. Tandlich, E. Prinsloo, M. Isaacs, H. Hoppe, R. Seldon, D. F. Warner, V. Steenkamp and P. T. Kaye, *ARKIVOC*, 2018, **2018**, 110-118.
2. G. Yan, L. Hao, Y. Niu, W. Huang, W. Wang, F. Xu, L. Liang, C. Wang, H. Jin and P. Xu, *European journal of medicinal chemistry*, 2017, **137**, 462-475.
3. C. Nitsche, V. N. Schreier, M. A. Behnam, A. Kumar, R. Bartenschlager and C. D. Klein, *Journal of Medicinal Chemistry*, 2013, **56**, 8389-8403.