SUPPORTING INFORMATION

Arylbiamidines: synthesis and structural studies *en route* to anticancer applications

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I. Synthetic procedures and characterization of all compounds

General Information

Solvents were purchased from Sigma Aldrich. DMF was dried by distillation under reduced pressure over $MgSO_4$ and the other solvents were used as received. All chemicals were purchased from Sigma-Aldrich, VWR or Alfa Aesar and used without further purification. Thin layer chromatography (TLC) was performed on precoated Merck 60 GF254 silica gel plates and revealed first by visualization under UV light (254 nm and 360 nm). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Advance 200 MHz spectrometer, a Bruker Advance 400 MHz or on a Bruker Advance 500 MHz. Mass spectra (ESI-MS) were recorded on a Bruker (Daltonics Esquire 3000+). HRMS spectra were recorded on a ThermoFisher Q Exactive (ESI-MS) at the resolution of 140 000 at m/z 200. IR spectra were recorded on a Jasco FT/IR-4600. The purity of compounds was further assayed by HPLC-*Method A* or UPLC-*Method B* as follow:

Method A: HPLC analysis on a JASCO PU-2089/AS4050 apparatus with a Supelco analytical column Ascentis Express C18, 100 mm × 46 mm 5 µM, employing the flowing method: 30% B for 1 min, 30% B to 100% B over 5 min, 100% B for 2.5 min then from 100% B to 30% B over 30 s, 30% B for 7 min (total time: 16 min). Solvent A: water with 1% formic acid. Solvent B: acetonitrile with 1% formic acid.

Method B: UHPLC analysis on a JASCO PU4180/AS4140 apparatus with a Agela Technologies analytical column Innoval C18 100 mm × 46 mm 1.9 µM, employing the flowing method: 5-95% B over 3 min; 95% B for 3 min, 95-5% B over 1 min, 5% B for 1 min; Solvent A: water with 0.1% formic acid; Solvent B: acetonitrile with 0.1% formic acid; 1 mL/min.

General Procedure for the guanidine intermediates: The key guanidine intermediates **6, 8** and **9** were prepared by using previously reported procedures.[1-5] Compound **7** has not been previously described and was synthesized by adapting the same procedure, where the α-haloketone **1** was replaced by **2**. Briefly, the 2-thiazolylguanidine derivatives **6-9** were afforded by thiazole-Hantzsch reaction from commercially available amidinothiourea (**5**) with the appropriately substituted α-haloketones (**1-4**) under reflux in ethanol for 2 h. After completion, the reaction mixture was concentrated under reduced pressure and washed with small amounts of cold methanol. For the compounds **7**, **8** and **9** the residues were additionally washed with 10% sodium hydroxide solution and water.

1-(4-methylthiazol-2-yl)guanidine hydrochloride (**6**) Synthesized following the general procedure using chloroacetone (10.2 g, 0.11 mol) and amidinothiourea (10.0 g, 0.85 mol) to afford the title compound as a white solid (12.1 g, 74%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.53 (s, 1H), 8.28 (s, 4H), 6.89 (s, 1H), 2.27 (d, *J* = 1.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.26, 154.33, 147.66, 107.68, 16.69.

1-(4,5-dimethylthiazol-2-yl)guanidine (**7**) Synthesized following the general procedure using 3-chlorobutan-2-one (14.1 g, 0.13 mol) and amidinothiourea (12.0 g, 0.10 mol) under reflux in ethanol for 2 h to afford the title compound as a white solid (12.1 g, 71%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.69 (s, 4H), 2.11 (d, *J* = 1.0 Hz, 3H), 2.04 (d, *J* = 1.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.62, 156.53, 141.66, 113.22, 14.59, 10.67.

1-(4-phenylthiazol-2-yl)guanidine (**8**) Synthesized following the general procedure using 2-bromo-acetophenone (19.9 g, 0.1 mol) and amidinothiourea (11.8 g, 0.1 mol) under reflux in ethanol for 2 h to afford the title compound as a white solid (17.0 g, 78%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.88 – 7.74 (m, 2H), 7.38 (t, *J* = 7.6 Hz, 2H), 7.27 (t, *J* = 7.3 Hz, 1H), 7.17 (s, 1H), 6.95 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.84, 156.94, 149.24, 134.81, 128.53 (2C), 127.24, 125.48 (2C), 103.24.

1-(4-(3-nitrophenyl)thiazol-2-yl)guanidine (**9**) Synthesized following the general procedure using 2-bromo-3'-nitroacetophenone (24.4 g, 0.1 mol) and amidinothiourea (11.8 g, 0.1 mol) under reflux in ethanol for 2 h to afford the title compound as an yellow solid (23.4 g, 89%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 (d, *J* = 1.7 Hz, 1H), 8.30 (d, *J* = 7.9 Hz, 1H), 8.15 – 8.07 (m, 1H), 7.67 (t, *J* = 8.0 Hz, 1H), 7.51 (s, 1H), 7.00 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 175.09, 156.98, 148.35, 146.99, 136.32, 131.81, 130.15, 121.74, 119.62, 106.18.

General Procedure for the synthesis of arylbiamidine compounds: Sodium hydride (60% dispersion in mineral oil, 1.5 eq.) was added to the solution of the corresponding guanidine (1.0 eq.) in *N*,*N*-dimethylformamide (25 mL/g of guanidine) at 0 °C and the mixture was stirred for 30 min at room temperature. The corresponding arylnitrile (1.0 eq.) was added in one portion to the reaction mixture and stirred at room temperature (2-6 h). The progress of the reaction was monitored by TLC. After the reaction was complete, the reaction mixture was treated employing the following methods:

Method 1 (optimized condition): the reaction mixture was slowly poured into cold water (300 mL/g of starting guanidine) under stirring and the precipitate was collected and washed with water (200 mL) and pentane (20 mL). The solid was then resuspended in ethanol and filtrated.

Method 2 (preliminary method, not optimized condition): the reaction mixture was slowly poured into water (200 mL/g of guanidine) under vigorous stirring, the precipitate was collected and washed with water, methanol and diethyl ether. This method leads to lower yields due to the solubility of arylbiamidines in diethyl ether.

N-(N-(4-methylthiazol-2-yl)carbamimidoyl)benzimidamide (**A1**) Synthesized following the general procedure using 1-(4-methylthiazol-2-yl)guanidine (1.00 g, 6.41 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 384 mg, 9.61 mmol) and benzonitrile (0.66 mL, 6.41 mmol) resulting in a pale yellow powder (1.09 g, 81%, method 1). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 10.42 (br. s, 1H), 9.11 (br. s, 1H), 8.61 (br. s, 1H), 8.02 – 7.96 (m, 2H), 7.57 – 7.52 (m, 1H), 7.85 – 7.39 (br. s, 4H), 7.51 – 7.46 (m, 2H), 6.57 (d, *J* = 1.0 Hz, 1H), 2.24 (d, *J* = 0.9 Hz, 3H). ^{13}C NMR (50 MHz, DMSO-*d6*): δ 172.99, 161.63, 160.69, 147.64, 135.50, 131.20, 128.21, 127.45, 105.64, 17.56. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{12}H_{13}N_5S^+$, 260.09644; Found: 260.09653. HPLC (method A, λ_{280}): Purity 95.7%; t_R: 5.167 min.

2-chloro-N-(N-(4-methylthiazol-2-yl)carbamimidoyl)benzimidamide (**A2**) Synthesized following the general procedure using 1-(4-methylthiazol-2-yl)guanidine (1.00 g, 6.41 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 384 mg, 9.61 mmol) and 2-chlorobenzonitrile (881 mg, 6.41 mmol) resulting in a light yellow powder (1.36 g, 89%, method 1). ^{1}H NMR (400 MHz, DMSO-*d6*): δ 10.27 (br. s, 1H), 9.06 (br. s, 1H), 8.63 (br. s, 1H), 7.63 (br. s, 1H), 7.46 (m, 4H), 6.57 (s, 1H), 2.24 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*): δ 172.87, 162.18, 160.47, 147.49, 136.79, 130.49, 130.27, 129.55, 129.40, 126.88, 105.69, 17.46. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{12}H_{12}ClN_5S^+$, 294.05747; Found: 294.05750. HPLC (method A, λ_{280}): Purity 95.0%; t_R: 5.500 min.

3-chloro-N-(N-(4-methylthiazol-2-yl)carbamimidoyl)benzimidamide (**A3**) Synthesized following the general procedure using 1-(4-methylthiazol-2-yl)guanidine (1.00 g, 6.41 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 384 mg, 9.61 mmol) and 3-chlorobenzonitrile (881 mg, 6.41 mmol) resulting in a white-yellowish powder (1.24 g, 66%, method 2). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 10.43 (br. s, 1H), 9.13 (br. s, 1H), 8.71 (br. s, 1H), 8.11 – 8.05 (m, 1H), 7.95 (d, *J* = 7.9 Hz, 1H), 7.64 – 7.59 (m, 1H), 7.58 (br.s, 1H), 7.53 (t, *J* = 7.9 Hz, 1H), 6.59 (d, *J* = 0.9 Hz, 1H), 2.25 (d, *J* = 0.5 Hz, 3H). ^{13}C NMR (50 MHz, DMSO-*d6*): δ 172.82, 160.44, 159.91, 147.64, 137.47, 133.18, 130.97, 130.15, 127.39, 126.00, 105.82, 17.53. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{12}H_{12}ClN_5S^+$, 294.05747; Found: 294.05768. HPLC (method A, λ_{280}): Purity 95.0%; t_R: 5.933 min.

4-chloro-N-(N-(4-methylthiazol-2-yl)carbamimidoyl)benzimidamide (**A4**) Synthesized following the general procedure using 1-(4-methylthiazol-2-yl)guanidine (1.00 g, 6.41 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 384 mg, 9.61 mmol) and 4-chlorobenzonitrile (881 mg, 6.41

mmol) resulting in a white-yellowish powder (1.66 mg, 88%, method 1). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 10.42 (br. s, 1H), 9.13 (br. s, 1H), 8.67 (br. s, 1H), 8.01 (d, *J* = 8.6 Hz, 2H), 7.57 (d, br. s *J* = 8.6 Hz, 3H), 6.57 (d, *J* = 1.0 Hz, 1H), 2.24 (d, *J* = 0.7 Hz, 3H). ^{13}C NMR (50 MHz, DMSO-*d6*): δ 172.87, 160.50, 160.37, 147.63, 136.08, 134.23, 129.30, 128.29, 105.74, 17.53. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{12}H_{12}ClN_5S^+$, 294.05747; Found: 294.05762. HPLC (method A, λ_{280}): Purity 95.3%; t_R: 5.958 min.

2-bromo-N-(N-(4-methylthiazol-2-yl)carbamimidoyl)benzimidamide (**A5**) Synthesized following the general procedure using 1-(4-methylthiazol-2-yl)guanidine (1.00 g, 6.41 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 384 mg, 9.61 mmol) and 2-bromobenzonitrile (1.17 g, 6.41 mmol) resulting in a pale beige/orange powder (1.73 g, 80%, method 1). ^{1}H NMR (400 MHz, DMSO-*d6*): δ 10.27 (br. s, 1H), 9.07 (br. s, 1H), 8.63 (br. s, 1H), 7.67 (d, br. s, *J* = 7.9 Hz, 2H), 7.46 – 7.43 (m, 2H), 7.36 (ddd, *J* = 8.1, 6.0, 3.2 Hz, 1H), 6.57 (s, 1H), 2.24 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*): δ 172.89, 163.29, 160.49, 147.50, 138.85, 132.49, 130.57, 129.44, 127.37, 119.62, 105.68, 17.46. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{12}H_{12}BrN_5S^+$, 338.00696; Found: 338.00705. HPLC (method A, λ_{280}): Purity 95.8%; t_R: 5.192 min.

3-bromo-N-(N-(4-methylthiazol-2-yl)carbamimidoyl)benzimidamide (**A6**) Synthesized following the general procedure using 1-(4-methylthiazol-2-yl)guanidine (1.00 g, 6.41 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 384 mg, 9.61 mmol) and 3-bromobenzonitrile (1.17 g, 6.41 mmol) resulting in a white-yellowish powder (1.11 g, 51%, method 2). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 10.42 (s, 1H), 9.12 (s, 1H), 8.72 (s, 1H), 8.21 (t, *J* = 1.7 Hz, 1H), 7.98 (d, *J* = 8.0 Hz, 1H), 7.75 (dd, br. s, *J* = 8.0, 1.1 Hz, 2H), 7.46 (t, *J* = 7.9 Hz, 1H), 6.59 (d, *J* = 0.9 Hz, 1H), 2.24 (d, *J* = 0.8 Hz, 3H). ^{13}C NMR (50 MHz, DMSO-*d6*): δ 172.80, 160.42, 159.83, 147.63, 137.63, 133.86, 130.38, 130.27, 126.35, 121.66, 105.81, 17.53. HRMS-ESI (m/z): $[M+H]^+$ calc. for $C_{12}H_{12}BrN_5S^+$, 338.00696; Found: 338.00760. HPLC (method A, λ_{280}): Purity 95.1%; t_R: 6.042 min.

3-methoxy-N-(N-(4-methylthiazol-2-yl)carbamimidoyl)benzimidamide (**A7**) Synthesized following the general procedure using 1-(4-methylthiazol-2-yl)guanidine (1.00 g, 6.41 mmol), sodium hydride (60 % dispersion in mineral oil, 1.5 eq., 384 mg, 9.61 mmol) and 3-methoxybenzonitrile (0.78 mL, 6.41 mmol) resulting in a beige powder (1.59 g, 86%, method 1). ^{1}H NMR (400 MHz, Acetone-d6) δ 10.86 (s, 1H), 9.43 (s, 1H), 7.90 (s, 1H), 7.67 – 7.54 (m, 2H), 7.37 (t, *J* = 8.0 Hz, 1H), 7.09 (ddd, *J* = 8.2, 2.6, 0.9 Hz, 1H), 6.83 (s, 1H), 6.47 (q, *J* = 1.1 Hz, 1H), 3.85 (s, 3H), 2.27 (d, *J* = 1.0 Hz, 3H). ^{13}C NMR (101 MHz, Acetone-*d6*) δ 174.37, 162.76, 161.97, 160.64, 149.04, 138.40, 130.15, 120.27, 117.64, 113.72, 106.27, 55.71, 17.80. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{13}H_{16}ON_5S^+$, 290.10701; Found: 290.10631. UPLC (method B, λ_{280}): Purity >99.9%; t_R: 3.767 min.

4-methoxy-N-(N-(4-methylthiazol-2-yl)carbamimidoyl)benzimidamide (**A8**) Synthesized following the general procedure using 1-(4-methylthiazol-2-yl)guanidine (1.00 g, 6.41 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 384 mg, 9.61 mmol) and 4-methoxybenzonitrile (0.853 mg, 6.41 mmol) resulting in a beige powder (1.65 mg, 89%, method 1). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 10.40 (br. s, 1H), 9.06 (br. s, 1H), 8.50 (br. s, 1H), 7.98 (d, *J* = 8.9 Hz, 2H), 7.49 (br. s, 1H), 7.02 (d, *J* = 8.9 Hz, 2H), 6.55 (d, *J* = 0.8 Hz, 1H), 3.83 (s, 3H), 2.24 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*): δ 172.97, 161.71, 160.99, 160.62, 147.51, 129.13, 127.43, 113.43, 105.33, 55.34, 17.48. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{13}H_{15}N_5OS^+$, 290.10701; Found: 290.10709. HPLC (method A, λ_{280}): Purity >99.9%; t_R: 5.308 min.

N-(N-(4-methylthiazol-2-yl)carbamimidoyl)picolinimidamide (**A9**) Synthesized following the general procedure using 1-(4-methylthiazol-2-yl)guanidine (1.00 g, 6.41 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 384 mg, 9.61 mmol) and 2-pyridinecarbonitrile (0.617 mL, 6.41 mmol) resulting in a yellow powder (1.05 g, 63%, method 2). ^{1}H NMR (200 MHz, DMSO-*d6*): δ 10.20 (s, 1H), 9.15 (s, 1H), 8.77 (s, 1H), 8.73 – 8.65 (m, 1H), 8.34 (d, *J* = 8.5 Hz, 1H), 8.00 (td, *J* = 7.8, 1.7 Hz, 1H), 7.61 (ddd, *J* = 7.4, 4.8, 1.1 Hz, 2H), 6.60 (d, *J* = 1.0 Hz, 1H), 2.25 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*): δ 172.90, 160.61, 158.17, 150.75, 148.52, 147.60, 137.32, 126.26, 121.87, 105.82, 17.46. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{11}H_{12}N_6S^+$, 261.09169; Found: 261.09174. HPLC (method A, λ_{280}): Purity 95.7%; t_R: 5.708 min.

N-(N-(4-methylthiazol-2-yl)carbamimidoyl)nicotinimidamide (**A10**) Synthesized following the general procedure using 1-(4-methylthiazol-2-yl)guanidine (1.00 g, 6.41 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 384 mg, 9.61 mmol) and 3-pyridinecarbonitrile (0.667 mg, 6.41 mmol) resulting in a yellow powder (751 mg, 45%, method 2). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 10.39 (br. s, 1H), 9.15 (d, br. s, *J* = 1.7 Hz, 2H), 8.77 (br. s, 1H), 8.71 (dd, *J* = 4.7, 1.5 Hz, 1H), 8.36 – 8.23 (m, 1H), 7.65 (br. s, 1H), 7.52 (dd, *J* = 7.5, 4.8 Hz, 1H), 6.59 (d, *J* = 0.7 Hz, 1H), 2.25 (s, 3H). ^{13}C NMR (50 MHz, DMSO-*d6*): δ 172.84, 160.46, 159.79, 151.75, 148.72, 147.67, 135.03, 131.09, 123.24, 105.89, 17.54. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{11}H_{12}N_6S^+$, 261.09169; Found: 261.09171. HPLC (method A, λ_{280}): Purity 97.1%; t_R: 5.508 min.

N-(N-(4-methylthiazol-2-yl)carbamimidoyl)isonicotinimidamide (**A11**) Synthesized following the general procedure using 1-(4-methylthiazol-2-yl)guanidine (1.00 g, 6.41 mmol), sodium hydride (60% dispersion in mineral oil, 384 mg, 9.61 mmol) and 4-pyridinecarbonitrile (0.667 mg, 6.41 mmol) resulting in a yellow powder (985 mg, 59%, method 2). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 10.37 (br. s, 1H), 9.15 (br. s, 1H), 8.80 (br. s, 1H), 8.74 (dd, *J* = 4.5, 1.6 Hz, 2H), 7.89 (dd, *J* = 4.5, 1.6 Hz, 2H), 7.68 (br. s, 1H), 6.60 (d, *J* = 0.9 Hz, 1H), 2.25 (d, *J* = 0.8 Hz, 3H). ^{13}C NMR (50 MHz, DMSO-*d6*): δ 172.77,

160.43, 159.58, 150.08, 147.74, 142.81, 121.45, 106.08, 17.54. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{11}H_{12}N_6S^+$, 261.09169; Found: 261.09174. HPLC (method A, λ_{280}): Purity 99.5%; t_R: 4.525 min.

N-(N-(4,5-dimethylthiazol-2-yl)carbamimidoyl)benzimidamide (**B1**) Synthesized following the general procedure using 1-(4,5-dimethylthiazol-2-yl)guanidine (1.00 g, 5.88 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 353 mg, 8.82 mmol) and benzonitrile (0.605 mL, 5.88 mmol) resulting in a light yellow powder (1.21 g, 81%, method 1). ^{1}H NMR (200 MHz, DMSO-*d6*): δ 10.45 (s, 1H), 8.94 (s, 1H), 8.61 (s, 1H), 7.98 (dd, *J* = 7.9, 1.7 Hz, 2H), 7.84 – 7.31 (m, 4H), 2.21 (d, *J* = 0.6 Hz, 3H), 2.15 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*): δ 169.26, 161.31, 160.33, 142.65, 135.47, 131.06, 128.12, 127.30, 116.88, 14.58, 10.70. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{13}H_{15}N_5S^+$, 274.11209; Found: 274.11212. HPLC (method A, λ_{280}): Purity 97.5%; t_R: 5.408 min.

2-chloro-N-(N-(4,5-dimethylthiazol-2-yl)carbamimidoyl)benzimidamide (**B2**) Synthesized following the general procedure using 1-(4,5-dimethylthiazol-2-yl)guanidine (1.00 g, 5.88 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 353 mg, 8.82 mmol) and 2-chlorobenzonitrile (809 mg, 5.88 mmol) resulting in a light yellow powder (1.10 mg, 73%, method 1). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 10.30 (br. s, 1H), 9.01 (br. s, 1H), 8.59 (br. s, 1H), 7.46 (m, 5H), 2.21 (s, 3H), 2.14 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*): δ 169.26, 162.01, 160.22, 142.63, 136.82, 130.47, 130.28, 129.56, 129.39, 126.88, 117.00, 14.58, 10.71. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{13}H_{14}ClN_5S^+$, 308.07312; Found: 308.07318. HPLC (method A, λ_{280}): Purity 95.4%; t_R: 5.425 min.

3-chloro-N-(N-(4,5-dimethylthiazol-2-yl)carbamimidoyl)benzimidamide (**B3**) Synthesized following the general procedure using 1-(4,5-dimethylthiazol-2-yl)guanidine (1.00 g, 5.88 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 353 mg, 8.82 mmol) and 3-chlorobenzonitrile (809 mg, 5.88 mmol) resulting in a pale yellow powder (1180 mg, 79%, method 1). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 10.44 (br. s, 1H), 9.04 (br. s, 1H), 8.68 (br. s, 1H), 8.08 – 8.03 (m, 1H), 7.93 (d, *J* = 7.9 Hz, 1H), 7.61 (ddd, *J* = 8.0, 2.1, 0.9 Hz, 1H), 7.78 – 7.39 (br. s, 1H), 7.52 (t, *J* = 7.9 Hz, 1H), 2.21 (s, 3H), 2.15 (s, 3H). ^{13}C NMR (50 MHz, DMSO-*d6*): δ 169.15, 160.14, 159.71, 142.73, 137.49, 133.13, 130.91, 130.15, 127.31, 125.93, 117.14, 14.62, 10.75. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{13}H_{14}ClN_5S^+$, 308.07312; Found: 308.07321. HPLC (method A, λ_{280}): Purity 95.2%; t_R: 6.075 min.

4-chloro-N-(N-(4,5-dimethylthiazol-2-yl)carbamimidoyl)benzimidamide (**B4**) Synthesized following the general procedure using 1-(4,5-dimethylthiazol-2-yl)guanidine (1.00 g, 5.88 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 353 mg, 8.82 mmol) 4-chlorobenzonitrile (809 mg, 5.88 mmol) resulting in a pale yellow powder (1.32 g, 88%, method 1). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 10.44 (br. s, 1H), 9.04 (br. s, 1H), 8.64 (br. s, 1H), 8.01 (d, *J* = 8.6 Hz, 2H), 7.57 (d, br. s, *J* = 8.6 Hz, 3H), 2.22 (s, 3H), 2.15 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*): δ 169.15, 160.16, 160.11, 142.66,

135.93, 134.23, 129.19 (2C), 128.22 (2C), 117.02, 14.57, 10.70. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{13}H_{14}ClN_5S^+$, 308.07312; Found: 308.07315. HPLC (method A, λ_{280}): Purity 98.0%; t_R: 6.267 min.

2-bromo-N-(N-(4,5-dimethylthiazol-2-yl)carbamimidoyl)benzimidamide (**B5**) Synthesized following the general procedure using 1-(4,5-dimethylthiazol-2-yl)guanidine (1.00 g, 5.88 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 353 mg, 8.82 mmol) and 2-bromobenzonitrile (1.07 g, 5.88 mmol) resulting in a yellow powder (1.33 g, 78%, method 1). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 10.28 (br. s, 1H), 9.01 (br. s, 1H), 8.58 (br. s, 1H), 7.70-7.30 (br. s, 1H), 7.67 (d, *J* = 7.9 Hz, 1H), 7.44 (dd, *J* = 8.1, 5.2 Hz, 2H), 7.40 – 7.33 (m, 1H), 2.21 (s, 3H), 2.14 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*): δ 169.26, 163.14, 160.22, 142.62, 138.85, 132.48, 130.54, 129.44, 127.36, 119.64, 116.98, 14.58, 10.71. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{13}H_{14}BrN_5S^+$, 352.02261; Found: 352.02289. HPLC (method A, λ_{280}): Purity 97.8%; t_R: 5.717 min.

2-bromo-N-(N-(4,5-dimethylthiazol-2-yl)carbamimidoyl)benzimidamide (**B6**) Synthesized following the general procedure using 1-(4,5-dimethylthiazol-2-yl)guanidine (1.00 g, 5.88 mmol), sodium hydride (60 % dispersion in mineral oil, 1.5 eq., 353 mg, 8.82 mmol) and 3-bromobenzonitrile (1.07 g, 5.88 mmol) resulting in a yellow powder (1.45 g, 85%, method 1). ^{1}H NMR (200 MHz, DMSO-*d6*): δ 10.45 (br. s, 1H), 9.01 (br. s, 1H), 8.70 (br. s, 1H), 8.20 (t, *J* = 1.7 Hz, 1H), 8.06-7.28 (br. s, 1H), 7.97 (d, *J* = 8.0 Hz, 1H), 7.74 (ddd, *J* = 7.9, 1.9, 0.8 Hz, 1H), 7.46 (t, *J* = 7.9 Hz, 1H), 2.21 (s, 3H), 2.15 (s, 3H). ^{13}C NMR (50 MHz, DMSO-*d6*): δ 169.14, 160.12, 159.63, 142.74, 137.65, 133.81, 130.41, 130.18, 126.29, 121.61, 117.13, 14.63, 10.76. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{13}H_{15}BrN_5S^+$, 352.02261; Found: 352.02377. HPLC (method A, λ_{280}): Purity 97.3%; t_R: 5.767 min.

N-(N-(4,5-dimethylthiazol-2-yl)carbamimidoyl)picolinimidamide (**B7**) Synthesized following the general procedure using 1-(4,5-dimethylthiazol-2-yl)guanidine (1.00 g, 5.88 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 353 mg, 8.82 mmol) and 2-pyridinecarbonitrile (0.566 mL, 5.88 mmol) resulting in a yellow powder (1.24 g, 94%, method 1). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 10.19 (br. s, 1H), 9.09 (br. s, 1H), 8.68 (d, br. s. *J* = 4.1 Hz, 2H), 8.33 (d, *J* = 7.9 Hz, 1H), 7.99 (td, *J* = 7.7, 1.3 Hz, 1H), 7.60 (dd, br. s, *J* = 6.5, 5.0 Hz, 2H), 2.21 (s, 3H), 2.15 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*): δ 169.29, 160.32, 158.01, 150.80, 148.50, 142.71, 137.28, 126.19, 121.80, 117.16, 14.56, 10.69. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{12}H_{14}N_6S^+$, 275.10734; Found: 275.10742. HPLC (method A, λ_{280}): Purity >99.9%%; t_R: 5.475 min.

N-(N-(4,5-dimethylthiazol-2-yl)carbamimidoyl)nicotinimidamide (**B8**) Synthesized following the general procedure using 1-(4,5-dimethylthiazol-2-yl)guanidine (1.00 g, 5.88 mmol), sodium hydride (60% dispersion in mineral oil, 353 mg, 8.82 mmol) and 3-pyridinecarbonitrile (612 mg, 5.88 mmol)

resulting in a yellow powder (742 mg, 46%, method 2). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 10.43 (br. s, 1H), 9.04 (br. s, 1H), 8.67 (br. s, 1H), 8.20 (t, *J* = 1.6 Hz, 1H), 7.97 (d, *J* = 8.0 Hz, 1H), 7.80 – 7.70 (m, 1H), 7.88 – 7.29 (br. s, 1H), 7.46 (t, *J* = 7.9 Hz, 1H), 2.21 (s, 3H), 2.15 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*): δ 169.13, 160.11, 159.55, 151.63, 148.60, 142.72, 134.89, 131.06, 123.17, 117.16, 14.57, 10.69. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{12}H_{14}N_6S^+$, 275.10734; Found: 275.10736. HPLC (method A, λ_{280}): Purity 97.0%; t_R: 5.458 min.

N-(N-(4,5-dimethylthiazol-2-yl)carbamimidoyl)isonicotinimidamide (**B9**) Synthesized following the general procedure using 1-(4,5-dimethylthiazol-2-yl)guanidine (1.00 g, 5.88 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 353 mg, 8.82 mmol) and 4-pyridinecarbonitrile (612 mg, 5.88 mmol) resulting in a yellow powder (548 mg, 34%, method 2). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 10.40 (br. s, 1H), 9.09 (br. s, 1H), 8.73 (d, br. s., *J* = 5.7 Hz, 3H), 7.88 (d, *J* = 5.5 Hz, 2H), 7.61 (br. s, 1H), 2.21 (s, 3H), 2.15 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*): δ 169.05, 160.06, 159.33, 149.98, 142.78, 121.31, 117.38, 14.55, 10.68. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{12}H_{14}N_6S^+$, 275.10734; Found: 275.10739. HPLC (method A, λ_{280}): Purity 99.5%; t_R: 5.108 min.

N-(N-(4-phenylthiazol-2-yl)carbamimidoyl)benzimidamide (**C1**) Synthesized following the general procedure using 1-(4-phenylthiazol-2-yl)guanidine (1.00 g, 4.58 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 275 mg, 6.88 mmol) and benzonitrile (0.47 mL, 4.58 mmol) resulting in a yellow powder (1.10 g, 87%, method 1). ^{1}H NMR (400 MHz, DMSO-d6) δ 10.32 (s, 1H), 9.11 (s, 1H), 8.67 (s, 1H), 8.06 – 7.96 (m, 2H), 7.91 – 7.84 (m, 2H), 7.71 (s, 1H), 7.59 – 7.46 (m, 4H), 7.43 (t, *J* = 7.6 Hz, 2H), 7.31 (t, *J* = 7.3 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 173.13, 161.62, 160.86, 149.77, 135.38, 134.53, 131.26, 128.70 (2C), 128.22 (2C), 127.58, 127.47 (2C), 125.56 (2C), 106.38. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{17}H_{16}N_5S^+$, 322.11209; Found: 322.11144. UPLC (method B, λ_{280}): Purity >99.9%; t_R: 3.767 min.

2-chloro-N-(N-(4-phenylthiazol-2-yl)carbamimidoyl)benzimidamide (**C2**) Synthesized following the general procedure using 1-(4-phenylthiazol-2-yl)guanidine (1.00 g, 4.58 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 275 mg, 6.88 mmol) and 2-chlorobenzonitrile (631 mg, 4.58 mmol) resulting in a beige powder (847 mg, 52%, method 2).^{1}H NMR (400 MHz, DMSO-d6) δ 10.20 (s, 1H), 9.11 (s, 1H), 8.69 (s, 1H), 7.86 (d, *J* = 7.2 Hz, 3H), 7.54 (s, 2H), 7.47 (d, *J* = 5.4 Hz, 2H), 7.43 (d, *J* = 6.3 Hz, 3H), 7.34 – 7.27 (m, 1H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 173.17, 162.43, 160.71, 149.72, 136.82, 134.51, 130.56, 130.30, 129.58, 129.43, 128.69 (2C), 127.56, 126.95, 125.53 (2C), 106.51. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{17}H_{15}N_5ClS^+$, 356.07312; Found: 356.07231. UPLC (method B, λ_{280}): Purity >99.9%; t_R: 3.693 min.

3-chloro-N-(N-(4-phenylthiazol-2-yl)carbamimidoyl)benzimidamide (**C3**) Synthesized following the general procedure using 1-(4-phenylthiazol-2-yl)guanidine (1.00 g, 4.58 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 275 mg, 6.87 mmol) and 3-chlorobenzonitrile (630 mg, 4.58 mmol) resulting in a white-yellowish powder (1.24 g, 89%, method 1). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 10.36 (br. s, 1H), 9.15 (br. s, 1H), 8.78 (br. s, 1H), 8.10 (t, *J* = 1.7 Hz, 1H), 7.98 (d, *J* = 7.9 Hz, 1H), 7.88 (d, br. s, *J* = 7.2 Hz, 3H), 7.63 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.54 (t, *J* = 7.9 Hz, 1H), 7.48 (s, 1H), 7.43 (t, *J* = 7.7 Hz, 2H), 7.31 (t, *J* = 7.3 Hz, 1H). ^{13}C NMR (50 MHz, DMSO-*d6*): δ 173.05, 160.66, 160.04, 149.85, 137.43, 134.51, 133.20, 131.03, 130.14, 128.69, 127.59, 127.46, 126.08, 125.58, 106.53. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{17}H_{14}ClN_5S^+$, 356.07312; Found: 356.07321. HPLC (method A, λ_{280}): Purity 98.0%; t_R: 7.008 min.

4-chloro-N-(N-(5-phenylthiazol-2-yl)carbamimidoyl)benzimidamide (**C4**) Synthesized following the general procedure using 1-(4-phenylthiazol-2-yl)guanidine (1.00 g, 4.58 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 275 mg, 6.87 mmol) and 4-chlorobenzonitrile (630 mg, 4.58 mmol) resulting in a pale yellow powder (1.26 mg, 90%, method 1). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 10.35 (br. s, 1H), 9.14 (br. s, 1H), 8.73 (br. s, 1H), 8.05 (d, *J* = 8.6 Hz, 2H), 7.87 (d, *J* = 7.2 Hz, 2H), 7.73 (br. s, 1H), 7.58 (d, *J* = 8.6 Hz, 2H), 7.47 (s, 1H), 7.43 (t, *J* = 7.7 Hz, 2H), 7.31 (t, *J* = 7.3 Hz, 1H). ^{13}C NMR (50 MHz, DMSO-*d6*): δ 173.07, 160.72, 160.43, 149.82, 136.13, 134.52, 134.17, 129.37, 128.69, 128.31, 127.58, 125.57, 106.47. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{17}H_{14}ClN_5S^+$, 356.07312; Found: 356.07318. HPLC (method A, λ_{280}): Purity 99.1%; t_R: 6.925 min.

3-bromo-N-(N-(4-phenylthiazol-2-yl)carbamimidoyl)benzimidamide (**C5**) Synthesized following the general procedure using 1-(4-phenylthiazol-2-yl)guanidine (1.00 g, 4.58 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 275 mg, 6.87 mmol) and 3-bromobenzonitrile (833 mg, 4.58 mmol) resulting in a pale yellow powder (1.44 mg, 92%, method 1). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 10.36 (br. s, 1H), 9.15 (br. s, 1H), 8.79 (br. s, 1H), 8.25 (s, 1H), 8.02 (d, *J* = 7.8 Hz, 1H), 7.88 (d, br. s, *J* = 7.3 Hz, 3H), 7.76 (dd, *J* = 7.9, 1.0 Hz, 1H), 7.47 (dd, *J* = 9.1, 6.6 Hz, 2H), 7.43 (t, *J* = 7.7 Hz, 2H), 7.31 (t, *J* = 7.3 Hz, 1H). ^{13}C NMR (50 MHz, DMSO-*d6*): δ 173.11, 160.69, 160.04, 149.88, 137.62, 134.52, 133.94, 130.38, 128.71, 127.61, 126.46, 125.61, 121.73, 106.53. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{17}H_{14}BrN_5S^+$, 400.02261; Found: 400.02213. HPLC (method A, λ_{280}): Purity 98.7%; t_R: 7.033 min.

4-methoxy-N-(N-(4-phenylthiazol-2-yl)carbamimidoyl)benzimidamide (**C6**) Synthesized following the general procedure using 1-(4-phenylthiazol-2-yl)guanidine (1.00 g, 4.58 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 275 mg, 6.87 mmol) and 4-methoxybenzonitrile (610 mg, 4.58 mmol) resulting in a beige powder (1.31 mg, 82%, method 1). ^{1}H NMR (400 MHz, DMSO-*d6*): δ 10.30 (br. s, 1H), 9.05 (br. s, 1H), 8.55 (s, 1H), 8.01 (d, *J* = 8.9 Hz, 2H), 7.87 (d, *J* = 7.3 Hz, 2H), 7.68 (br. s,

1H), 7.46 (s, 1H), 7.43 (t, *J* = 7.7 Hz, 1H), 7.31 (t, *J* = 7.3 Hz, 1H), 7.04 (d, *J* = 8.9 Hz, 2H), 3.83 (s, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*): δ 173.16, 161.77, 161.07, 160.84, 149.72, 134.54, 129.21, 128.64, 127.50, 127.36, 125.51, 113.46, 106.11, 55.37, 38.89. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{18}H_{17}N_5OS^+$, 352.12266; Found: 352.12268. HPLC (method A, λ_{280}): Purity 95.1%; t_R: 6.842 min.

N-(N-(4-phenylthiazol-2-yl)carbamimidoyl)picolinimidamide (**C7**) Synthesized following the general procedure using 1-(4-phenylthiazol-2-yl)guanidine (1.00 g, 4.58 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 275 mg, 6.87 mmol) and 2-pyridinecarbonitrile (441 mL, 4.58 mmol) resulting in a yellow powder (1.28 g, 87%, method 1). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 10.16 (br. s, 1H), 9.19 (br. s, 1H), 8.79 (br. s, 1H), 8.70 (d, *J* = 3.6 Hz, 1H), 8.38 (d, *J* = 7.7 Hz, 1H), 8.01 (t, *J* = 7.4 Hz, 1H), 8.19 – 7.70 (m, 4H), 7.88 (d, br. s, *J* = 7.4 Hz, 4H), 7.66 – 7.57 (m, 1H), 7.50 (s, 1H), 7.43 (t, *J* = 7.3 Hz, 2H), 7.32 (t, *J* = 7.0 Hz, 1H). ^{13}C NMR (50 MHz, DMSO-*d6*): δ 173.20, 160.91, 158.35, 150.73, 149.86, 148.61, 137.40, 134.50, 128.70, 127.61, 126.38, 125.59, 122.04, 106.61. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{16}H_{14}N_6S^+$, 323.10734; Found: 323.10770. HPLC (method A, λ_{280}): Purity 98.0%; t_R: 6.692 min.

N-(N-(4-phenylthiazol-2-yl)carbamimidoyl)nicotinimidamide (**C8**) Synthesized following the general procedure using 1-(4-phenylthiazol-2-yl)guanidine (1.00 g, 4.58 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 275 mg, 6.87 mmol) and 3-cyanopyridine (477 mg, 4.58 mmol) resulting in a yellowish powder (694 mg, 47%, method 2). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 10.30 (br. s, 1H), 9.17 (d, br. s, *J* = 1.6 Hz, 2H), 8.82 (br. s, 1H), 8.73 (dd, *J* = 4.7, 1.4 Hz, 1H), 8.33 (d, *J* = 8.0 Hz, 1H), 7.88 (d, br. s, *J* = 7.3 Hz, 3H), 7.54 (dd, *J* = 7.8, 4.8 Hz, 1H), 7.49 (s, 1H), 7.43 (t, *J* = 7.6 Hz, 2H), 7.32 (t, *J* = 7.3 Hz, 1H). ^{13}C NMR (50 MHz, DMSO-*d6*): δ 173.07, 160.68, 159.88, 151.80, 149.86, 148.77, 135.10, 134.52, 131.05, 128.71, 127.60, 125.59, 123.25, 106.58. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{16}H_{14}N_6S^+$, 323.10734; Found: 323.10764. HPLC (method A, λ_{280}): Purity 96.9%; t_R: 6.075 min.

N-(N-(4-phenylthiazol-2-yl)carbamimidoyl)isonicotinimidamide (**C9**) Synthesized following the general procedure using 1-(4-phenylthiazol-2-yl)guanidine (1.00 g, 4.58 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 275 mg, 6.87 mmol) and 4-pyridinecarbonitrile (477 mg, 4.58 mmol) resulting in a yellowish powder (871 mg, 59%, method 2). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 10.30 (br. s, 1H), 9.20 (br. s, 1H), 8.86 (br. s, 1H), 8.75 (dd, *J* = 4.5, 1.6 Hz, 2H), 7.92 (dd, *J* = 4.5, 1.6 Hz, 2H), 7.88 (d, br. s, *J* = 7.2 Hz, 3H), 7.50 (s, 1H), 7.43 (t, *J* = 7.7 Hz, 2H), 7.32 (t, *J* = 7.3 Hz, 1H). ^{13}C NMR (50 MHz, DMSO-*d6*): δ 173.00, 160.64, 159.63, 150.09, 149.90, 142.74, 134.50, 128.72, 127.63, 125.61, 121.49, 106.75. HRMS-ESI (m/z): $[M+H]^+$ calc. for $C_{16}H_{14}N_6S^+$, 323.10734; Found: 323.10757. HPLC (method A, λ_{280}): Purity 95.2%; t_R: 6.058 min.

N-(N-(4-(3-nitrophenyl)thiazol-2-yl)carbamimidoyl)benzimidamide (**D1**) Synthesized following the general procedure A using 1-(4-(3-nitrophenyl)thiazol-2-yl)guanidine (1.00 g, 3.80 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 228 mg, 5.70 mmol) and benzonitrile (0.39 mL, 5.20 mmol) resulting in a orange powder (933 mg, 67%, method 2).^{1}H NMR (400 MHz, DMSO-d6) δ 10.17 (s, 1H), 8.63 (t, *J* = 2.0 Hz, 3H), 8.34 (d, *J* = 8.1 Hz, 1H), 8.16 (dd, *J* = 8.3, 1.9 Hz, 1H), 8.14 – 7.81 (m, 3H), 7.81 (s, 1H), 7.73 (t, *J* = 8.0 Hz, 1H), 7.59 – 7.44 (m, 3H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 173.40, 161.58, 161.01, 148.39, 147.43, 136.09, 135.28, 131.84, 131.30, 130.32, 128.23 (2C), 127.51 (2C), 122.05, 119.77, 109.19. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{17}H_{15}O_2N_6S^+$, 367.09717; Found: 367.09622. UPLC (method B, λ_{280}): Purity >99.9%%; t_R: 3.733 min. $[M+H]^+$ calc. for $C_{17}H_{15}O_2N_6S^+$, 367.09717; Found: 367.09622.

2-chloro-N-(N-(4-(3-nitrophenyl)thiazol-2-yl)carbamimidoyl)benzimidamide (**D2**) Synthesized following the general procedure A using 1-(4-(3-nitrophenyl)thiazol-2-yl)guanidine (1.00 g, 3.80 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 228 mg, 5.70 mmol) and 2-chlorobenzonitrile (523 mg, 3.80 mmol) resulting in an orange powder (1.26 g, 82%, method 1). ^{1}H NMR (400 MHz, DMSO-*d6*): δ 10.08 (br. s, 1H), 8.83 (br. s, 1H), 8.70 (br. s, 1H), 8.62 (s, 1H), 8.34 (d, *J* = 7.7 Hz, 1H), 8.15 (d, *J* = 7.4 Hz, 1H), 8.03-7.64 (br. s, 1H), 7.81 (s, 1H), 7.72 (t, *J* = 7.7 Hz, 1H), 7.52 (t, *J* = 7.8 Hz, 2H), 7.49 – 7.37 (m, 2H). ^{13}C NMR (101 MHz, DMSO-*d6*): δ 173.55, 162.25, 160.72, 148.36, 147.36, 136.69, 136.00, 131.76, 130.52, 130.25 (2C), 129.51, 129.39, 126.87, 121.95, 119.70, 109.25. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{17}H_{13}ClN_6O_2S^+$, 401.05820; Found: 401.05820. HPLC (method A, λ_{280}): Purity 95.2%; t_R: 6.858 min.

3-chloro-N-(N-(4-(3-nitrophenyl)thiazol-2-yl)carbamimidoyl)benzimidamide (**D3**) Synthesized following the general procedure using 1-(4-(3-nitrophenyl)thiazol-2-yl)guanidine (1.00 g, 3.80 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 228 mg, 5.70 mmol) and 3-chlorobenzonitrile (523 mg, 3.80 mmol) resulting in a yellow powder (1.28 g, 84%, method 1). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 10.22 (br. s, 1H), 8.77 (br. s, 1H), 8.62 (s, 1H), 8.34 (d, *J* = 7.7 Hz, 1H),8.23-7.91 (br. s, 1H), 8.15 (d, *J* = 8.0 Hz, 1H), 8.09 (s, 1H), 7.97 (d, *J* = 7.7 Hz, 1H), 7.80 (s, 1H), 7.72 (t, *J* = 8.0 Hz, 1H), 7.63 (d, *J* = 7.9 Hz, 1H), 7.54 (t, *J* = 7.9 Hz, 1H). ^{13}C NMR (50 MHz, DMSO-*d6*): δ 173.31, 160.75, 160.02, 148.34, 147.48, 137.34, 136.02, 133.17, 131.83, 131.05, 130.24, 130.17, 127.45, 126.11, 122.02, 119.78, 109.32. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{17}H_{13}ClN_6O_2S^+$, 401.05820; Found: 401.05835. HPLC (method A, λ_{280}): Purity 95.5%; t_R: 7.025 min.

4-chloro-N-(N-(4-(3-nitrophenyl)thiazol-2-yl)carbamimidoyl)benzimidamide (**D4**) Synthesized following the general procedure using 1-(4-(3-nitrophenyl)thiazol-2-yl)guanidine (1.00 g, 3.80 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 228 mg, 5.70 mmol) and 4-chlorobenzonitrile

(523 mg, 3.80 mmol) resulting in a dark yellow powder (1.09 mg, 81%, method 1). ^{1}H NMR (200 MHz, DMSO-*d6*): δ 10.15 (br. s, 1H), 8.74 (br. s, 2H), 8.68 – 8.60 (m, 1H), 8.55-7.90 (br. s, 1H), 8.39 – 8.30 (m, 1H), 8.16 (ddd, *J* = 8.2, 2.3, 0.8 Hz, 1H), 8.10 – 7.97 (m, 2H), 7.82 (s, 1H), 7.72 (t, *J* = 8.0 Hz, 1H), 7.66 – 7.48 (m, 2H). ^{13}C NMR (50 MHz, DMSO-*d6*): δ 173.37, 160.84, 160.46, 148.30, 147.47, 136.17, 136.04, 134.10, 131.77, 130.16, 129.40 (2C), 128.29 (2C), 121.96, 119.77, 109.19. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{17}H_{13}ClN_6O_2S^+$, 401.05820; Found: 401.05853. HPLC (method A, λ_{280}): Purity 96.7%; t_R: 7.033 min.

3-bromo-N-(N-(4-(3-nitrophenyl)thiazol-2-yl)carbamimidoyl)benzimidamide (**D5**) Synthesized following the general procedure using 1-(4-(3-nitrophenyl)thiazol-2-yl)guanidine (1.00 g, 3.80 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 228 mg, 5.70 mmol) and 3-bromobenzonitrile (692 mg, 3.80 mmol) resulting in a yellow powder (1.61 g, 95%, method 1). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 10.21 (s, 1H), 8.78 (s, 2H), 8.62 (s, 1H), 8.34 (d, *J* = 7.5 Hz, 1H), 8.23 (s, 1H), 8.19 (br. s, 1H), 8.15 (d, *J* = 7.8 Hz, 1H), 8.01 (d, *J* = 7.5 Hz, 1H), 7.80 (s, 1H), 7.76 (d, *J* = 7.7 Hz, 1H), 7.72 (t, *J* = 7.9 Hz, 1H), 7.48 (t, *J* = 7.8 Hz, 1H). ^{13}C NMR (50 MHz, DMSO-*d6*): δ 173.32, 160.75, 159.98, 148.31, 147.48, 137.51, 136.01, 133.95, 131.81, 130.37, 130.20, 126.47, 121.99, 121.66, 119.77, 109.28. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{17}H_{13}BrN_6O_2S^+$, 445.00768; Found: 445.00806. HPLC (method A, λ_{280}): Purity 99.1%; t_R: 7.100 min.

N-(N-(4-(3-nitrophenyl)thiazol-2-yl)carbamimidoyl)picolinimidamide (**D6**) Synthesized following the general procedure A using 1-(4-(3-nitrophenyl)thiazol-2-yl)guanidine (1.00 g, 3.80 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 228 mg, 5.70 mmol) and 2-pyridinecarbonitrile (366 mL, 3.80 mmol) resulting in a yellow powder (1.26 g, 90%, method 1). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 10.03 (br. s, 1H), 9.08 (br. s, 1H), 8.78 (br. s, 1H), 8.71 (d, *J* = 4.2 Hz, 1H), 8.66 – 8.61 (m, 1H), 8.38 (d, *J* = 7.9 Hz, 1H), 8.35 (d, *J* = 7.9 Hz, 1H), 8.16 (dd, *J* = 8.1, 1.5 Hz, 1H), 8.02 (td, *J* = 7.8, 1.6 Hz, 1H), 7.91 (br. s, 1H), 7.82 (s, 1H), 7.73 (t, *J* = 8.0 Hz, 1H), 7.63 (ddd, *J* = 7.4, 4.8, 1.0 Hz, 1H). ^{13}C NMR (50 MHz, DMSO-*d6*): δ 173.49, 161.02, 158.37, 150.67, 148.58, 148.29, 147.51, 137.39, 135.99, 131.78, 130.16, 126.39, 122.10, 121.99, 119.78, 109.31. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{16}H_{13}N_7O_2S^+$, 368.09242; Found: 368.09274. HPLC (method A, λ_{280}): Purity 99.2%; t_R: 6.850 min.

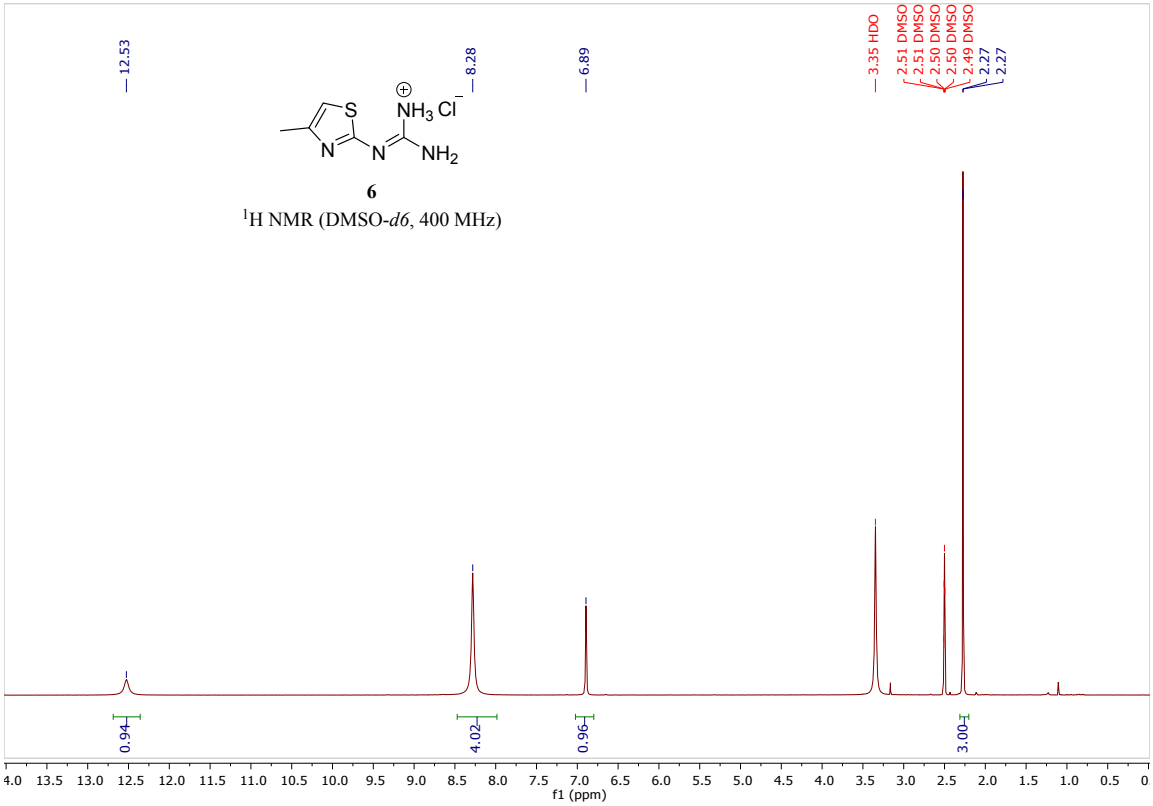
N-(N-(4-(3-nitrophenyl)thiazol-2-yl)carbamimidoyl)nicotinimidamide (**D7**) Synthesized following the general procedure using 1-(4-(3-nitrophenyl)thiazol-2-yl)guanidine (1.00 g, 3.80 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 228 mg, 5.70 mmol) and 3-pyridinecarbonitrile (395 mg, 3.80 mmol) resulting in a yellow powder (656 mg, 47%, method 2). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 10.14 (br s, 1H), 8.84 (br. s, 2H), 8.75 (d, *J* = 5.8 Hz, 2H), 8.63 (s, 1H), 8.34 (d, *J* = 7.8 Hz, 1H), 8.15 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.91 (d, *J* = 5.8 Hz, 2H), 7.86 (br. s, 1H), 7.82 (s, 1H), 7.72 (t, *J* = 8.0 Hz,

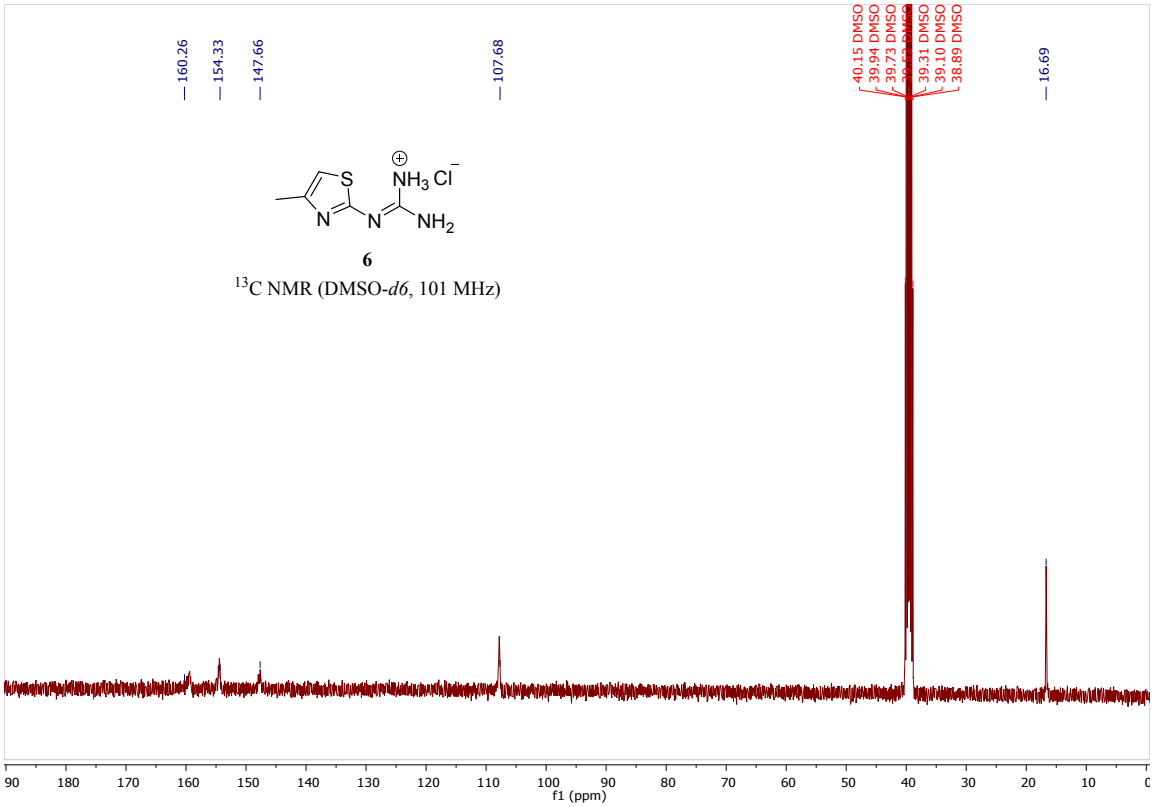
1H). ^{13}C NMR (50 MHz, DMSO-*d6*): δ 173.23, 160.74, 159.55, 150.07, 148.33, 147.49, 142.64, 136.00, 131.80, 130.20, 122.01, 121.49, 119.79, 109.48. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{16}H_{13}N_7O_2S^+$, 368.09242; Found: 368.09283. HPLC (method A, λ_{280}): Purity 95.1%; t_R: 6.350 min.

N-(N-(4-(3-nitrophenyl)thiazol-2-yl)carbamimidoyl)isonicotinimidamide (**D8**) Synthesized following the general procedure using 1-(4-(3-nitrophenyl)thiazol-2-yl)guanidine (1.00 g, 3.80 mmol), sodium hydride (60% dispersion in mineral oil, 1.5 eq., 228 mg, 5.70 mmol) and 4-pyridinecarbonitrile (395 mg, 3.80 mmol) resulting in a yellow powder (544 mg, 39%, method 2). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 10.14 (br. s, 1H), 9.17 (d, *J* = 1.6 Hz, 1H), 8.81 (br. s, 2H), 8.73 (dd, *J* = 4.7, 1.4 Hz, 1H), 8.63 (s, 1H), 8.33 (t, *J* = 8.4 Hz, 2H), 8.15 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.81 (s, 1H), 7.76 (br. s, 1H), 7.72 (t, *J* = 8.0 Hz, 1H), 7.54 (dd, *J* = 7.8, 4.8 Hz, 1H). ^{13}C NMR (50 MHz, DMSO-*d6*): δ 173.30, 160.78, 159.84, 151.81, 148.78, 148.32, 147.48, 136.02, 135.13, 131.79, 130.97, 130.19, 123.25, 121.99, 119.79, 109.31. HRMS-ESI *(m/z)*: $[M+H]^+$ calc. for $C_{16}H_{13}N_7O_2S^+$, 368.09242; Found: 368.09283. HPLC (method A, λ_{280}): Purity 96.8%; t_R: 6.367 min.

II. Spectra for all compounds

1-(4-methylthiazol-2-yl)guanidine hydrochloride (**6**)

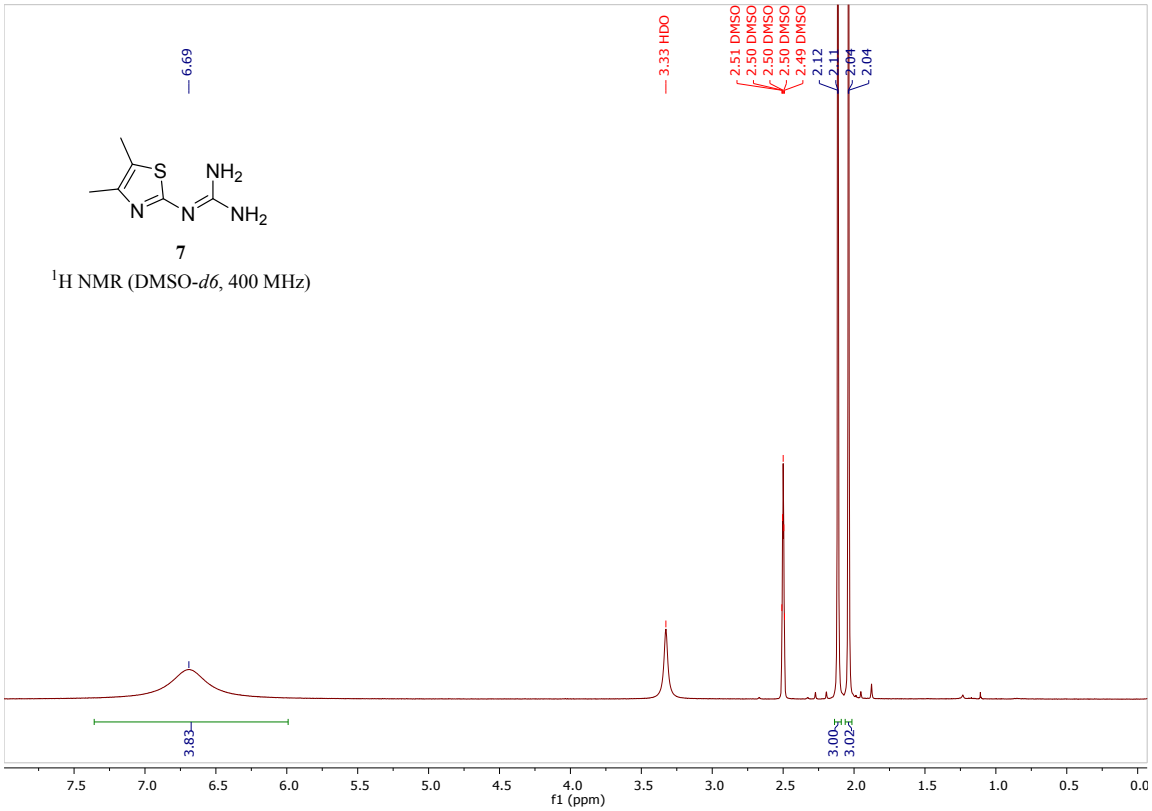




6

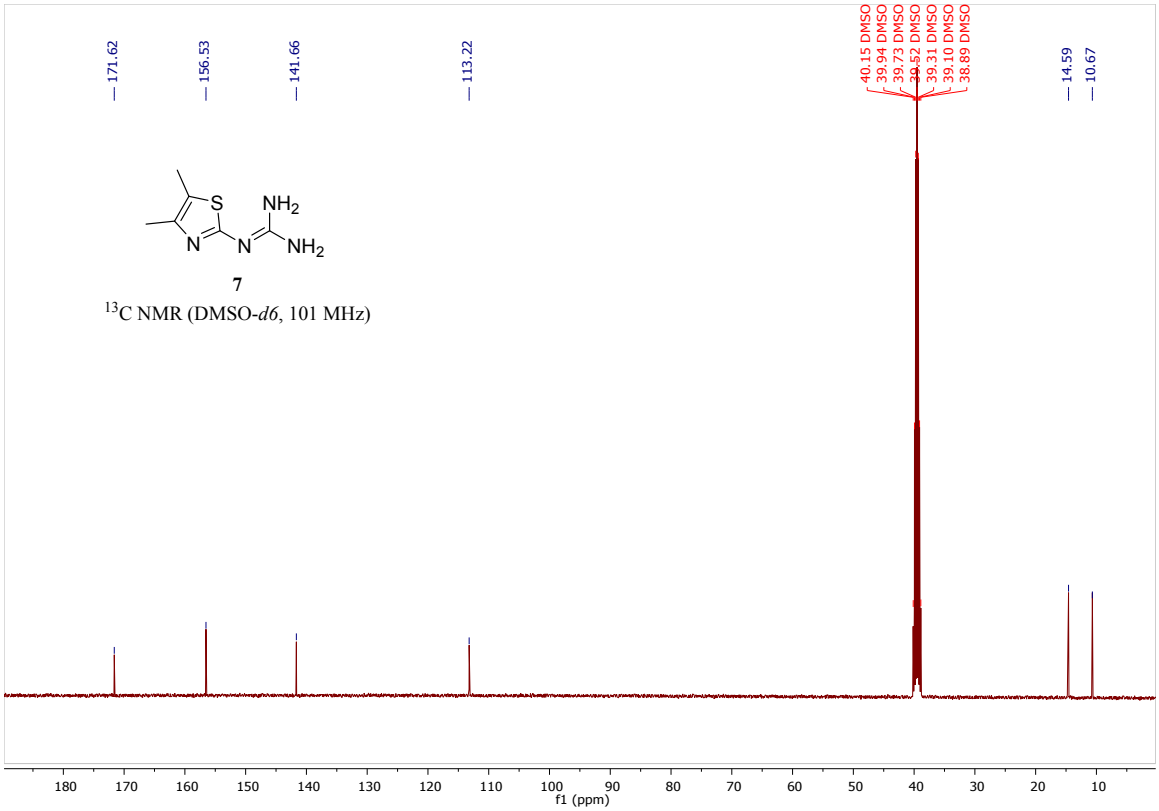
^{13}C NMR (DMSO-*d6*, 101 MHz)

1-(4,5-dimethylthiazol-2-yl)guanidine (**7**)



7

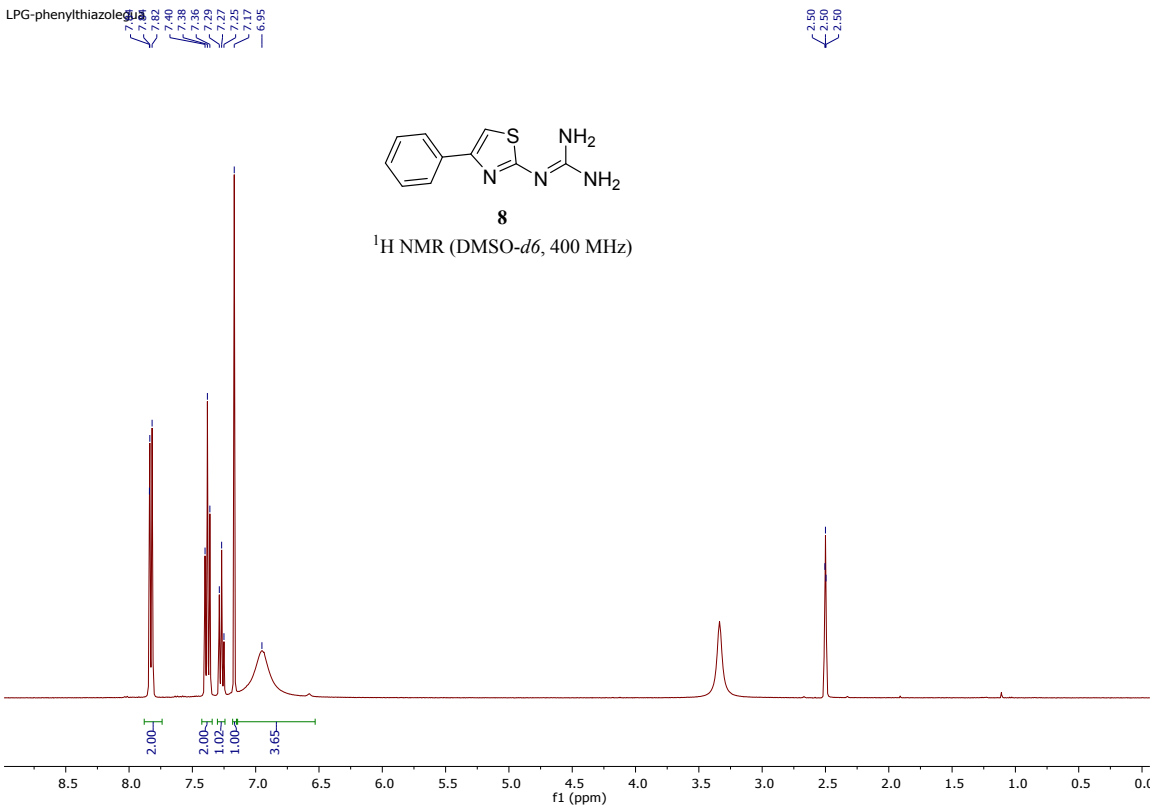
^{1}H NMR (DMSO-*d6*, 400 MHz)



7

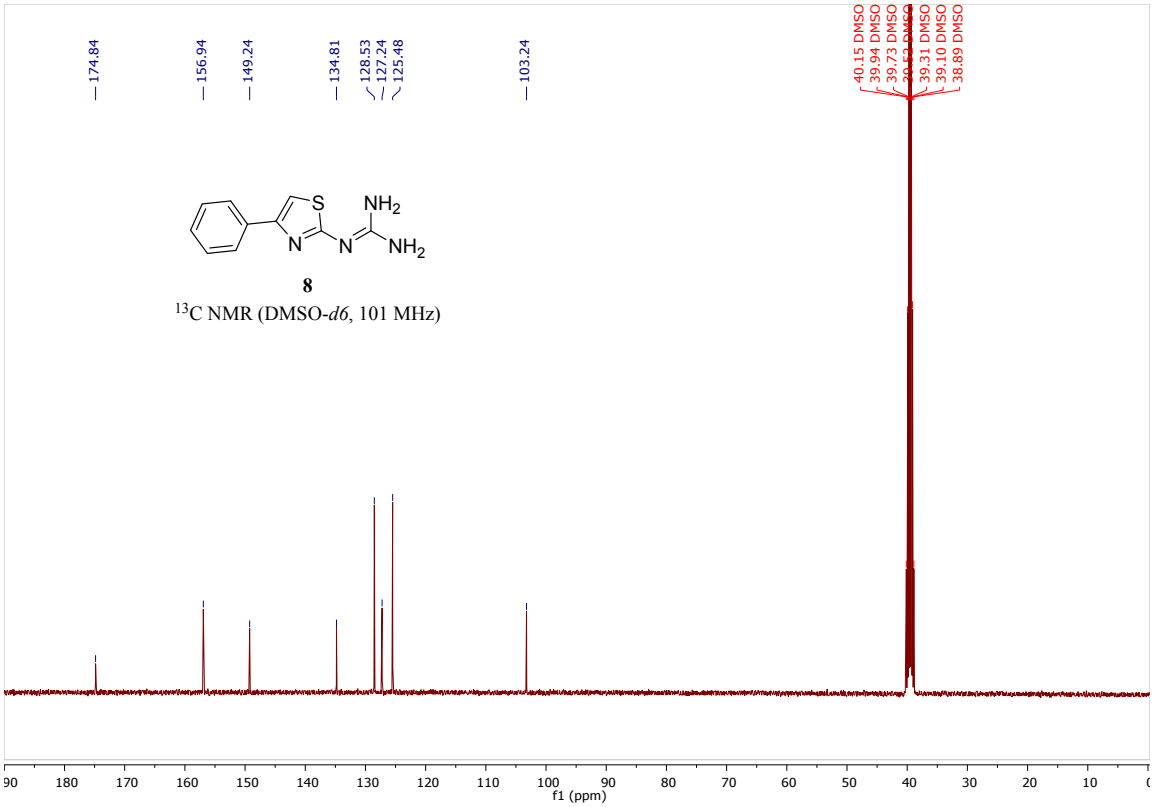
^{13}C NMR (DMSO-*d6*, 101 MHz)

1-(4-phenylthiazol-2-yl)guanidine (**8**)



8

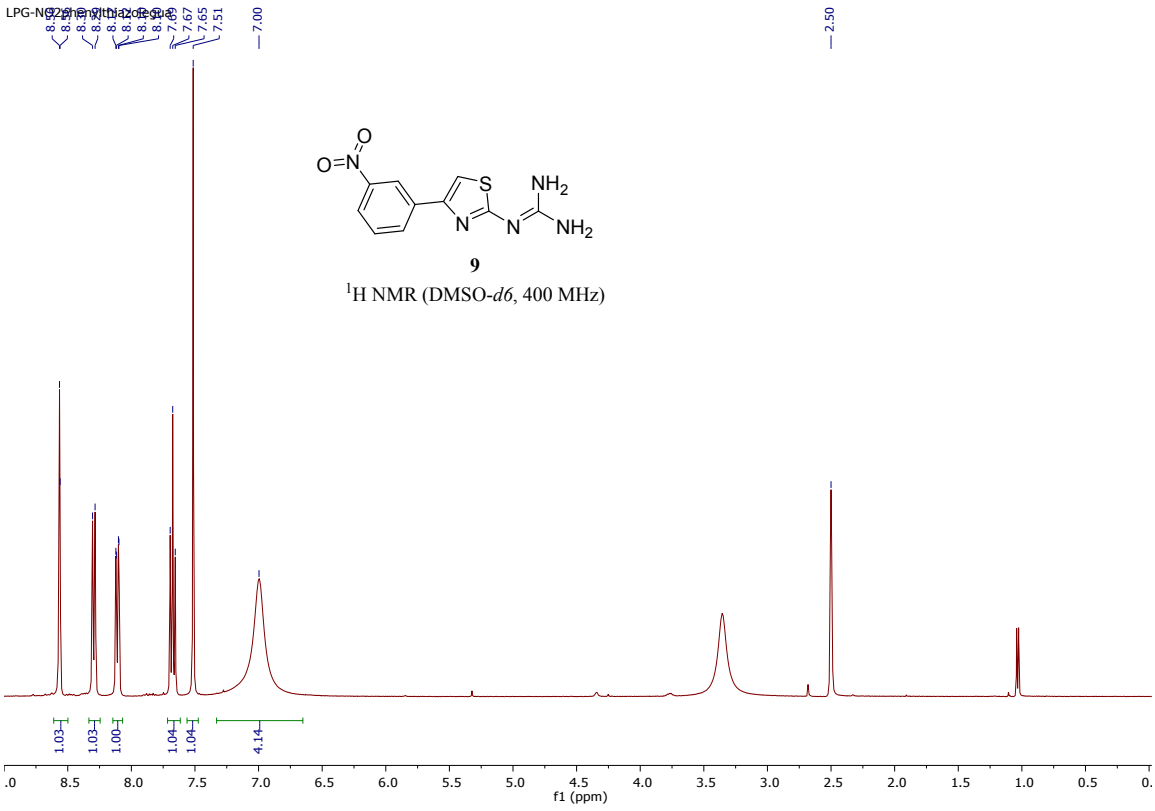
^{1}H NMR (DMSO-*d6*, 400 MHz)



8

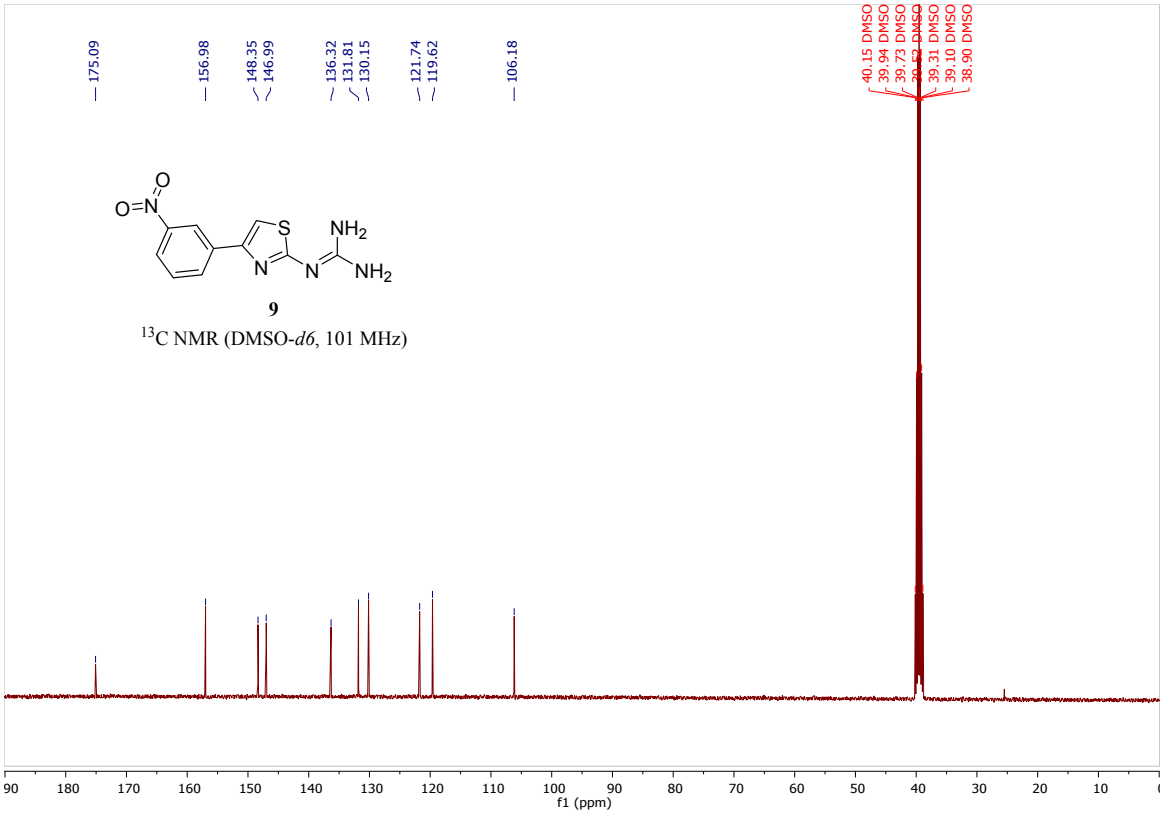
^{13}C NMR (DMSO-*d6*, 101 MHz)

1-(4-(3-nitrophenyl)thiazol-2-yl)guanidine (**9**)



9

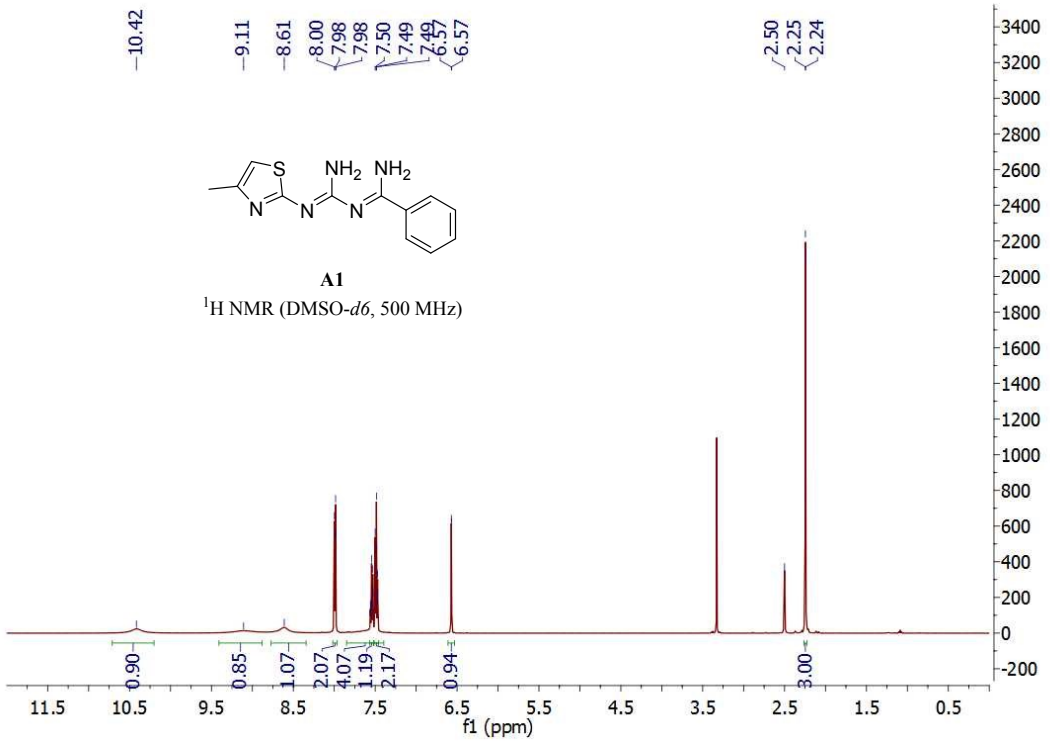
^{1}H NMR (DMSO-*d6*, 400 MHz)



9

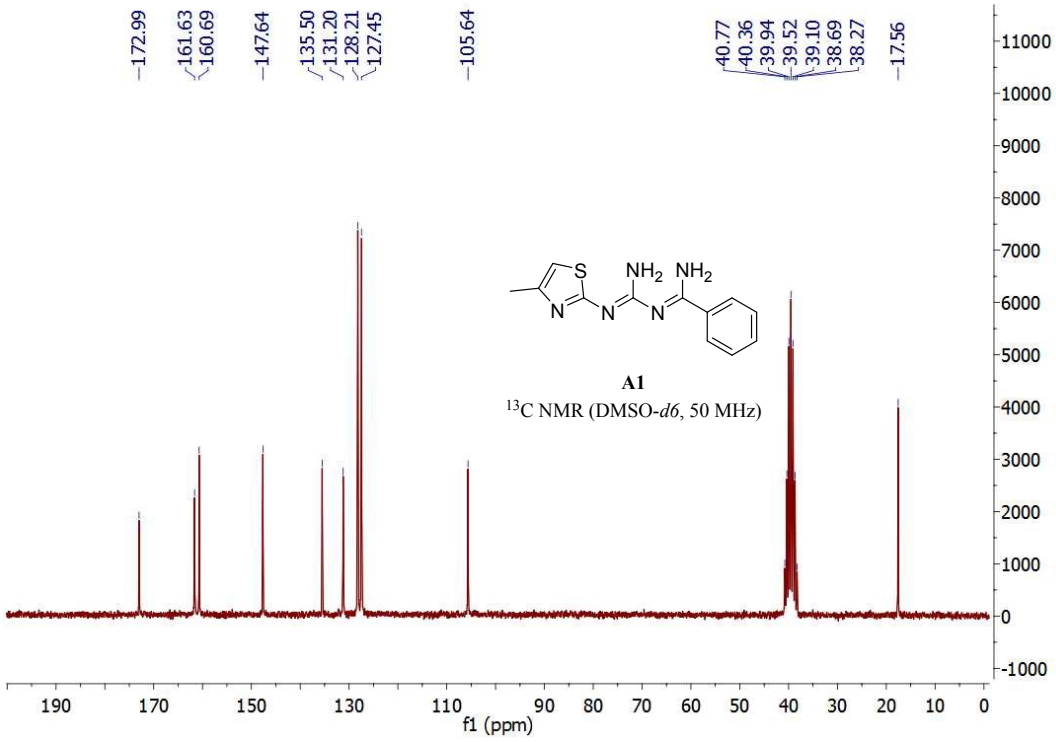
^{13}C NMR (DMSO-*d6*, 101 MHz)

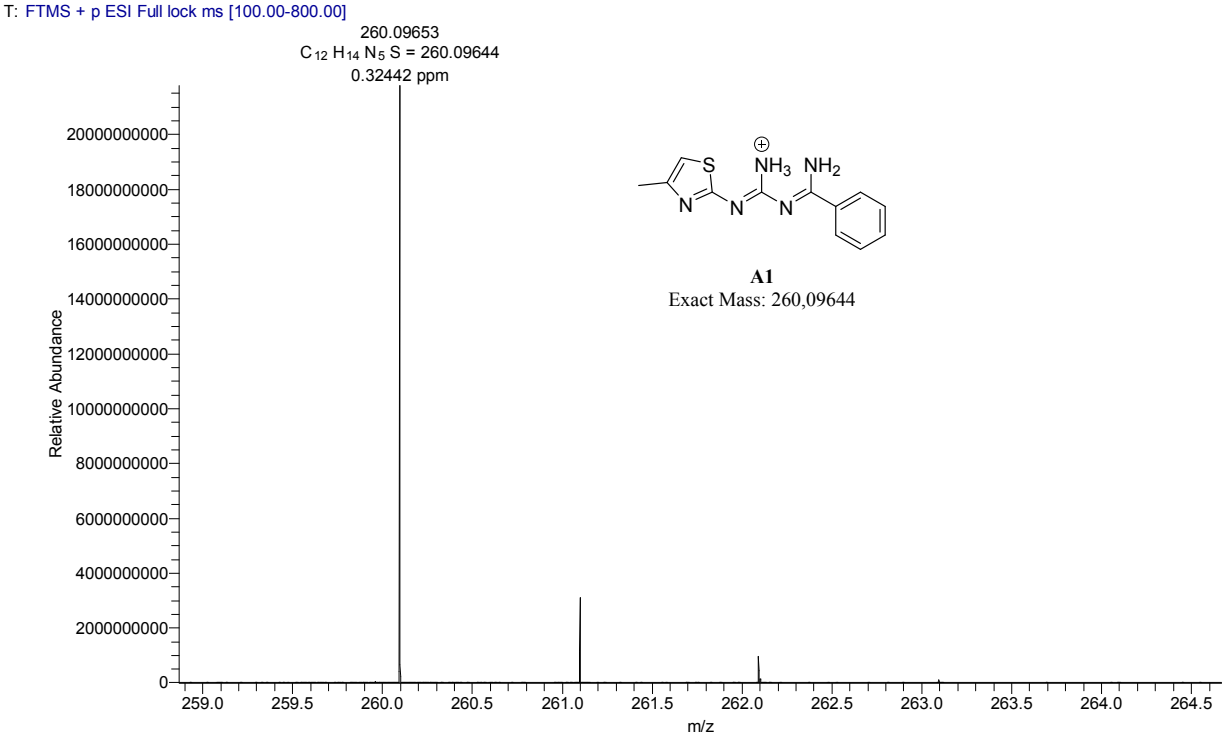
N-(N-(4-methylthiazol-2-yl)carbamimidoyl)benzimidamide **(A1)**

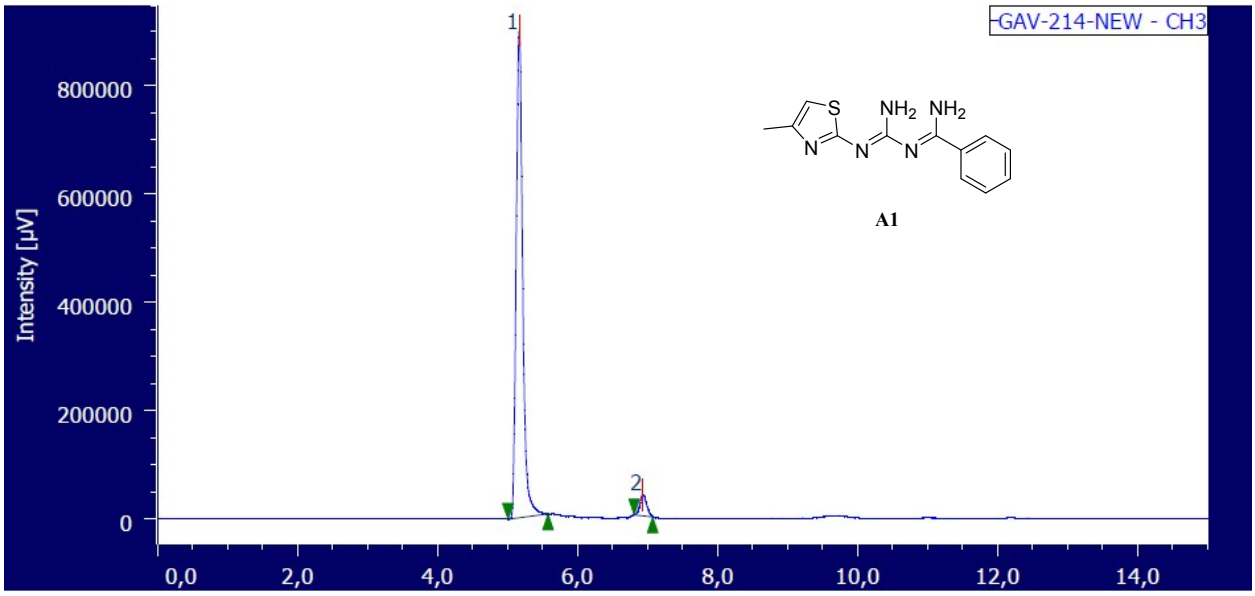


A1

^{1}H NMR (DMSO-*d6*, 500 MHz)


172.99
161.63
160.69
147.64
135.50
131.20
128.21
127.45
105.64
40.77
40.36
39.94
39.52
39.10
38.69
38.27
17.56
NH2
NH2
S
N
N
N
A1
13C NMR (DMSO-d6, 50 MHz)
f1 (ppm)

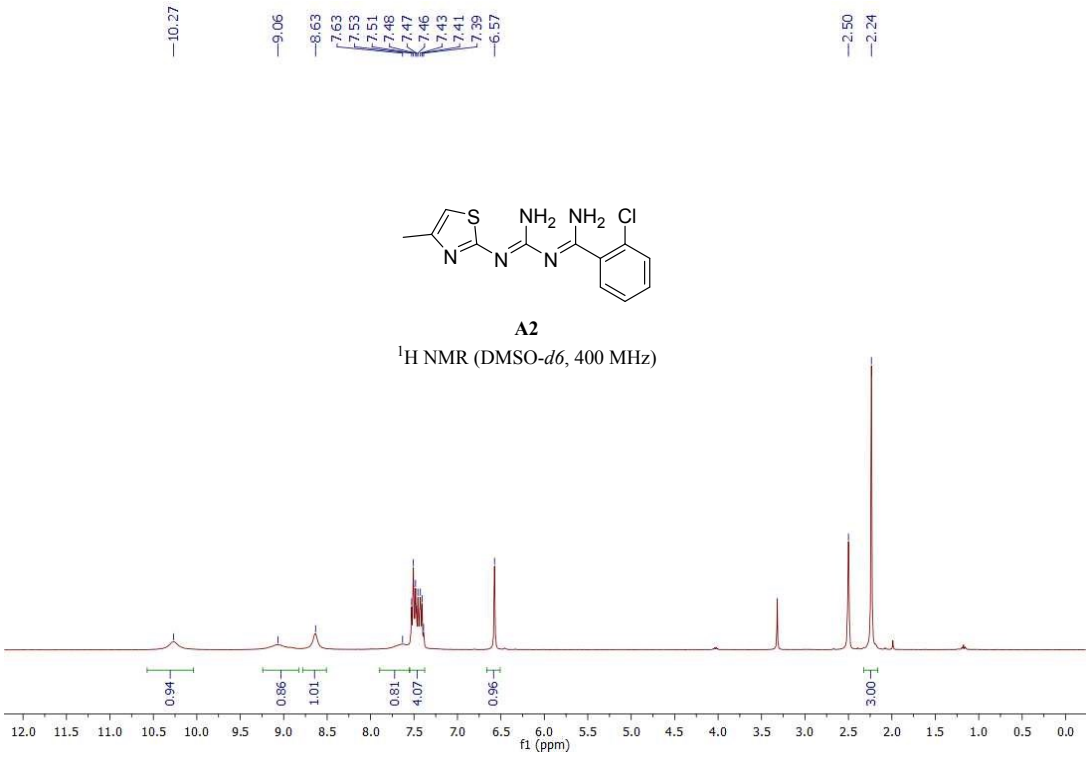

230217icn28 #23 RT: 0.22 AV: 1 NL: 2.18E10
T: FTMS + p ESI Full lock ms [100.00-800.00]
260.09653
C12 H14 N5 S = 260.09644
0.32442 ppm
NH3
NH2
S
N
N
N
A1
Exact Mass: 260,09644
Relative Abundance
m/z



A1

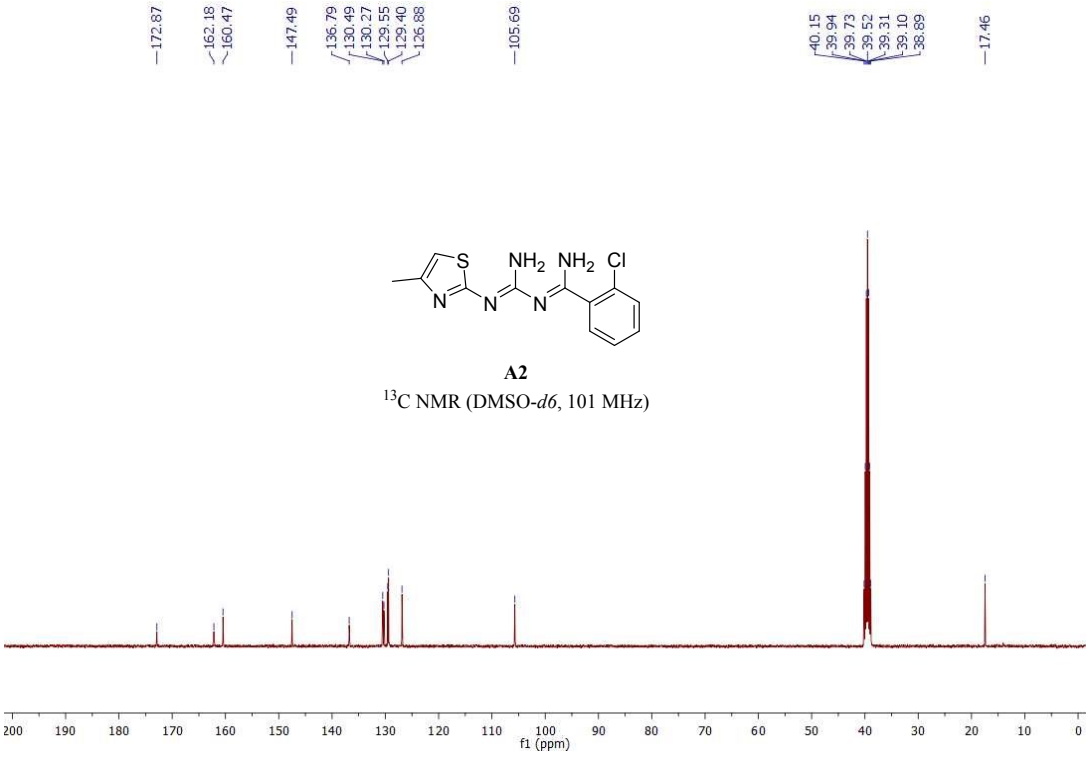
#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,167	5805587	898742	95,683	95,856	N/A	16218	10,371	1,229	
2	Unknown	3	6,933	261955	38851	4,317	4,144	N/A	23921	N/A	1,113	

2-chloro-N-(N-(4-methylthiazol-2-yl)carbamimidoyl)benzimidamide **(A2)**



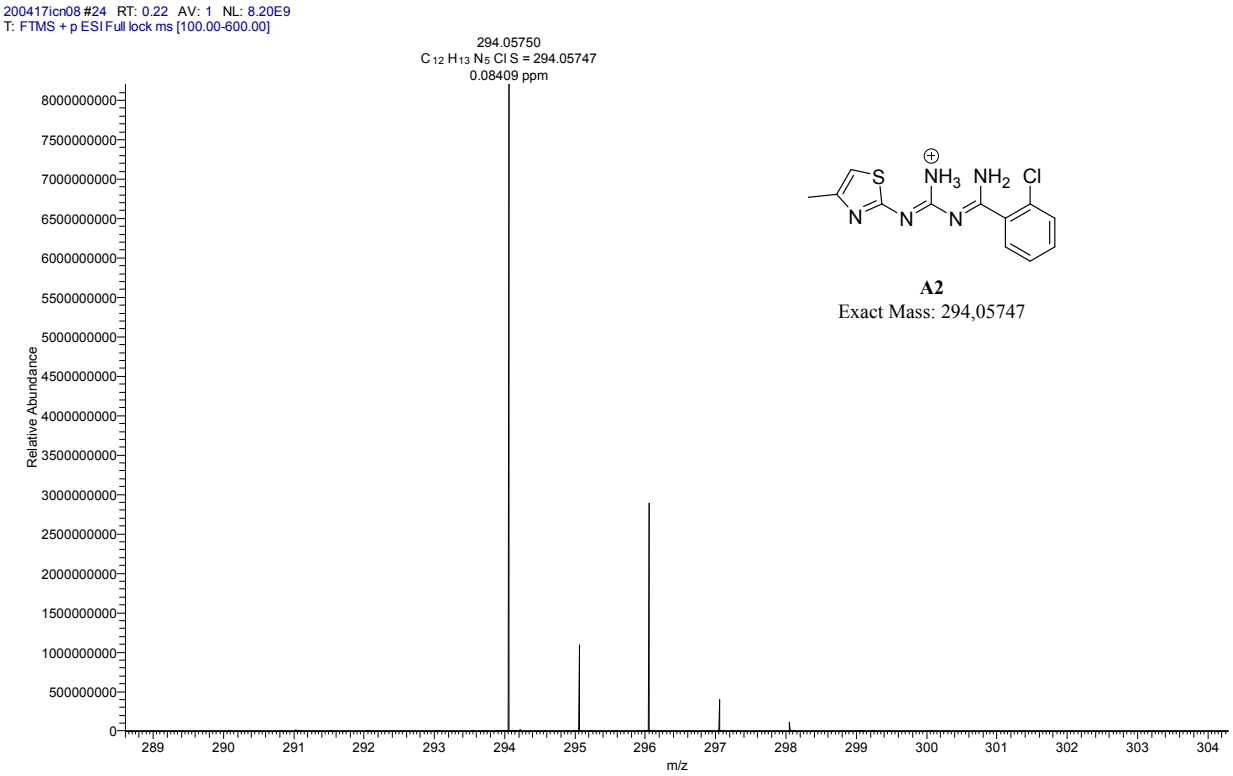
A2

^{1}H NMR (DMSO-*d6*, 400 MHz)



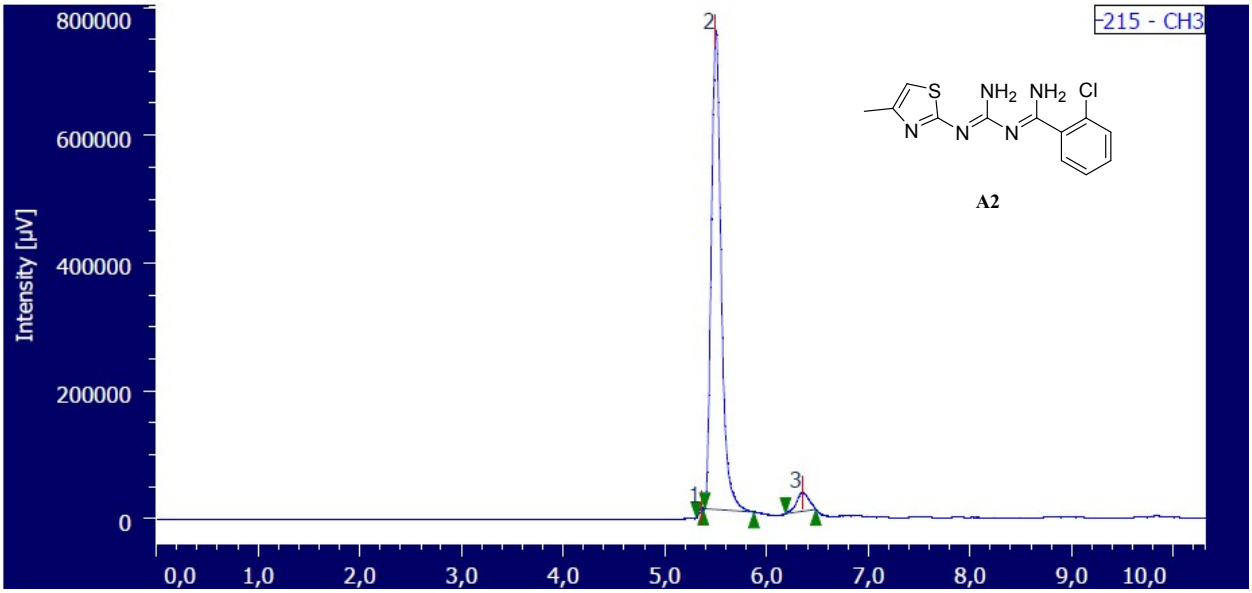
A2

^{13}C NMR (DMSO-*d6*, 101 MHz)



A2

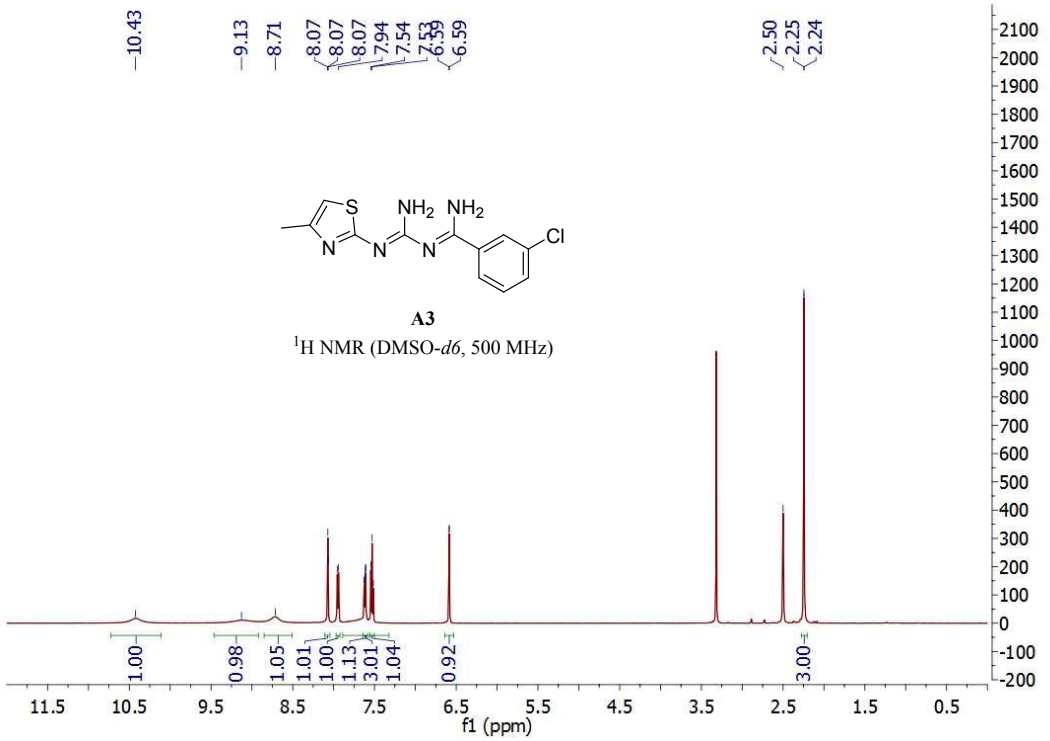
Exact Mass: 294,05747



A2

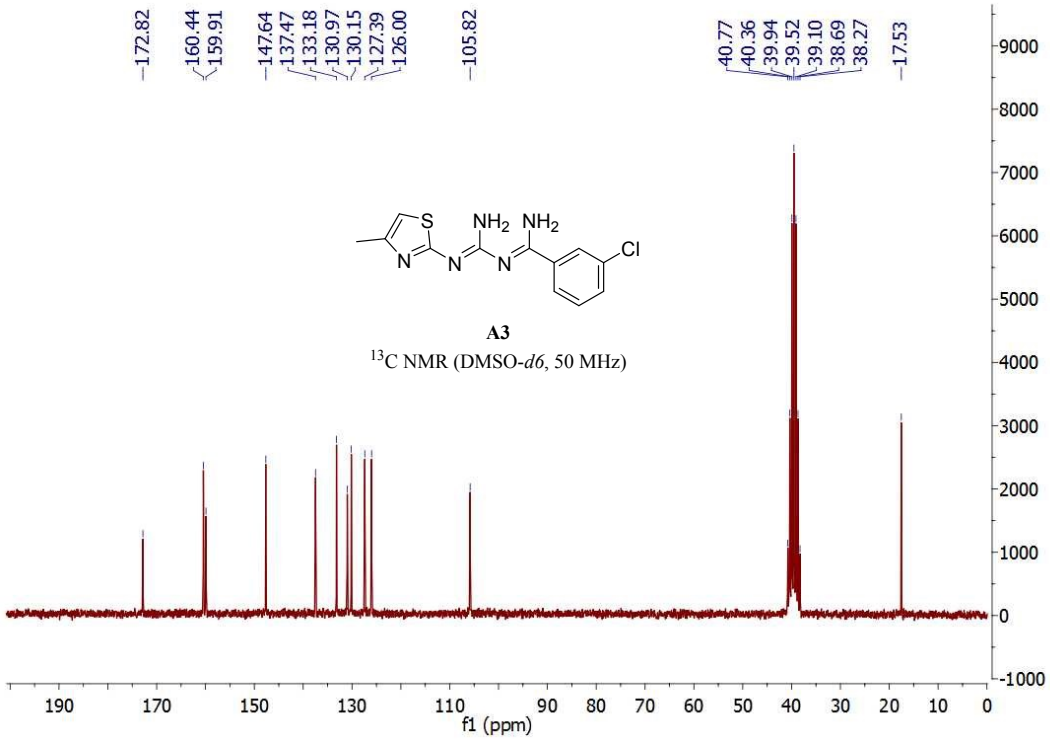
#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,358	14698	6177	0,277	0,787	N/A	N/A	N/A	N/A	
2	Unknown	3	5,500	5045816	749265	95,033	95,441	N/A	16460	4,303	1,336	
3	Unknown	3	6,350	249007	29614	4,690	3,772	N/A	12788	N/A	0,922	

3-chloro-N-(N-(4-methylthiazol-2-yl)carbamimidoyl)benzimidamide (**A3**)



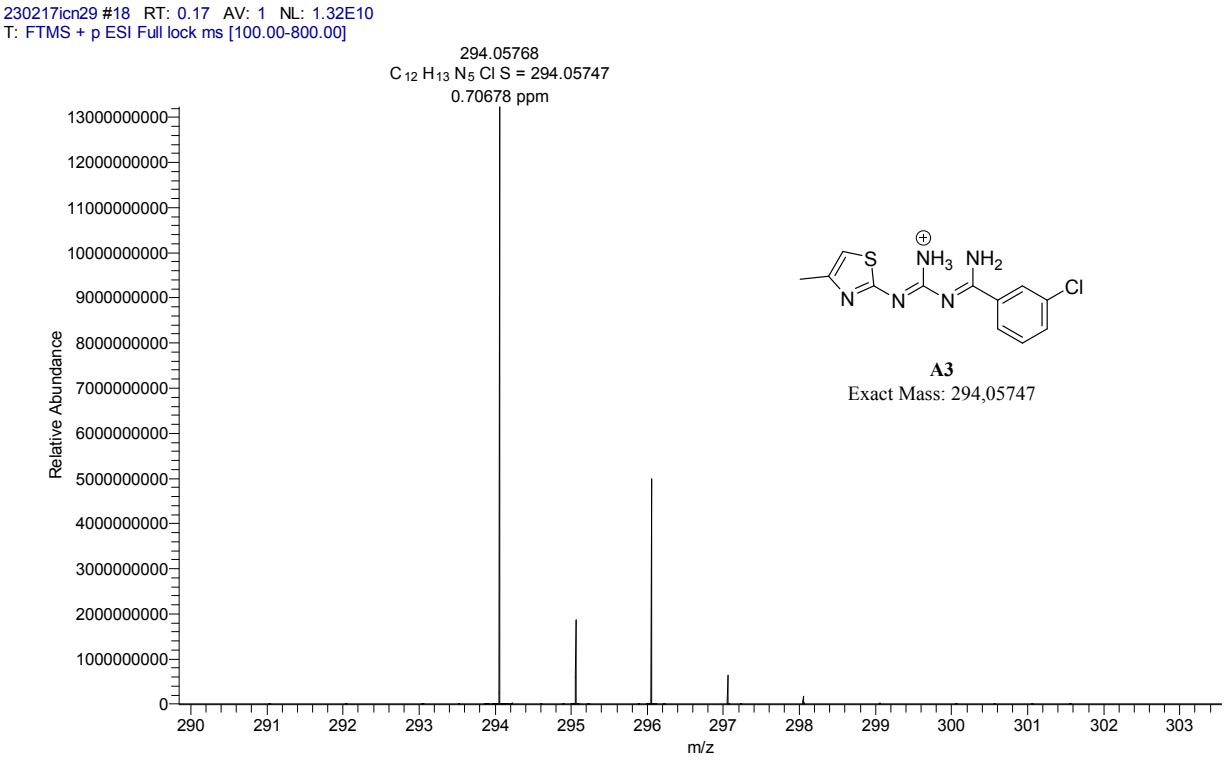
A3

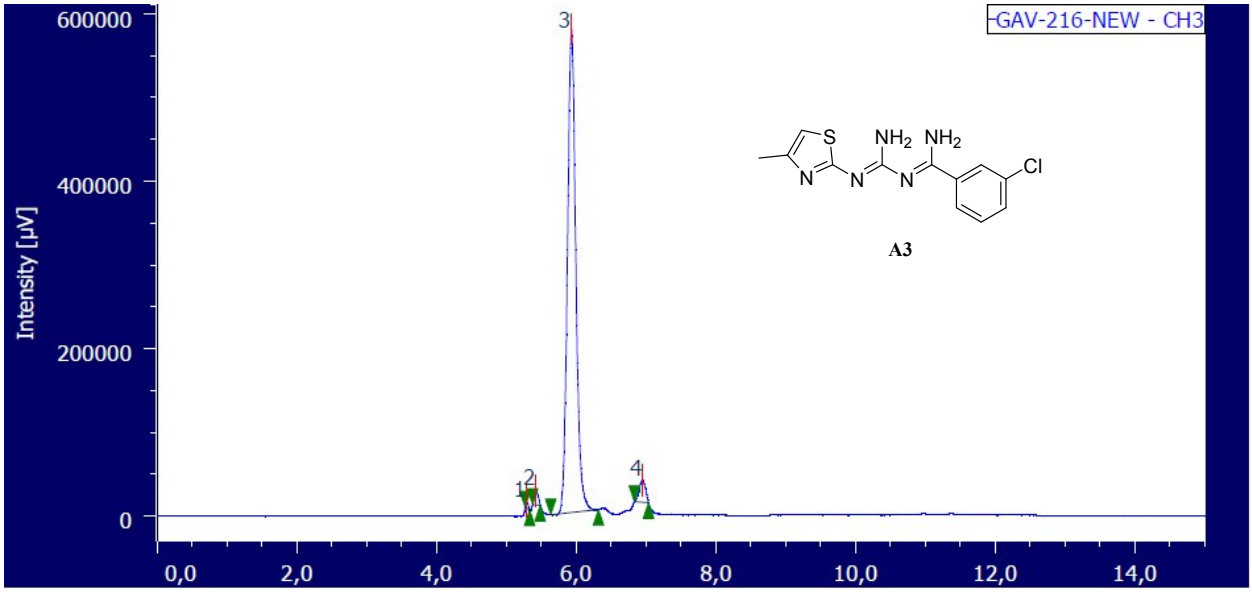
^{1}H NMR (DMSO-*d6*, 500 MHz)



A3

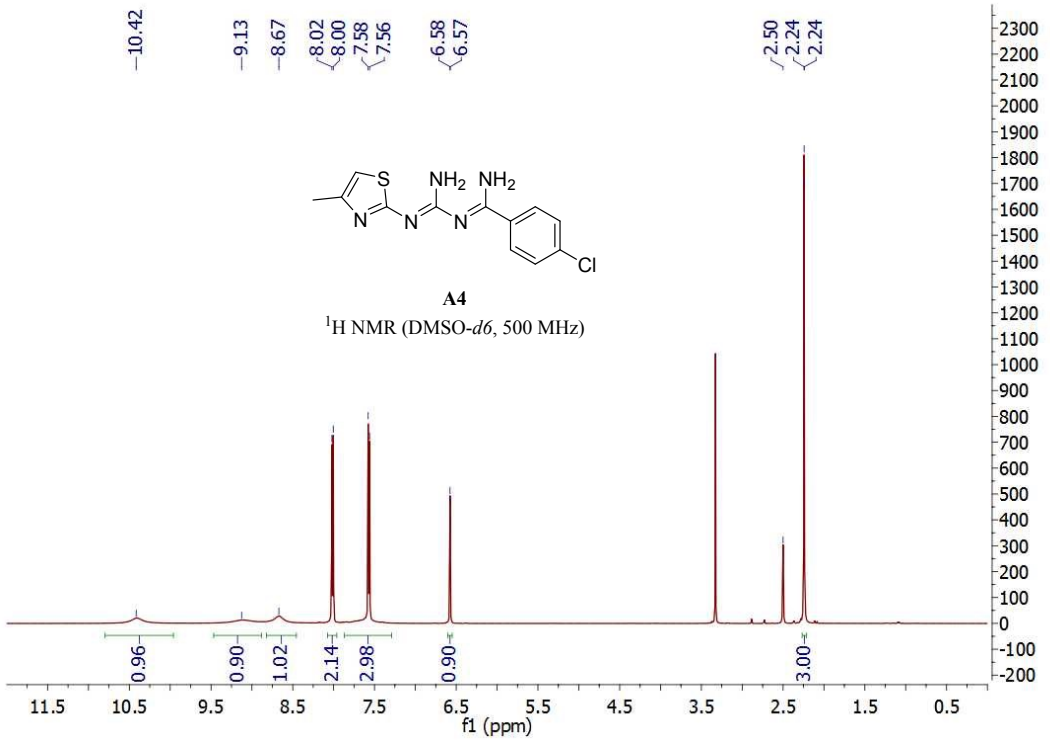
^{13}C NMR (DMSO-*d6*, 50 MHz)





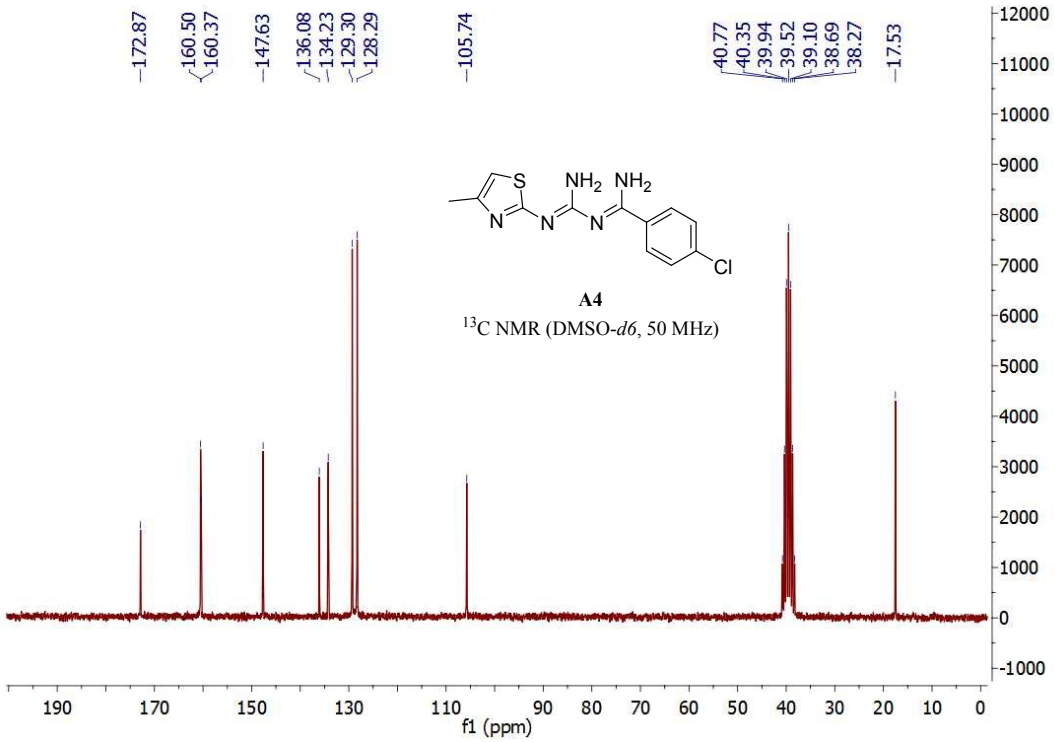
#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,292	16977	7307	0,337	1,166	N/A	103362	1,474	1,329	
2	Unknown	3	5,425	67340	17005	1,336	2,713	N/A	35263	3,102	1,078	
3	Unknown	3	5,933	4786716	576955	95,002	92,035	N/A	12404	5,172	1,117	
4	Unknown	3	6,950	167526	25622	3,325	4,087	N/A	23569	N/A	0,872	

4-chloro-N-(N-(4-methylthiazol-2-yl)carbamimidoyl)benzimidamide (**A4**)



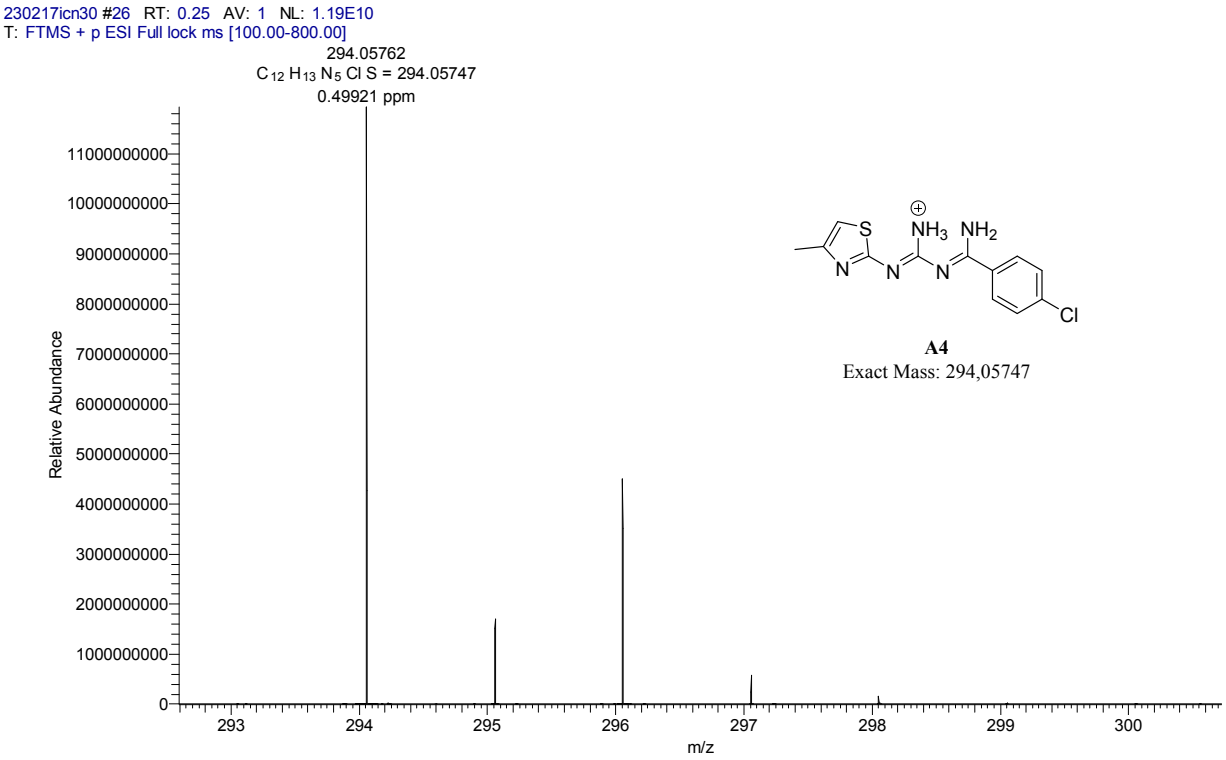
A4

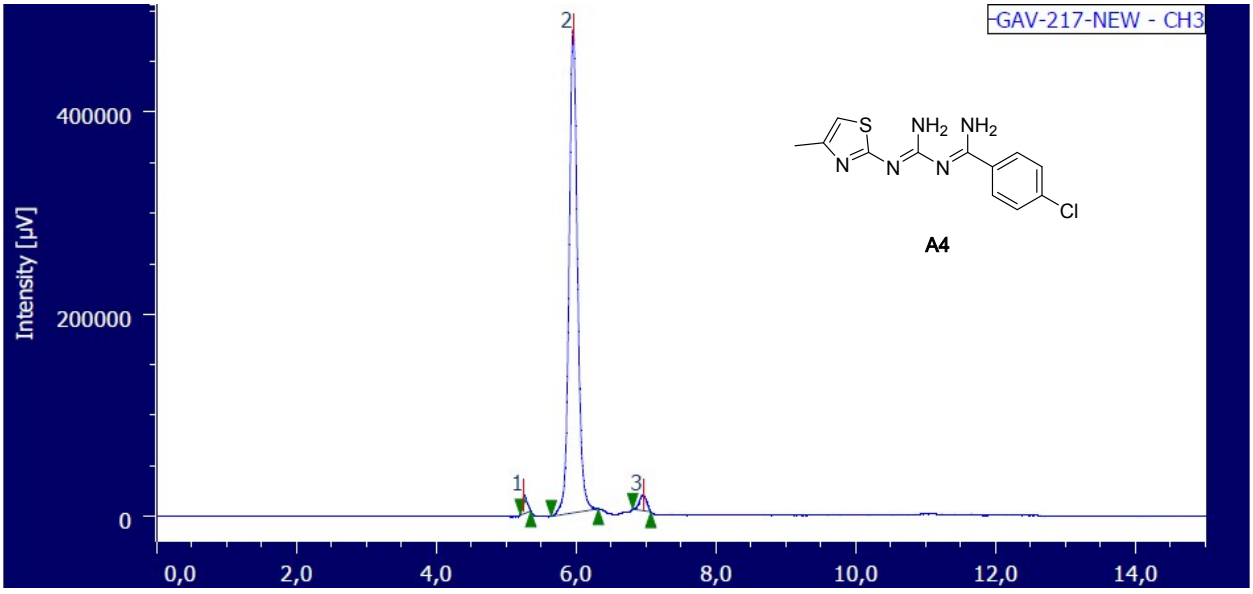
^{1}H NMR (DMSO-*d6*, 500 MHz)



A4

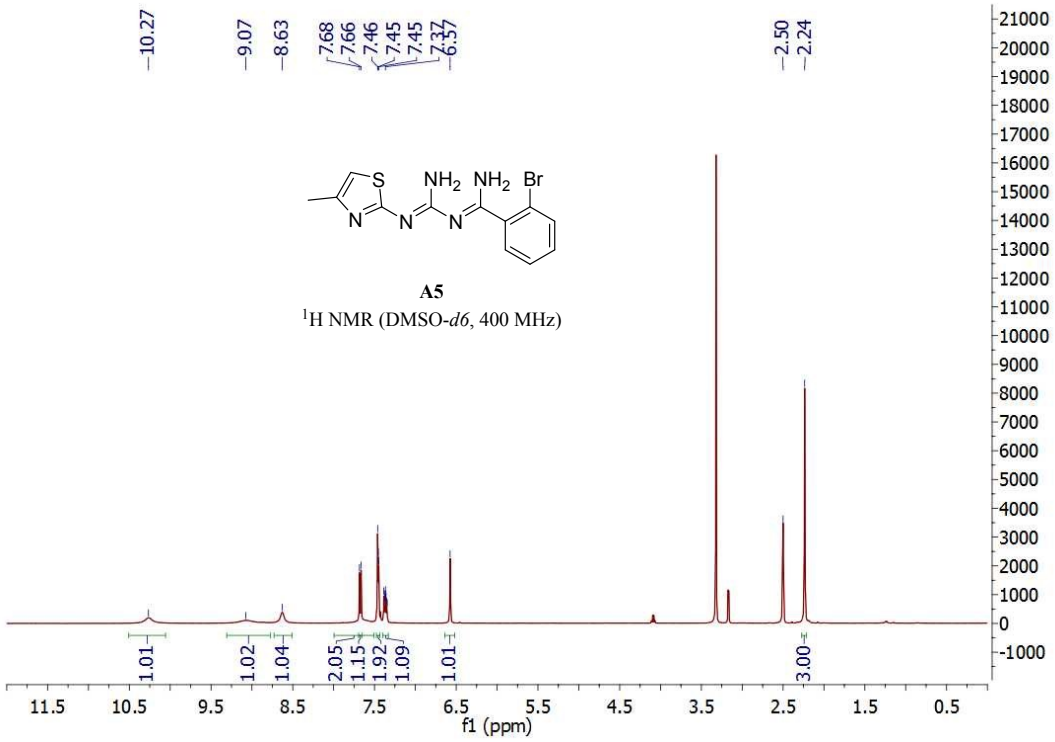
^{13}C NMR (DMSO-*d6*, 50 MHz)





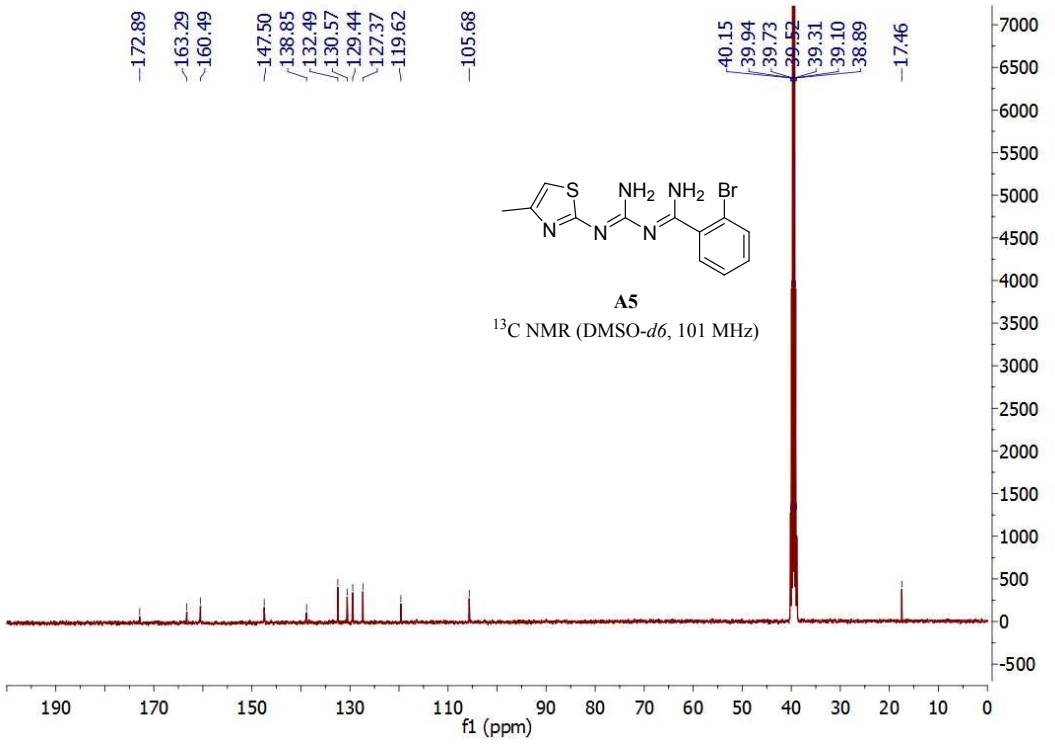
#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,258	87537	17926	2,079	3,505	N/A	23196	4,023	1,273	
2	Unknown	3	5,958	4010732	477888	95,260	93,448	N/A	12778	4,941	1,070	
3	Unknown	3	6,958	112030	15579	2,661	3,046	N/A	20370	N/A	0,884	

2-bromo-N-(N-(4-methylthiazol-2-yl)carbamimidoyl)benzimidamide (**A5**)



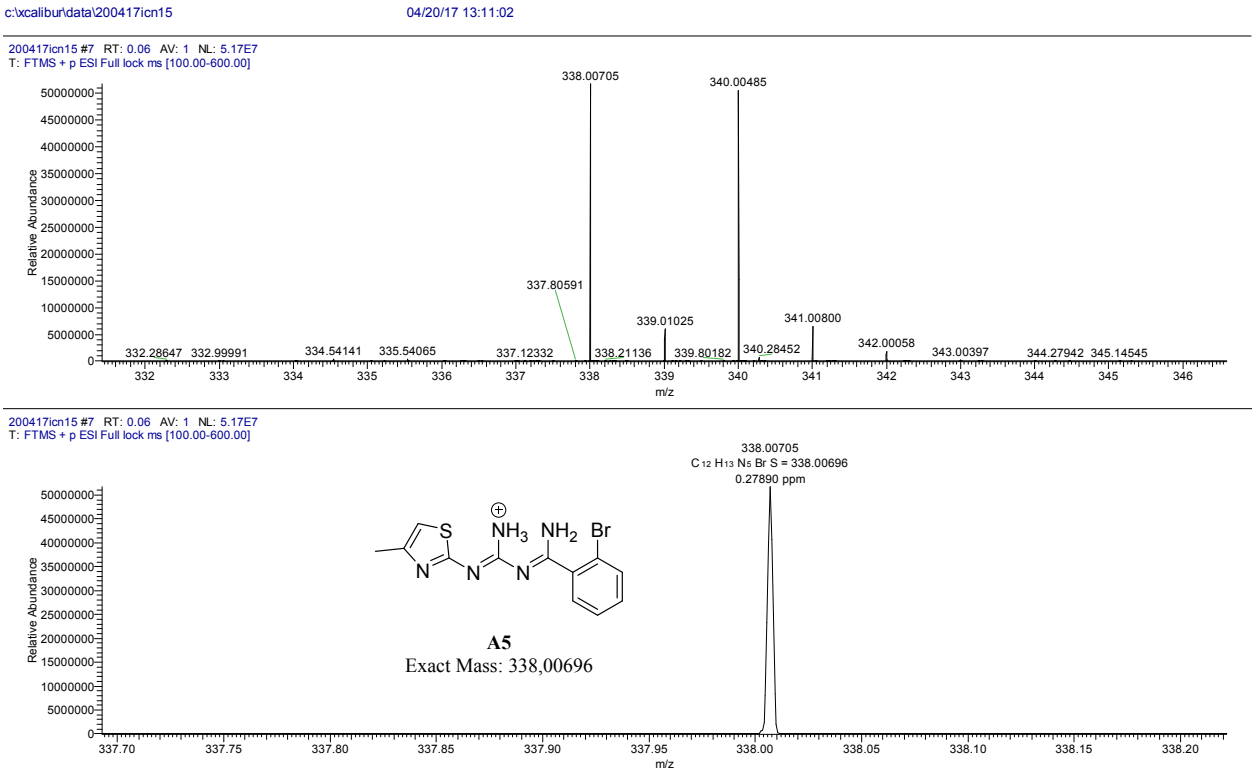
A5

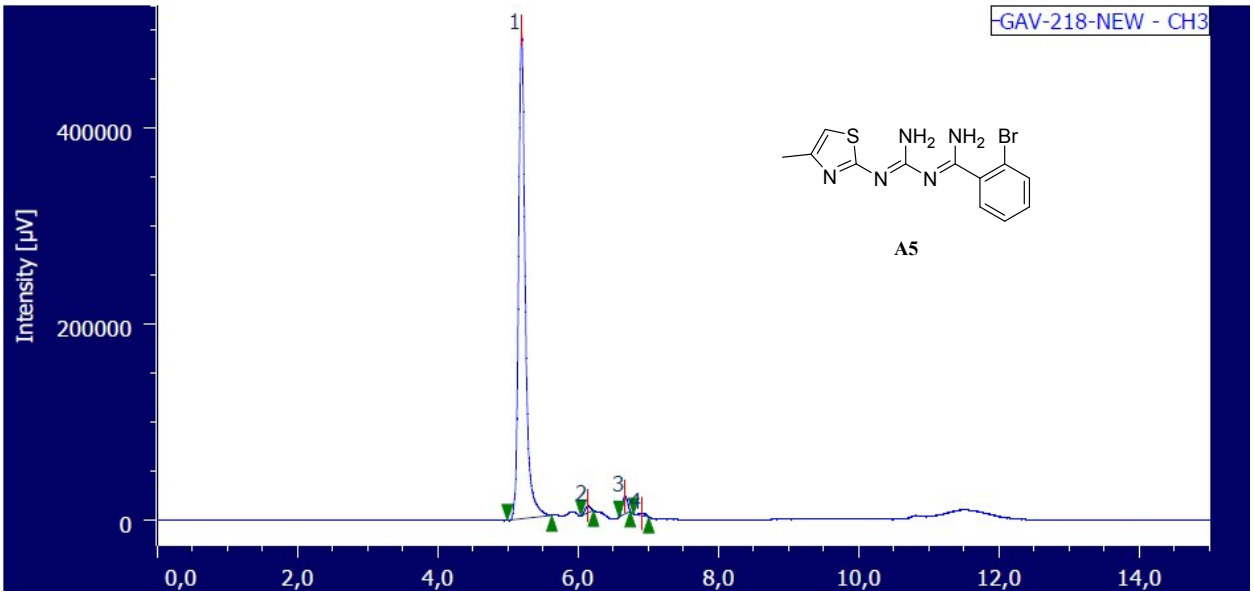
^{1}H NMR (DMSO-*d6*, 400 MHz)



A5

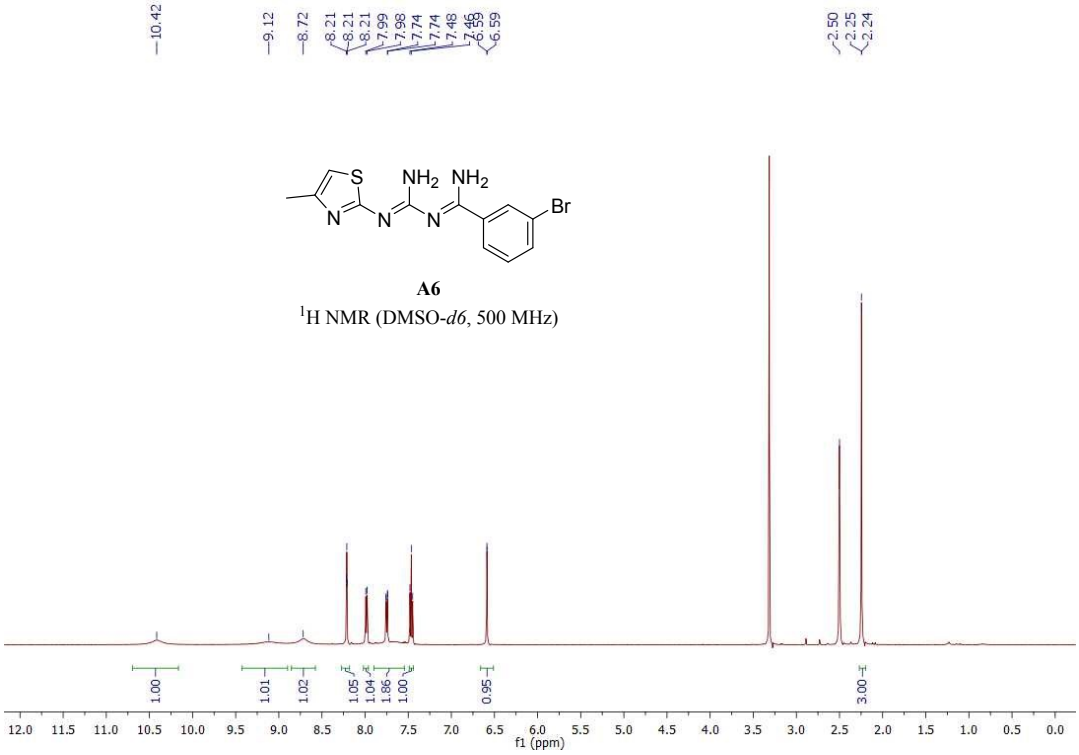
^{13}C NMR (DMSO-*d6*, 101 MHz)





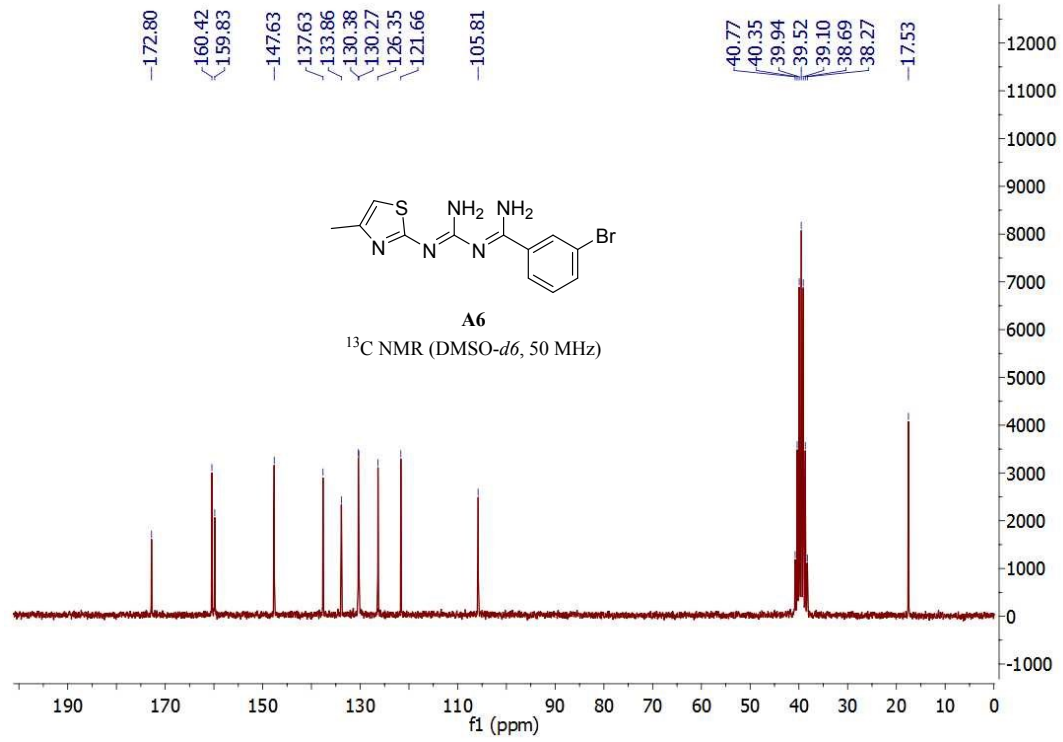
#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,192	3311790	496803	95,794	94,820	N/A	16760	5,944	1,348	
2	Unknown	3	6,142	37674	6724	1,090	1,283	N/A	23543	3,459	0,909	
3	Unknown	3	6,667	92069	17991	2,663	3,434	N/A	34157	1,498	0,962	
4	Unknown	3	6,908	15660	2426	0,453	0,463	N/A	23785	N/A	1,093	

3-bromo-N-(N-(4-methylthiazol-2-yl)carbamimidoyl)benzimidamide (**A6**)



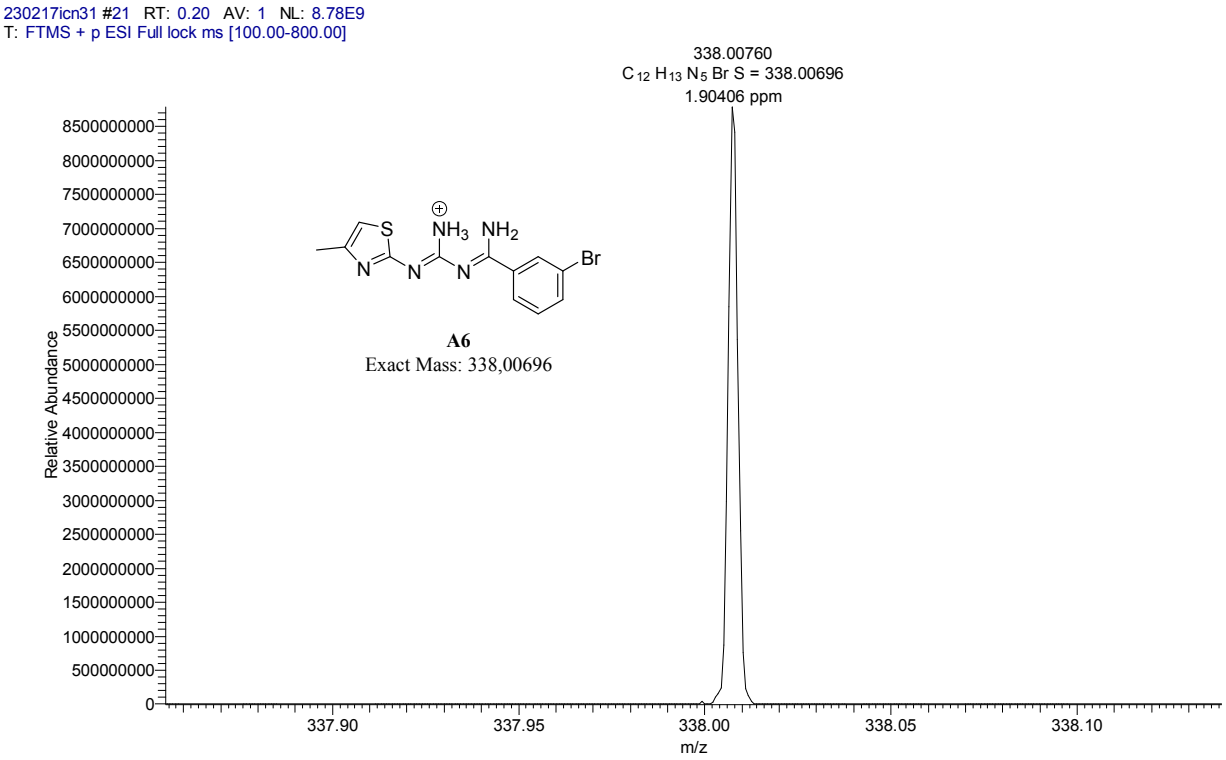
A6

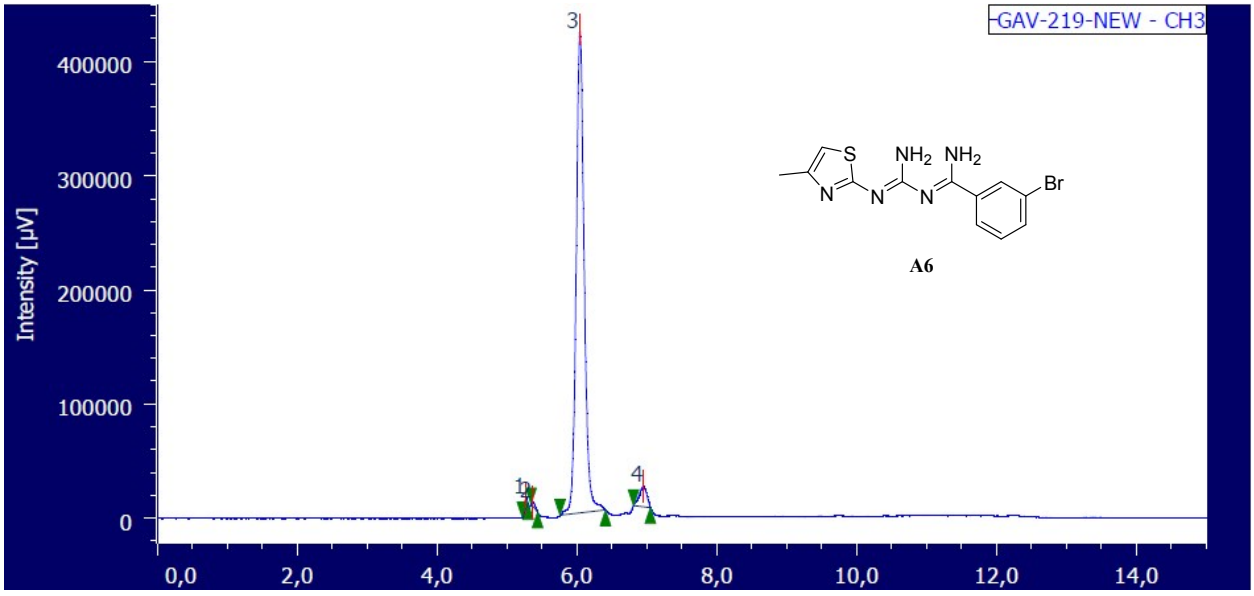
^{1}H NMR (DMSO-*d6*, 500 MHz)



A6

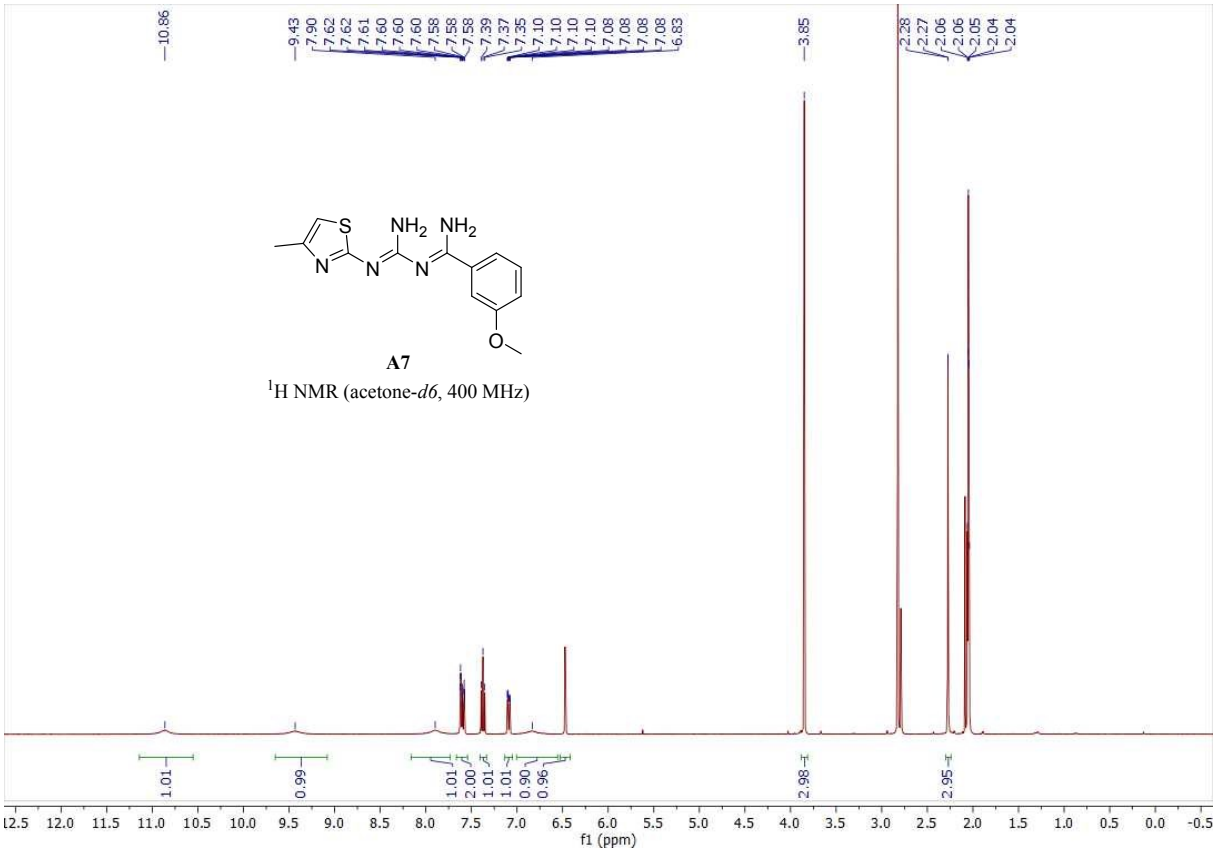
^{13}C NMR (DMSO-*d6*, 50 MHz)





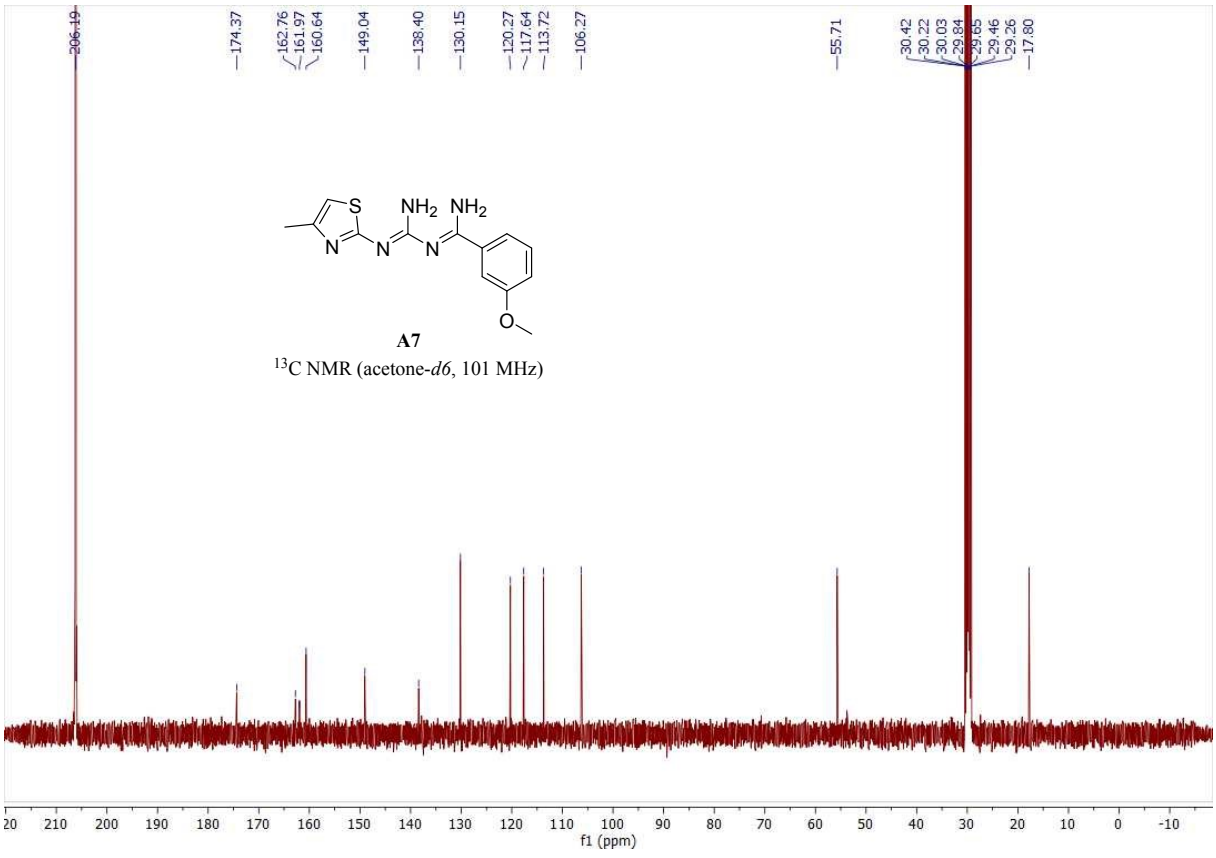
#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,275	22023	8550	0,626	1,888	N/A	87339	1,079	0,788	
2	Unknown	3	5,367	13079	3604	0,372	0,796	N/A	47090	4,558	1,807	
3	Unknown	3	6,042	3344686	423308	95,111	93,452	N/A	14887	4,403	1,121	
4	Unknown	3	6,950	136808	17504	3,890	3,864	N/A	16626	N/A	0,852	

3-methoxy-N-(N-(4-methylthiazol-2-yl)carbamimidoyl)benzimidamide (**A7**)



A7

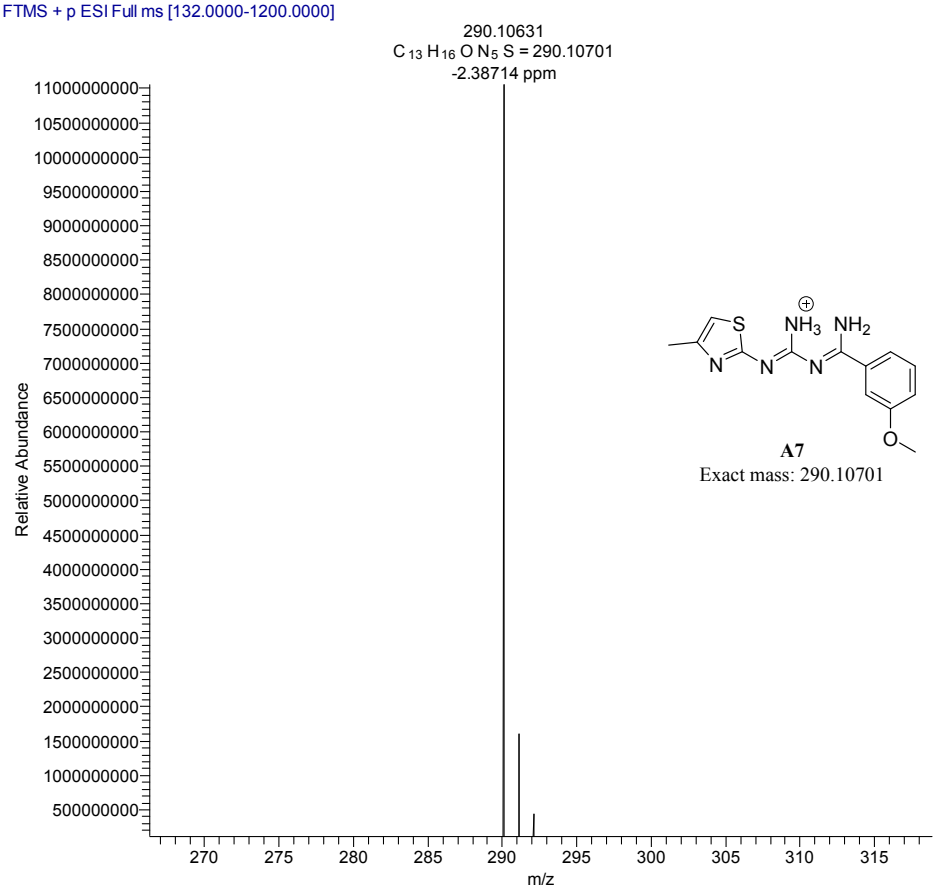
^{1}H NMR (acetone-*d6*, 400 MHz)



A7

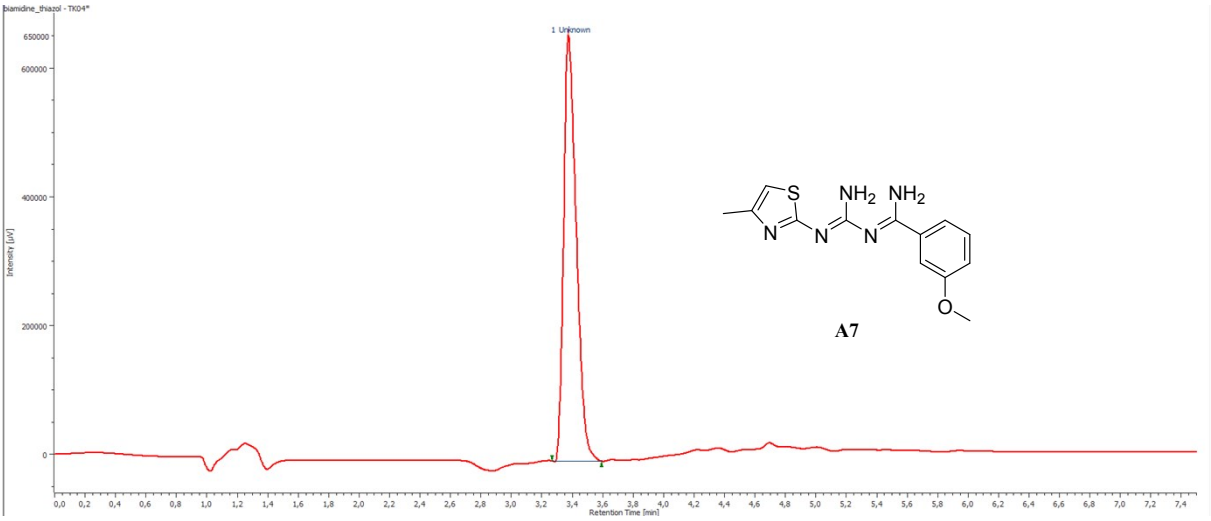
^{13}C NMR (acetone-*d6*, 101 MHz)

MTF560 #762-833 RT: 6.07-6.59 AV: 36 NL: 1.10E10
T: FTMS + p ESI Full ms [132.0000-1200.0000]



A7

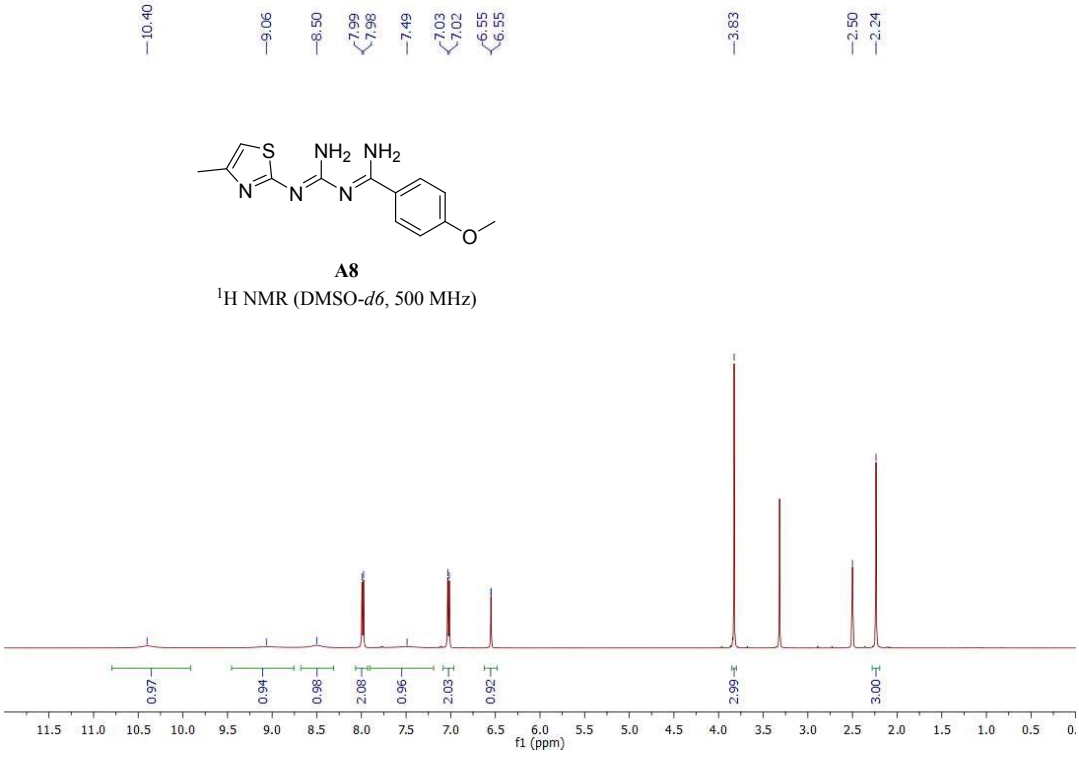
Exact mass: 290.10701



A7

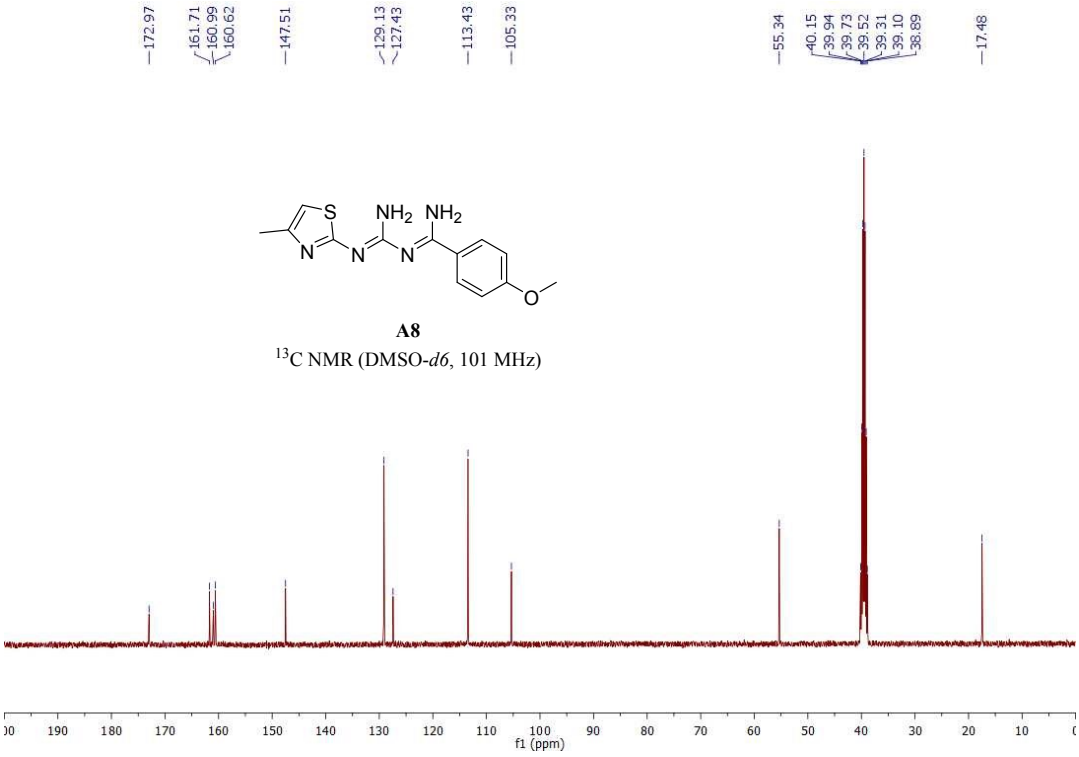
#	Peak Name	tR	Area	Height	Area%	Height%	Peak Start	Peak End	Base Start	Base End	Peak Mark
1	Unknown	3,373	3802726	661093	100,000	100,000	3,267	3,590	3,267	3,590	Manual

4-methoxy-N-(N-(4-methylthiazol-2-yl)carbamimidoyl)benzimidamide (**A8**)



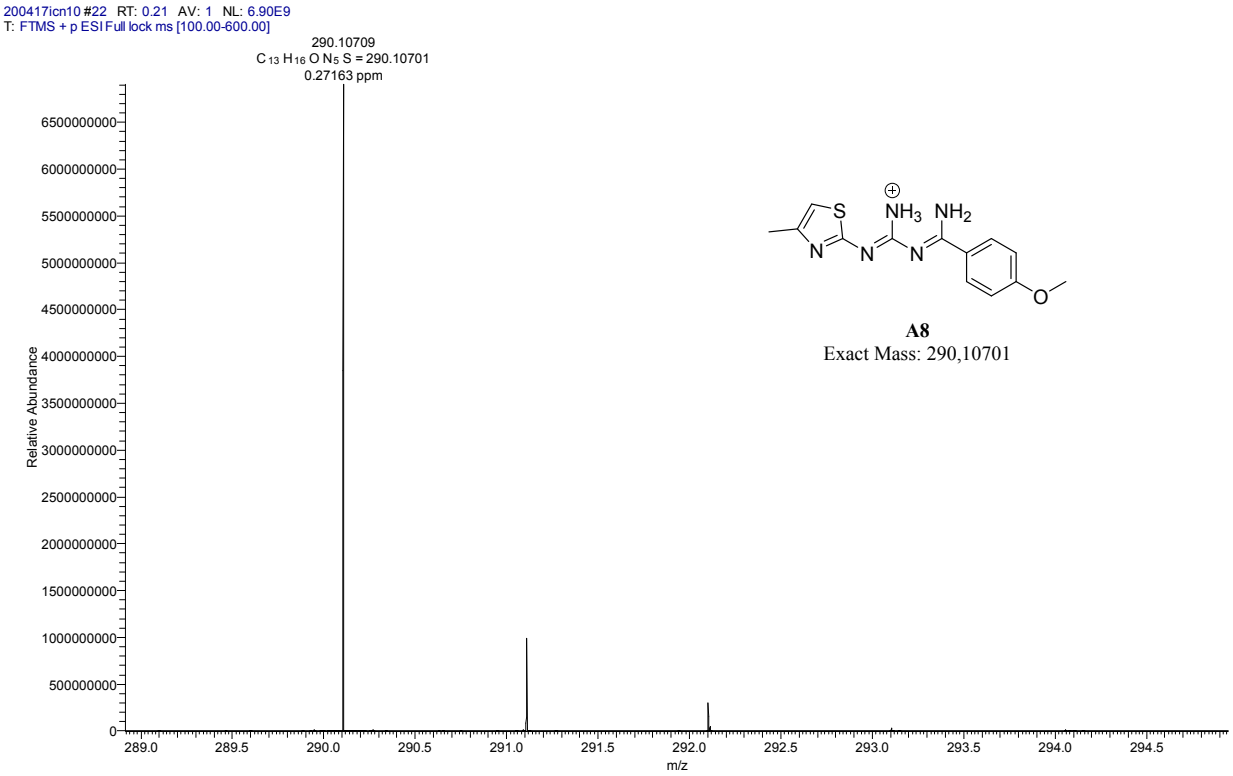
A8

^{1}H NMR (DMSO-*d6*, 500 MHz)

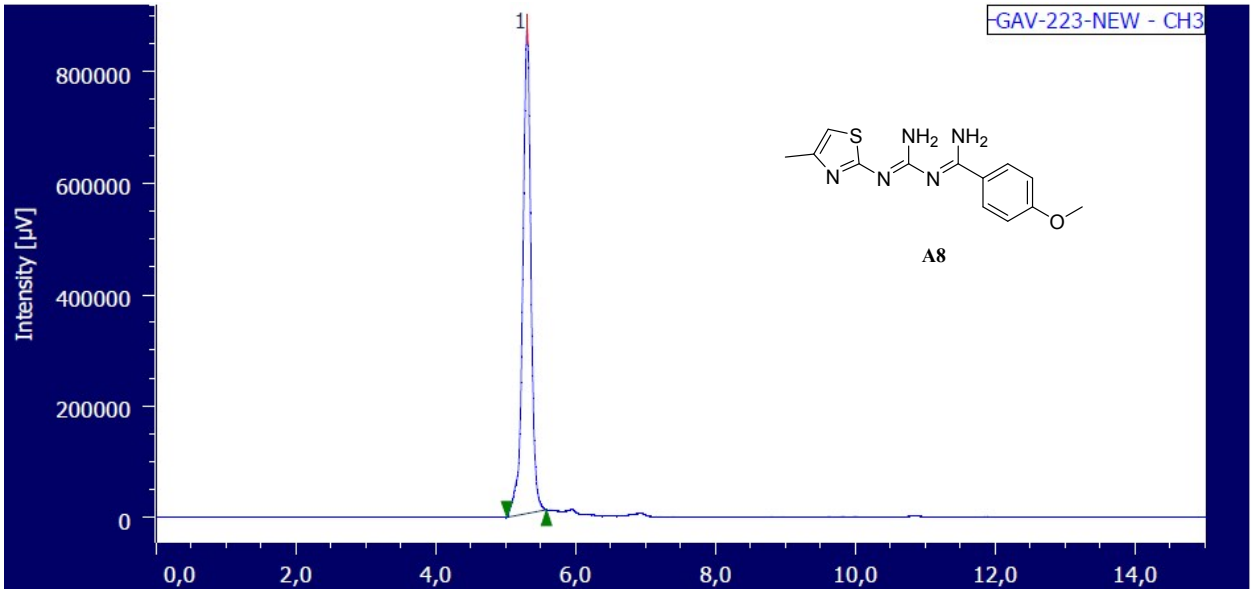


A8

^{13}C NMR (DMSO-*d6*, 101 MHz)



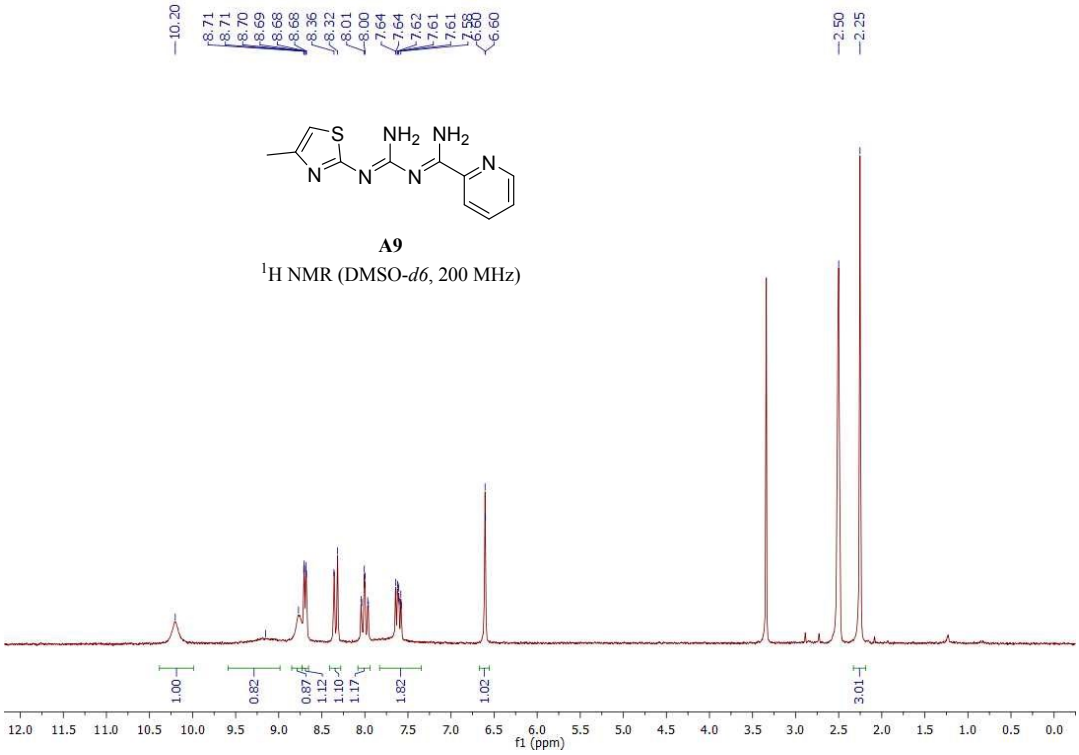
A8
Exact Mass: 290,10701



A8

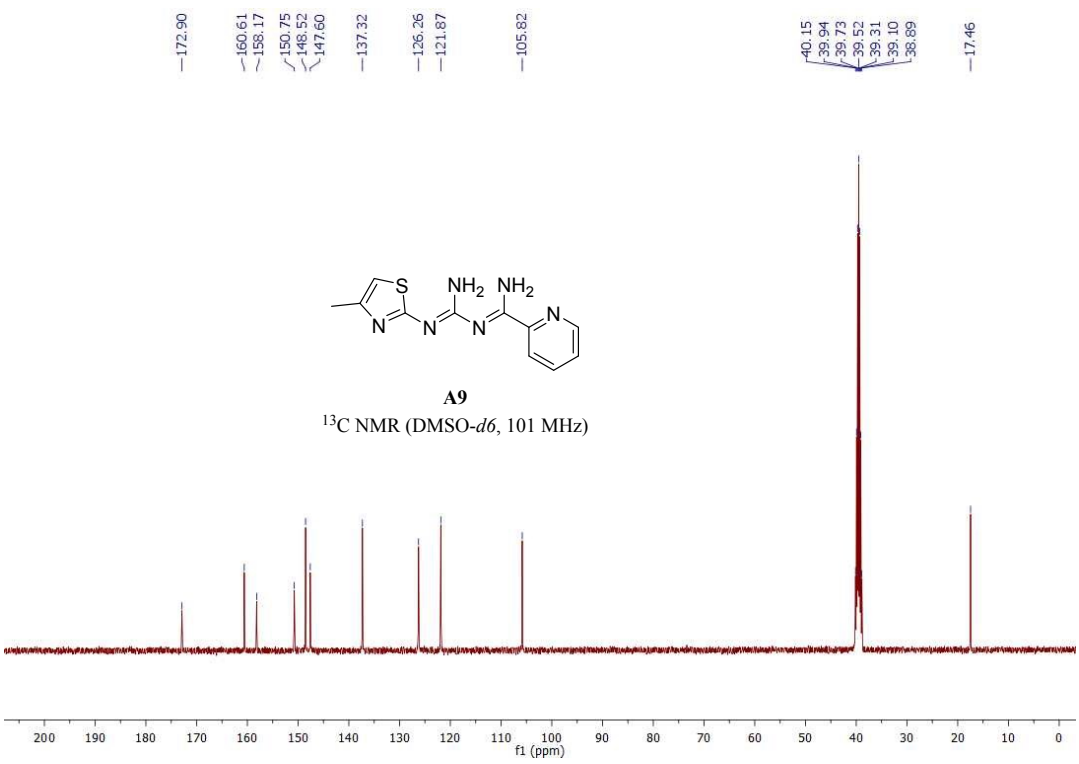
#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,308	7121286	867612	100,000	100,000	N/A	11095	N/A	0,868	

N-(N-(4-methylthiazol-2-yl)carbamimidoyl)picolinimidamide (**A9)**



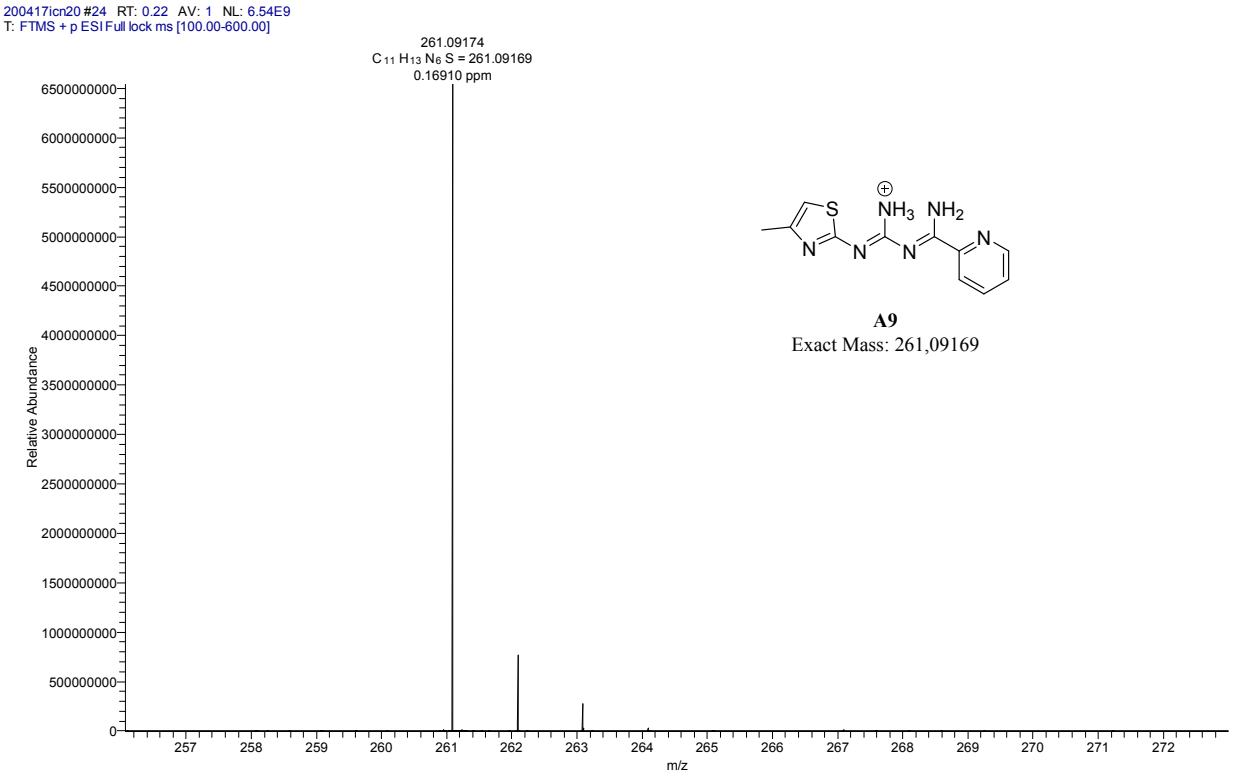
A9

^{1}H NMR (DMSO-*d6*, 200 MHz)



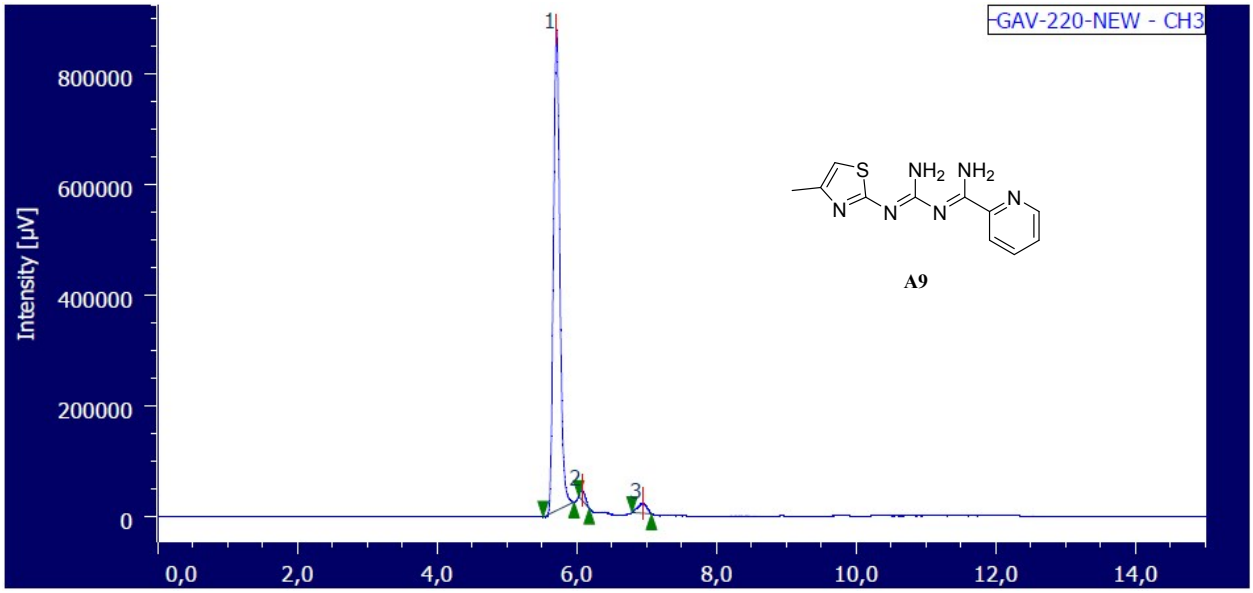
A9

^{13}C NMR (DMSO-*d6*, 101 MHz)



A9

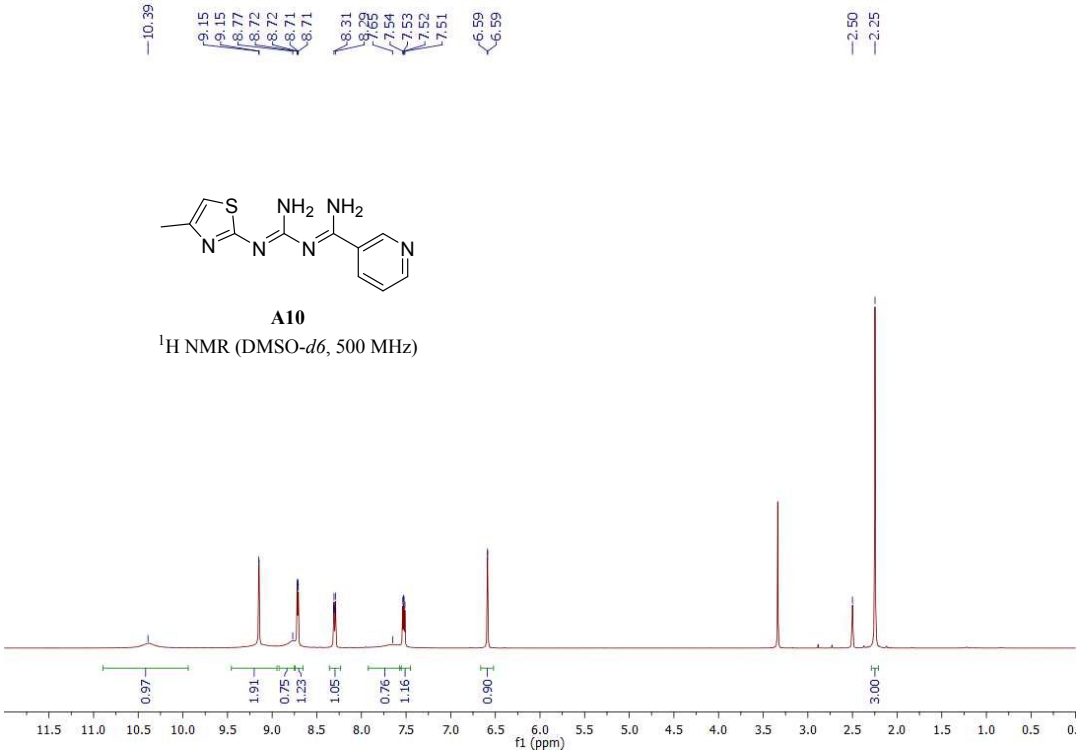
Exact Mass: 261,09169



A9

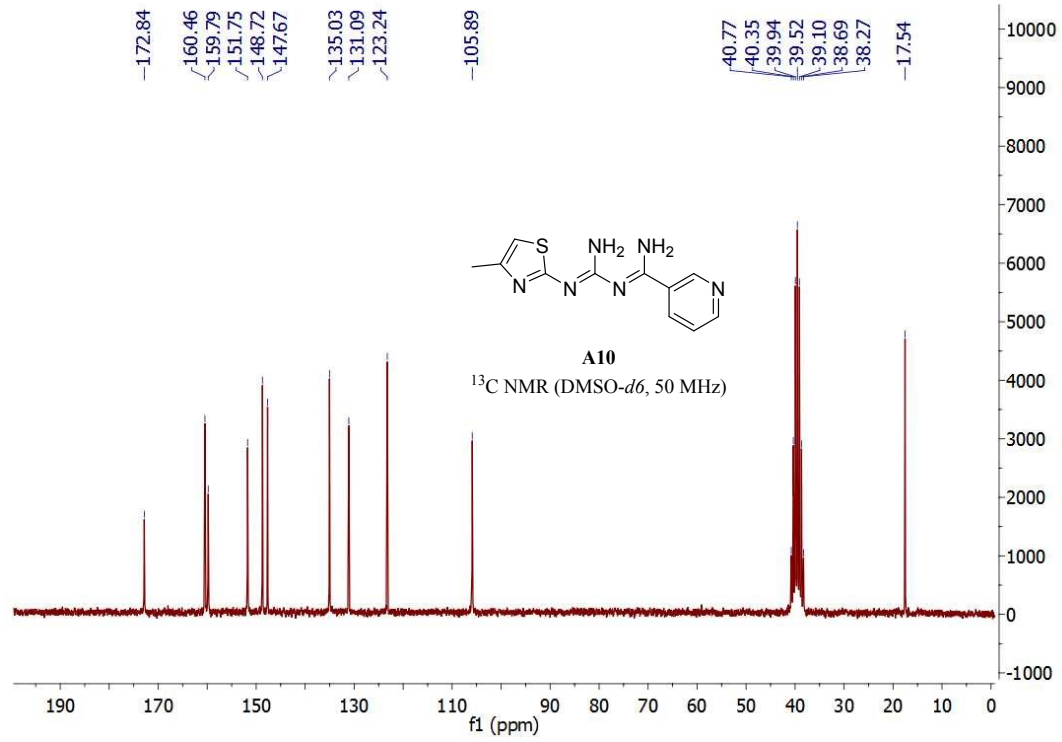
#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,708	5507245	867717	95,670	96,041	N/A	19151	2,398	1,167	
2	Unknown	3	6,075	91703	18400	1,593	2,037	N/A	29422	4,355	1,425	
3	Unknown	3	6,942	157565	17370	2,737	1,923	N/A	11635	N/A	0,907	

N-(N-(4-methylthiazol-2-yl)carbamimidoyl)nicotinimidamide (**A10**)



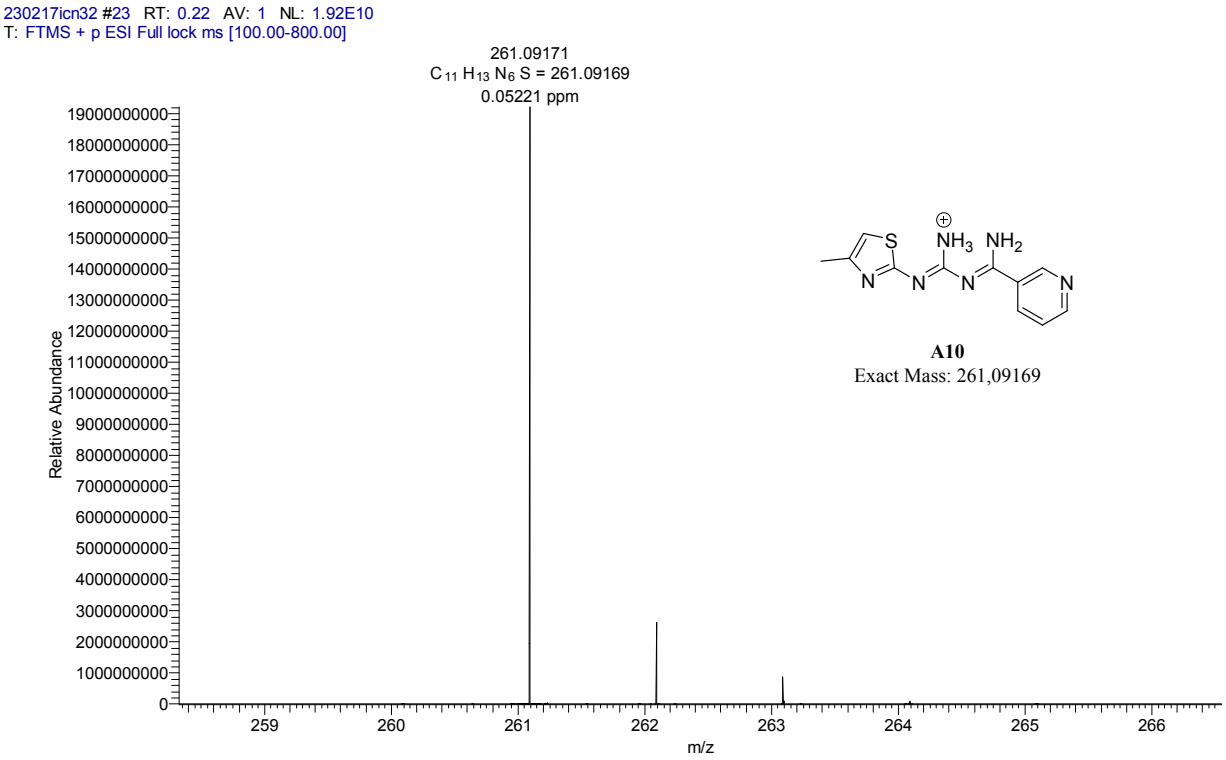
A10

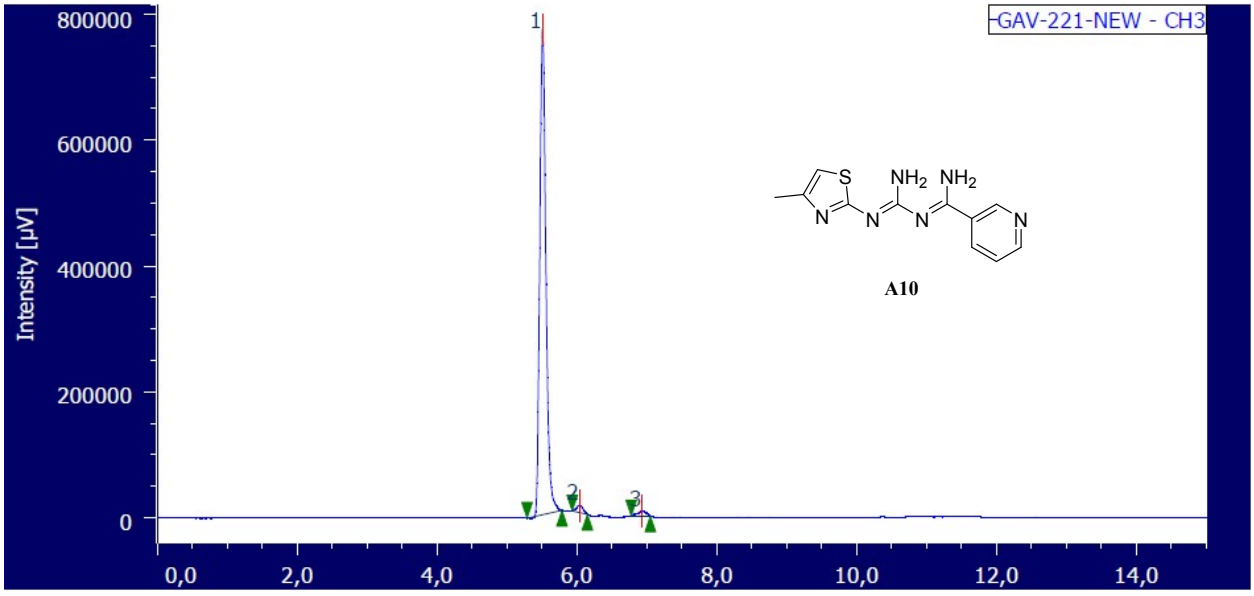
^{1}H NMR (DMSO-*d6*, 500 MHz)



A10

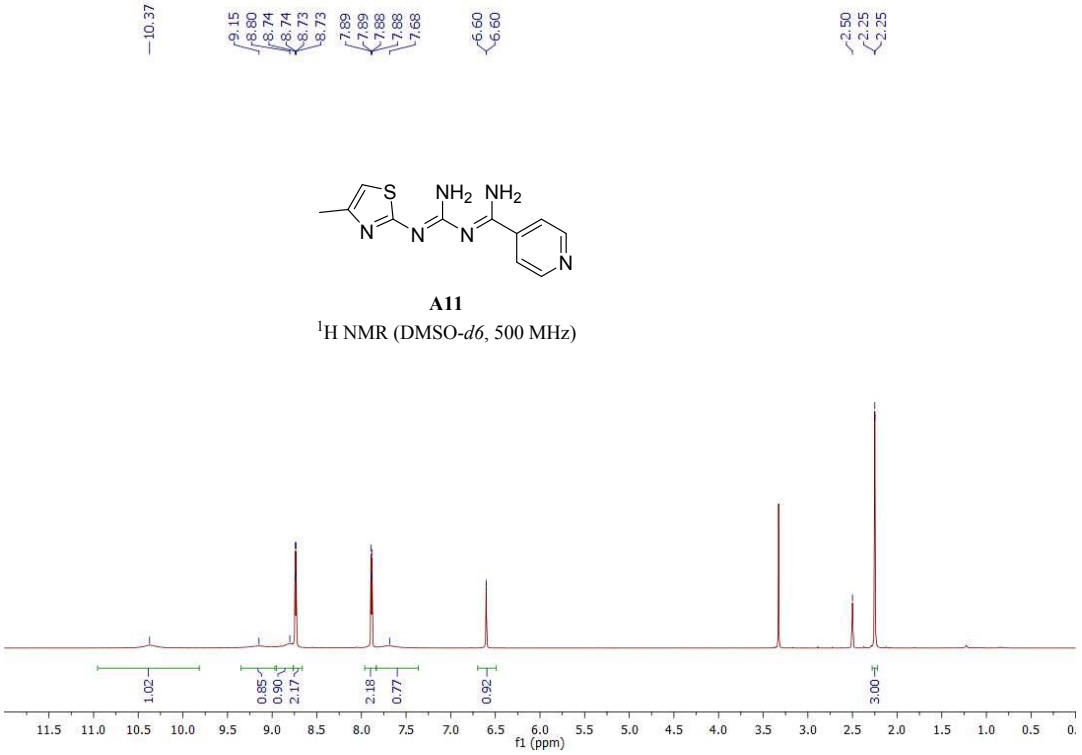
^{13}C NMR (DMSO-*d6*, 50 MHz)





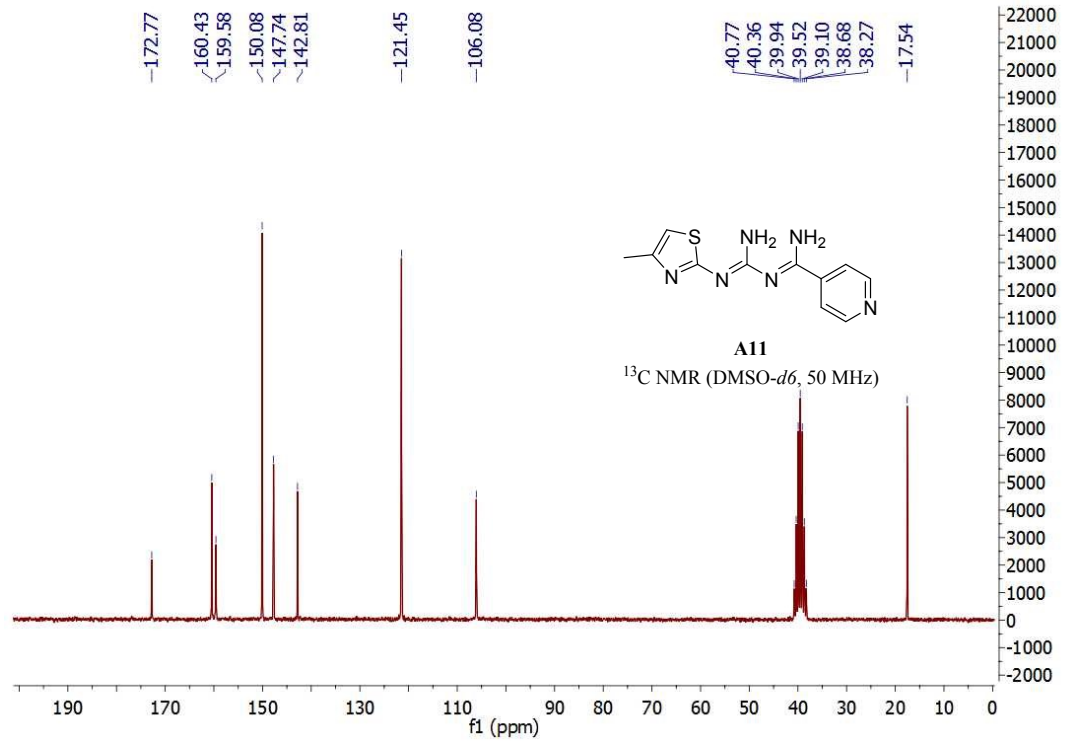
#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,508	4716422	769997	97,090	97,647	N/A	19325	3,062	1,227	
2	Unknown	3	6,033	76563	11356	1,576	1,440	N/A	16949	4,246	1,083	
3	Unknown	3	6,933	64806	7197	1,334	0,913	N/A	13389	N/A	0,863	

N-(N-(4-methylthiazol-2-yl)carbamimidoyl)isonicotinimidamide (**A11**)



A11

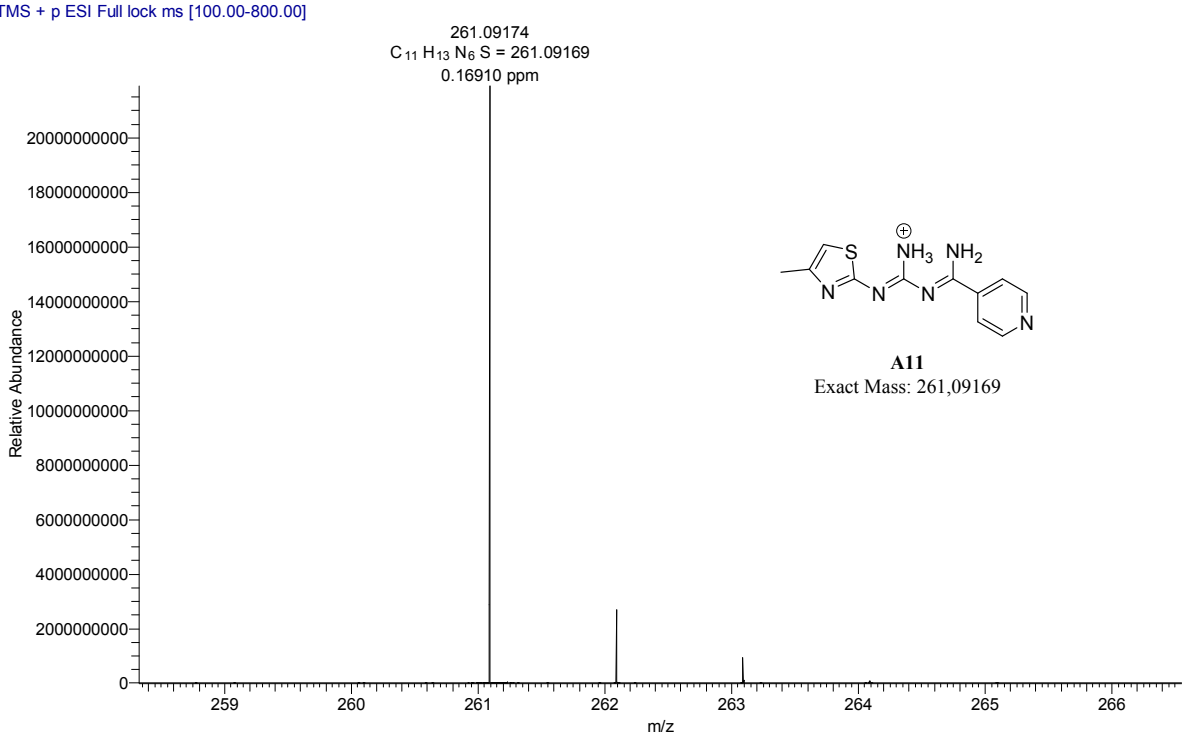
^{1}H NMR (DMSO-*d6*, 500 MHz)



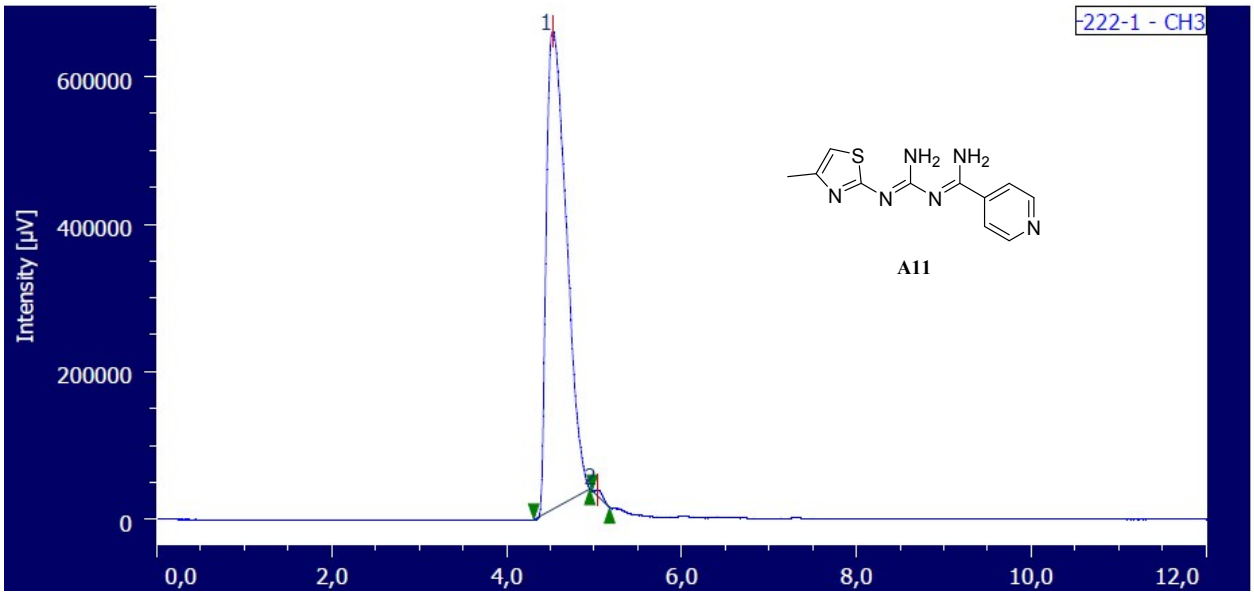
A11

^{13}C NMR (DMSO-*d6*, 50 MHz)

230217icn33 #11 RT: 0.10 AV: 1 NL: 2.19E10
T: FTMS + p ESI Full lock ms [100.00-800.00]



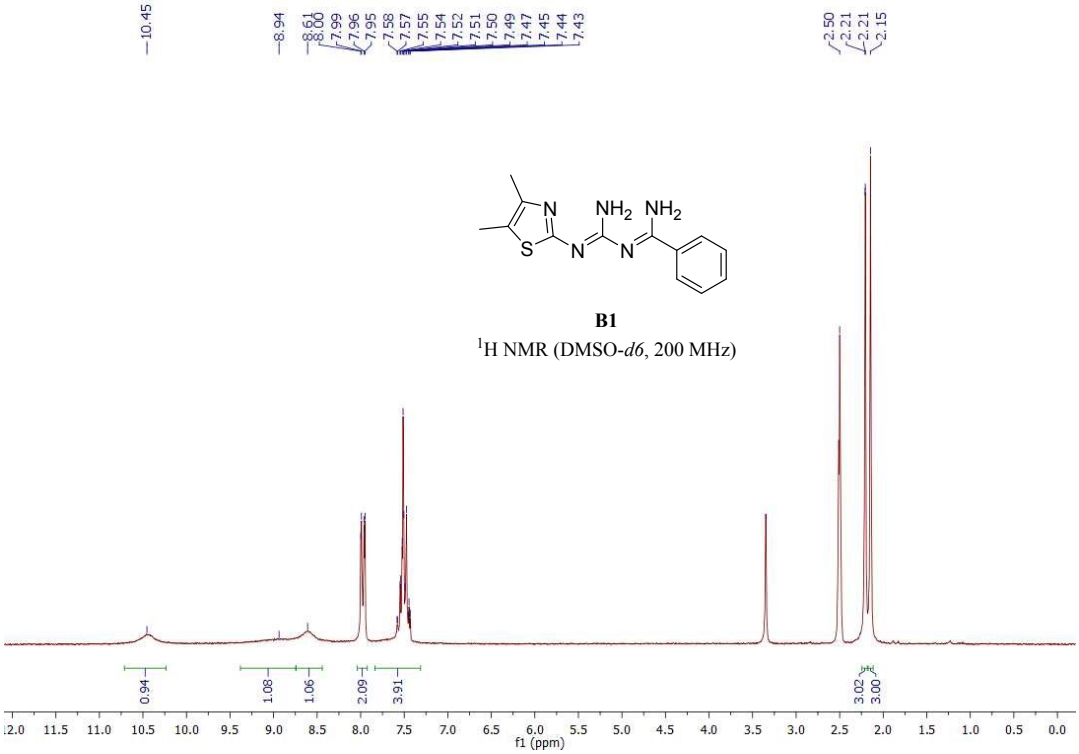
A11
Exact Mass: 261,09169



A11

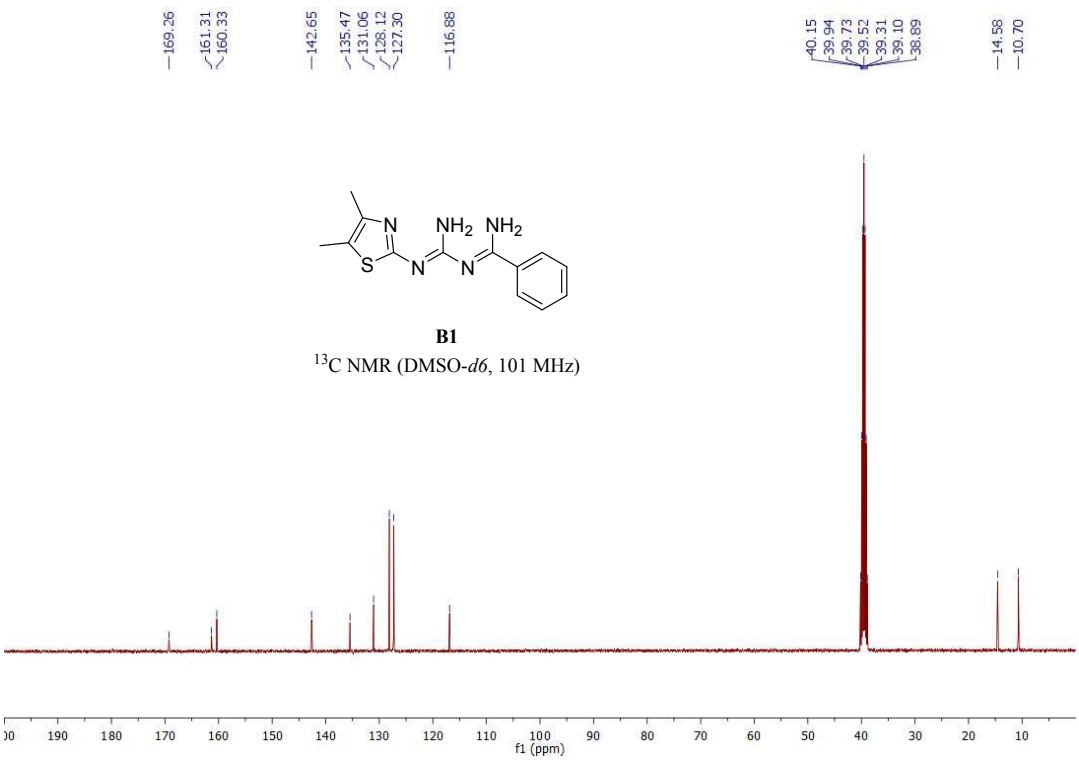
#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	4,525	10292860	647626	99,466	98,783	N/A	1653	1,628	1,851	
2	Unknown	3	5,033	55304	7981	0,534	1,217	N/A	12366	N/A	1,594	

N-(N-(4,5-dimethylthiazol-2-yl)carbamimidoyl)benzimidamide (**B1**)



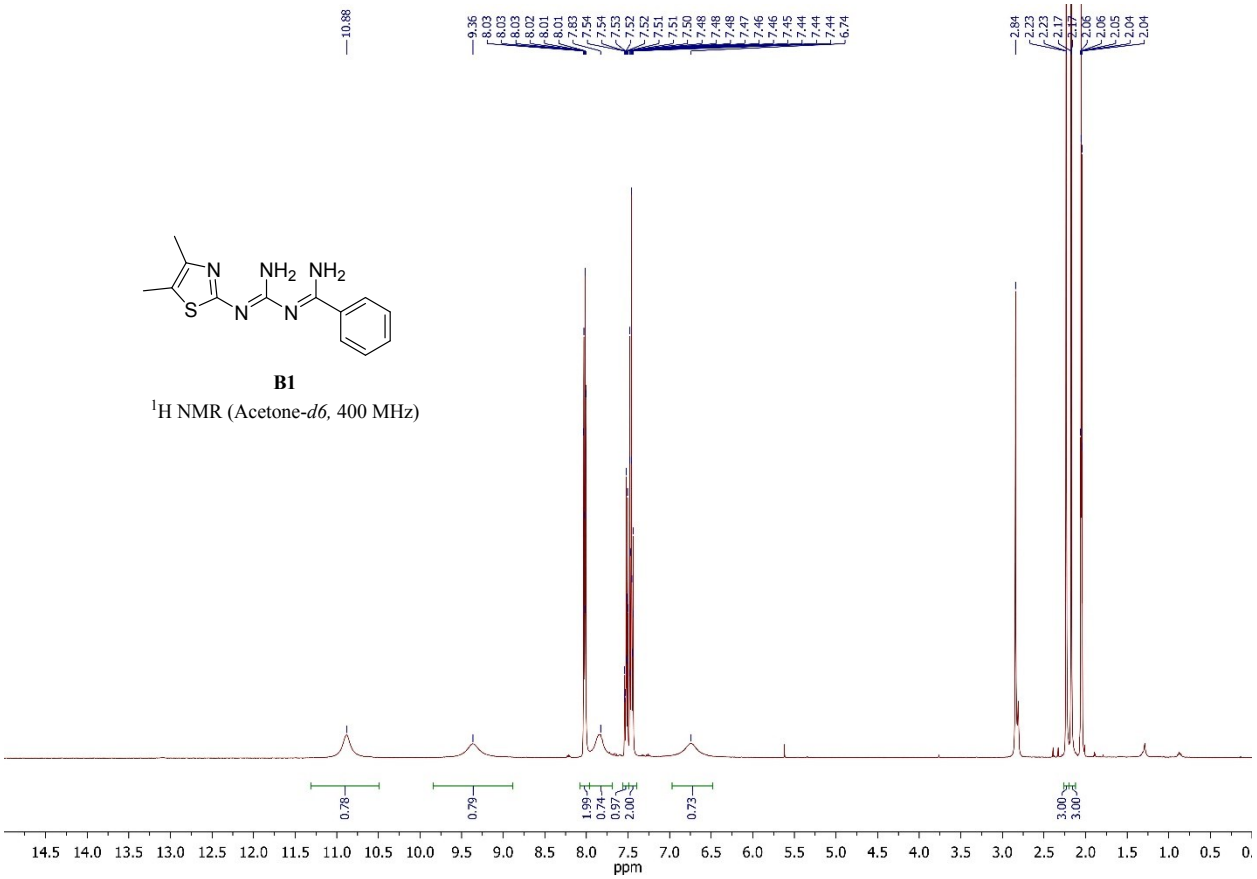
B1

^{1}H NMR (DMSO-*d6*, 200 MHz)



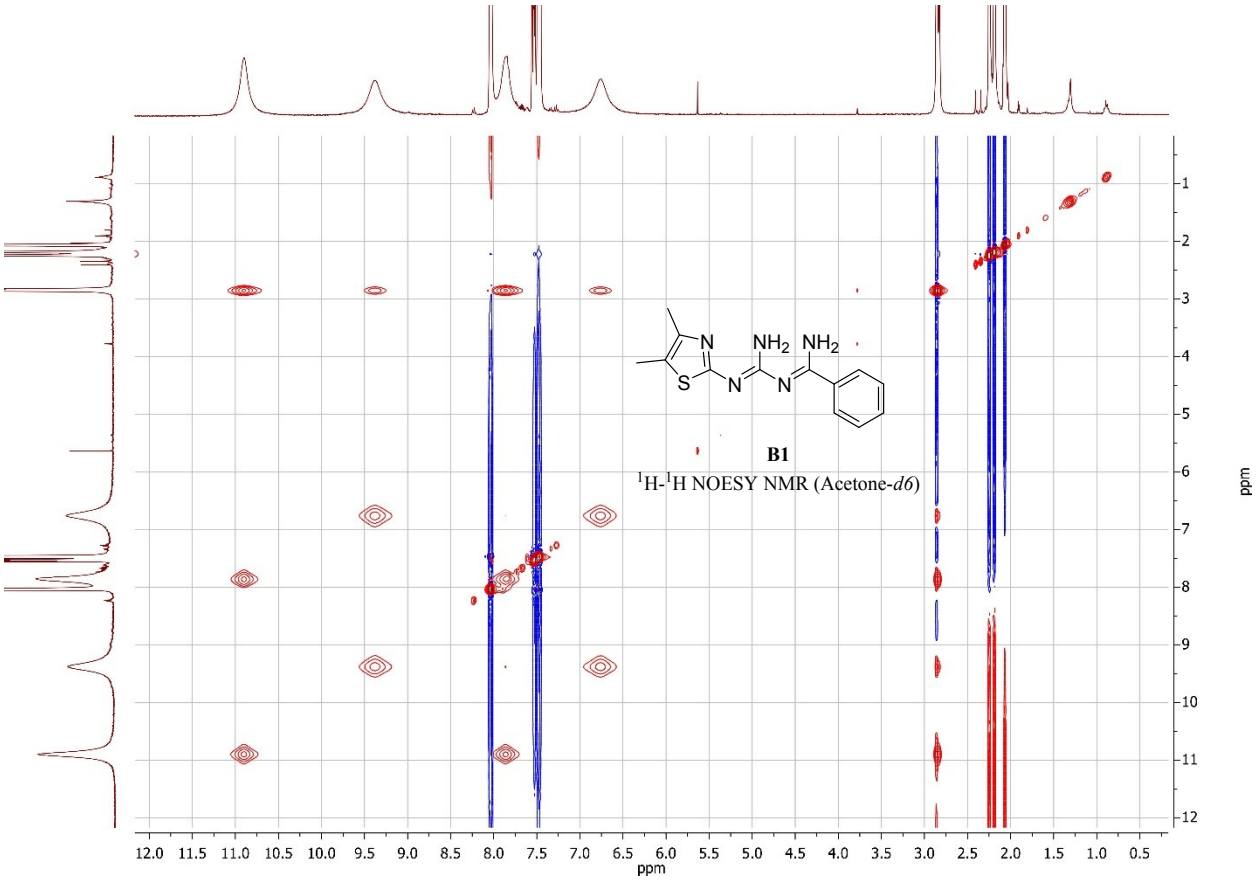
B1

^{13}C NMR (DMSO-*d6*, 101 MHz)



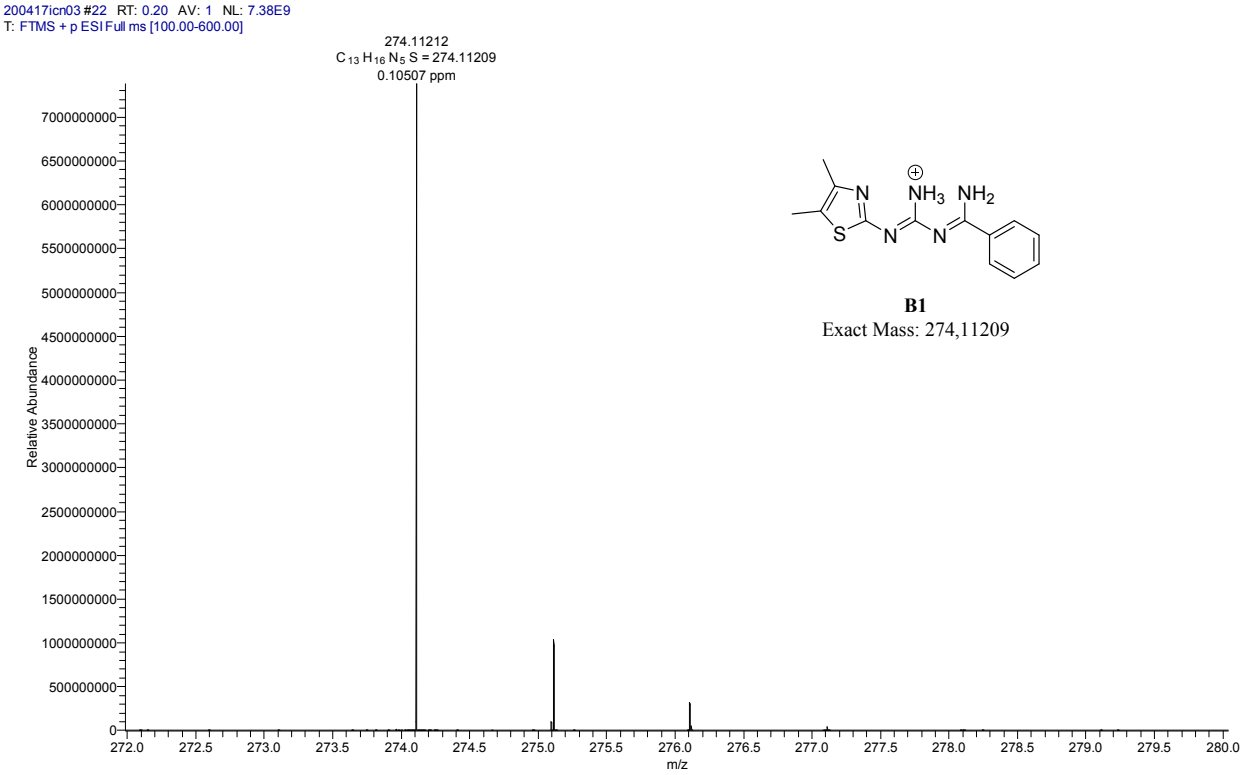
B1

^{1}H NMR (Acetone-*d6,* 400 MHz)

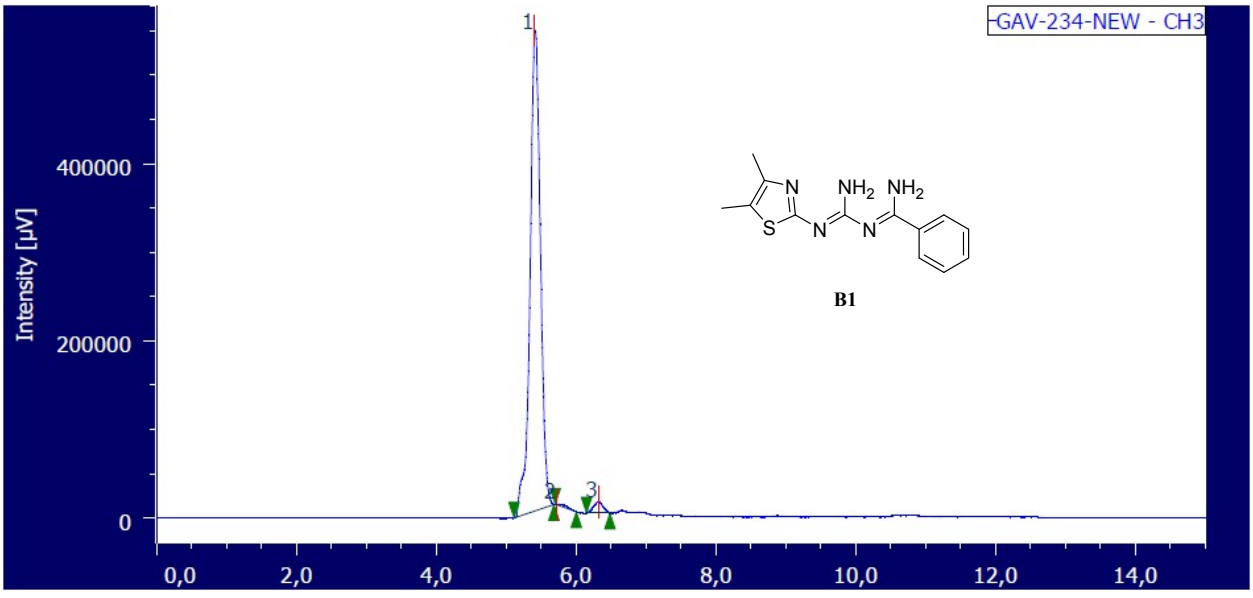


B1

^{1}H-^{1}H NOESY NMR (Acetone-*d6*)



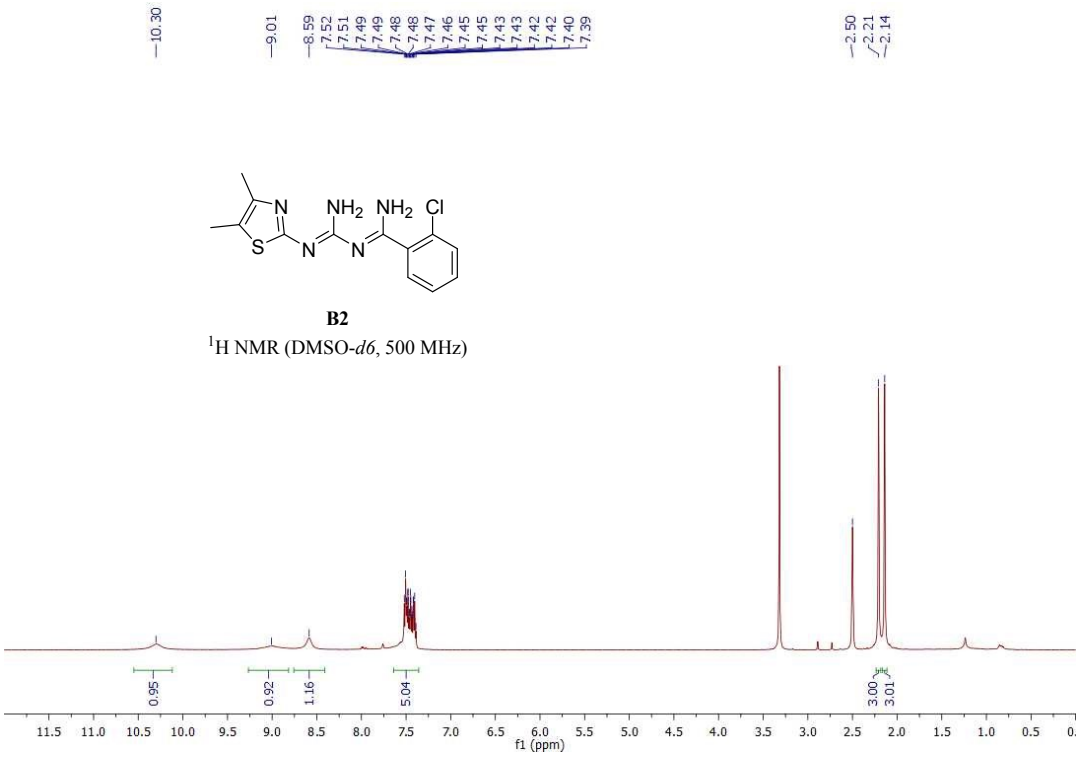
B1
Exact Mass: 274,11209



B1

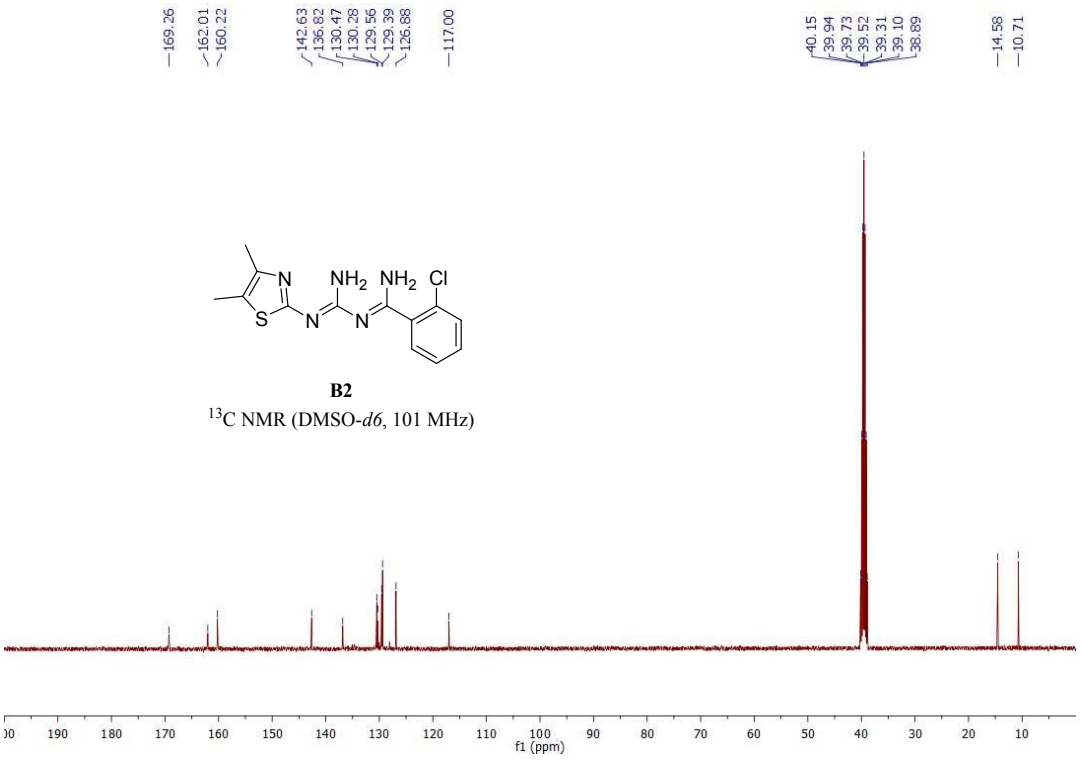
#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,408	5572413	541832	97,478	97,604	N/A	6991	0,953	0,901	
2	Unknown	3	5,725	24739	925	0,433	0,167	N/A	3152	1,772	5,977	
3	Unknown	3	6,317	119432	12378	2,089	2,230	N/A	9308	N/A	0,982	

2-chloro-N-(N-(4,5-dimethylthiazol-2-yl)carbamimidoyl)benzimidamide (**B2**)



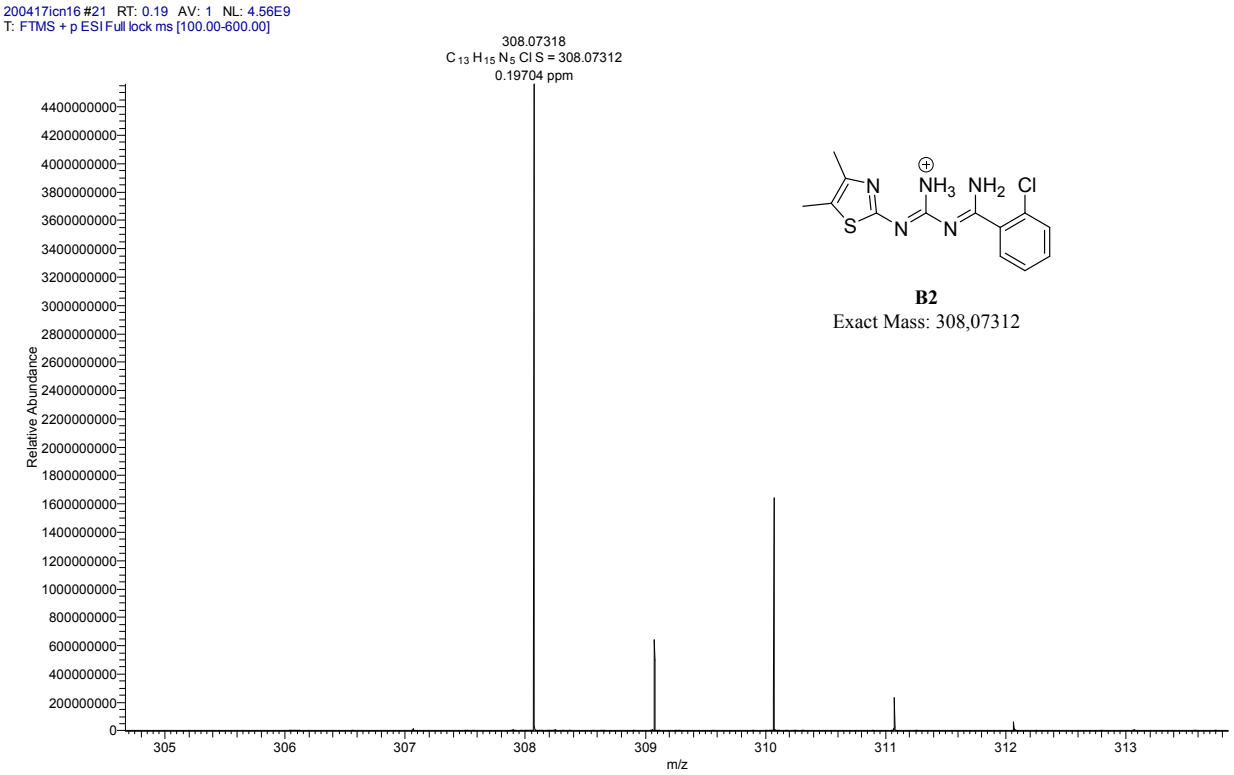
B2

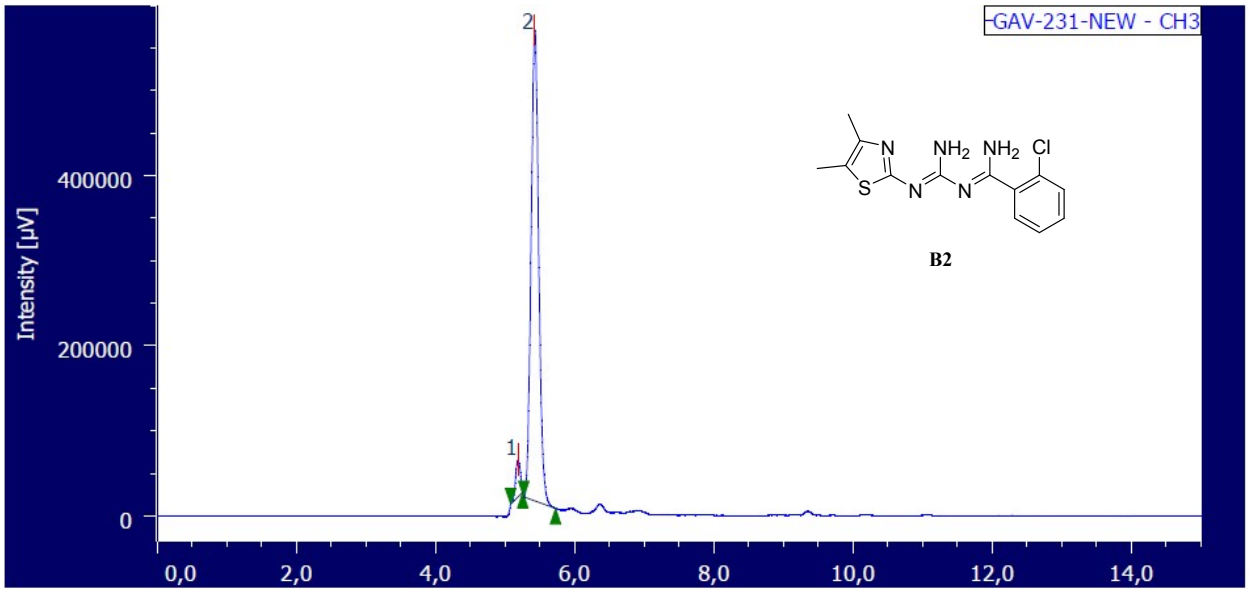
^{1}H NMR (DMSO-*d6*, 500 MHz)



B2

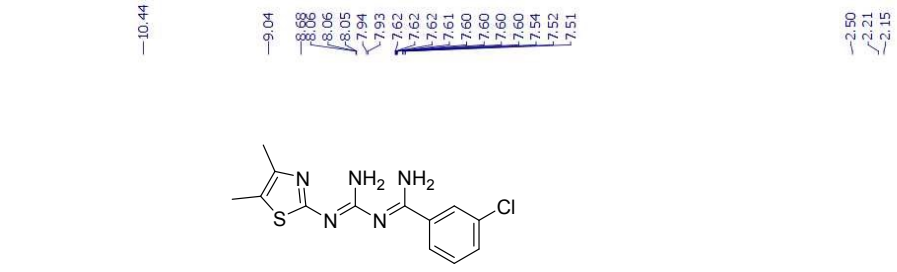
^{13}C NMR (DMSO-*d6*, 101 MHz)





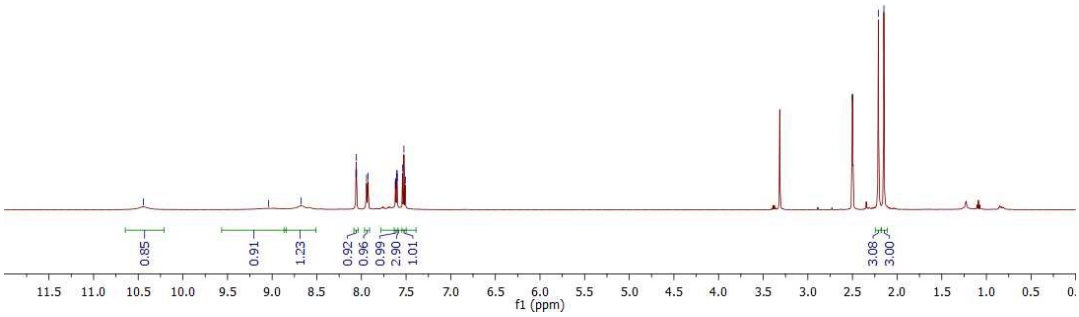
#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,183	204215	43052	4,640	7,238	N/A	24462	1,464	0,831	
2	Unknown	3	5,425	4196779	551721	95,360	92,762	N/A	11950	N/A	1,100	

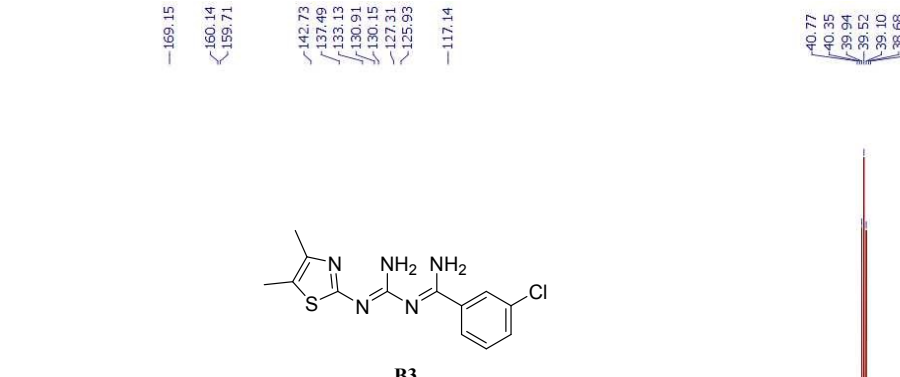
3-chloro-N-(N-(4,5-dimethylthiazol-2-yl)carbamimidoyl)benzimidamide (**B3**)



B3

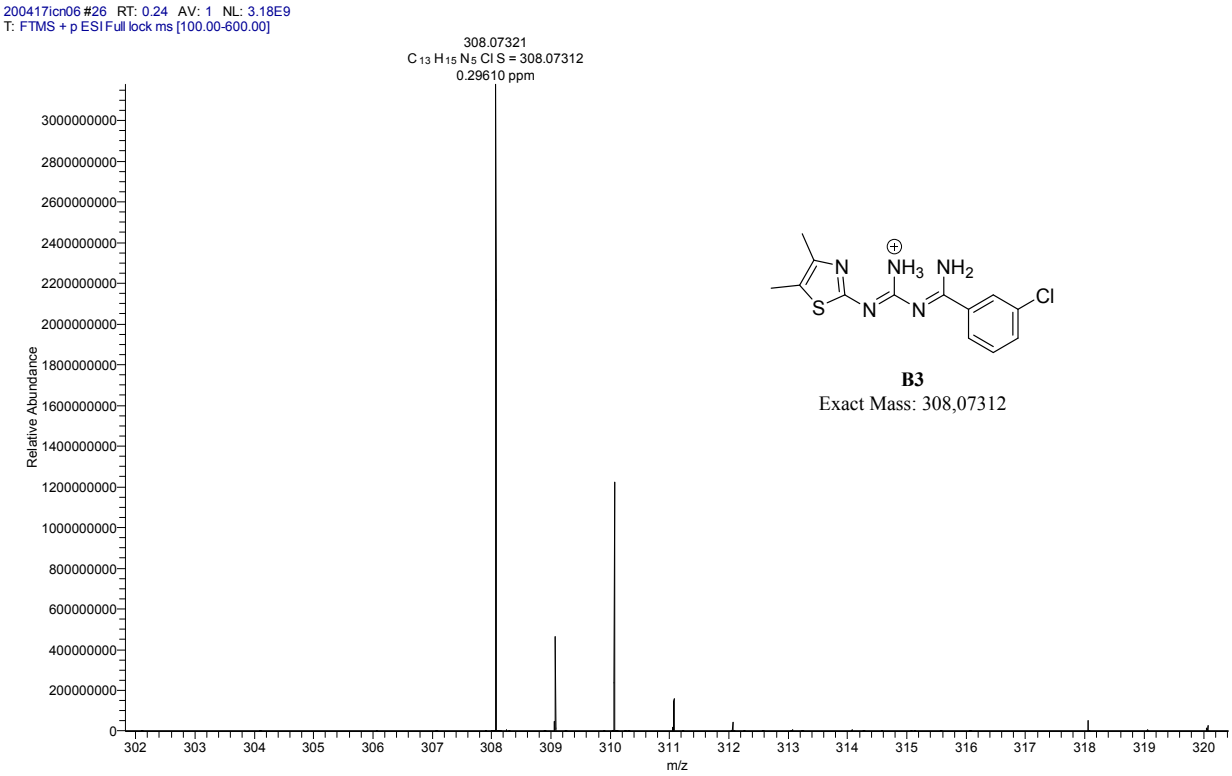
^{1}H NMR (DMSO-*d6*, 500 MHz)

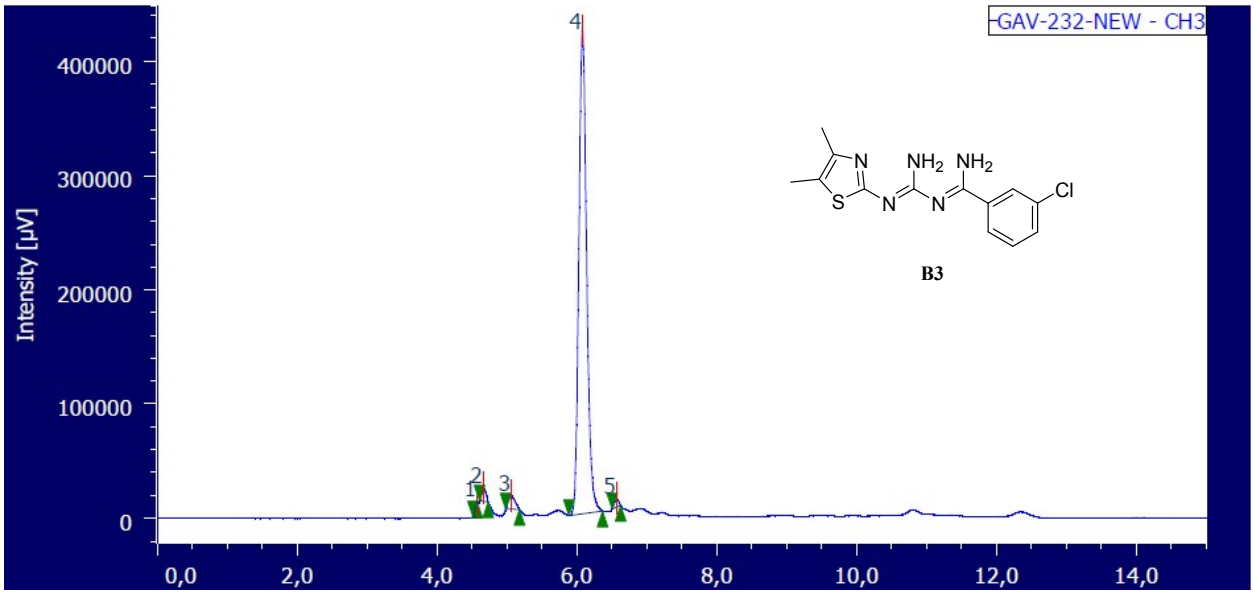




B3

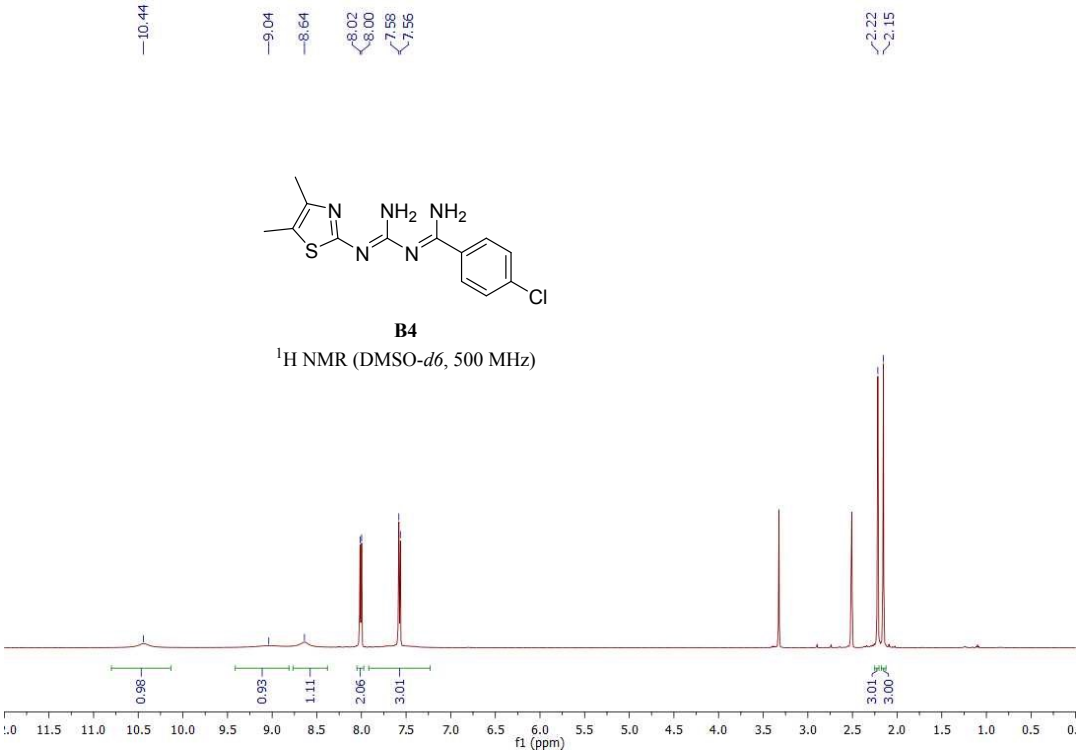
^{13}C NMR (DMSO-*d6*, 50 MHz)





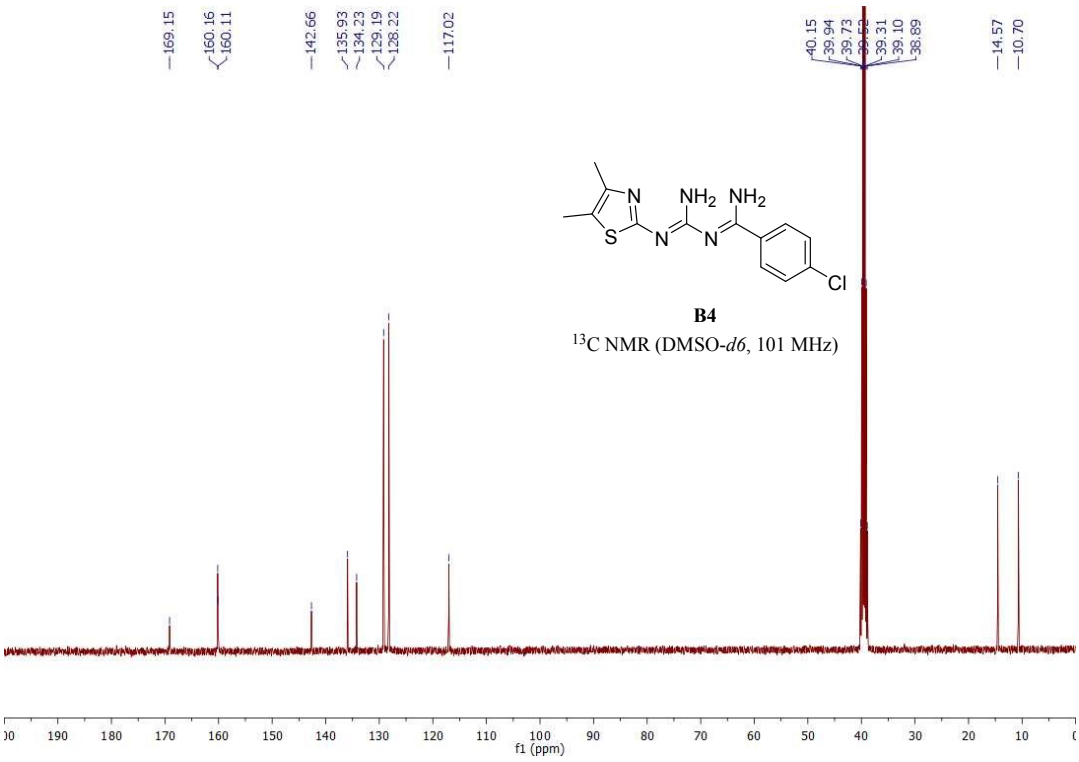
#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	4,567	6009	2281	0,175	0,501	N/A	N/A	N/A	N/A	
2	Unknown	3	4,658	52382	11982	1,523	2,630	N/A	21542	2,512	1,255	
3	Unknown	3	5,058	75503	11303	2,195	2,481	N/A	11057	5,198	1,269	
4	Unknown	3	6,075	3274337	422741	95,195	92,773	N/A	14787	3,066	1,221	
5	Unknown	3	6,567	31393	7364	0,913	1,616	N/A	46552	N/A	0,946	

4-chloro-N-(N-(4,5-dimethylthiazol-2-yl)carbamimidoyl)benzimidamide (**B4**)



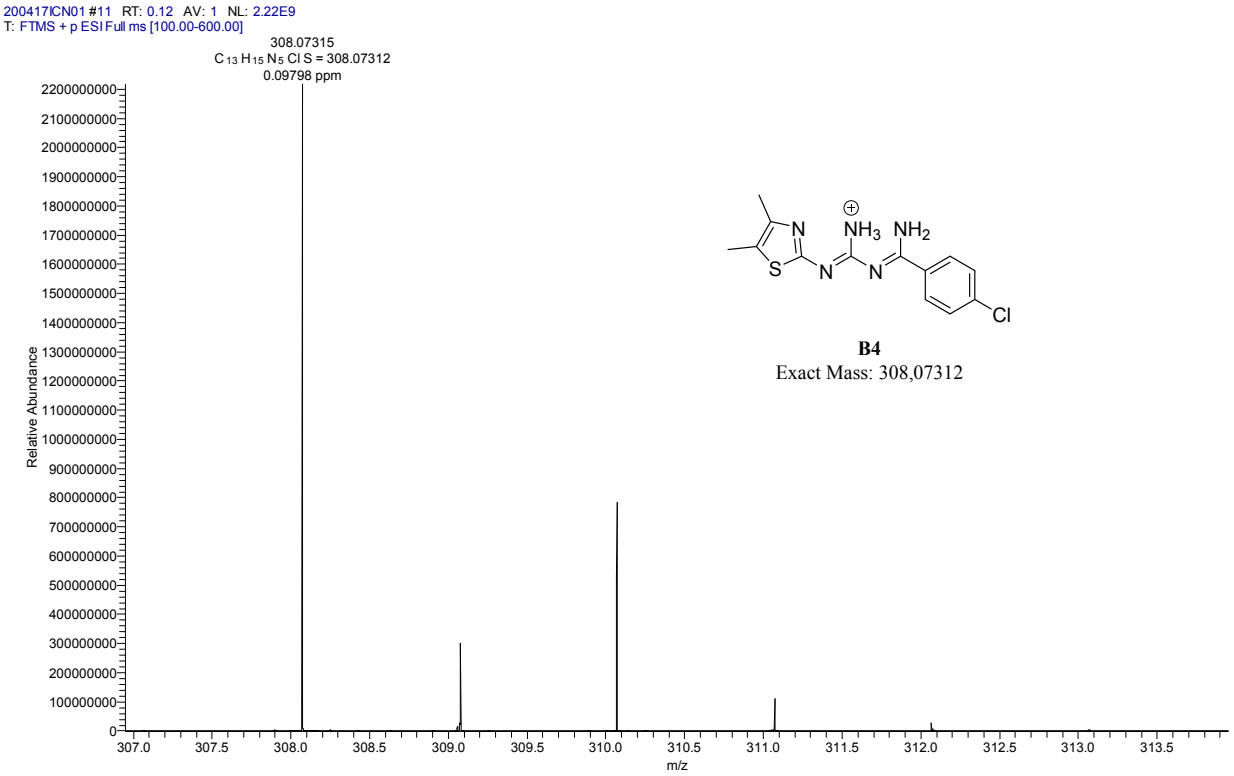
B4

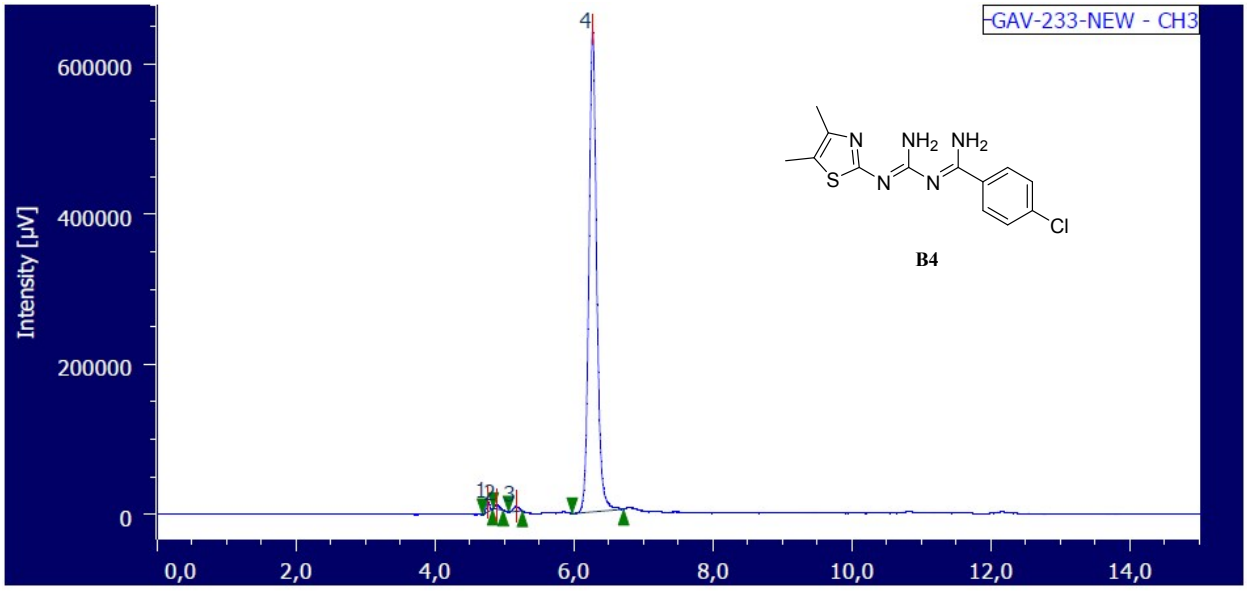
^{1}H NMR (DMSO-*d6*, 500 MHz)



B4

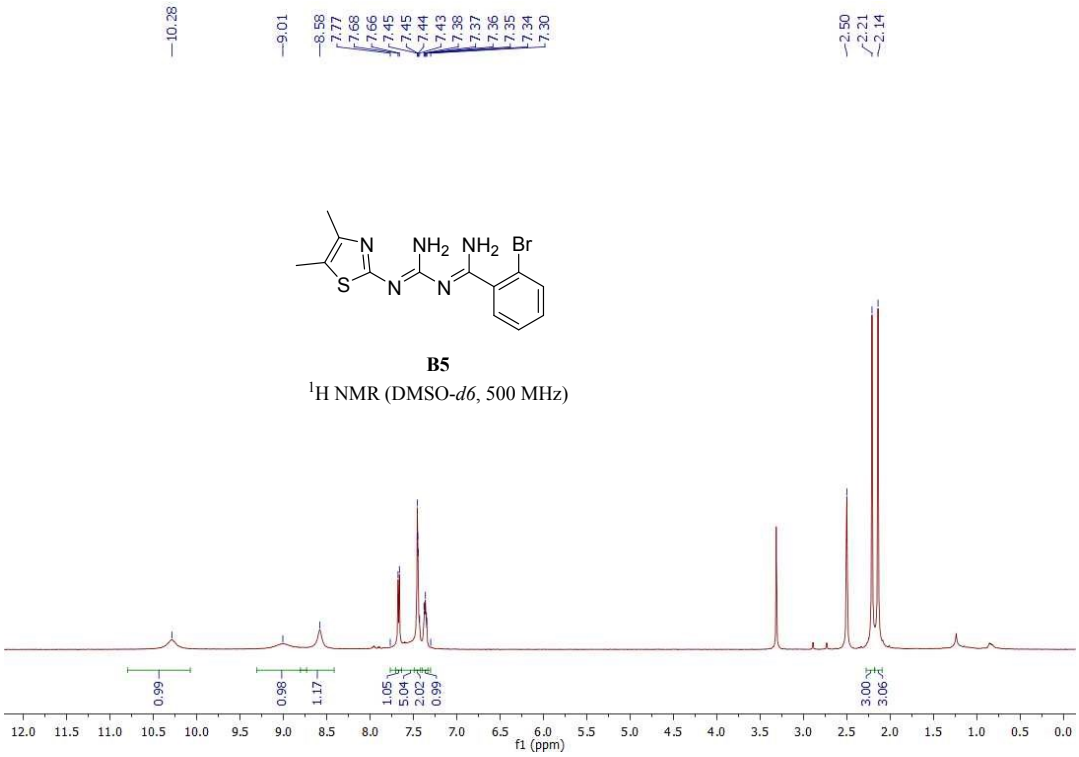
^{13}C NMR (DMSO-*d6*, 101 MHz)





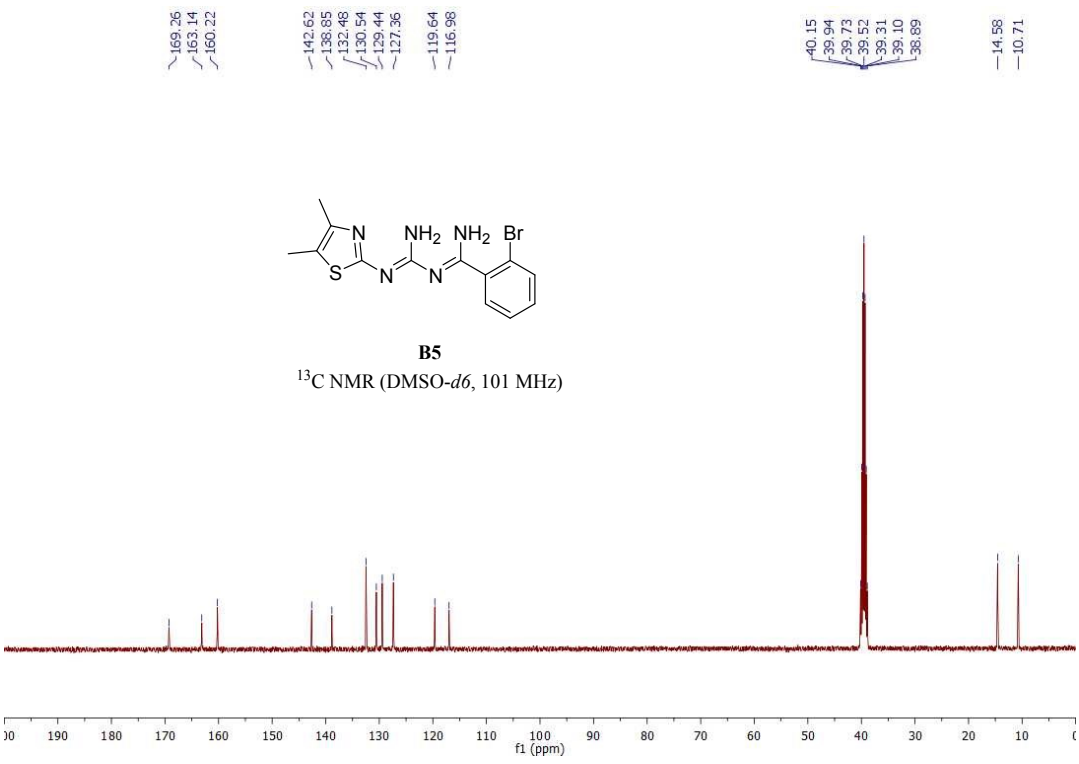
#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	4,758	42106	13013	0,821	1,953	N/A	45020	1,180	1,137	
2	Unknown	3	4,883	22193	5062	0,433	0,760	N/A	25299	1,922	1,614	
3	Unknown	3	5,167	38533	6314	0,751	0,948	N/A	14291	5,960	1,019	
4	Unknown	3	6,267	5026068	641830	97,995	96,339	N/A	16151	N/A	1,127	

2-bromo-N-(N-(4,5-dimethylthiazol-2-yl)carbamimidoyl)benzimidamide (**B5**)



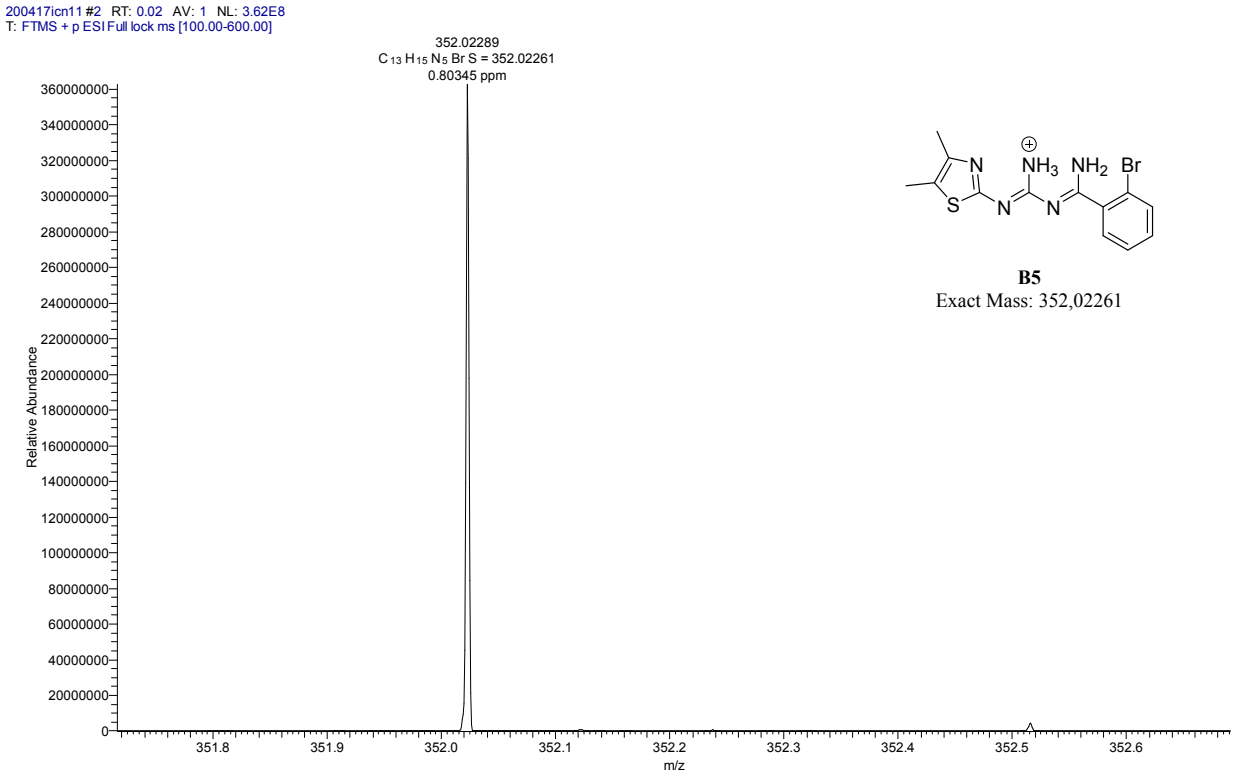
B5

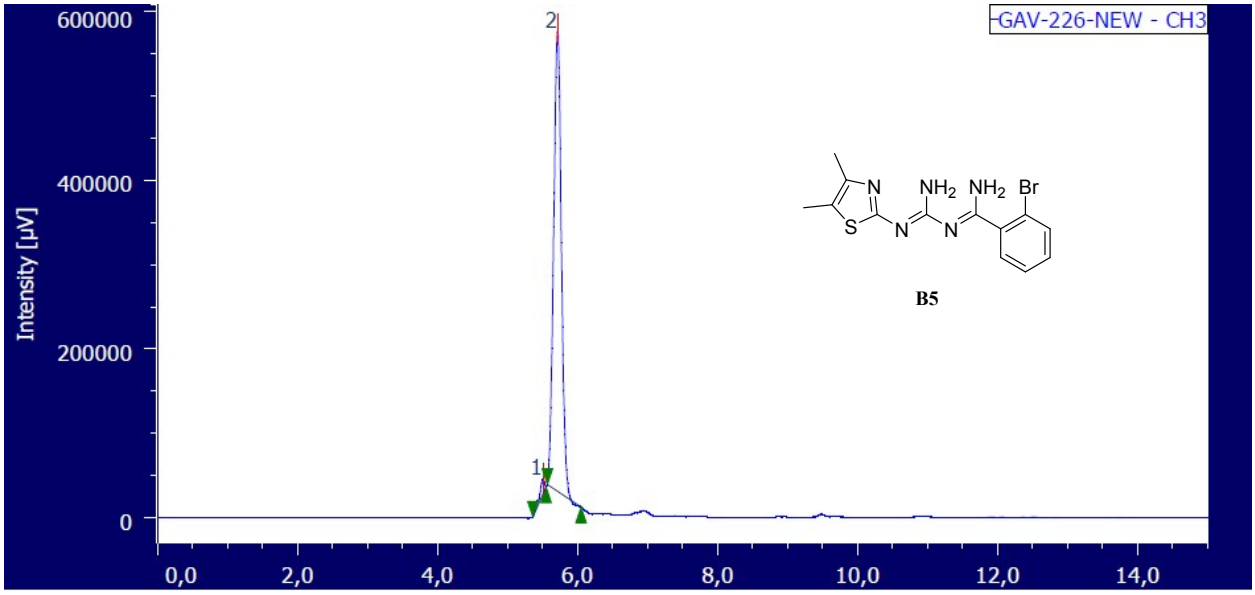
^{1}H NMR (DMSO-*d6*, 500 MHz)



B5

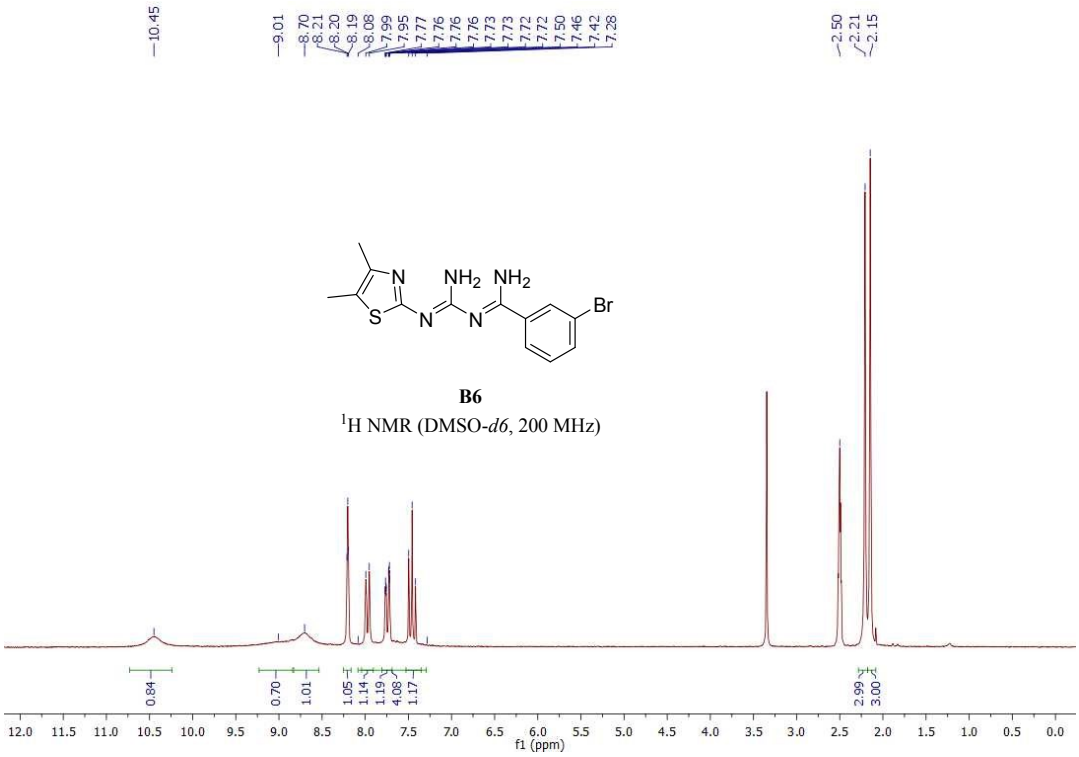
^{13}C NMR (DMSO-*d6*, 101 MHz)





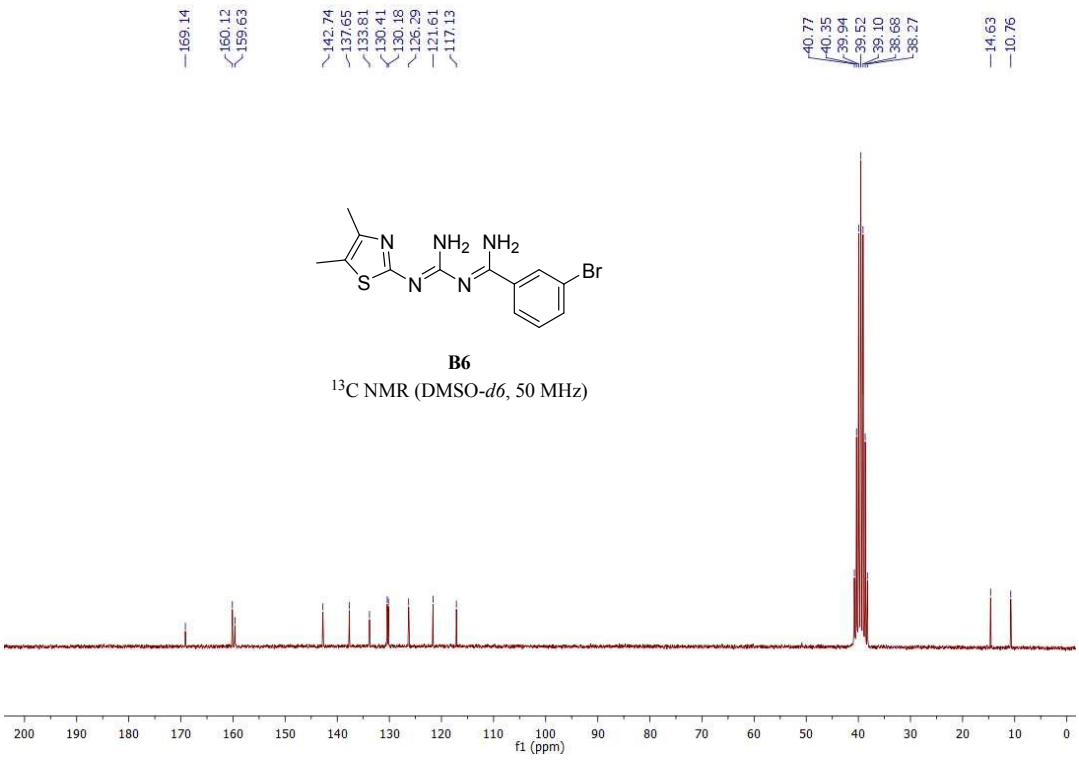
#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,508	95594	15968	2,237	2,834	N/A	9640	0,982	0,642	
2	Unknown	3	5,717	4177556	547429	97,763	97,166	N/A	12922	N/A	1,011	

2-bromo-N-(N-(4,5-dimethylthiazol-2-yl)carbamimidoyl)benzimidamide (**B6**)



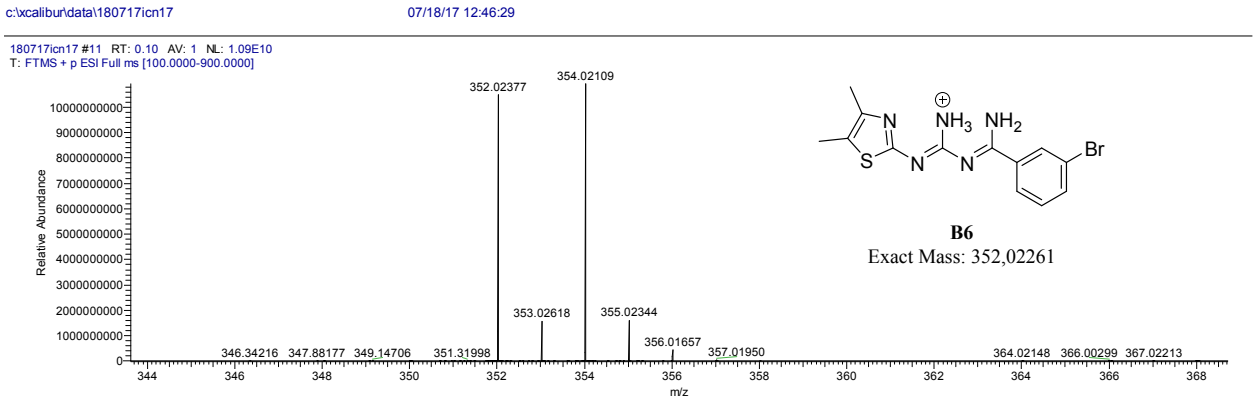
B6

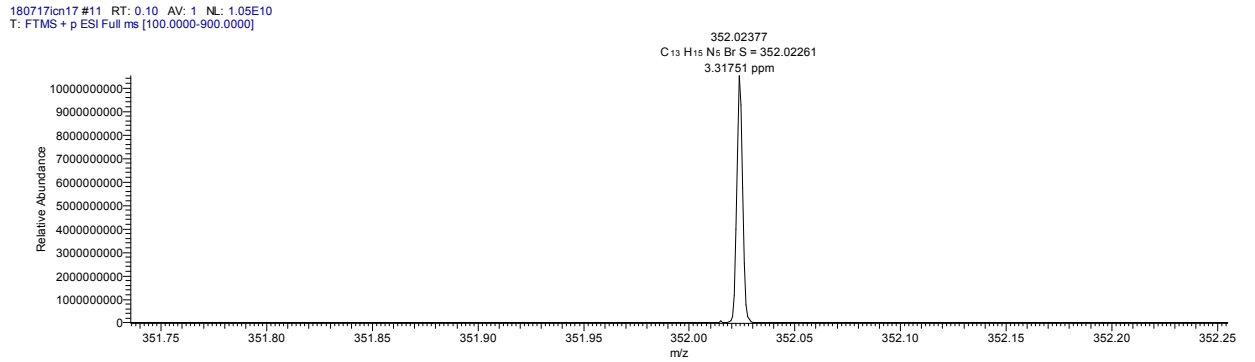
^{1}H NMR (DMSO-*d6*, 200 MHz)

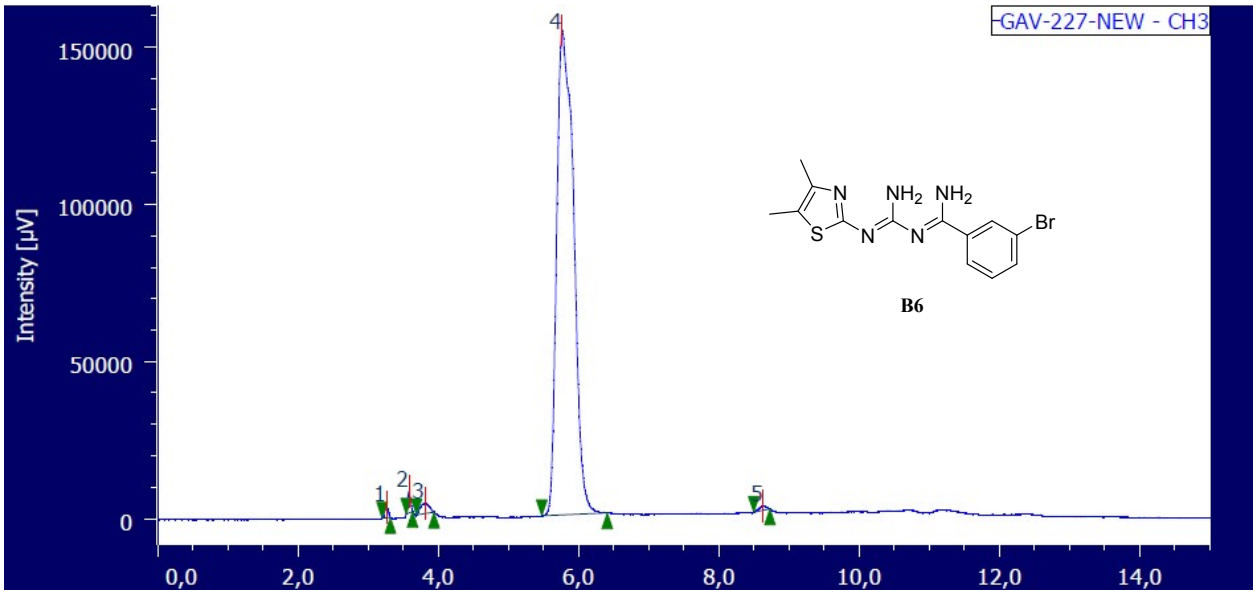


B6

^{13}C NMR (DMSO-*d6*, 50 MHz)

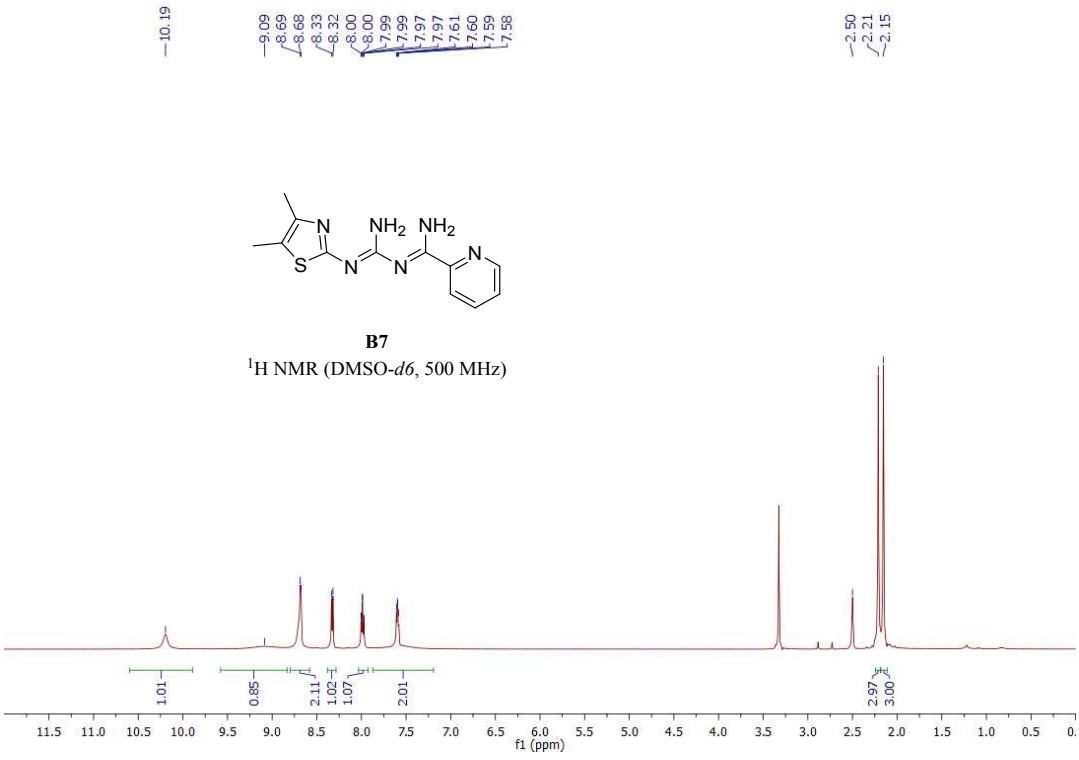






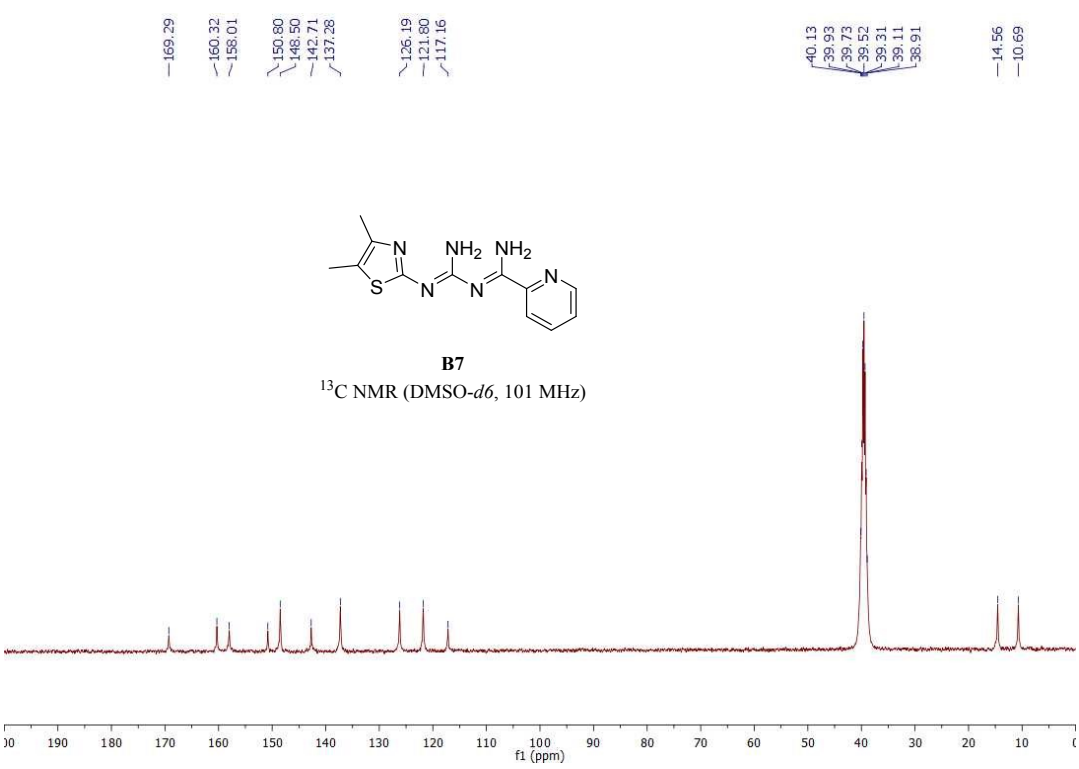
#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	3,258	11563	3422	0,438	2,032	N/A	19464	3,636	1,036	
2	Unknown	3	3,583	20391	6714	0,772	3,986	N/A	27906	1,304	1,092	
3	Unknown	3	3,808	29177	3260	1,105	1,935	N/A	3429	5,378	1,052	
4	Unknown	3	5,767	2569094	153823	97,315	91,322	N/A	2408	8,203	1,464	
5	Unknown	3	8,633	9741	1222	0,369	0,726	N/A	22406	N/A	0,855	

N-(N-(4,5-dimethylthiazol-2-yl)carbamimidoyl)picolinimidamide (**B7**)



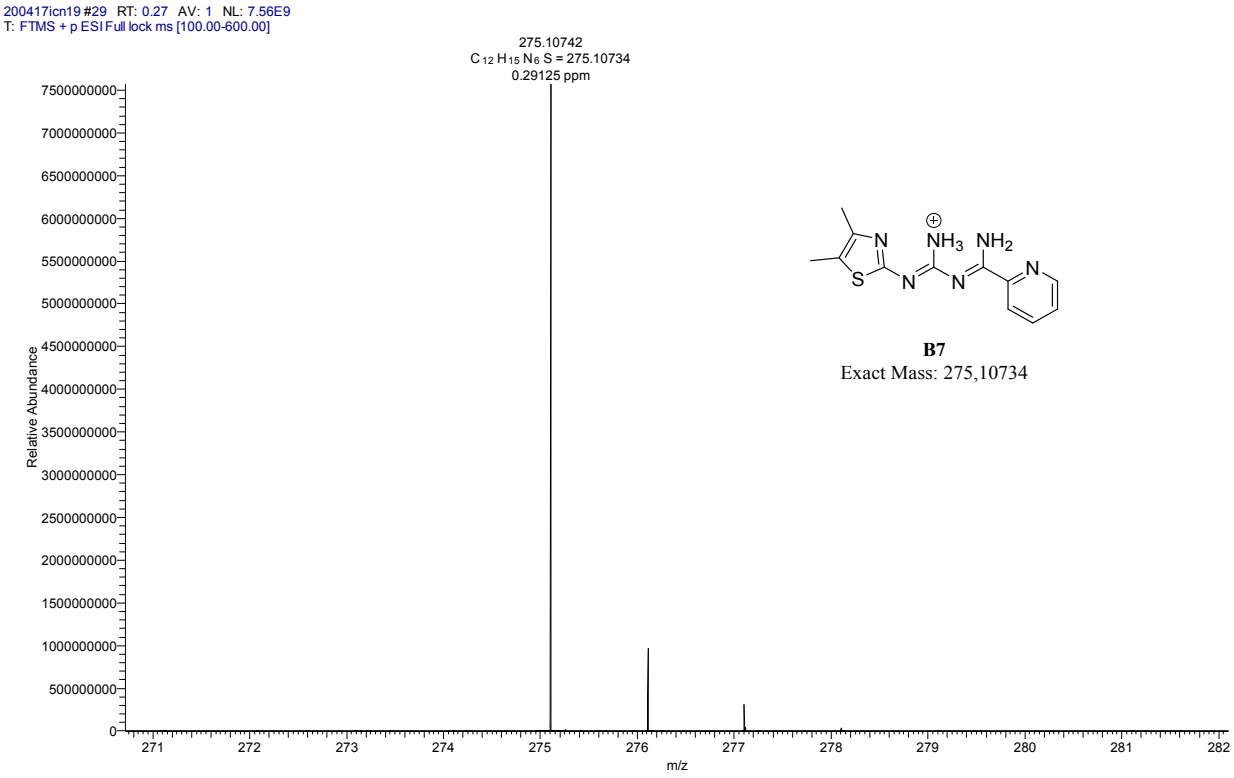
B7

^{1}H NMR (DMSO-*d6*, 500 MHz)



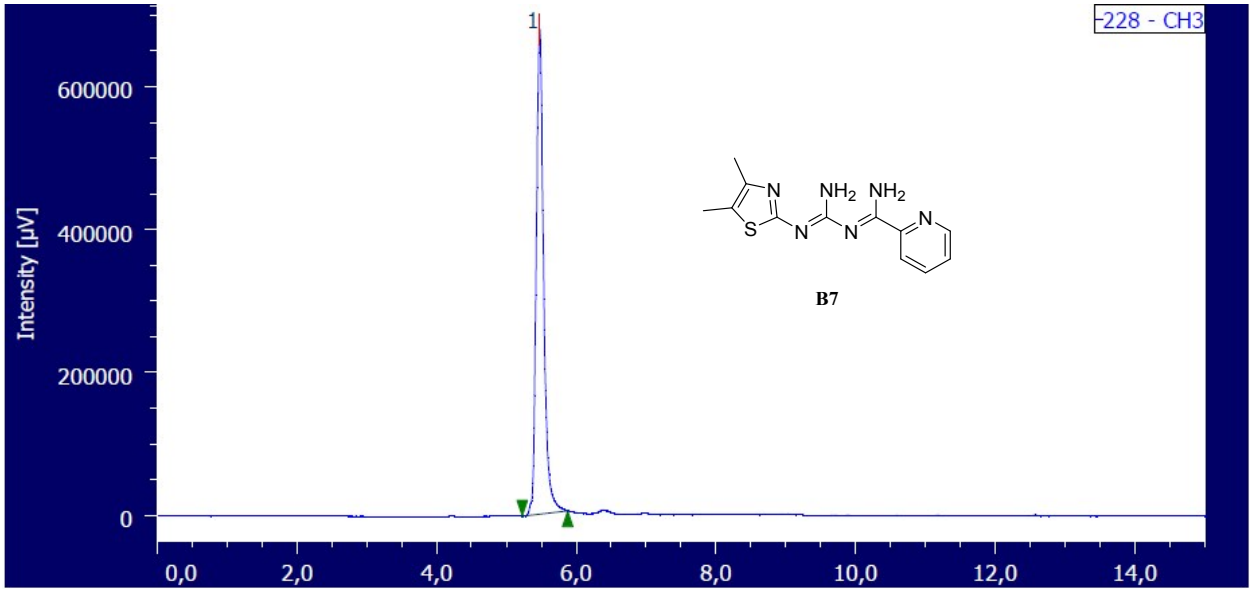
B7

^{13}C NMR (DMSO-*d6*, 101 MHz)



B7

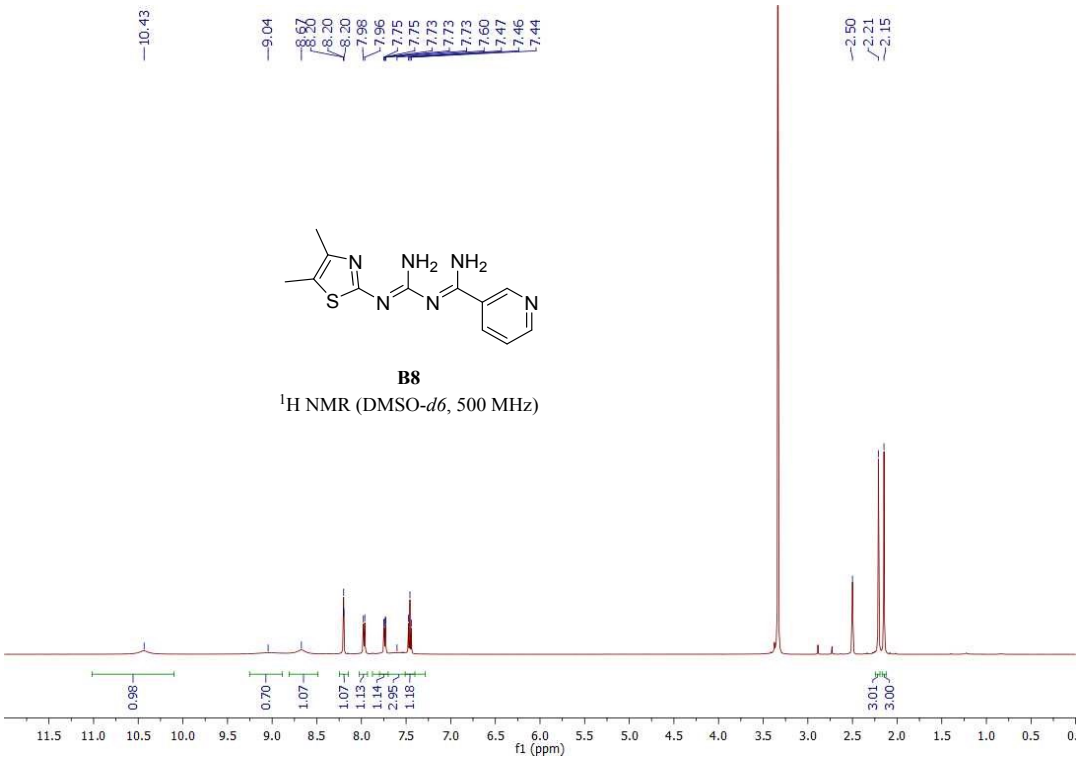
Exact Mass: 275,10734



B7

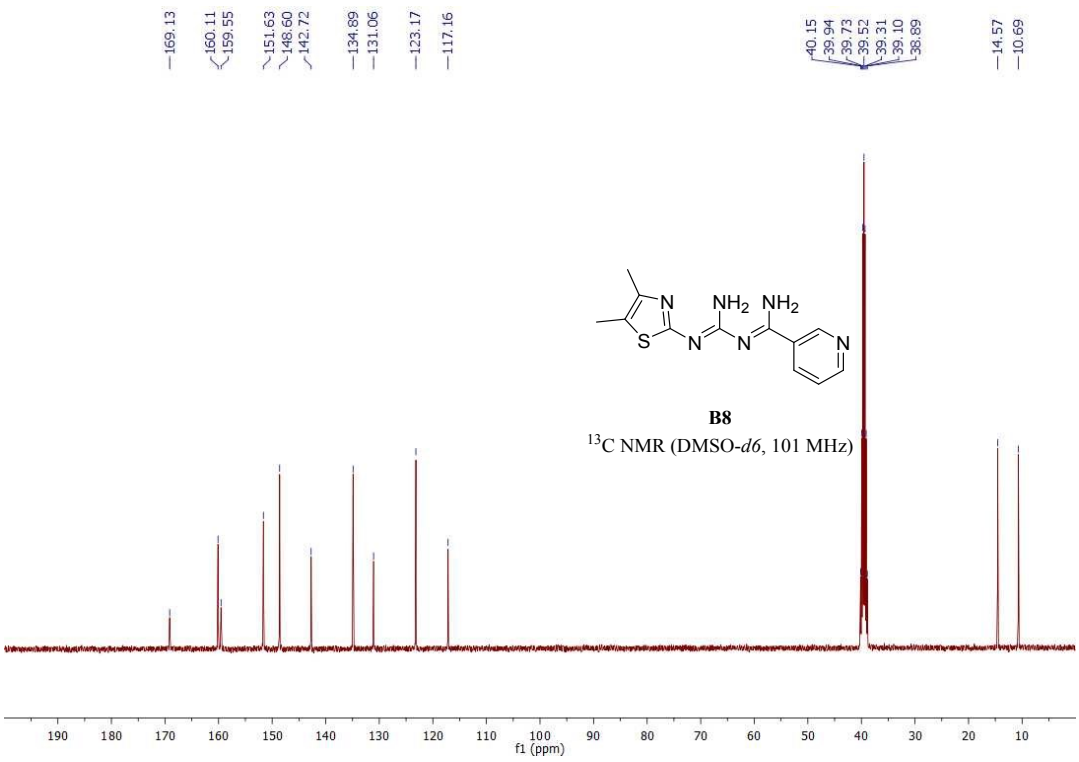
#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,475	5015551	677513	100,000	100,000	N/A	13922	N/A	1,254	

N-(N-(4,5-dimethylthiazol-2-yl)carbamimidoyl)nicotinimidamide (**B8**)



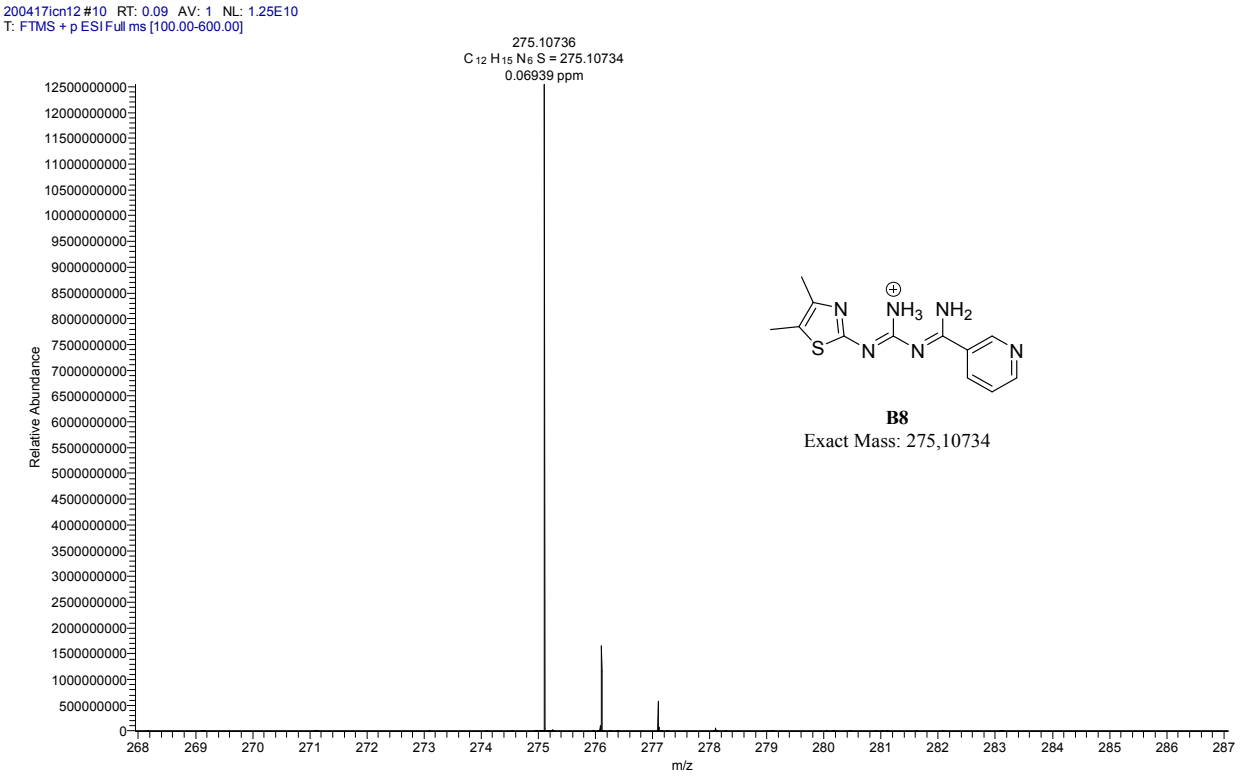
B8

^{1}H NMR (DMSO-*d6*, 500 MHz)

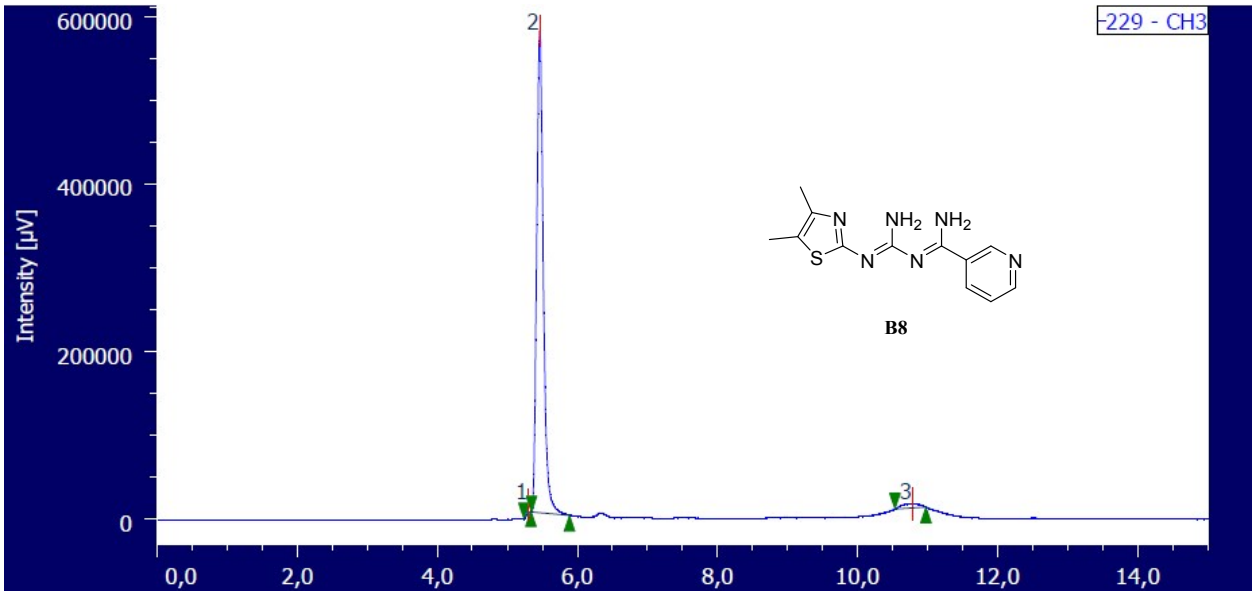


B8

^{13}C NMR (DMSO-*d6*, 101 MHz)



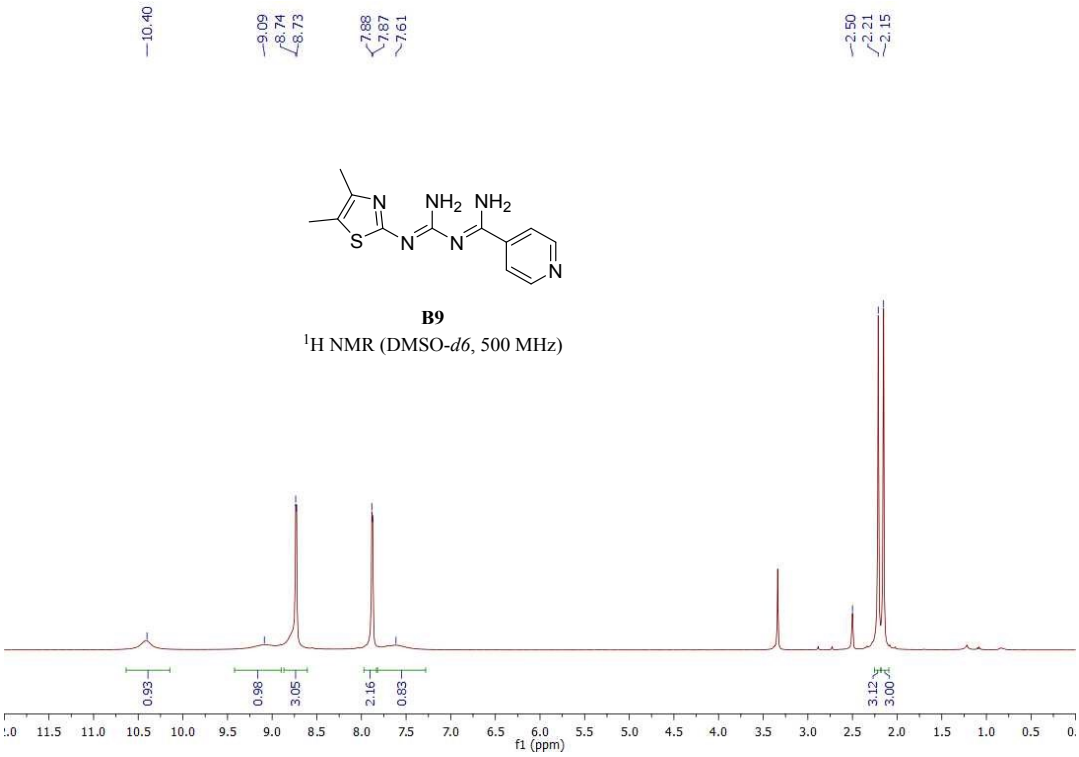
B8
Exact Mass: 275,10734



B8

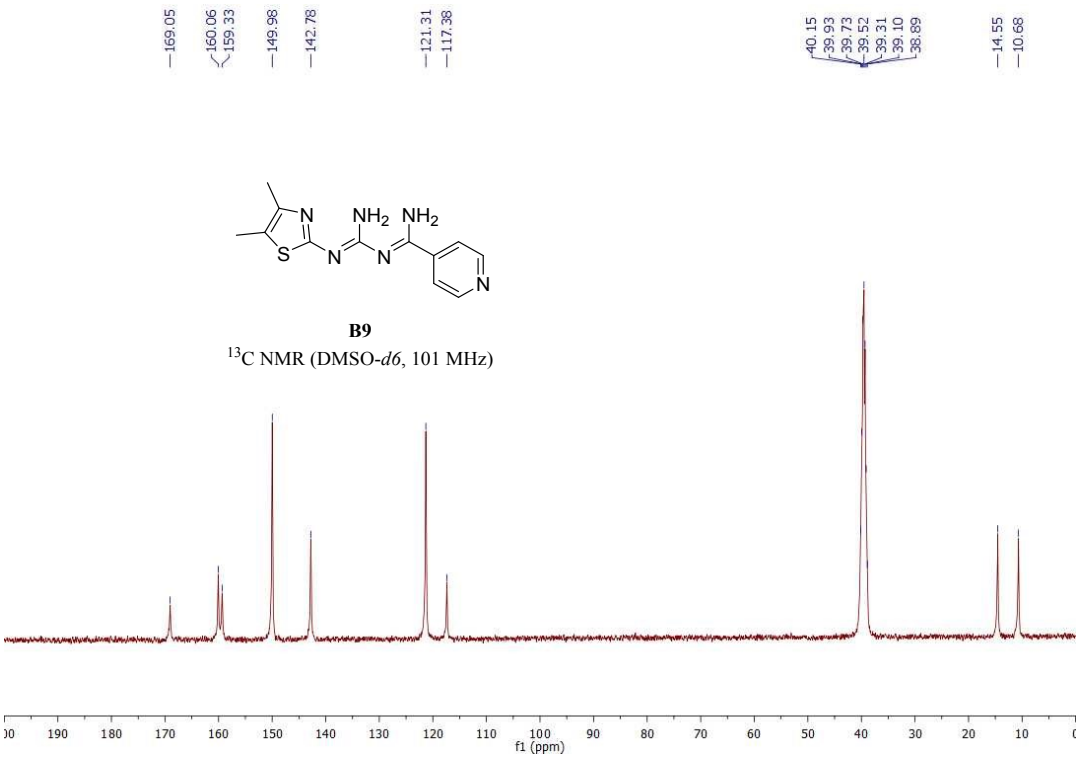
#	Peak Name	CH	tR [min]	Area [μV·sec]	Height [μV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,300	27180	10288	0,695	1,744	N/A	79762	1,300	0,905	
2	Unknown	3	5,458	3794018	574792	96,958	97,410	N/A	16670	14,965	1,269	
3	Unknown	3	10,783	91859	4995	2,347	0,847	N/A	6276	N/A	0,886	

N-(N-(4,5-dimethylthiazol-2-yl)carbamimidoyl)isonicotinimidamide (**B9**)



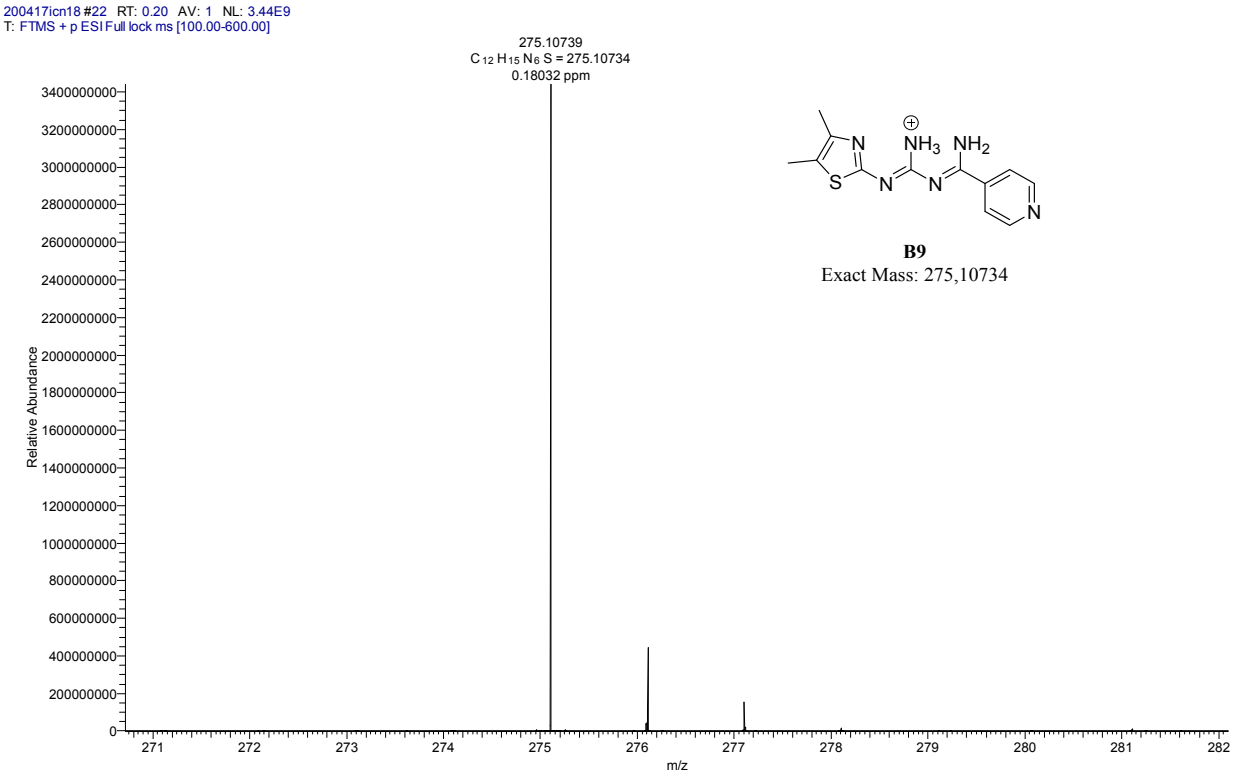
B9

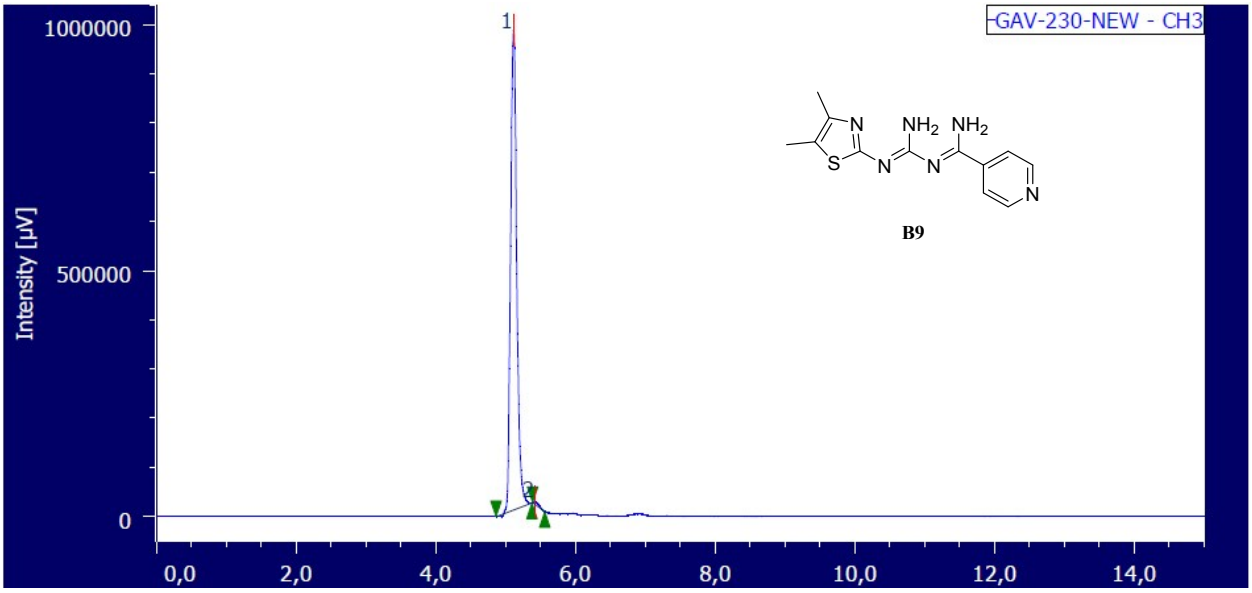
^{1}H NMR (DMSO-*d6*, 500 MHz)



B9

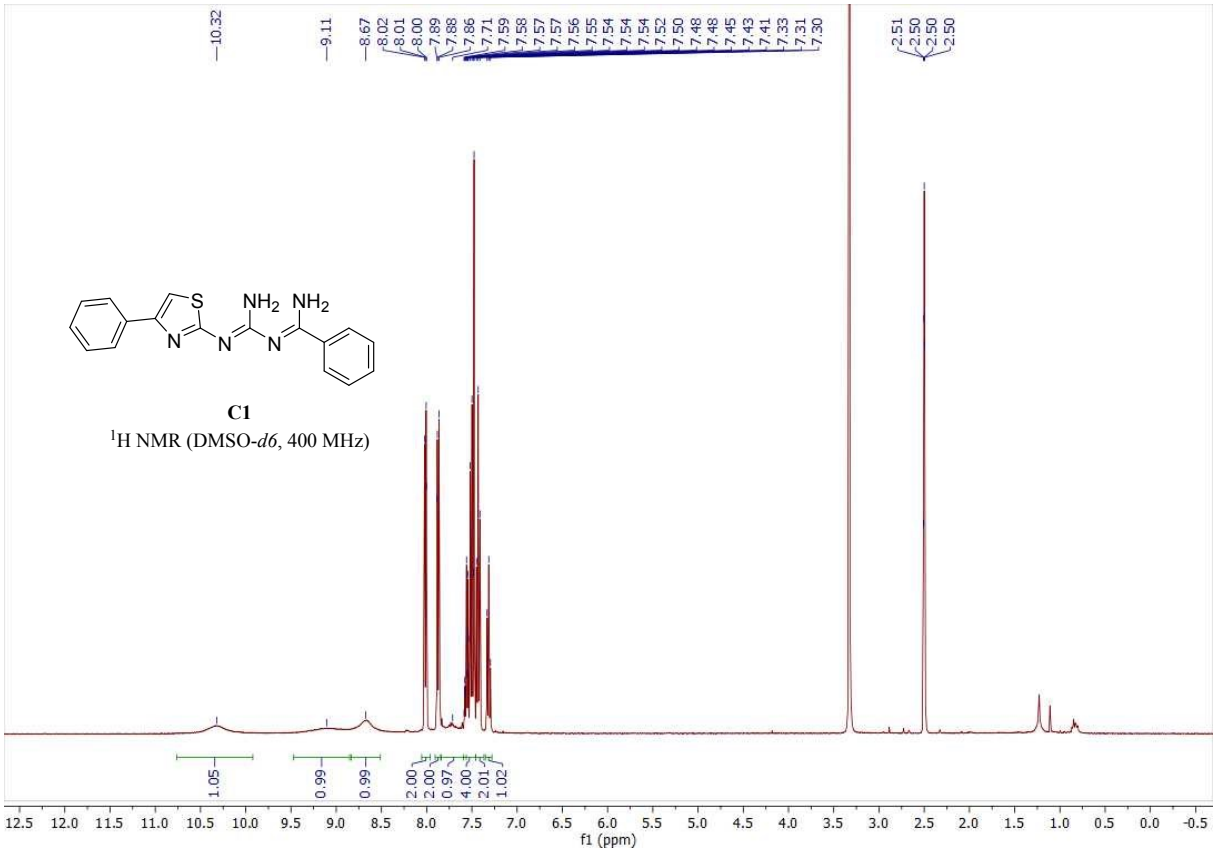
^{13}C NMR (DMSO-*d6*, 101 MHz)





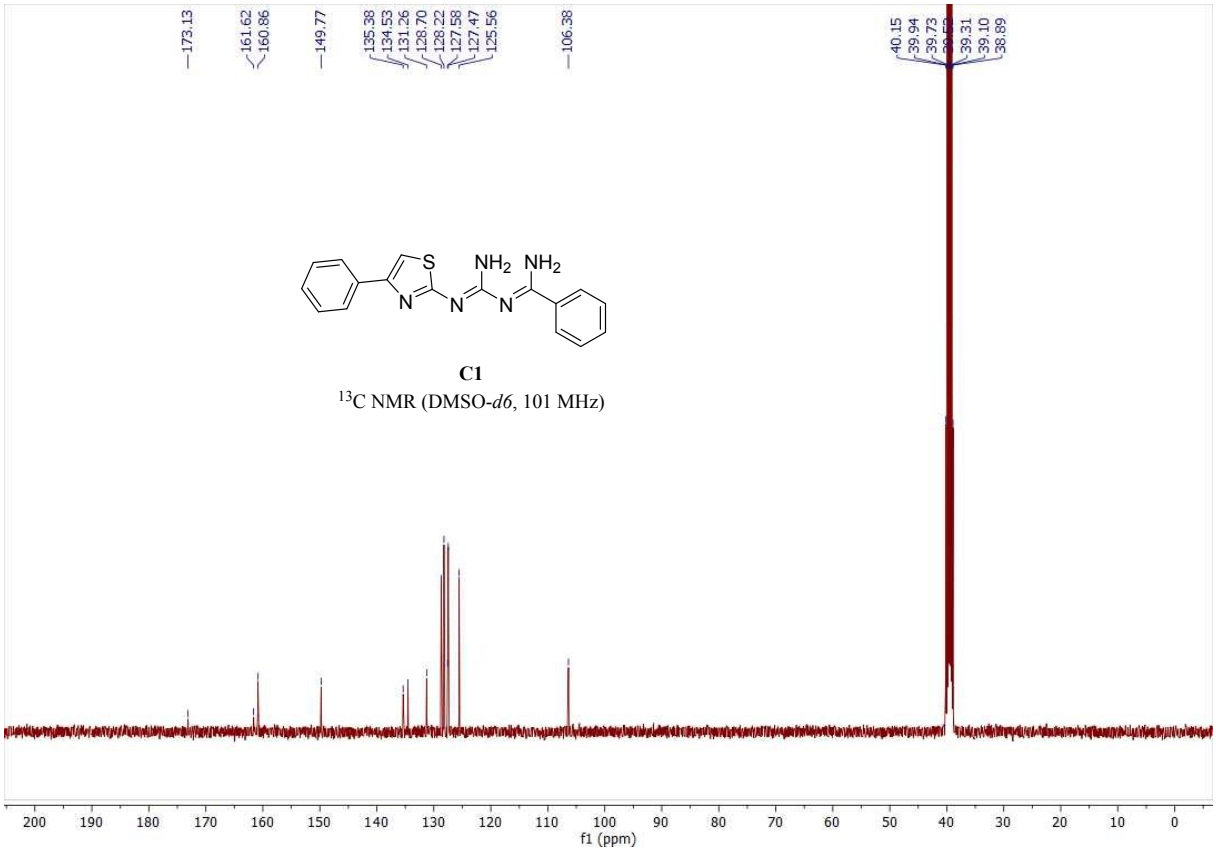
#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,108	5961561	974675	99,493	99,494	N/A	16462	1,936	1,249	
2	Unknown	3	5,417	30355	4958	0,507	0,506	N/A	18315	N/A	2,186	

N-(N-(4-phenylthiazol-2-yl)carbamimidoyl)benzimidamide (**C1**)



C1

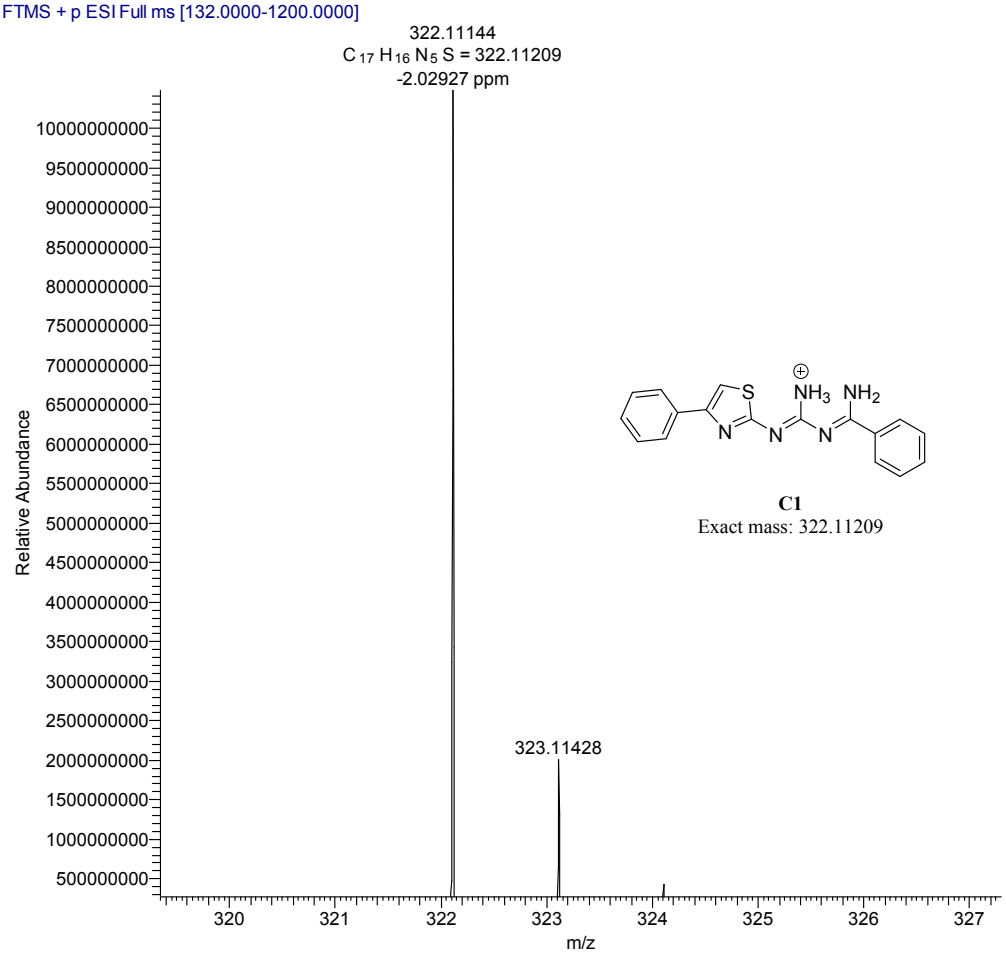
^{1}H NMR (DMSO-*d6*, 400 MHz)



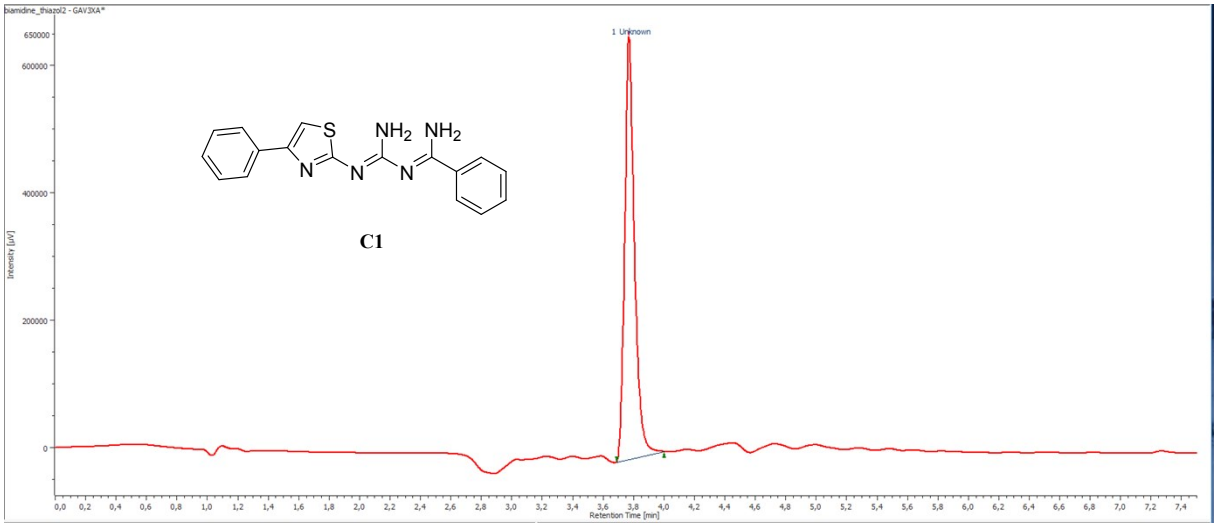
C1

^{13}C NMR (DMSO-*d6*, 101 MHz)

MTF561 #1047-1100 RT: 8.31-8.71 AV: 27 NL: 1.05E10
T: FTMS + p ESI Full ms [132.0000-1200.0000]



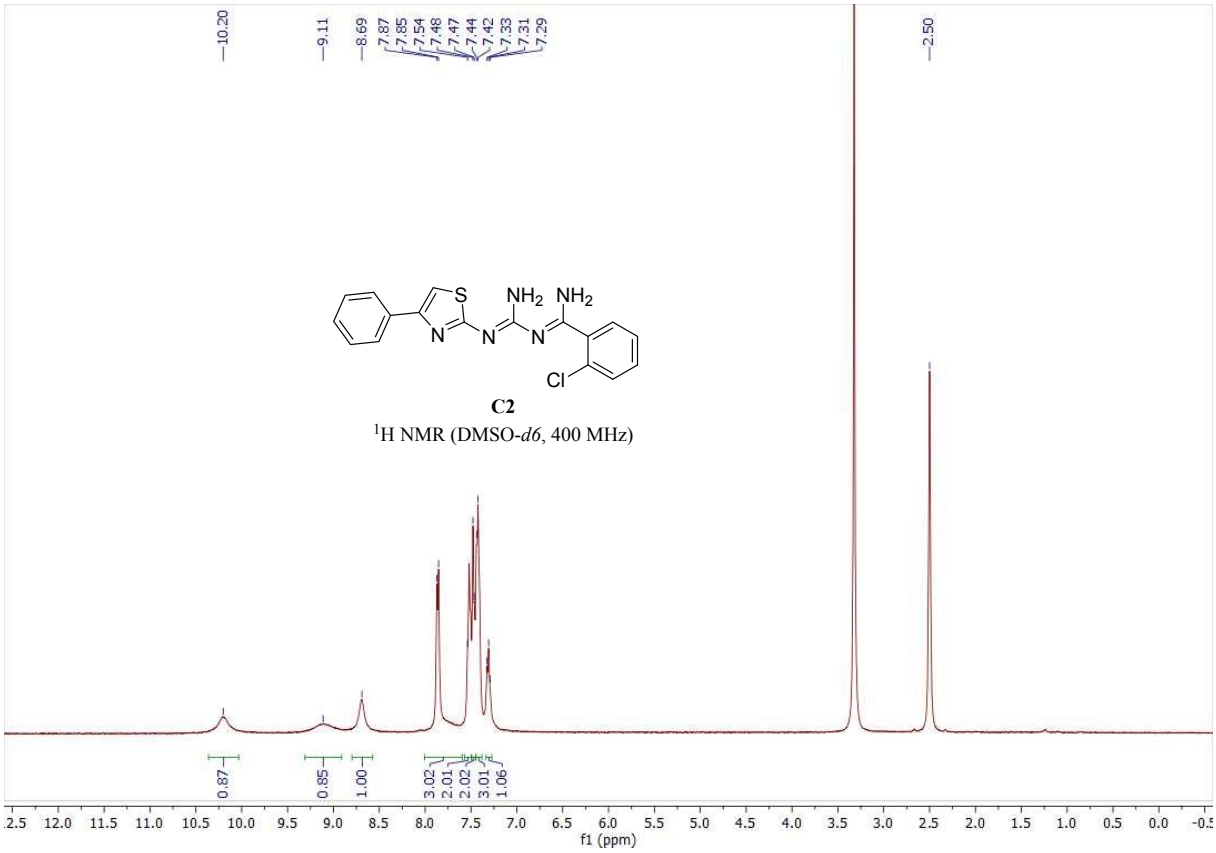
C1
Exact mass: 322.11209



C1

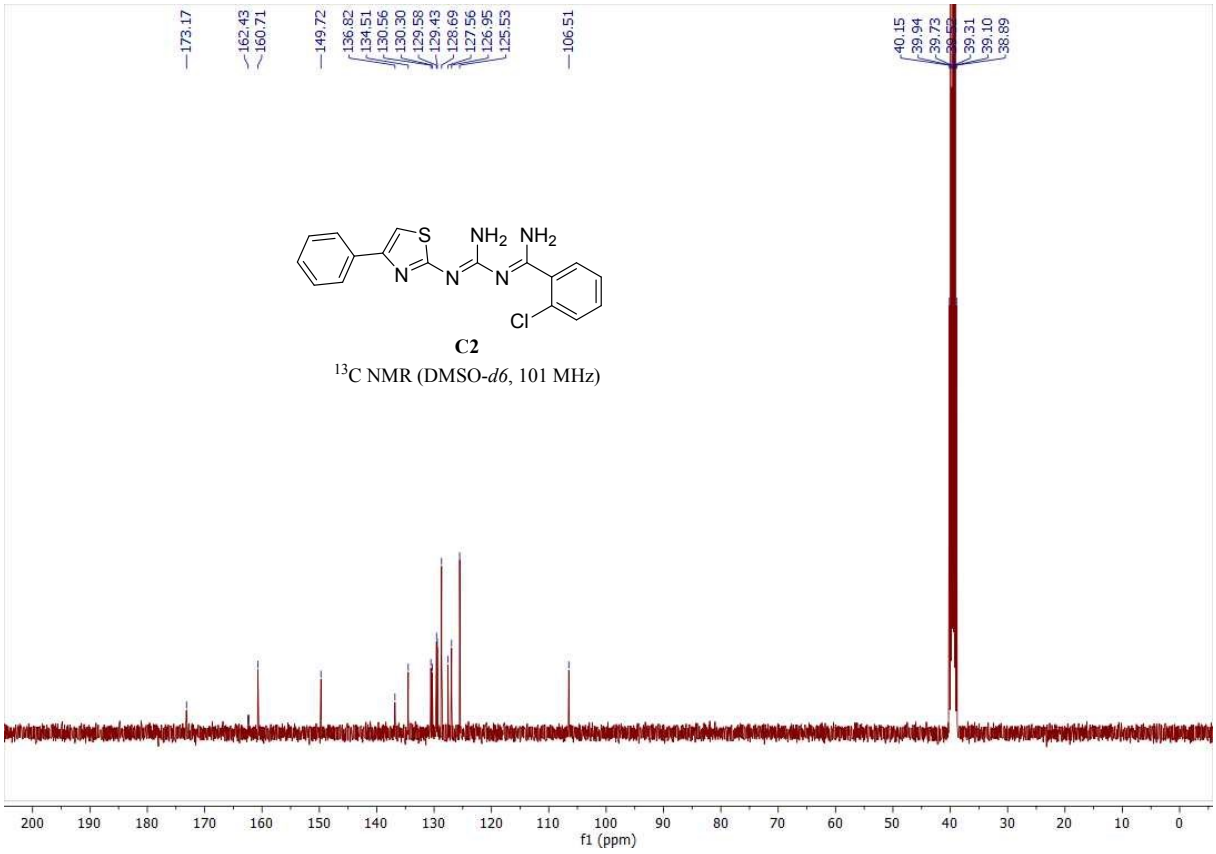
#	Peak Name	tR	Area	Height	Area%	Height%	Peak Start	Peak End	Base Start	Base End	Peak Mark
1	Unknown	3,767	2949370	662892	100,000	100,000	3,687	3,997	3,687	3,997	Manual

2-chloro-N-(N-(4-phenylthiazol-2-yl)carbamimidoyl)benzimidamide (**C2**)



C2

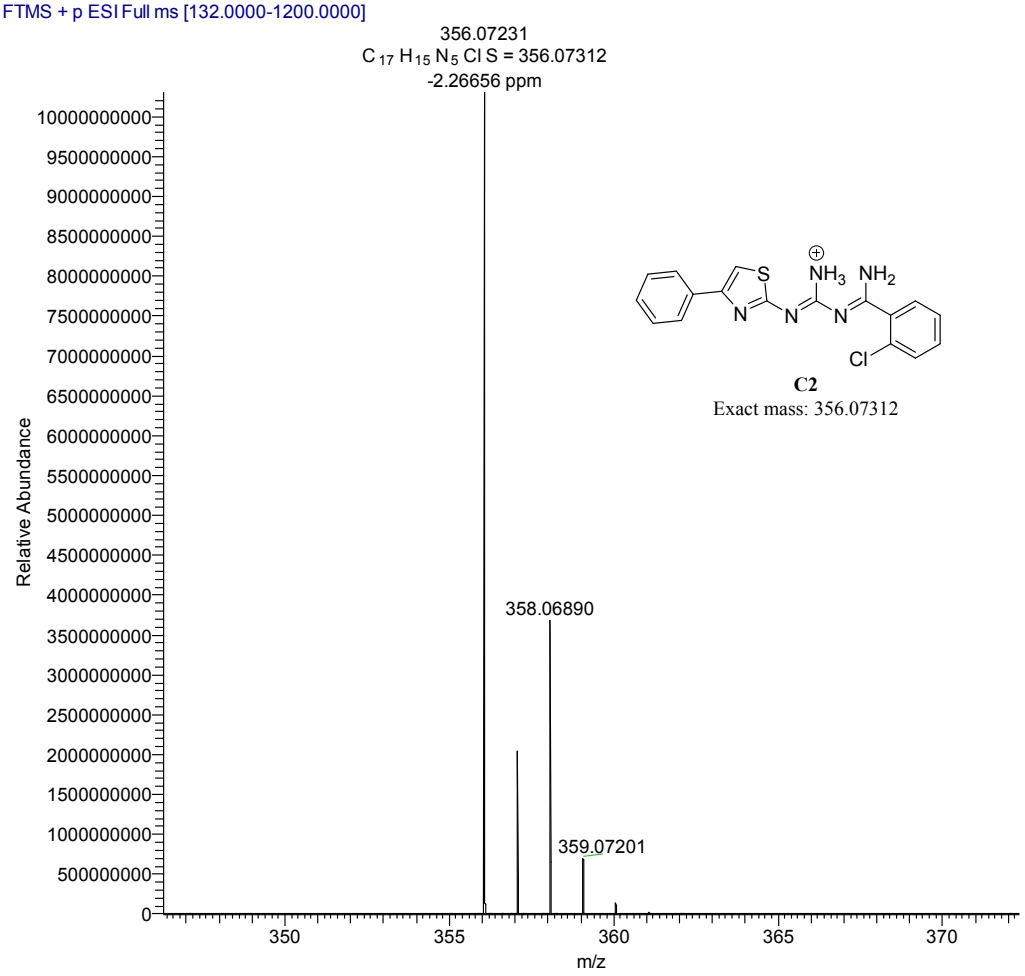
^{1}H NMR (DMSO-*d6*, 400 MHz)

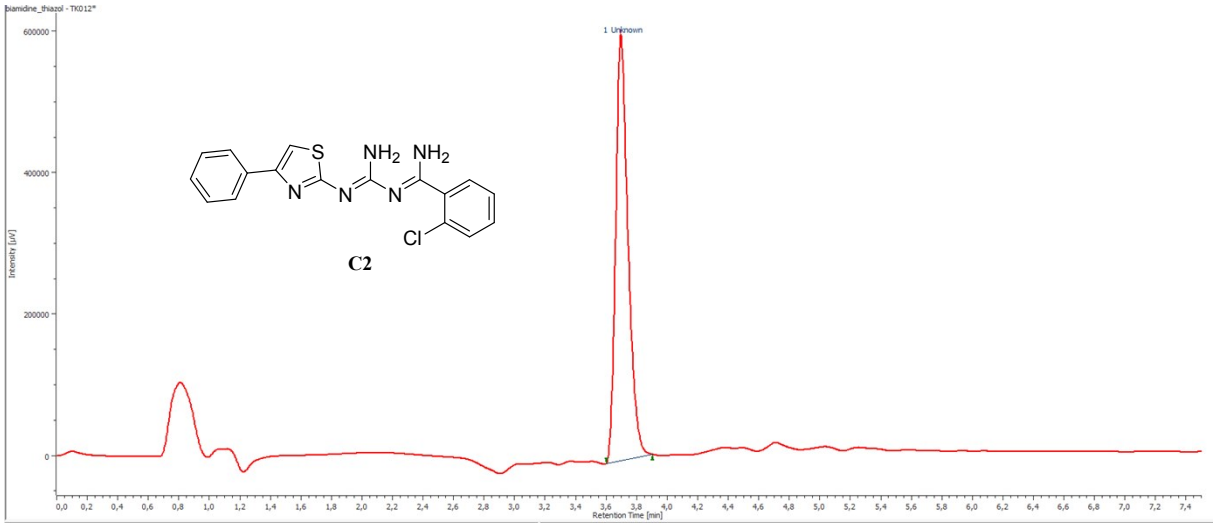


C2

^{13}C NMR (DMSO-*d6*, 101 MHz)

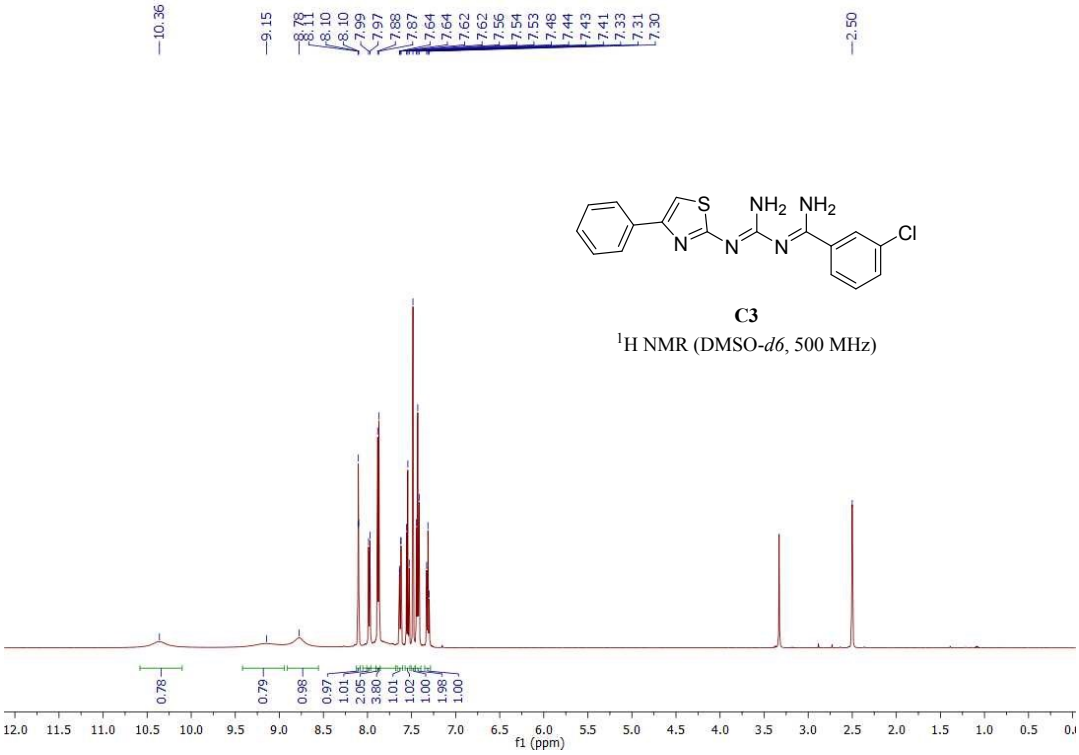
MTF562 #1029-1105 RT: 8.20-8.77 AV: 39 NL: 1.03E10
T: FTMS + p ESI Full ms [132.0000-1200.0000]





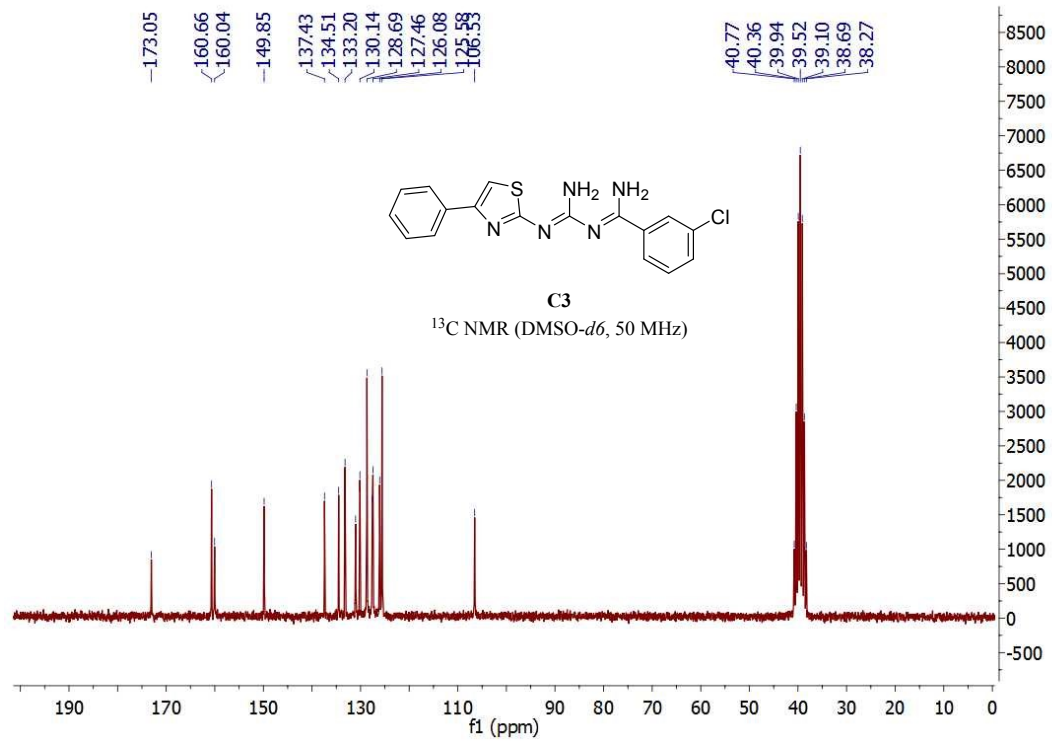
#	Peak Name	tR	Area	Height	Area%	Height%	Peak Start	Peak End	Base Start	Base End	Peak Mark
1	Unknown	3,693	3374754	600658	100,000	100,000	3,600	3,900	3,600	3,900	Manual

3-chloro-N-(N-(4-phenylthiazol-2-yl)carbamimidoyl)benzimidamide (**C3**)



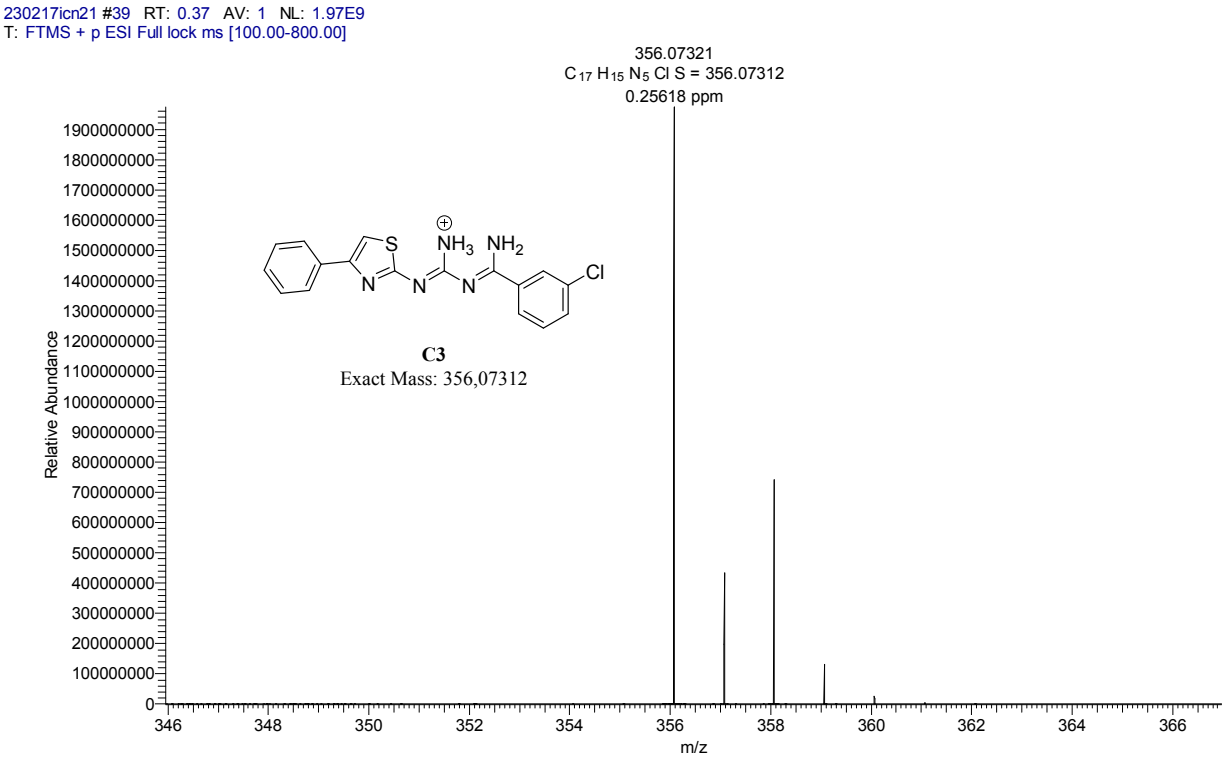
C3

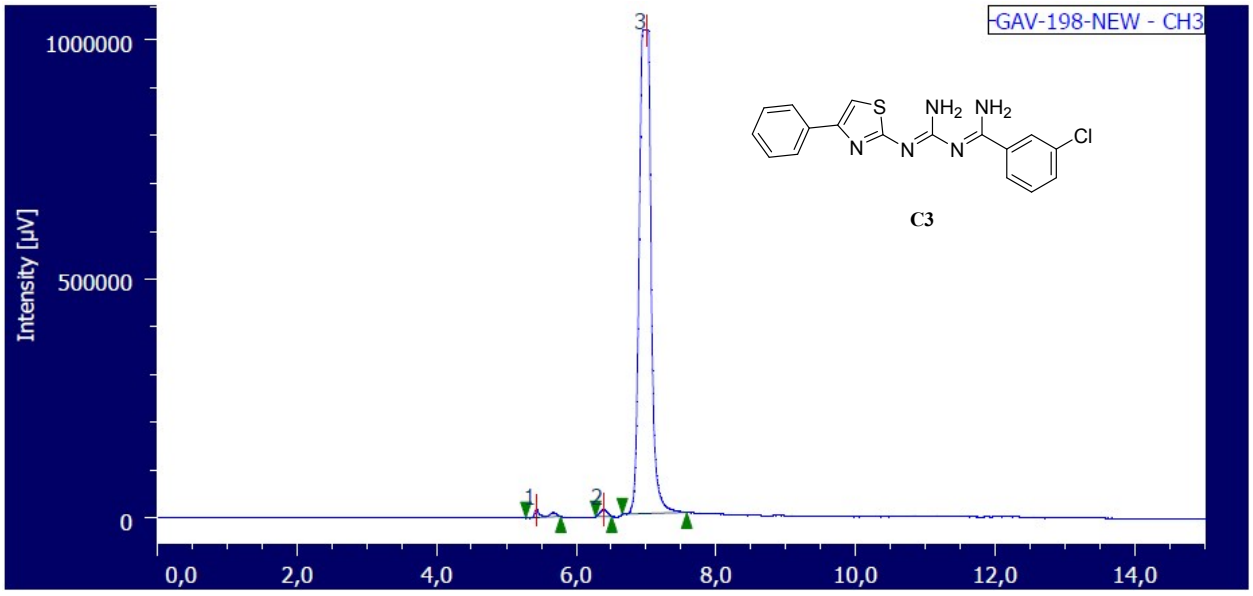
^{1}H NMR (DMSO-*d6*, 500 MHz)



C3

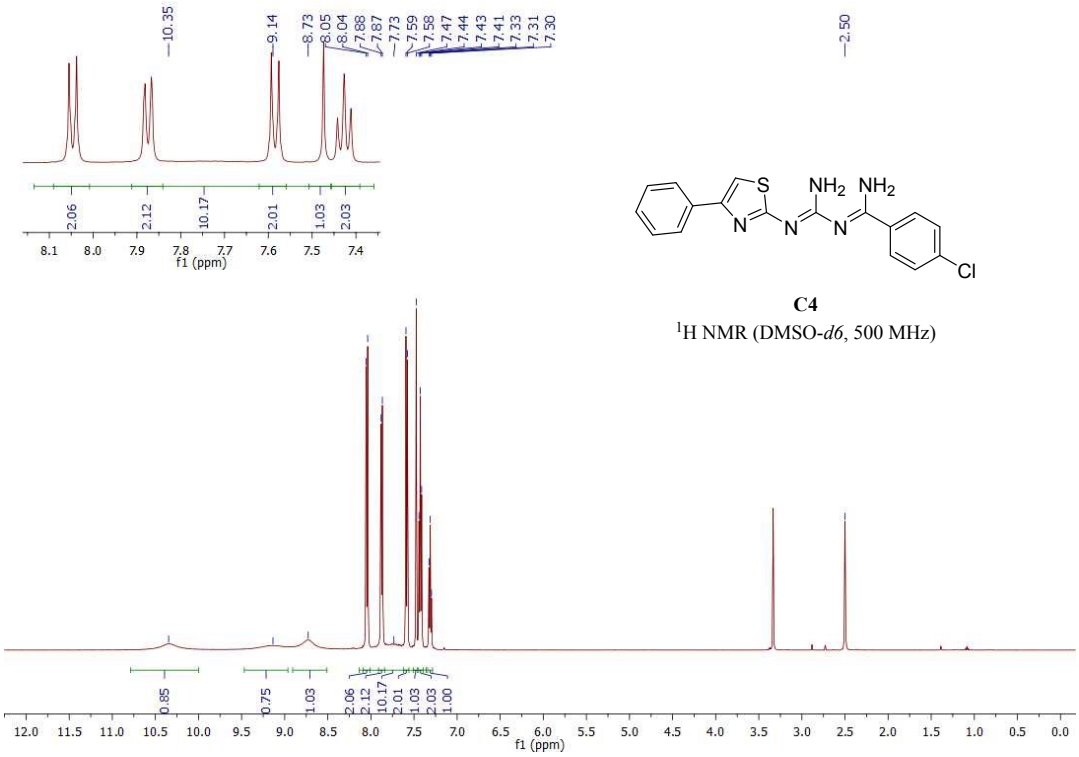
^{13}C NMR (DMSO-*d6*, 50 MHz)





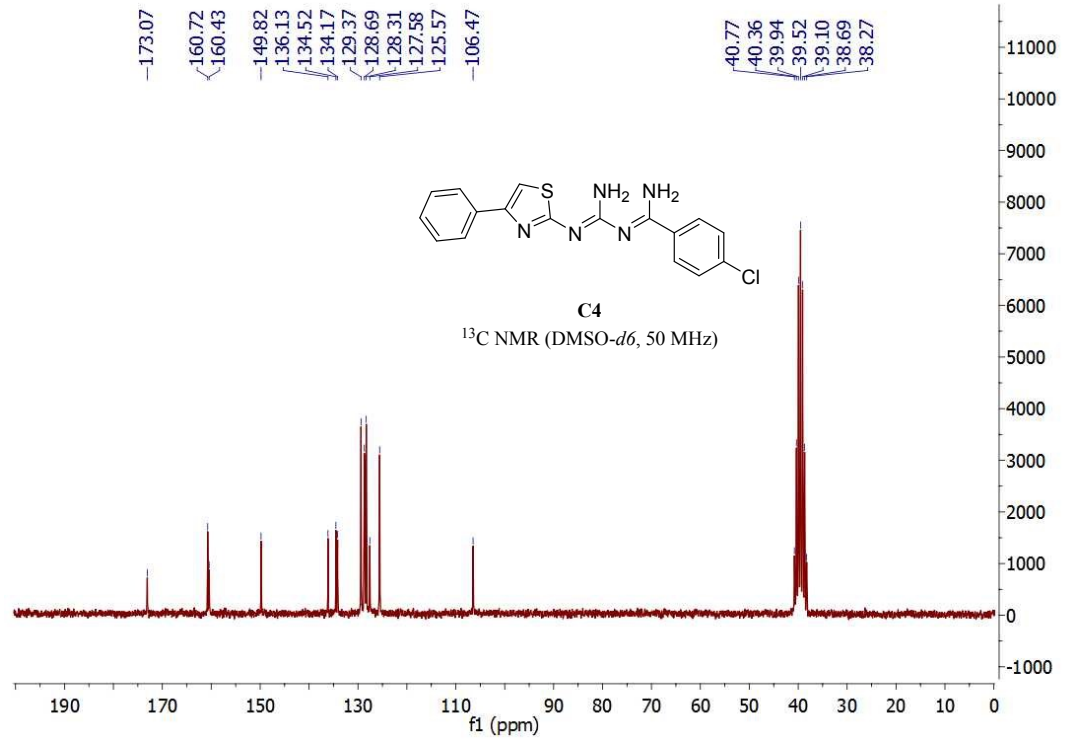
#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,425	134061	16040	1,095	1,542	N/A	2031	2,835	3,710	
2	Unknown	3	6,392	106558	14686	0,870	1,412	N/A	15957	2,431	1,009	
3	Unknown	3	7,008	12003230	1009665	98,035	97,047	N/A	8378	N/A	0,967	

4-chloro-N-(N-(5-phenylthiazol-2-yl)carbamimidoyl)benzimidamide (**C4**)



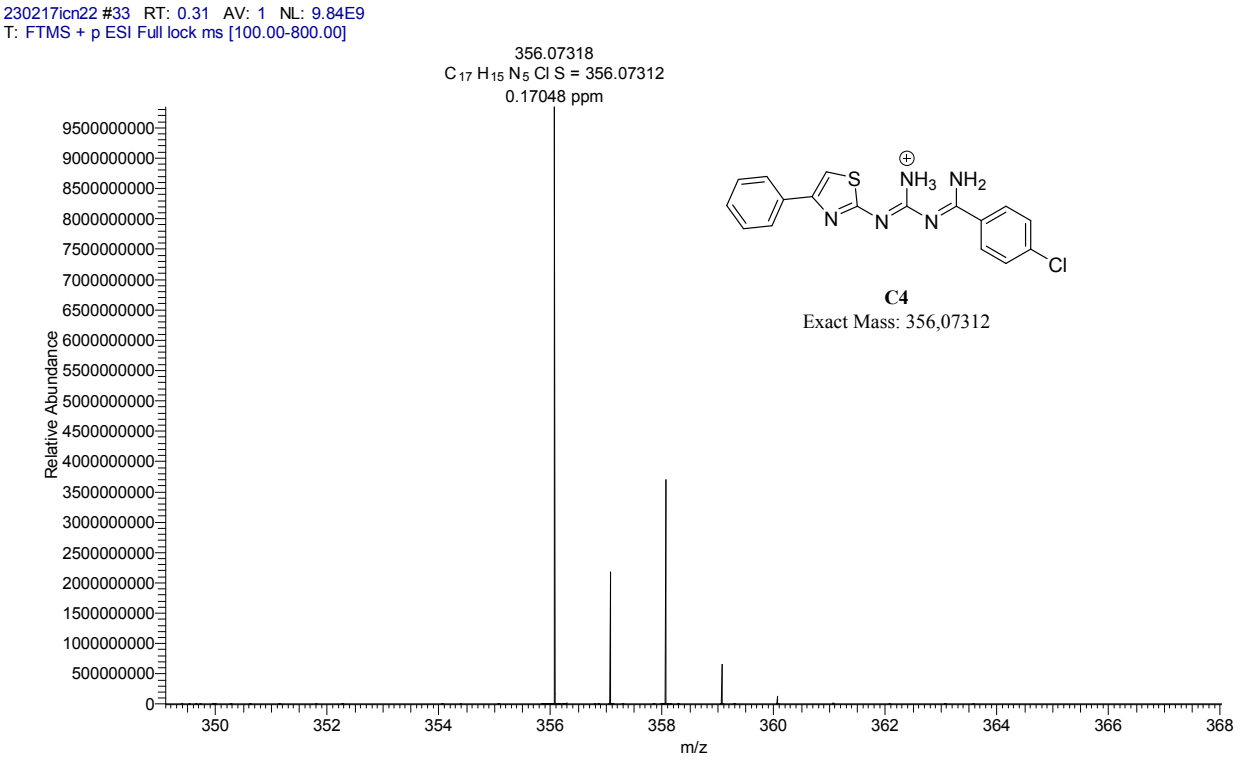
C4

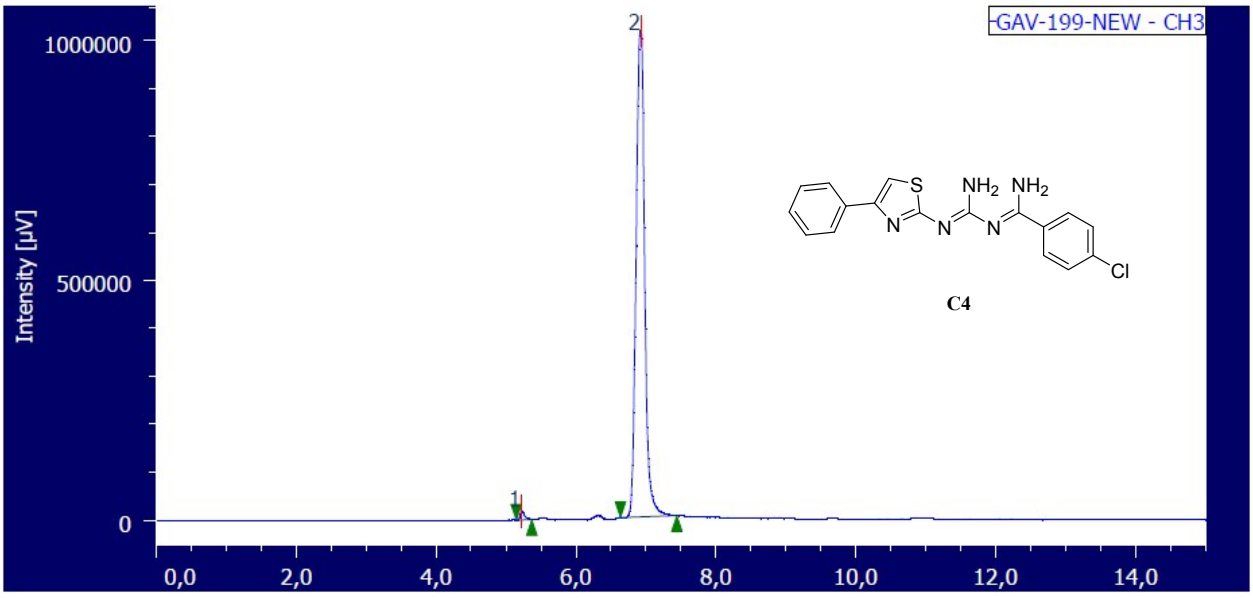
^{1}H NMR (DMSO-*d6*, 500 MHz)



C4

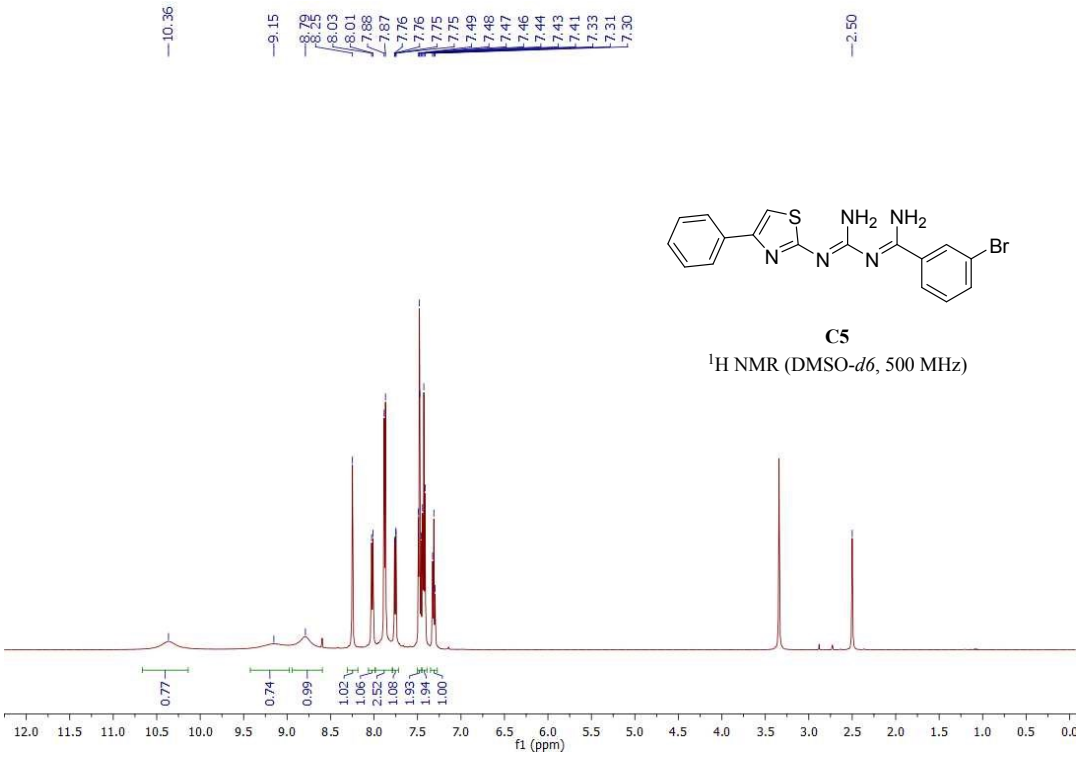
^{13}C NMR (DMSO-*d6*, 50 MHz)





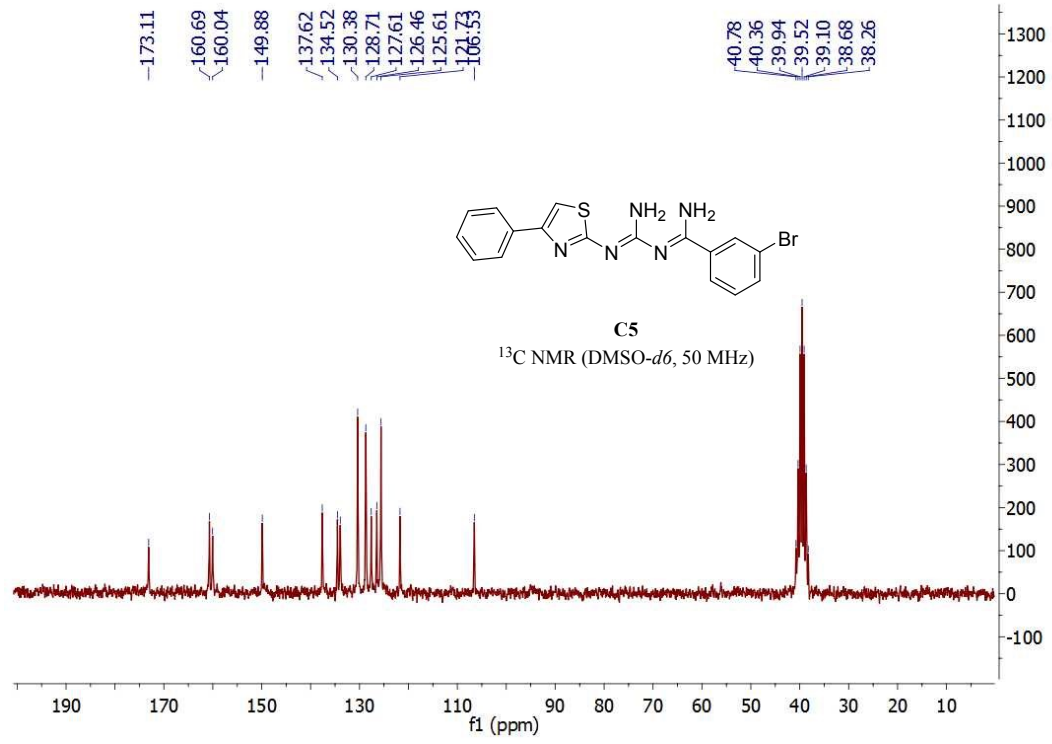
#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,225	79840	17887	0,910	1,737	N/A	36065	10,354	1,623	
2	Unknown	3	6,925	8698276	1012148	99,090	98,263	N/A	15971	N/A	1,078	

3-bromo-N-(N-(4-phenylthiazol-2-yl)carbamimidoyl)benzimidamide (**C5**)



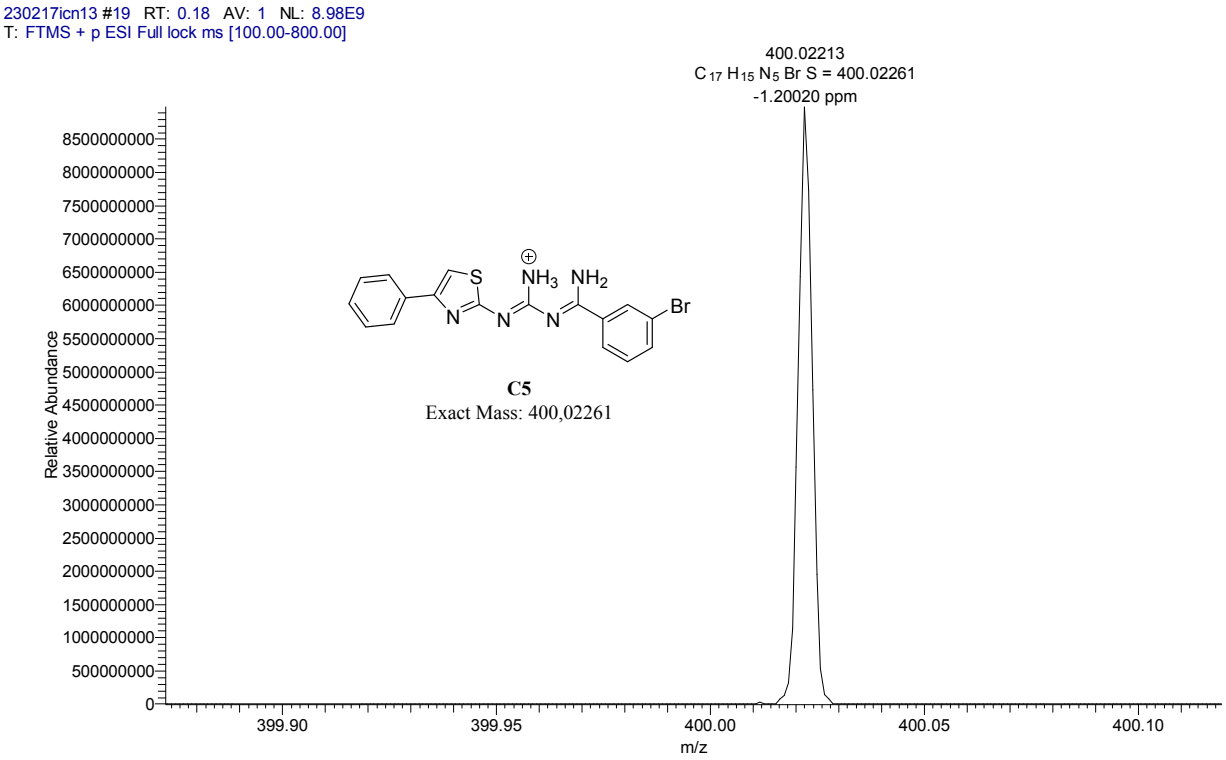
C5

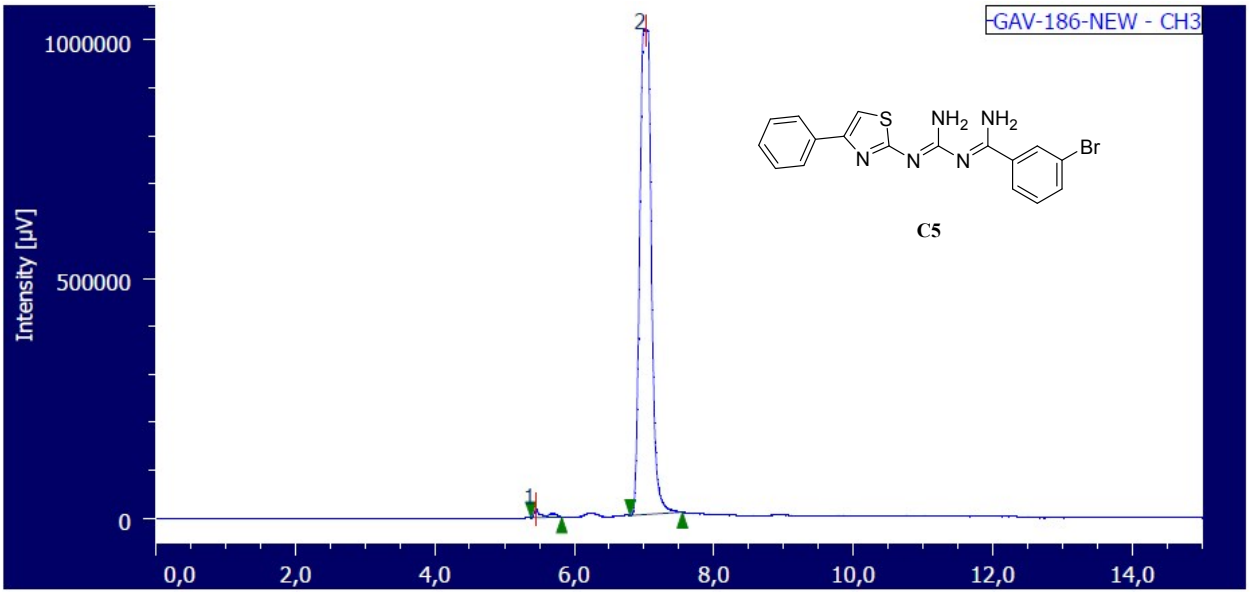
^{1}H NMR (DMSO-*d6*, 500 MHz)



C5

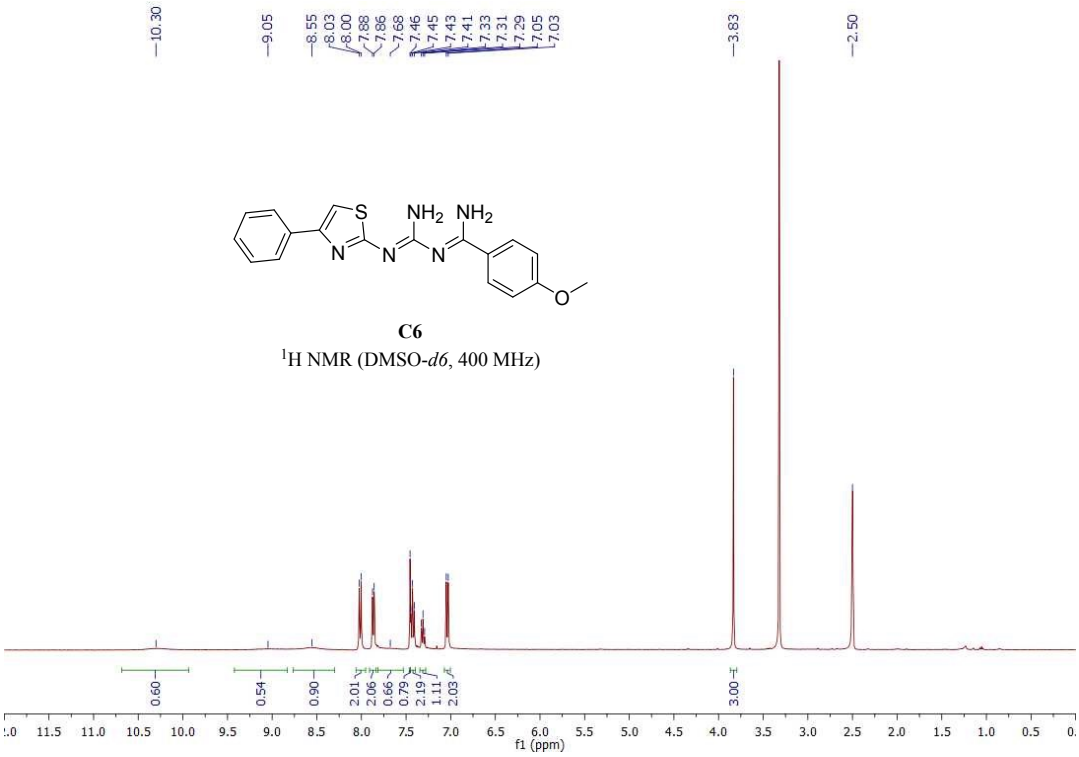
^{13}C NMR (DMSO-*d6*, 50 MHz)





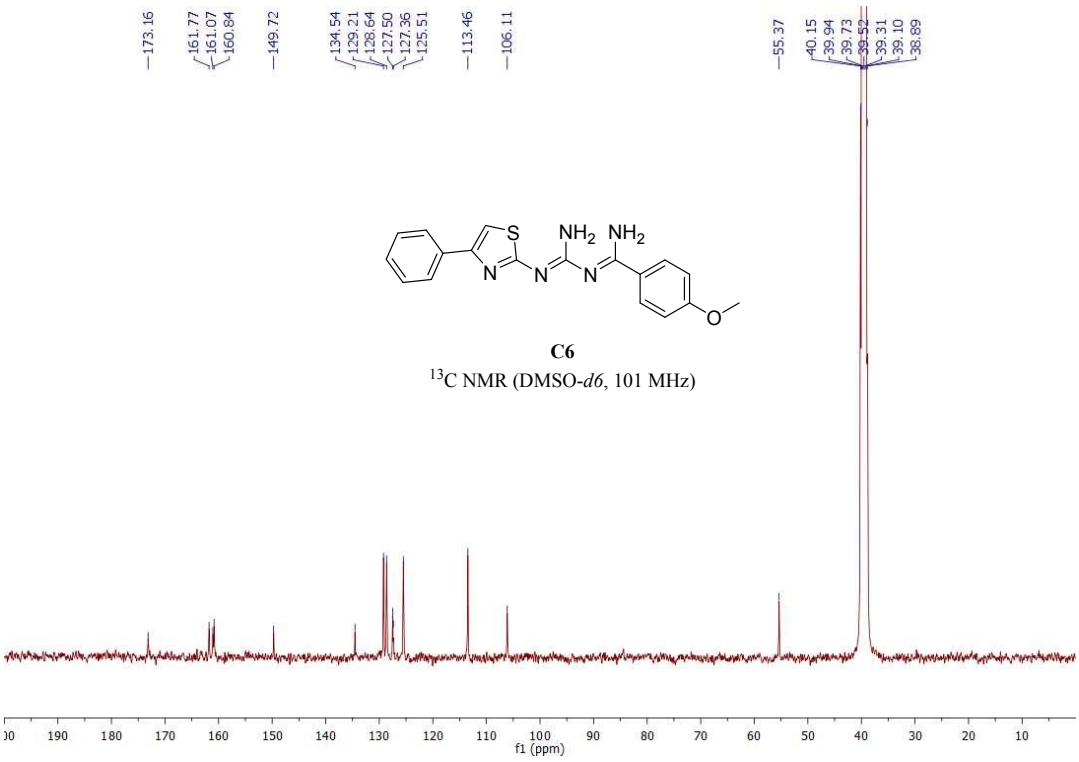
#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,450	160357	17477	1,338	1,698	N/A	34404	7,537	3,493	
2	Unknown	3	7,033	11822478	1012009	98,662	98,302	N/A	8578	N/A	1,039	

4-methoxy-N-(N-(4-phenylthiazol-2-yl)carbamimidoyl)benzimidamide (**C6**)



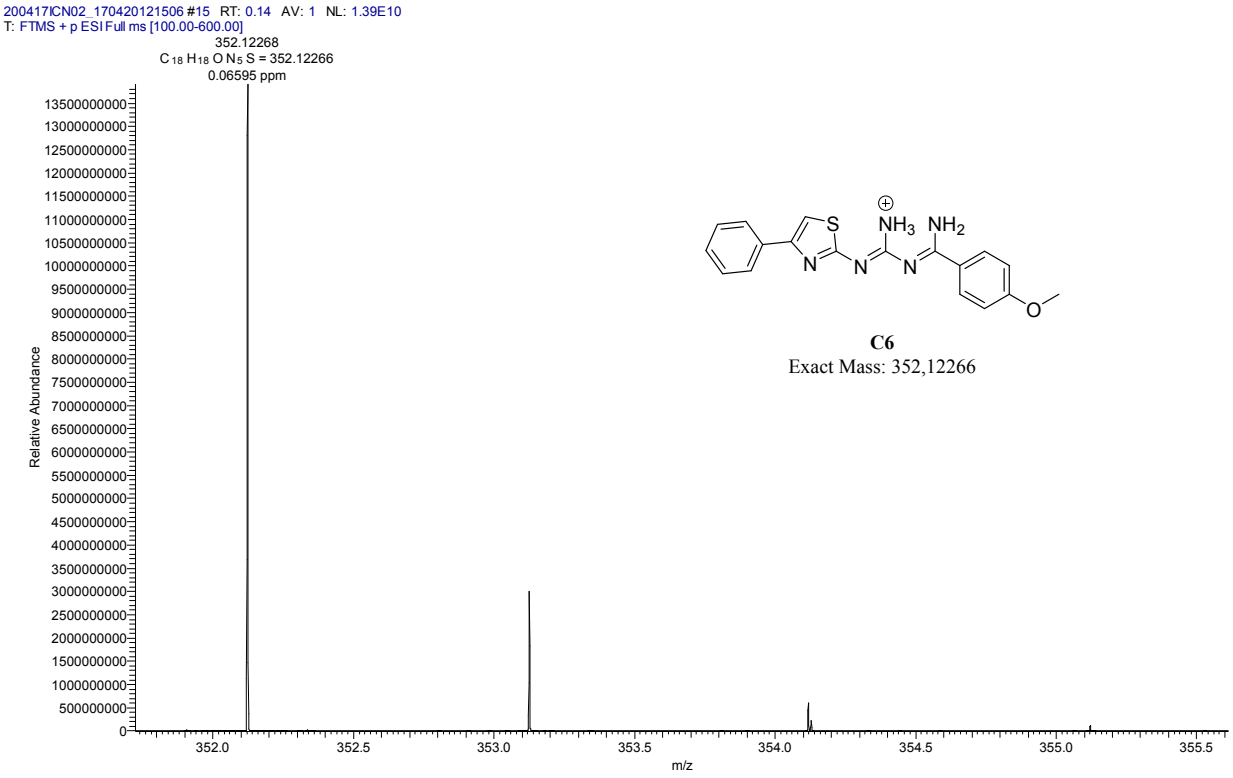
C6

^{1}H NMR (DMSO-*d6*, 400 MHz)



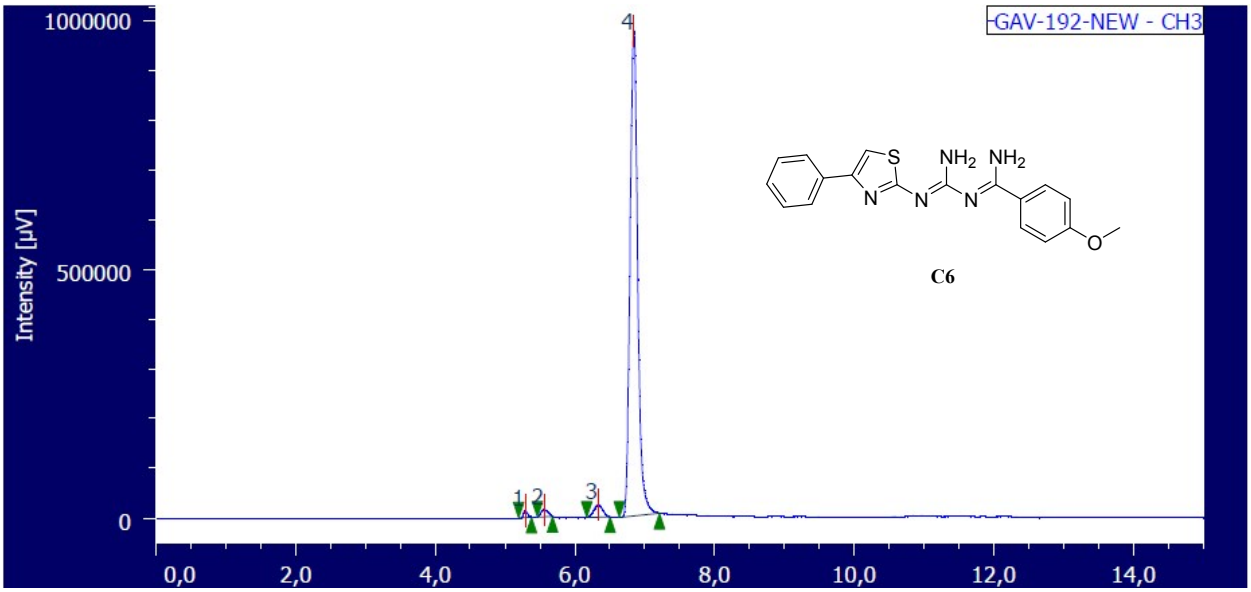
C6

^{13}C NMR (DMSO-*d6*, 101 MHz)



C6

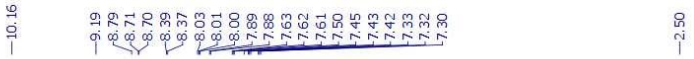
Exact Mass: 352,12266

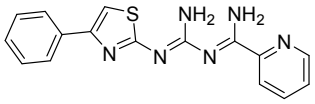


C6

#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,283	55871	13981	0,714	1,360	N/A	38724	1,808	1,344	
2	Unknown	3	5,558	99412	14224	1,271	1,384	N/A	12652	3,514	1,105	
3	Unknown	3	6,333	225406	24858	2,882	2,419	N/A	10728	2,318	0,994	
4	Unknown	3	6,842	7440325	974742	95,132	94,837	N/A	19671	N/A	1,170	

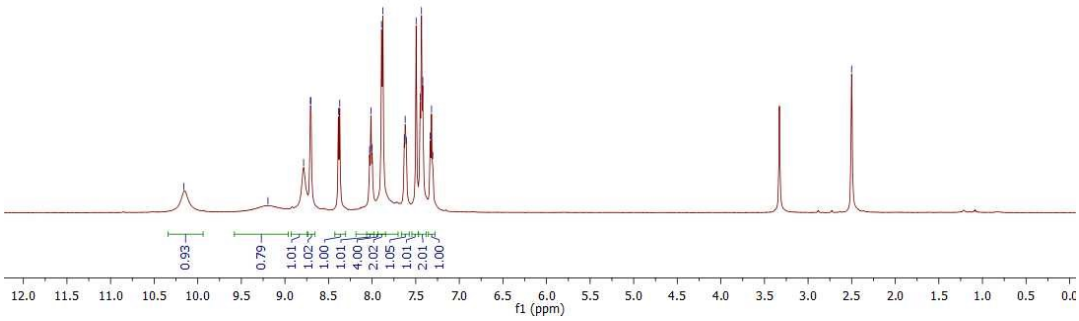
N-(N-(4-phenylthiazol-2-yl)carbamimidoyl)picolinimidamide (**C7**)

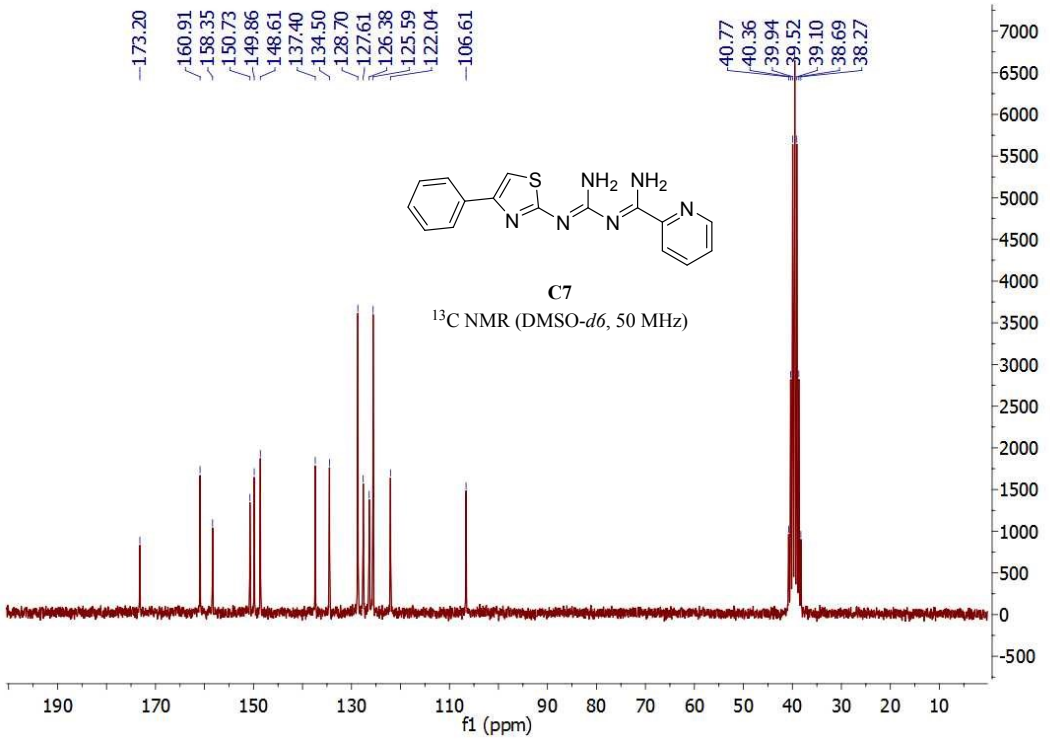




C7

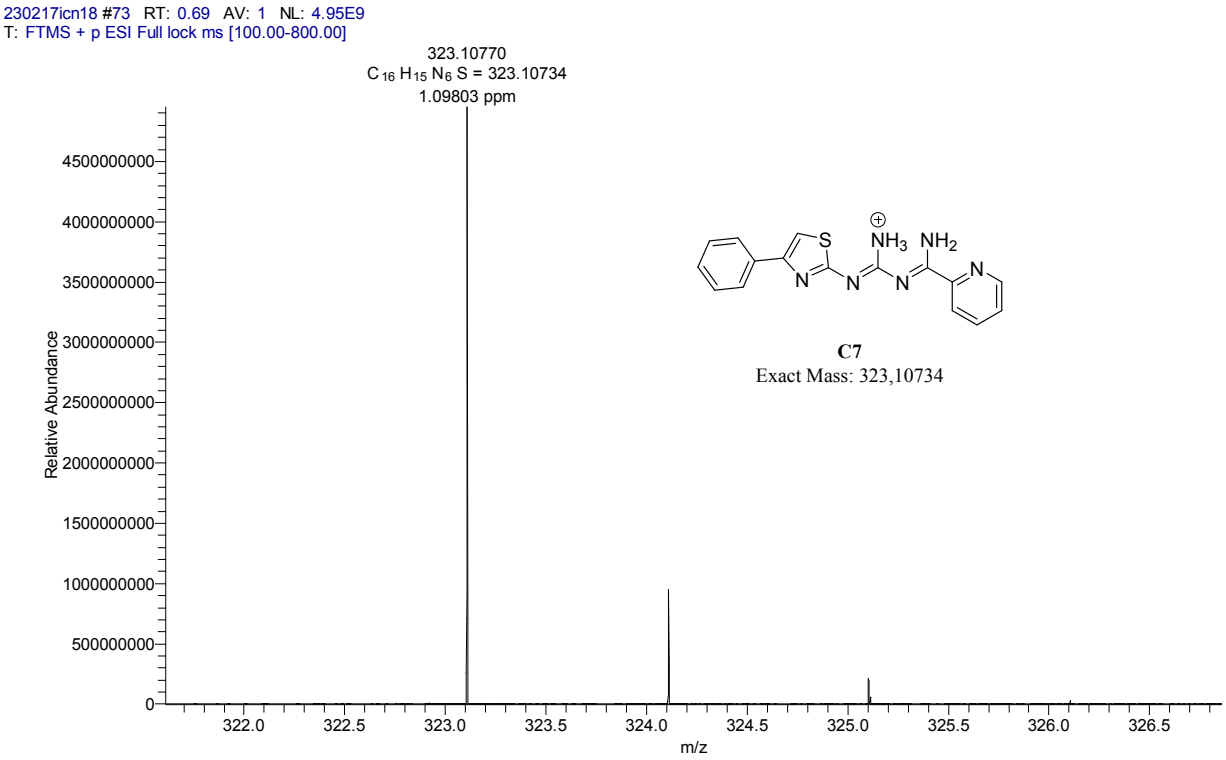
^{1}H NMR (DMSO-*d6*, 400 MHz)

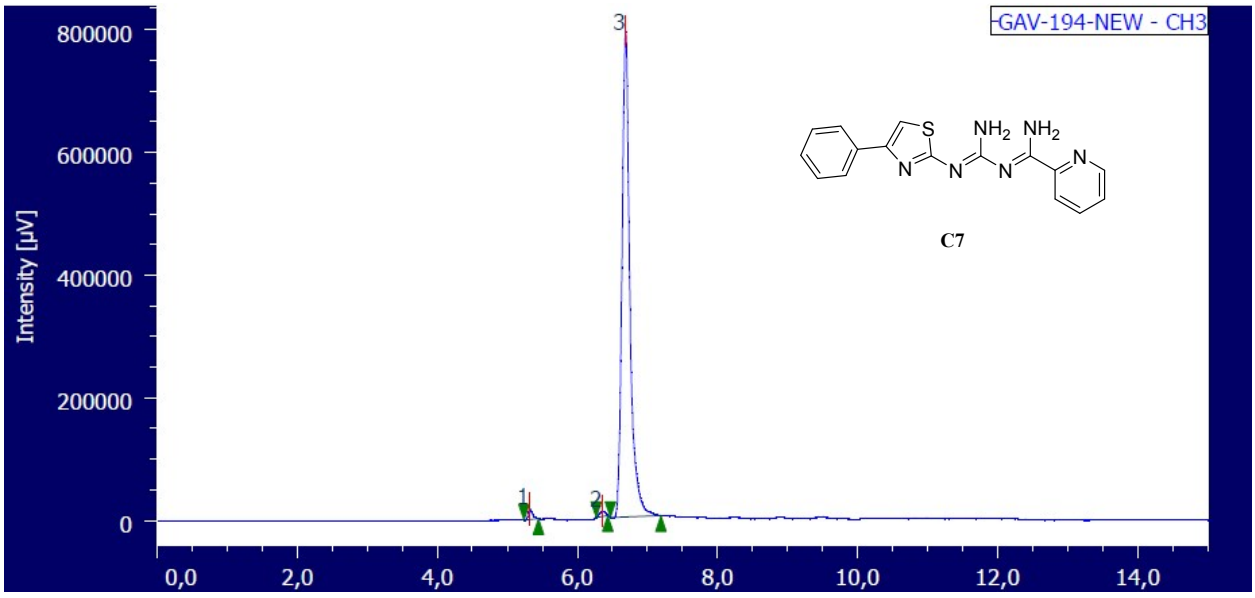




C7

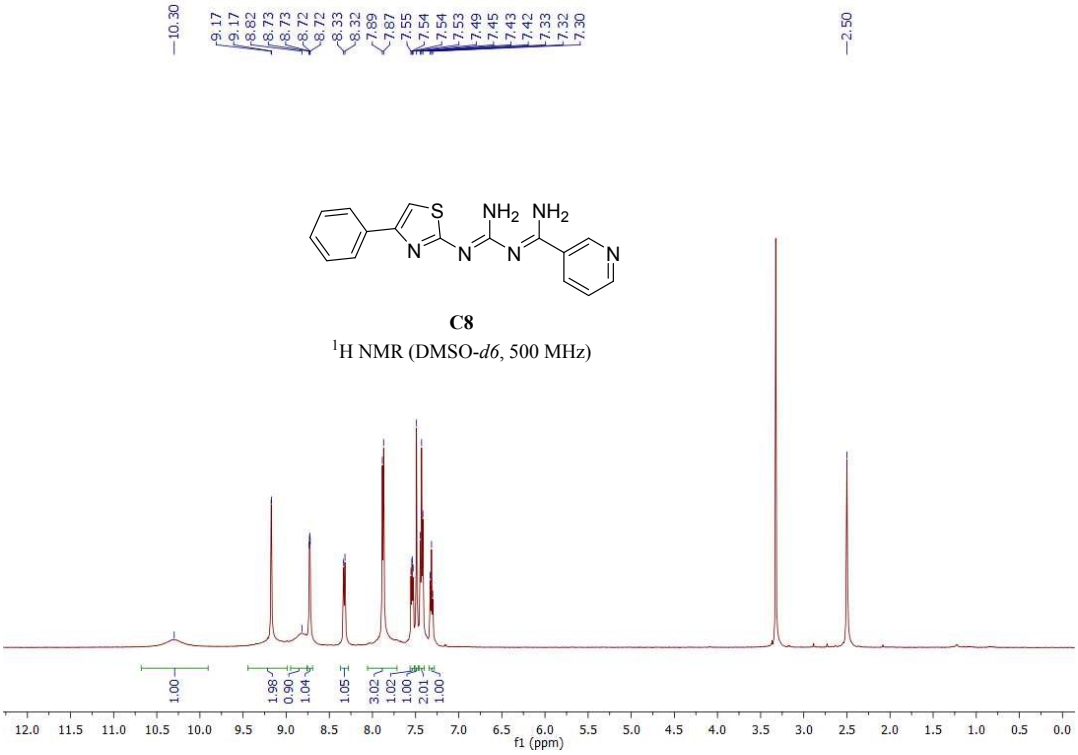
^{13}C NMR (DMSO-*d6*, 50 MHz)

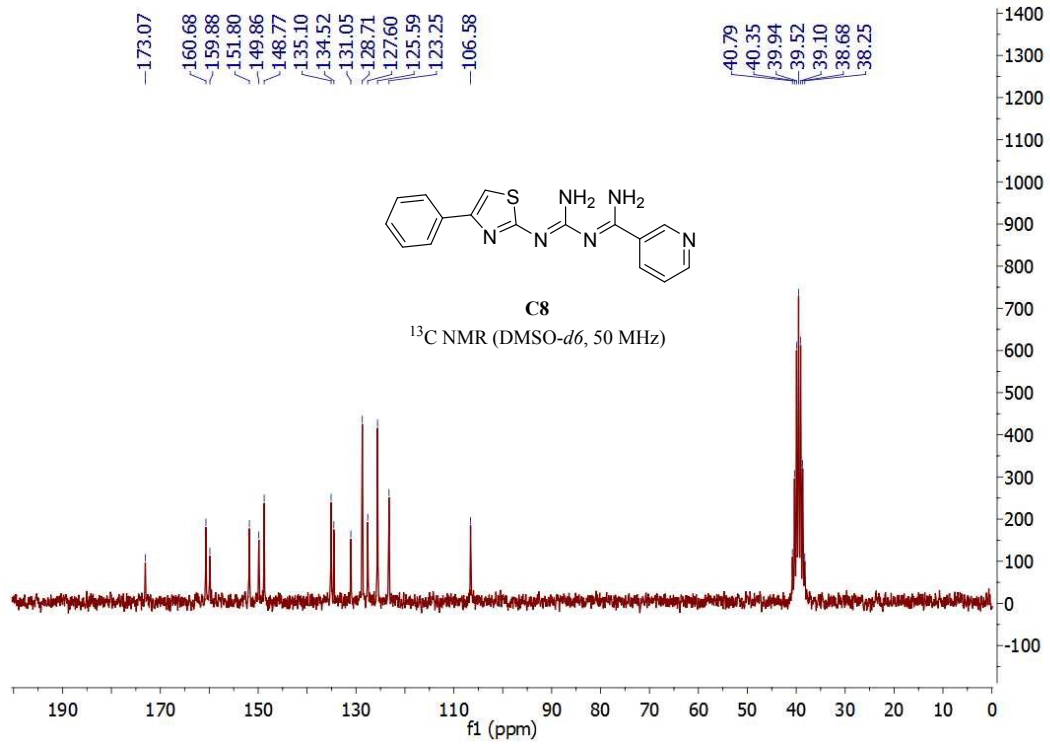


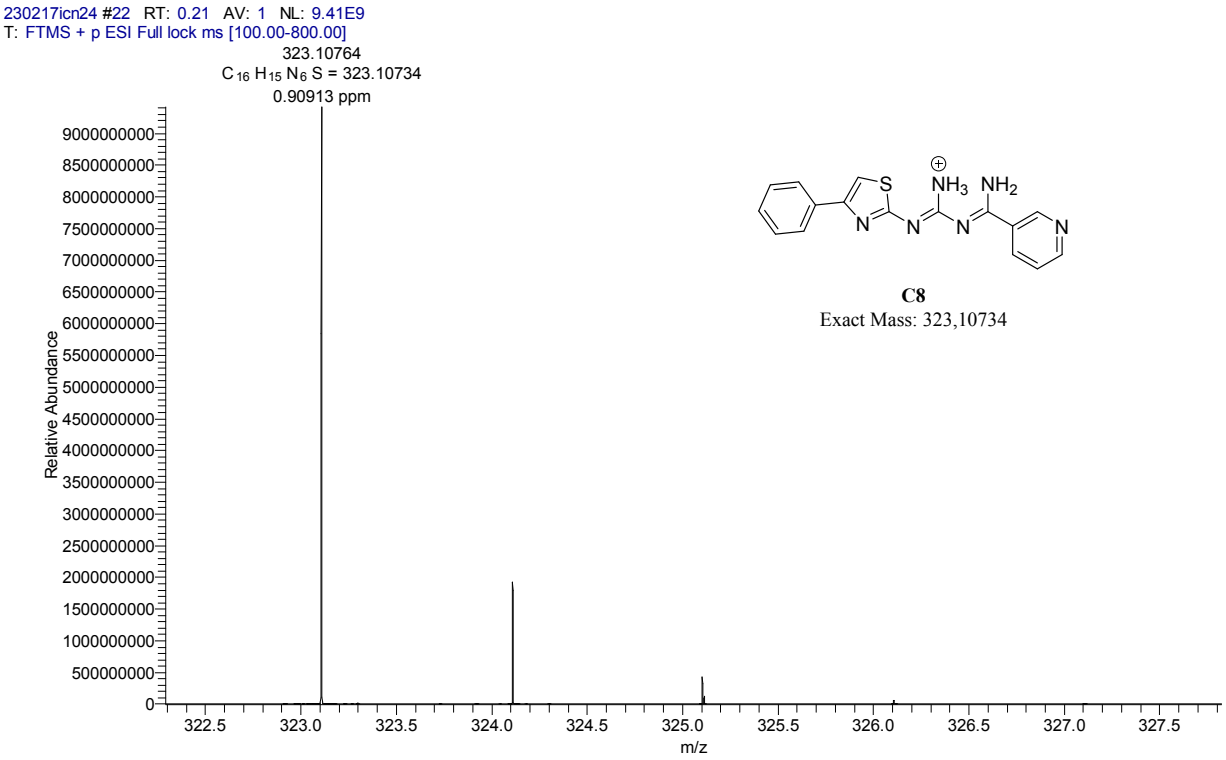


#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,317	76108	17257	1,202	2,112	N/A	35770	7,374	1,485	
2	Unknown	3	6,358	49867	8451	0,788	1,034	N/A	22163	1,874	0,907	
3	Unknown	3	6,692	6203732	791297	98,010	96,853	N/A	20761	N/A	1,293	

N-(N-(4-phenylthiazol-2-yl)carbamimidoyl)nicotinimidamide (**C8**)

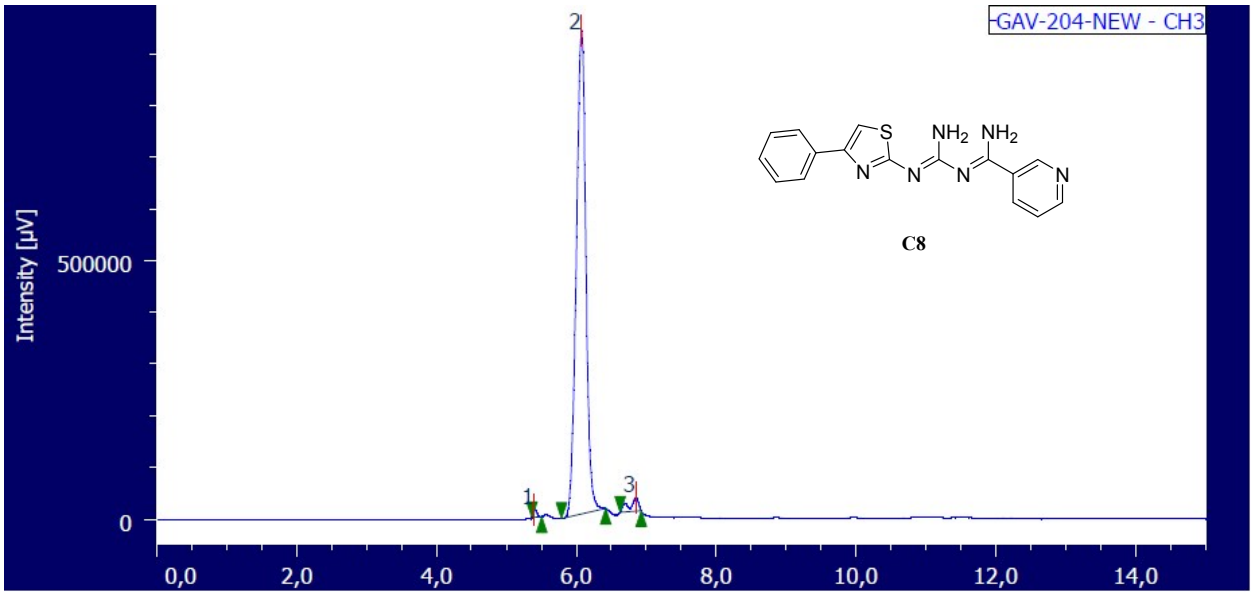






C8

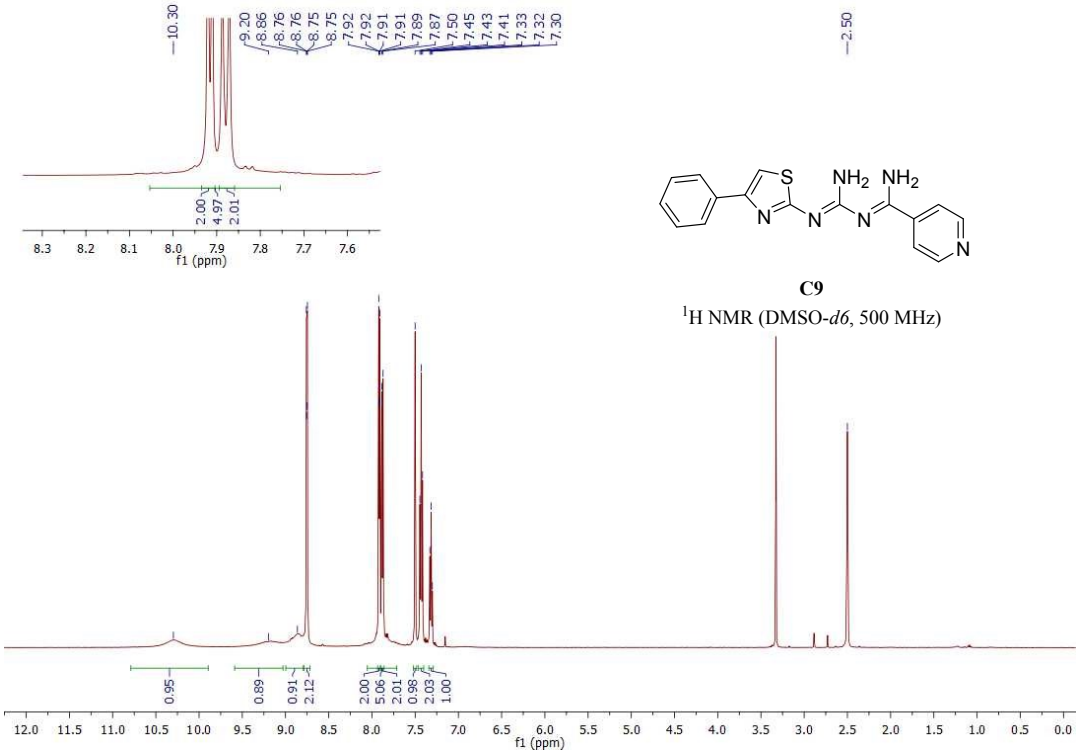
Exact Mass: 323,10734



C8

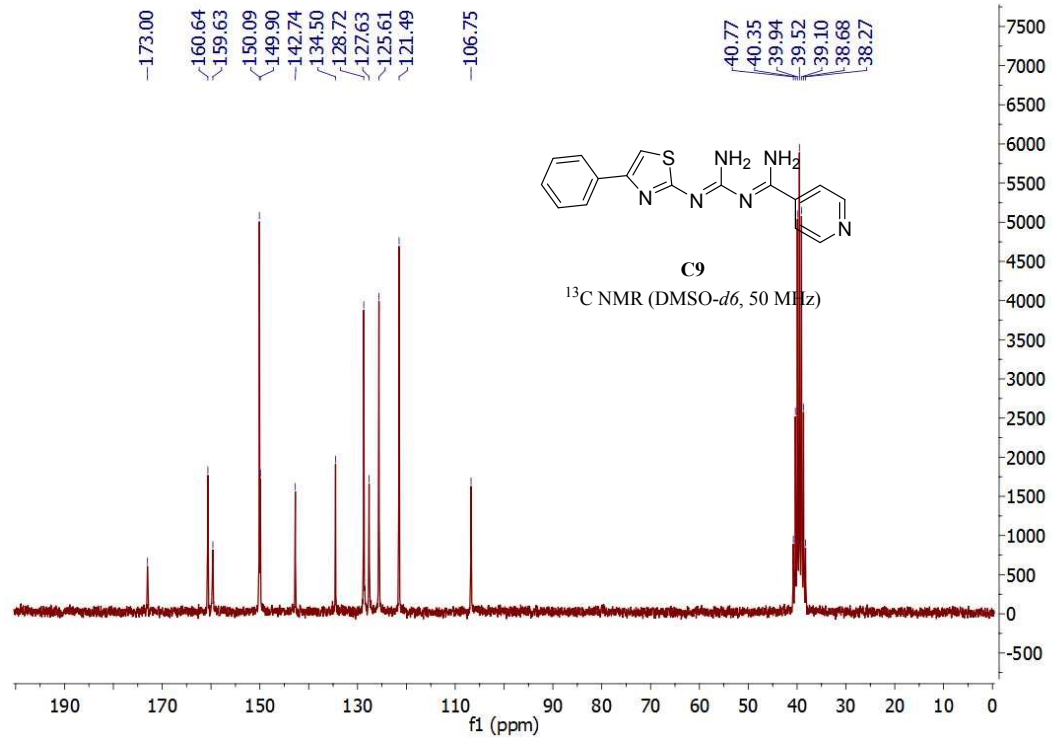
#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,400	52503	15340	0,571	1,571	N/A	54517	4,004	1,323	
2	Unknown	3	6,075	8902575	934825	96,858	95,757	N/A	9792	2,459	0,946	
3	Unknown	3	6,850	236312	26086	2,571	2,672	N/A	5027	N/A	0,659	

N-(N-(4-phenylthiazol-2-yl)carbamimidoyl)isonicotinimidamide (**C9**)



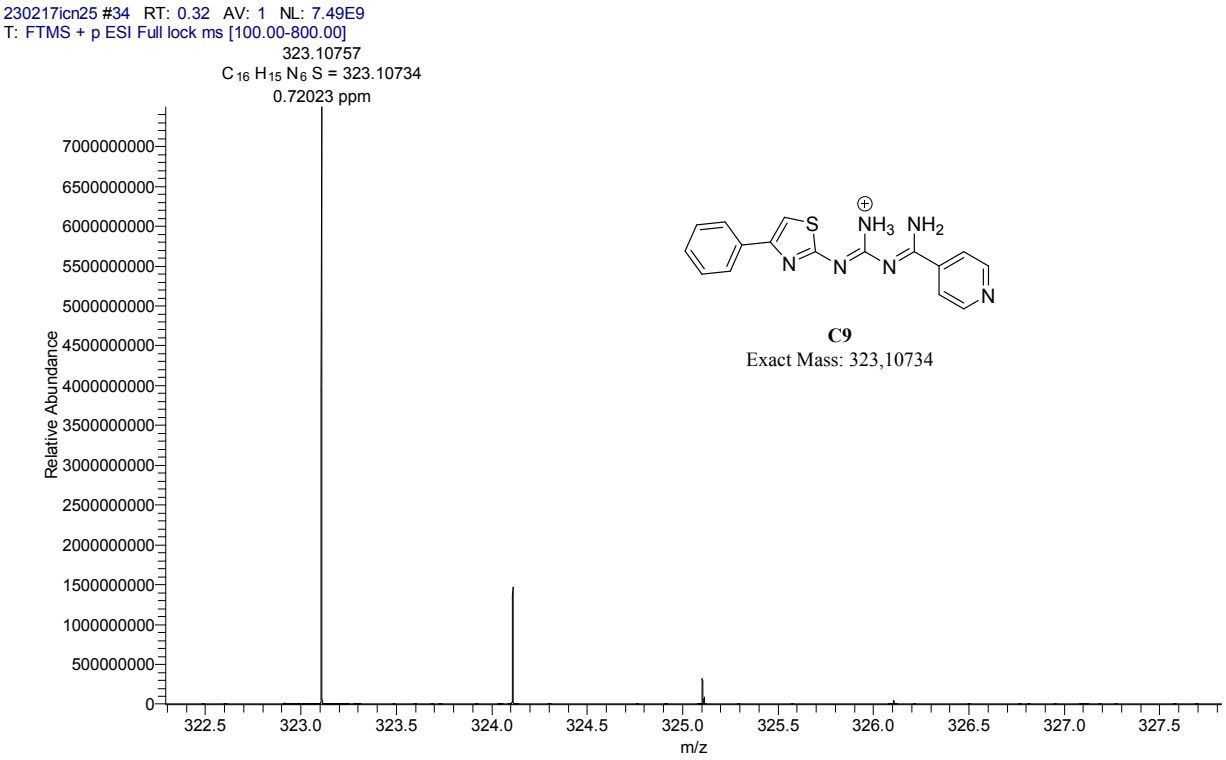
C9

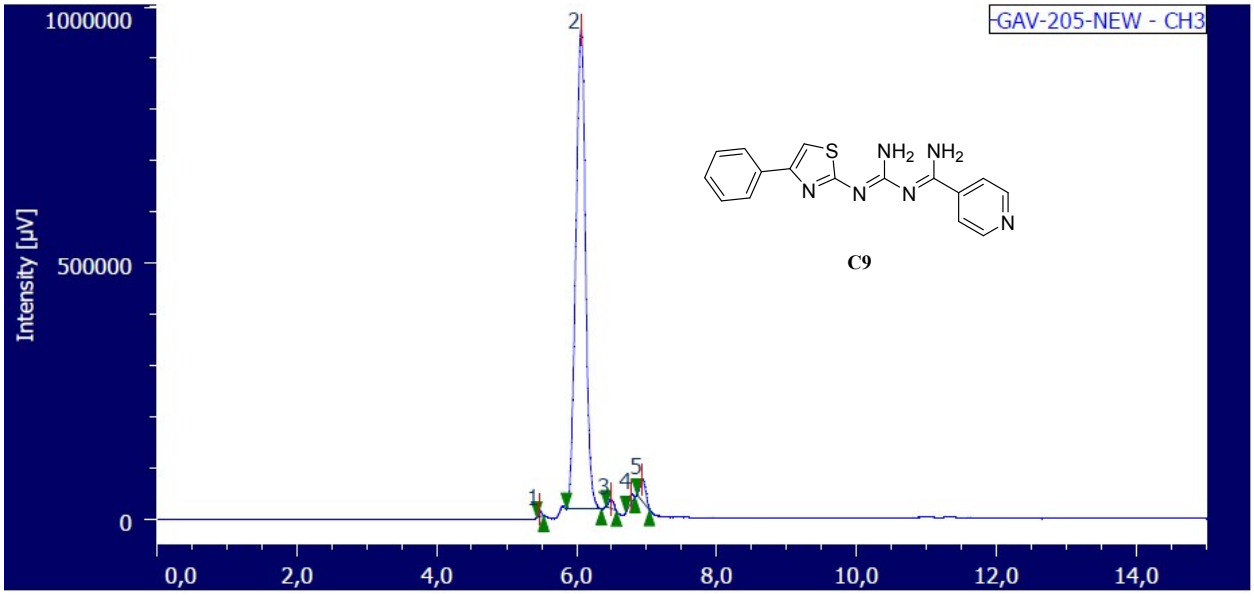
^{1}H NMR (DMSO-*d6*, 500 MHz)



C9

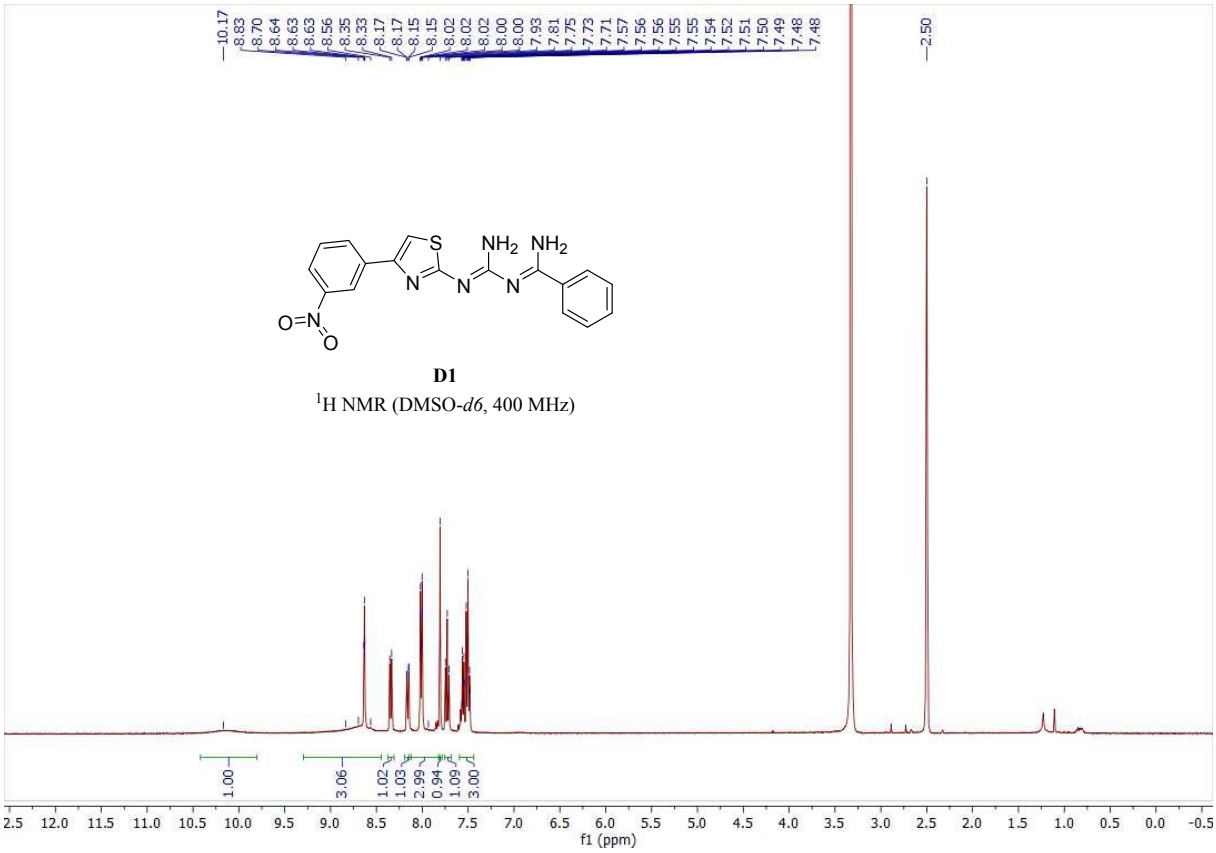
^{13}C NMR (DMSO-*d6*, 50 MHz)





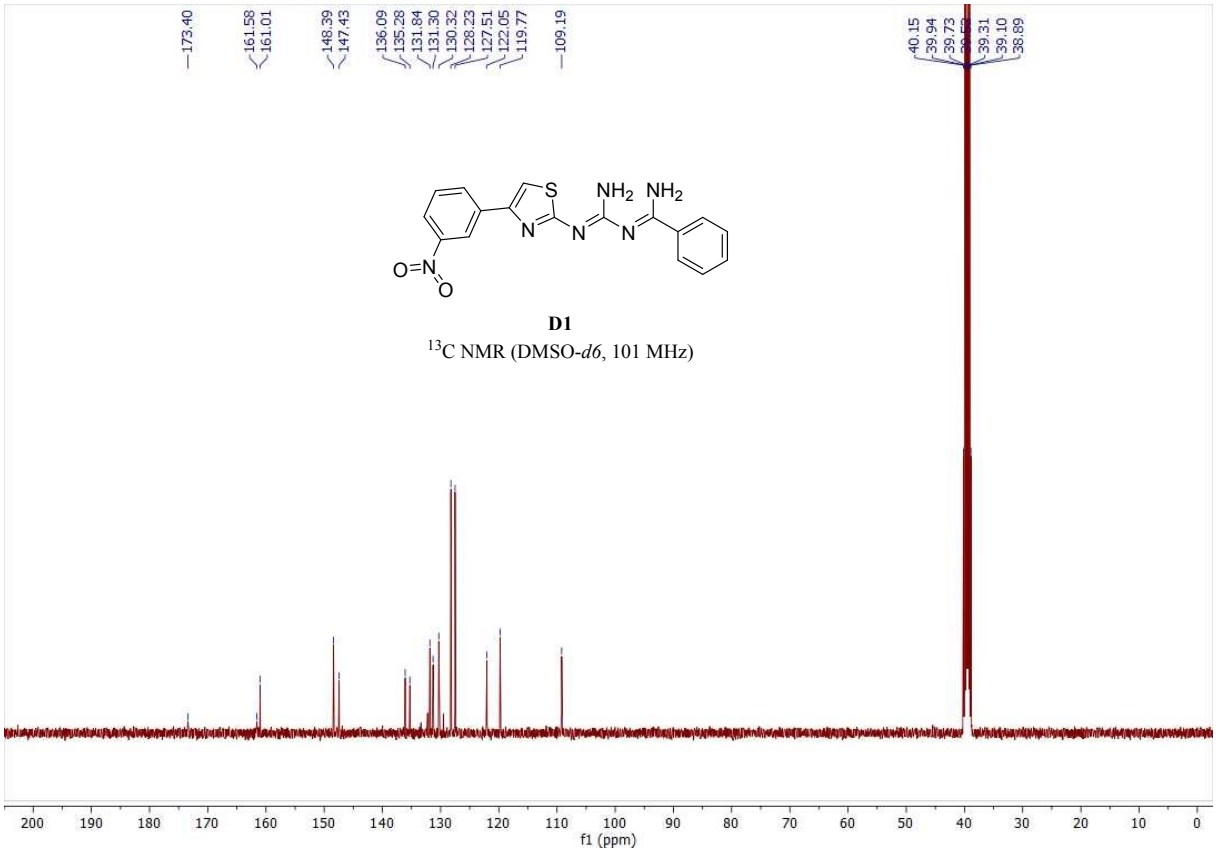
#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,467	42825	13521	0,450	1,317	N/A	61259	3,460	1,166	
2	Unknown	3	6,058	9067626	935895	95,232	91,188	N/A	9063	2,128	1,005	
3	Unknown	3	6,483	90365	17475	0,949	1,703	N/A	31595	2,186	1,147	
4	Unknown	3	6,783	73903	16308	0,776	1,589	N/A	44015	1,095	0,809	
5	Unknown	3	6,942	246938	43137	2,593	4,203	N/A	29910	N/A	1,075	

N-(N-(4-(3-nitrophenyl)thiazol-2-yl)carbamimidoyl)benzimidamide (**D1**)



D1

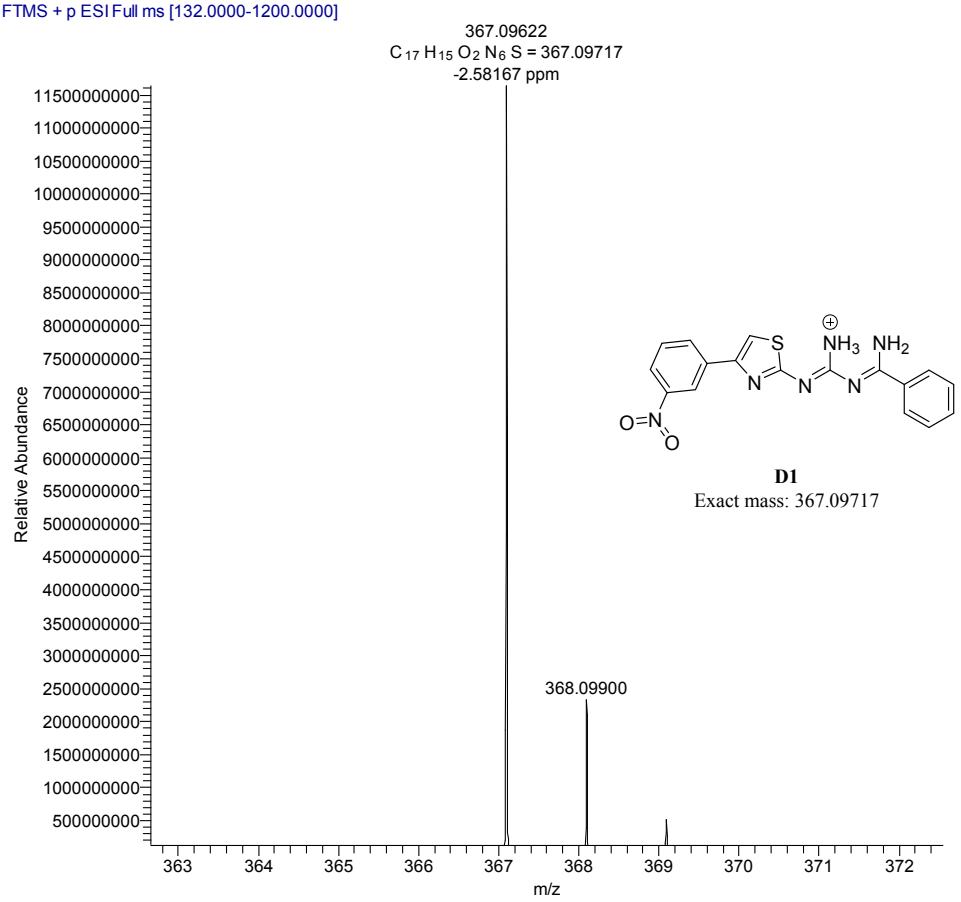
^{1}H NMR (DMSO-*d6*, 400 MHz)

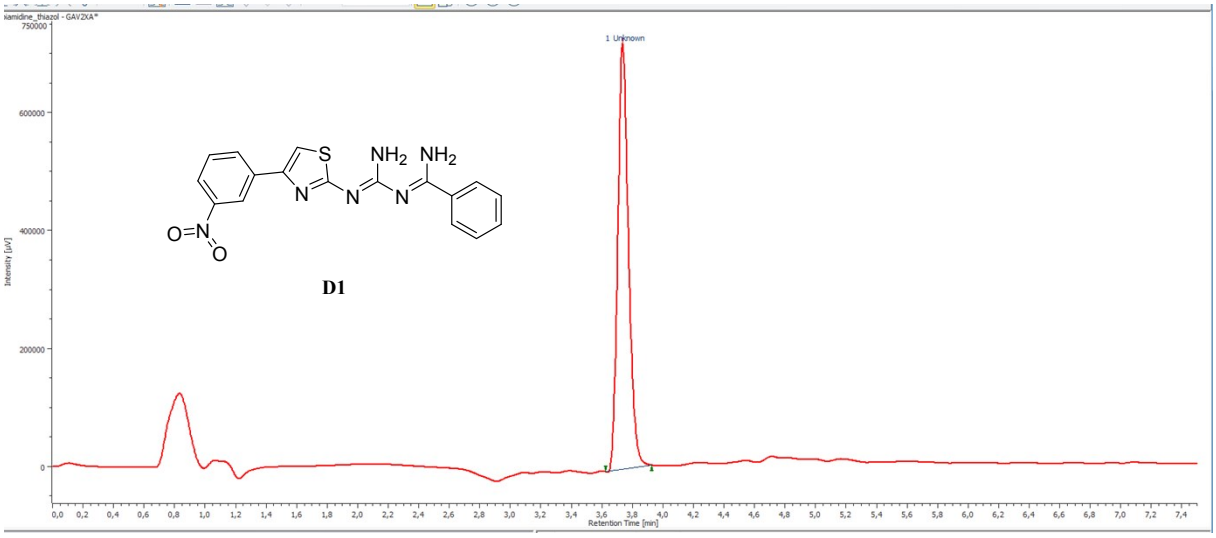


D1

^{13}C NMR (DMSO-*d6*, 101 MHz)

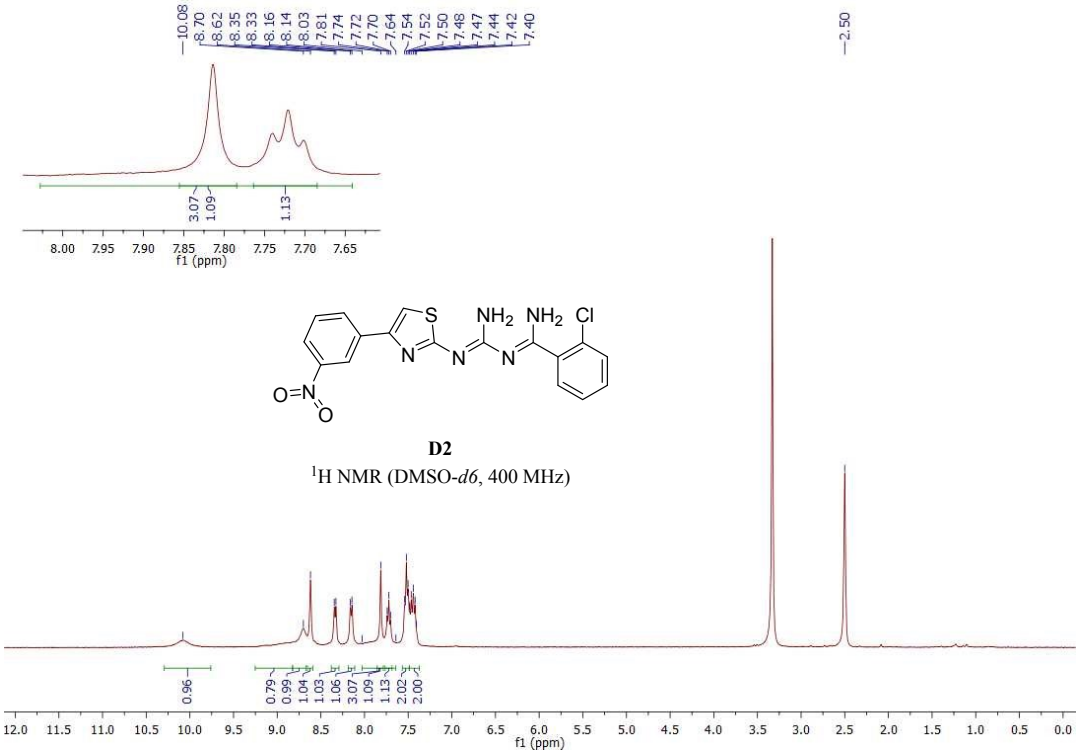
MTF563 #1034-1099 RT: 8.26-8.74 AV: 33 NL: 1.16E10
T: FTMS + p ESI Full ms [132.0000-1200.0000]





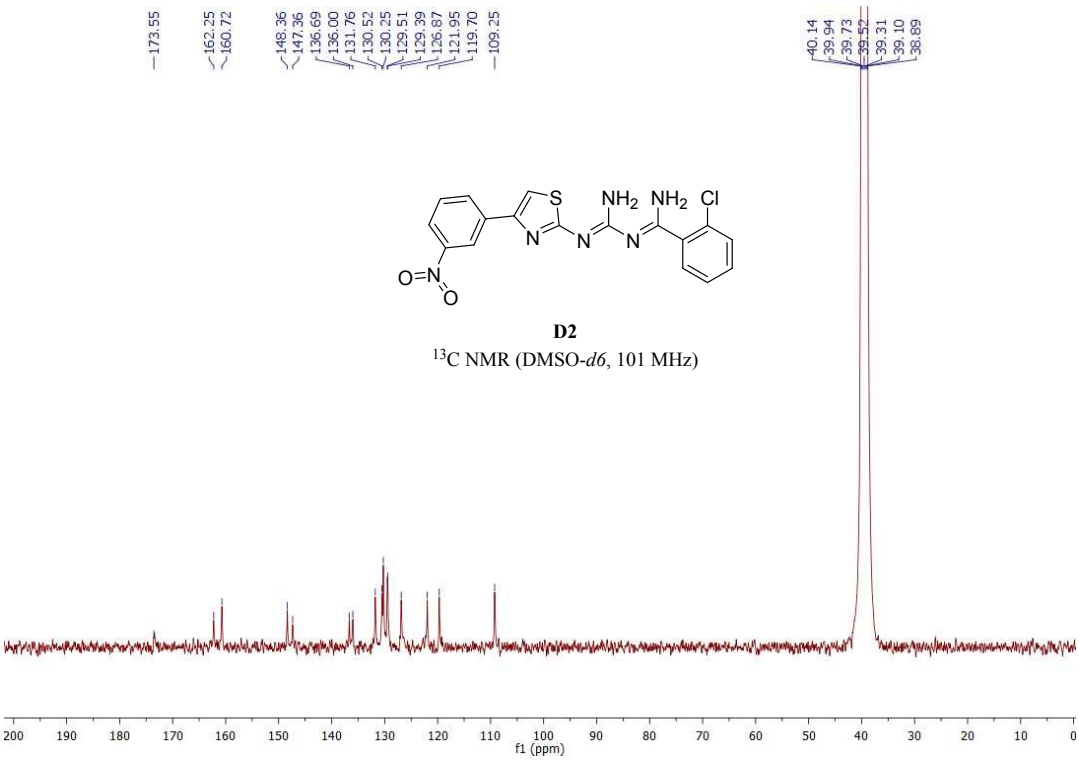
#	Peak Name	tR	Area	Height	Area%	Height%	Peak Start	Peak End	Base Start	Base End	Peak Mark
1	Unknown	3,733	3520863	721186	100,000	100,000	3,623	3,923	3,623	3,923	Manual

2-chloro-N-(N-(4-(3-nitrophenyl)thiazol-2-yl)carbamimidoyl)benzimidamide (**D2**)



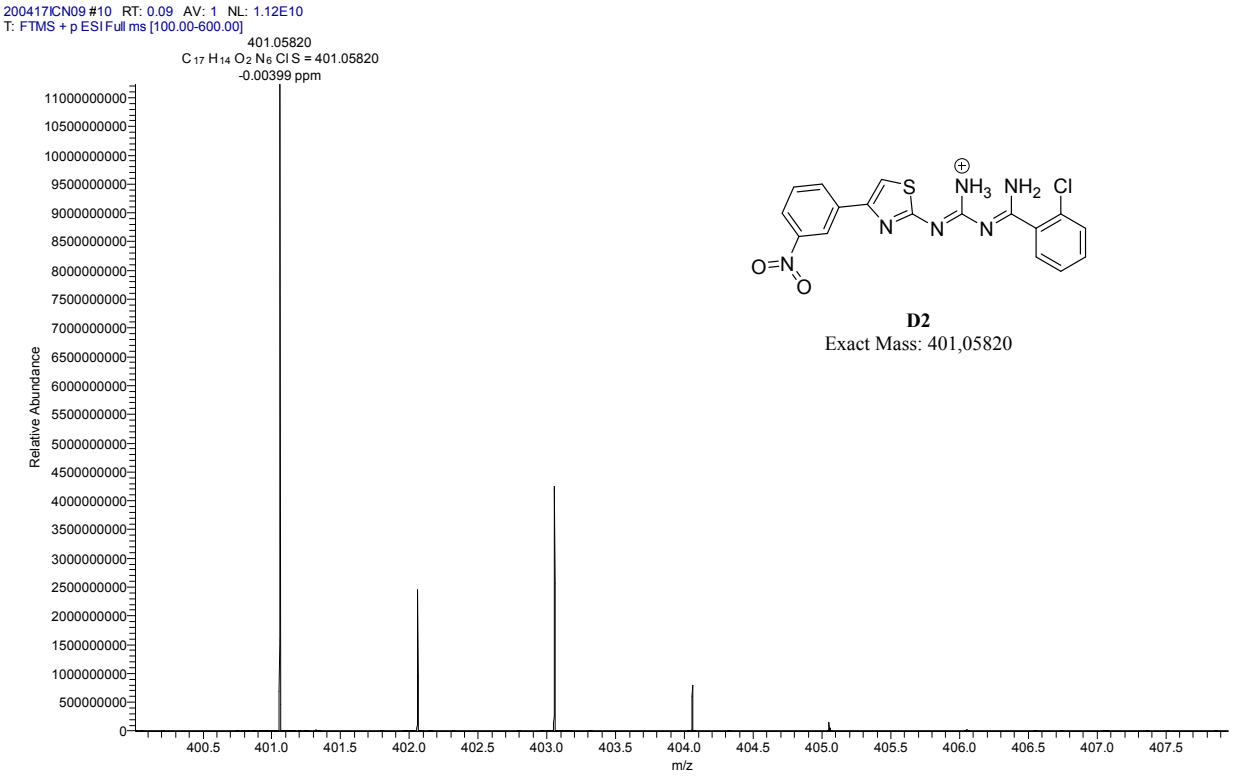
D2

^{1}H NMR (DMSO-*d6*, 400 MHz)



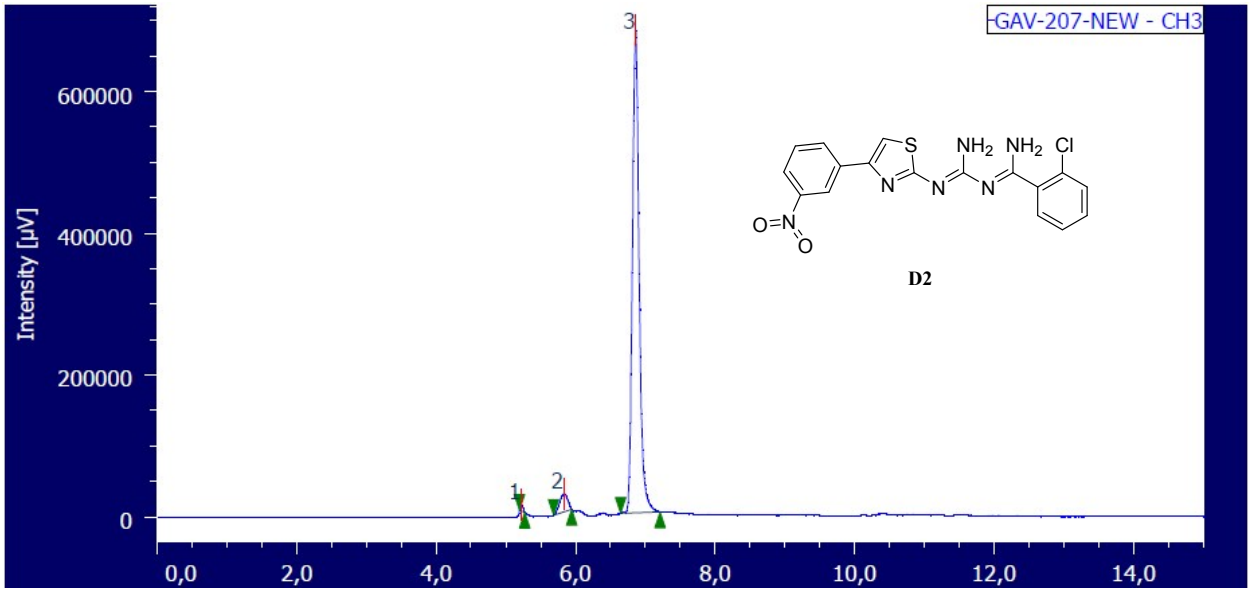
D2

^{13}C NMR (DMSO-*d6*, 101 MHz)



D2

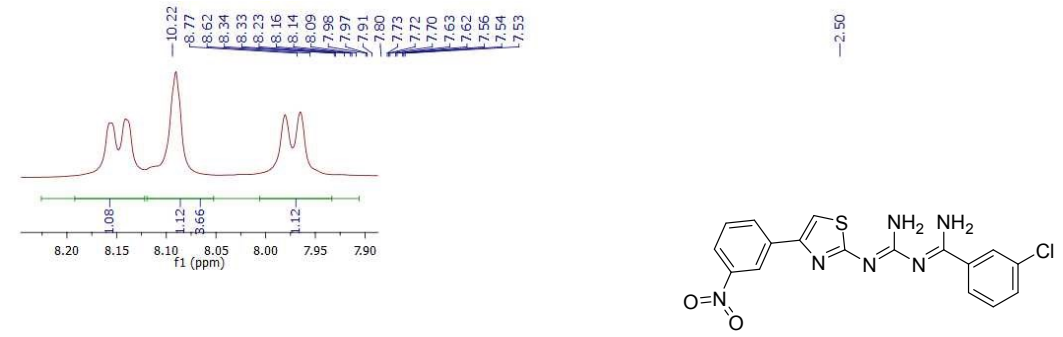
Exact Mass: 401,05820



D2

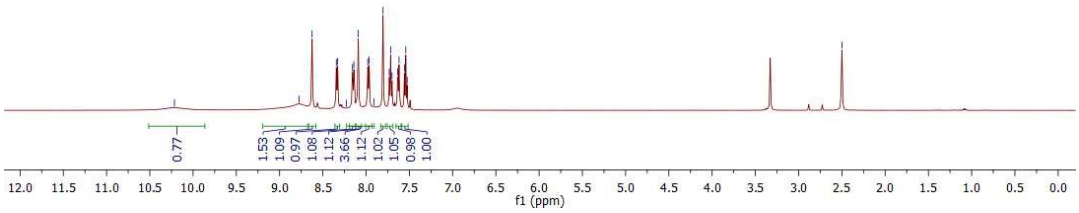
#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,217	23319	9117	0,487	1,275	N/A	83783	4,129	1,471	
2	Unknown	3	5,833	204887	25219	4,278	3,528	N/A	10526	5,183	0,876	
3	Unknown	3	6,858	4560596	680471	95,235	95,196	N/A	26307	N/A	1,208	

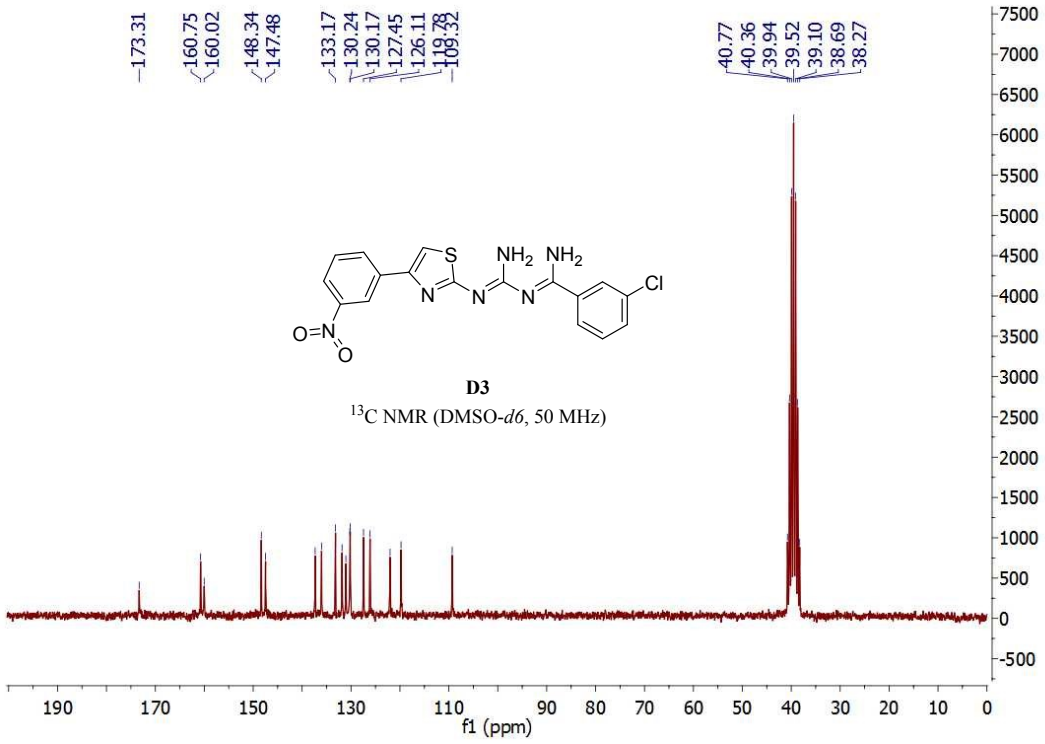
3-chloro-N-(N-(4-(3-nitrophenyl)thiazol-2-yl)carbamimidoyl)benzimidamide (**D3**)



D3

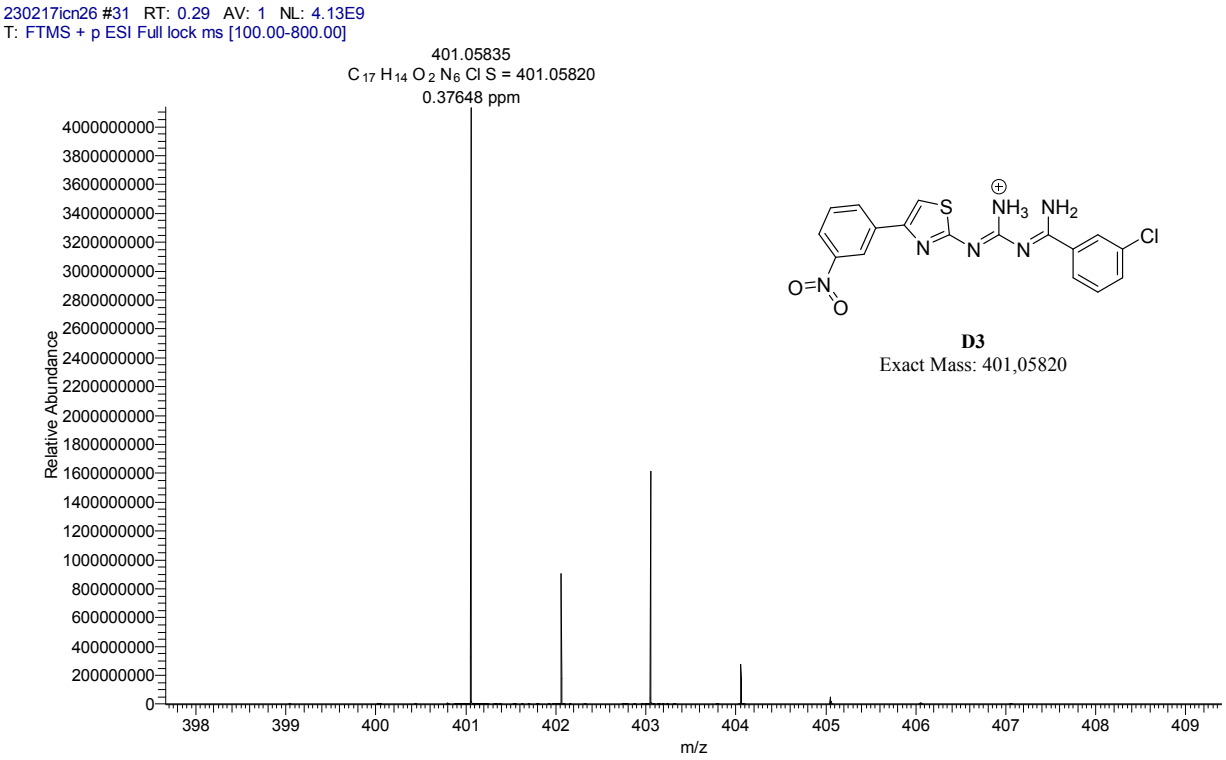
^{1}H NMR (DMSO-*d6*, 500 MHz)

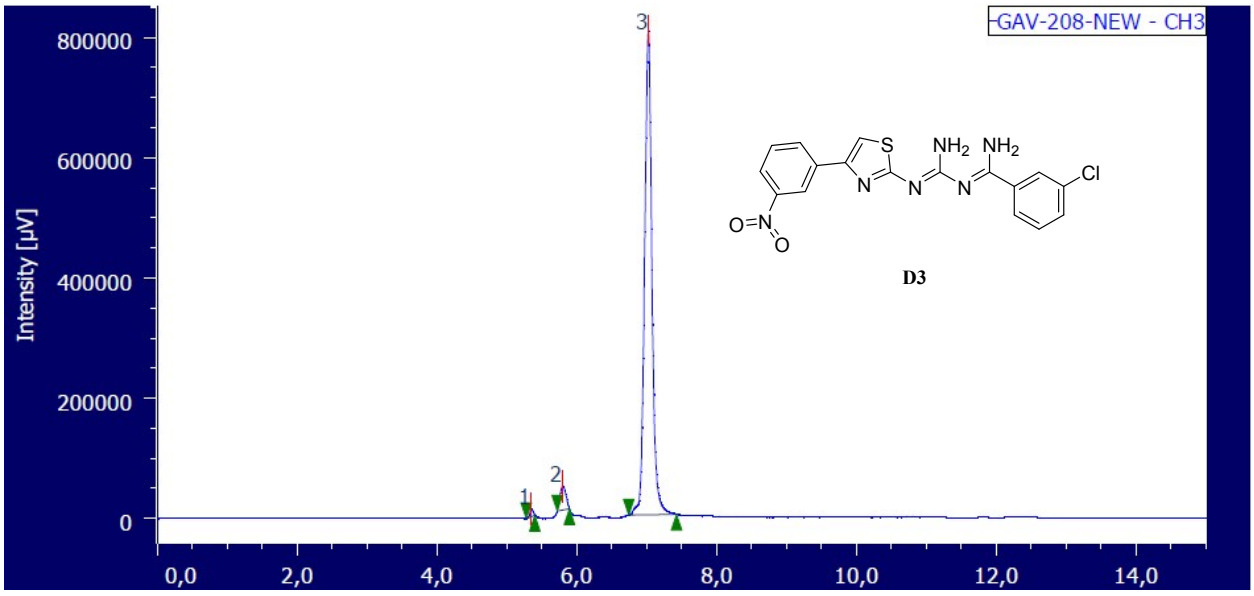




D3

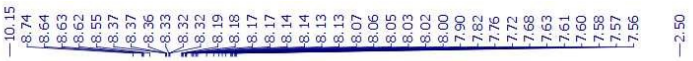
^{13}C NMR (DMSO-*d6*, 50 MHz)

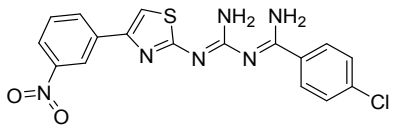




#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,350	39671	12830	0,642	1,497	N/A	60419	3,482	1,056	
2	Unknown	3	5,800	236849	39342	3,832	4,592	N/A	18168	6,867	1,047	
3	Unknown	3	7,025	5903917	804621	95,526	93,911	N/A	22921	N/A	1,069	

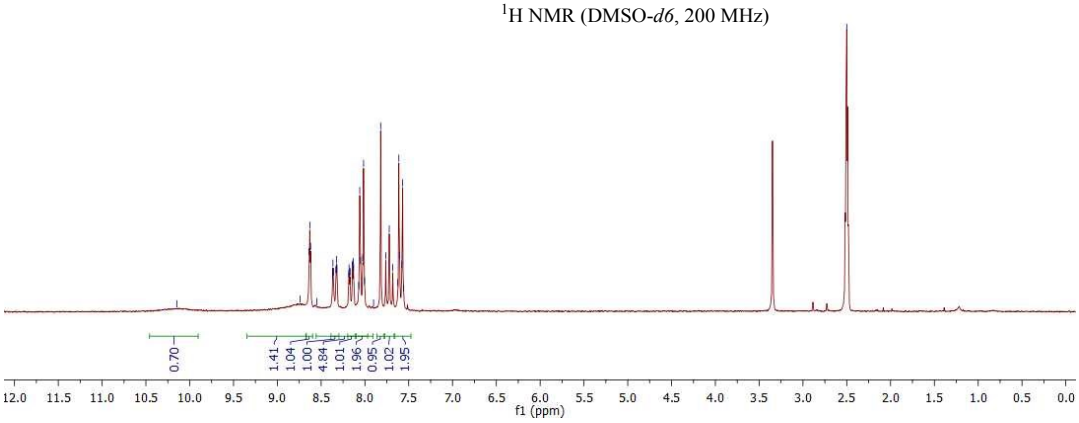
4-chloro-N-(N-(4-(3-nitrophenyl)thiazol-2-yl)carbamimidoyl)benzimidamide **(D4)**

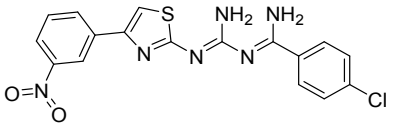




D4

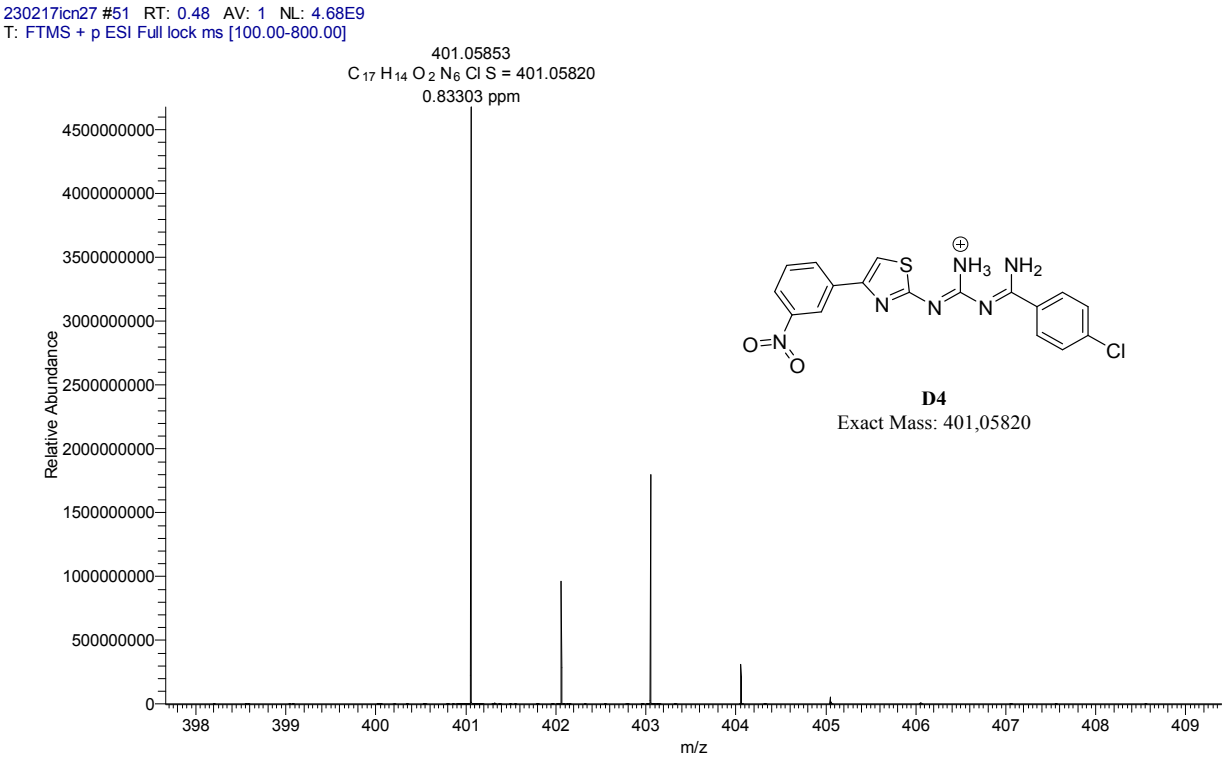
^{1}H NMR (DMSO-*d6*, 200 MHz)

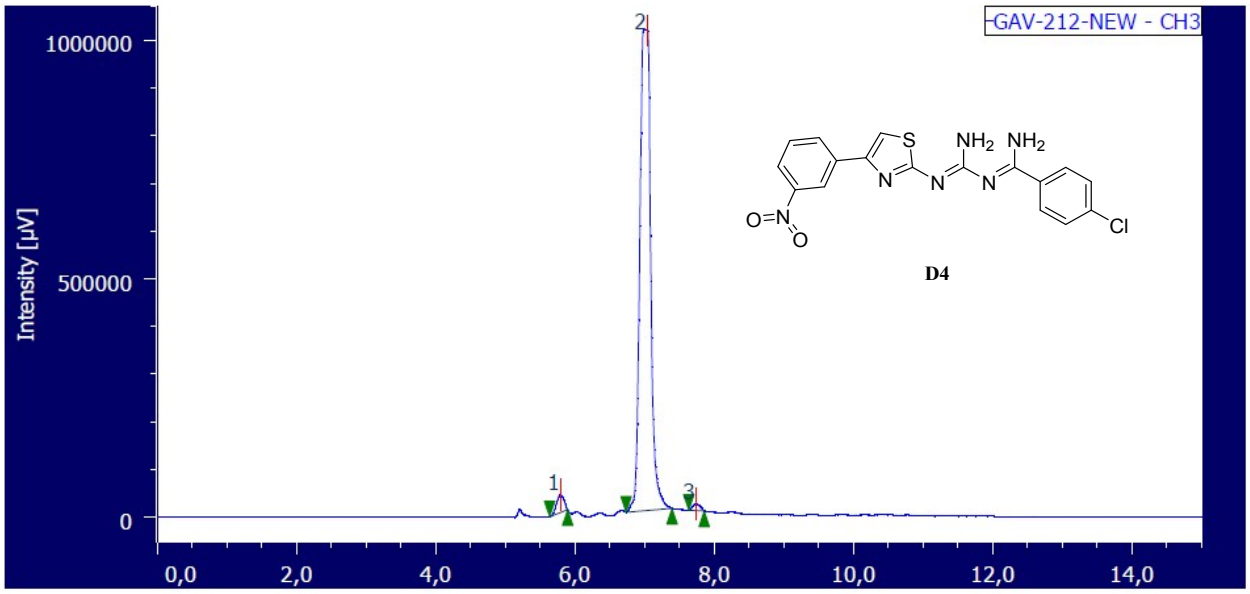




D4

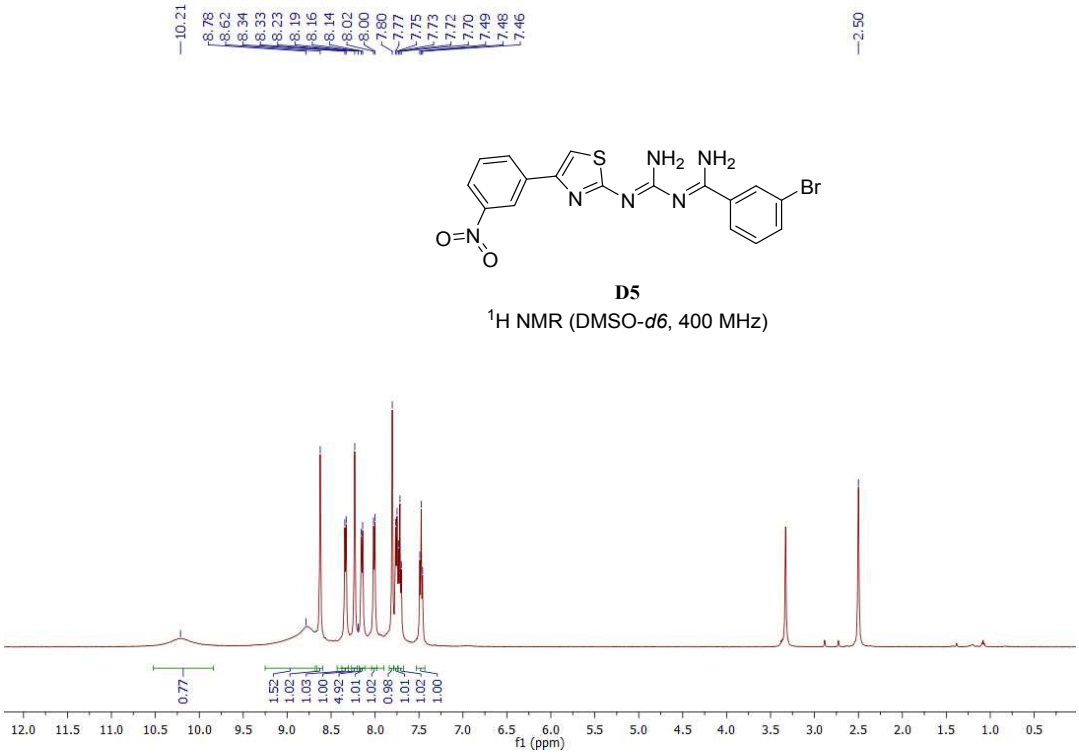
^{13}C NMR (DMSO-*d6*, 50 MHz)





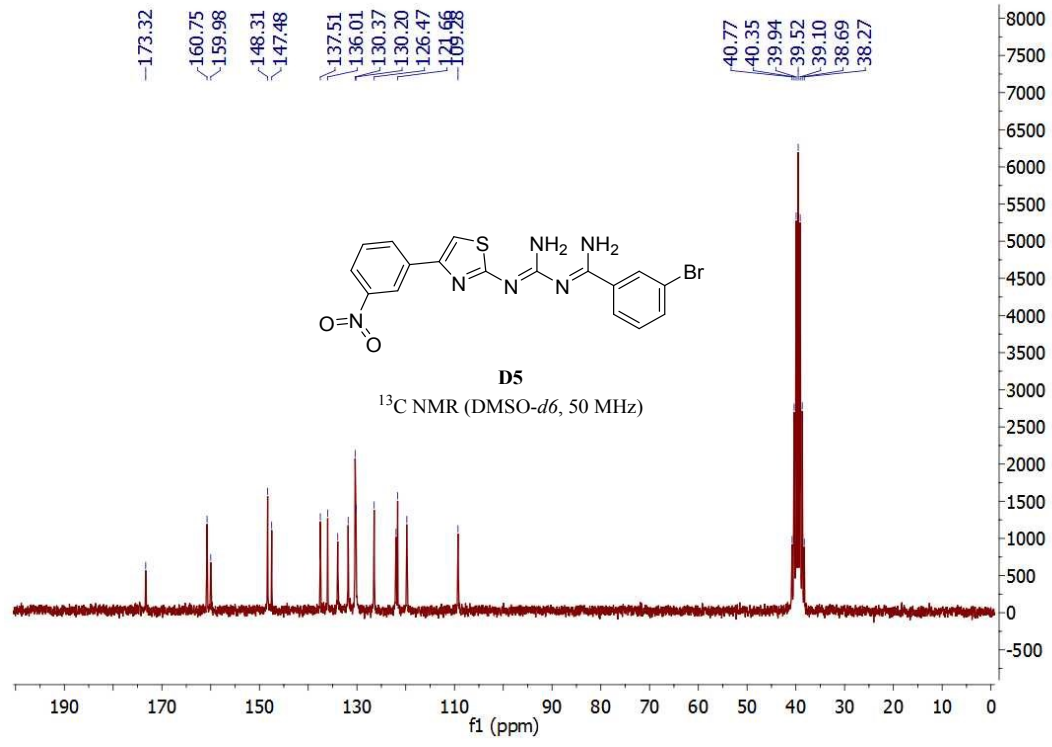
#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,792	271786	36613	2,405	3,471	N/A	12441	5,090	0,903	
2	Unknown	3	7,033	10927440	1004501	96,683	95,228	N/A	9986	2,836	0,963	
3	Unknown	3	7,733	103074	13723	0,912	1,301	N/A	21001	N/A	1,149	

3-bromo-N-(N-(4-(3-nitrophenyl)thiazol-2-yl)carbamimidoyl)benzimidamide (**D5**)



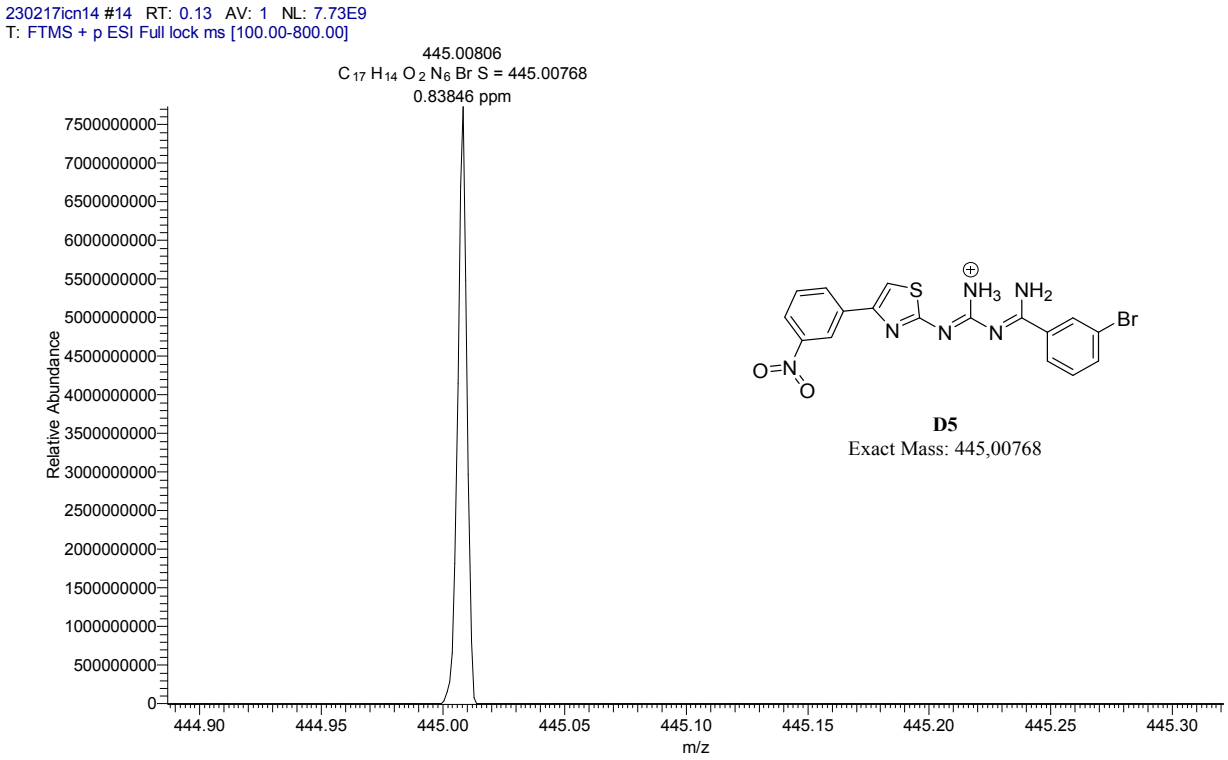
D5

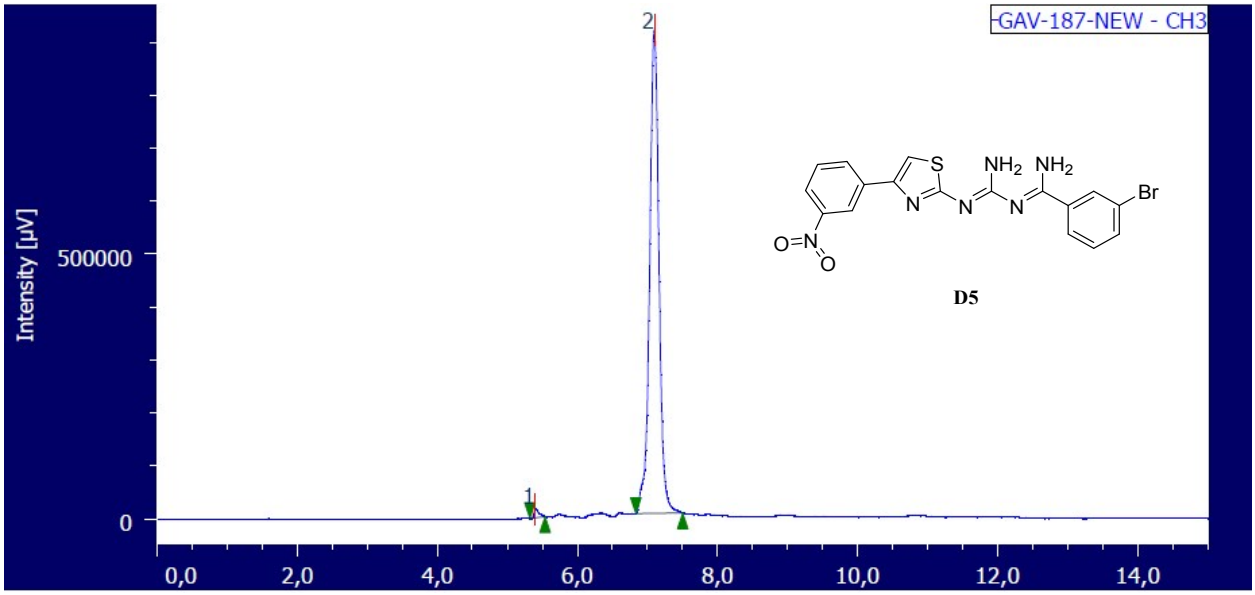
^{1}H NMR (DMSO-*d6*, 400 MHz)



D5

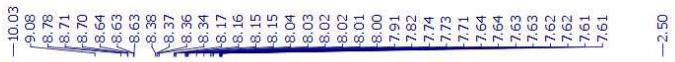
^{13}C NMR (DMSO-*d6*, 50 MHz)

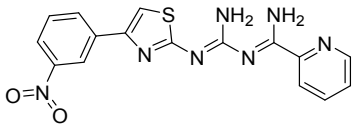




#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,400	81626	16945	0,949	1,823	N/A	35173	9,820	1,738	
2	Unknown	3	7,100	8523367	912606	99,051	98,177	N/A	14989	N/A	0,911	

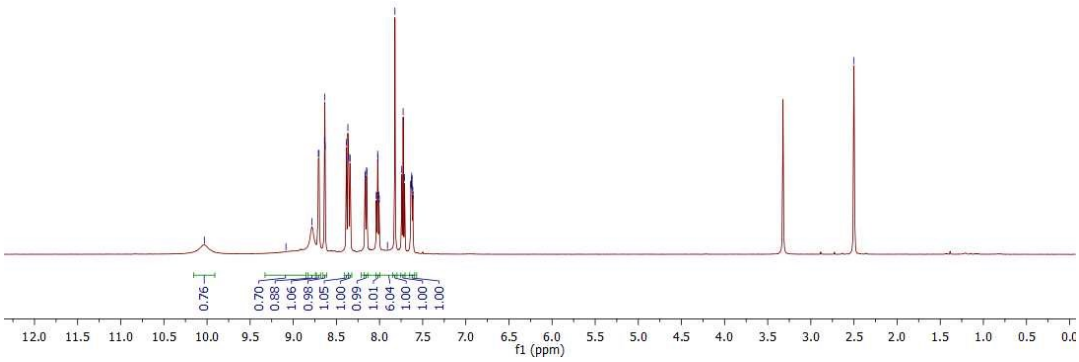
N-(N-(4-(3-nitrophenyl)thiazol-2-yl)carbamimidoyl)picolinimidamide (**D6**)

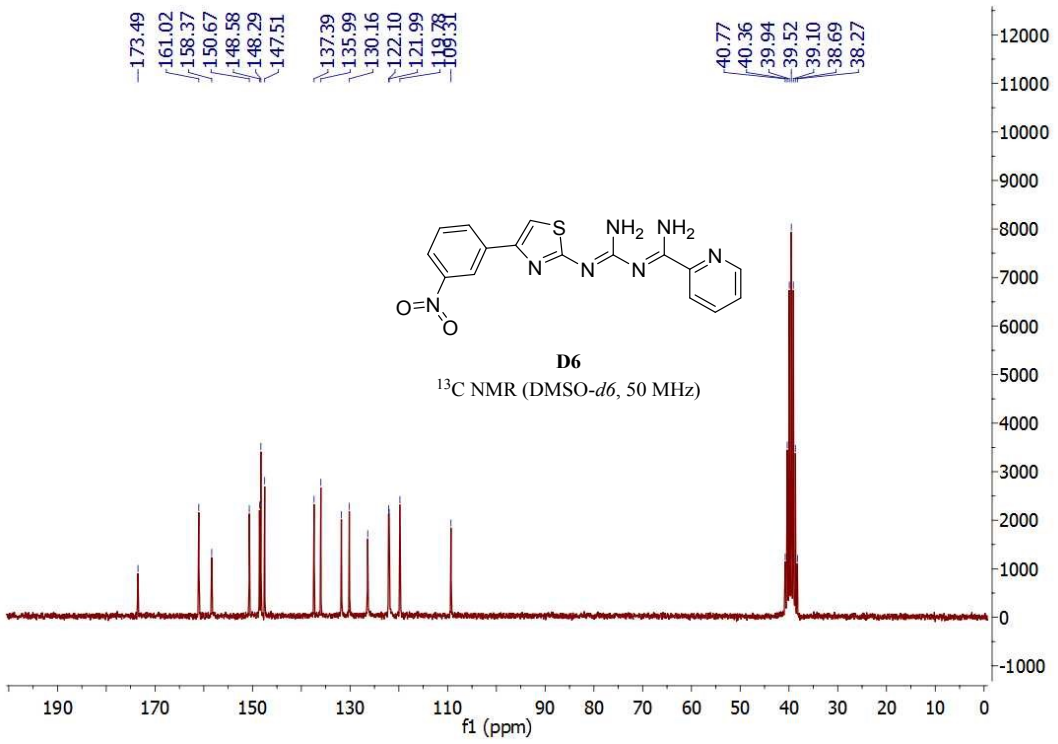




D6

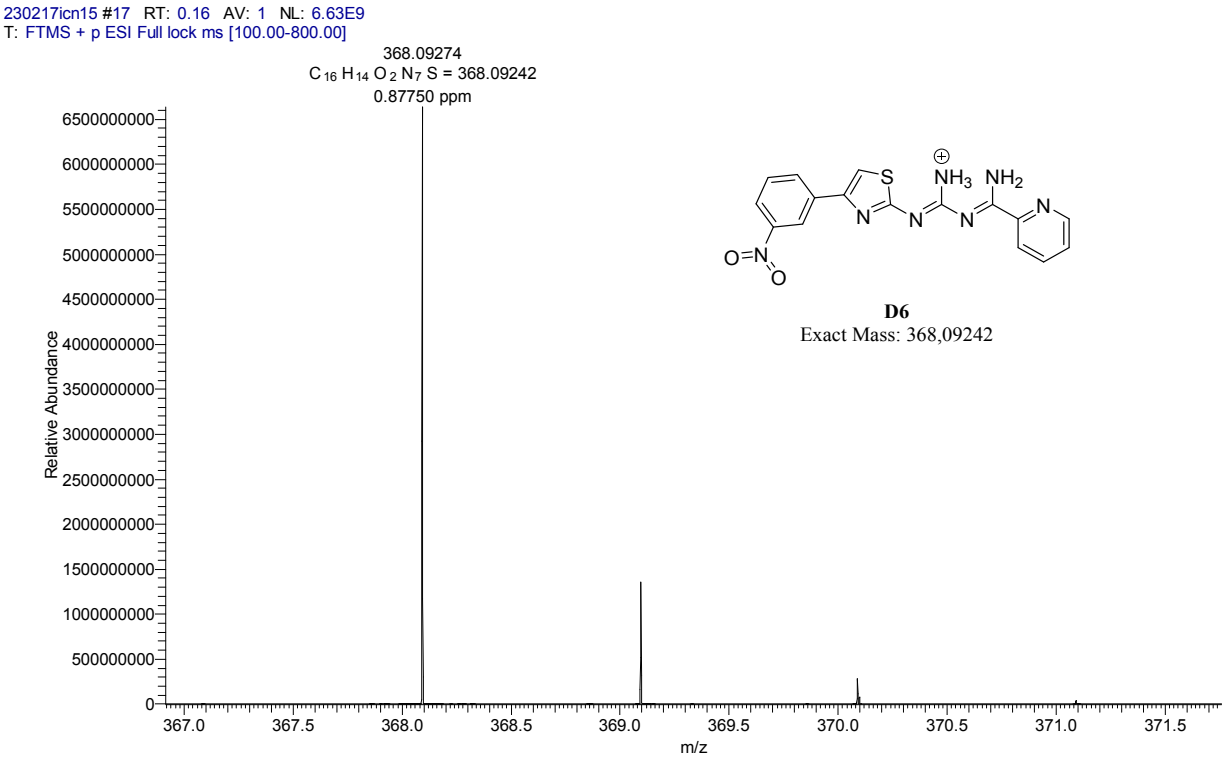
^{1}H NMR (DMSO-*d6*, 500 MHz)

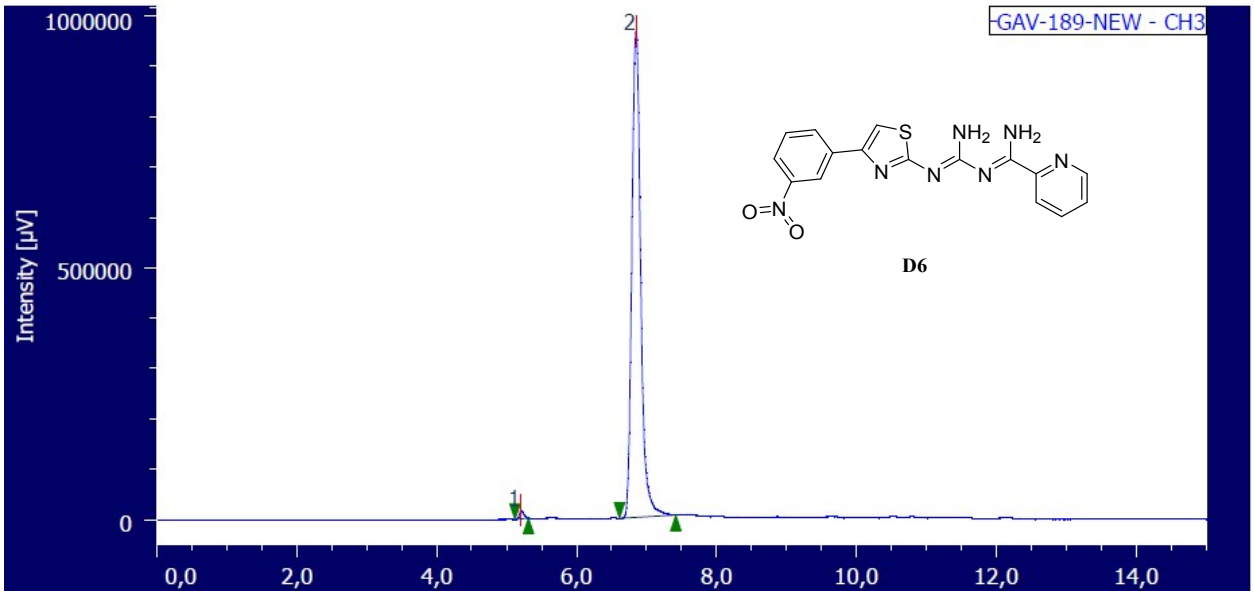




D6

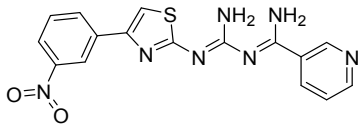
^{13}C NMR (DMSO-*d6*, 50 MHz)





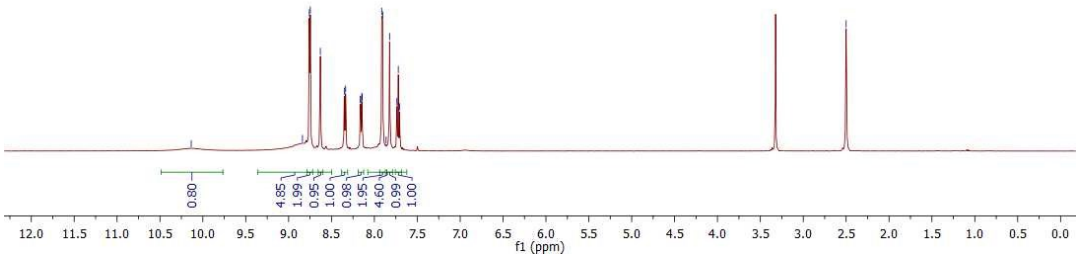
#	Peak Name	CH	tR [min]	Area [µV sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,200	71299	16603	0,831	1,692	N/A	34392	9,868	1,463	
2	Unknown	3	6,850	8508048	964790	99,169	98,308	N/A	15078	N/A	1,211	

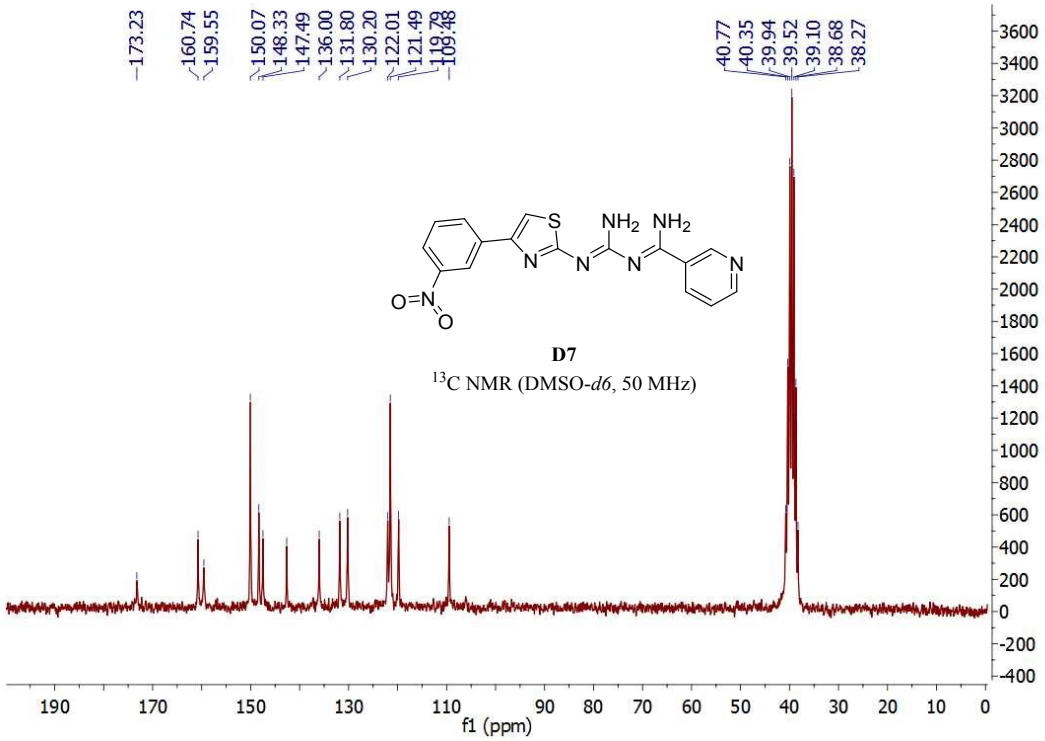
N-(N-(4-(3-nitrophenyl)thiazol-2-yl)carbamimidoyl)nicotinimidamide (**D7**)



D7

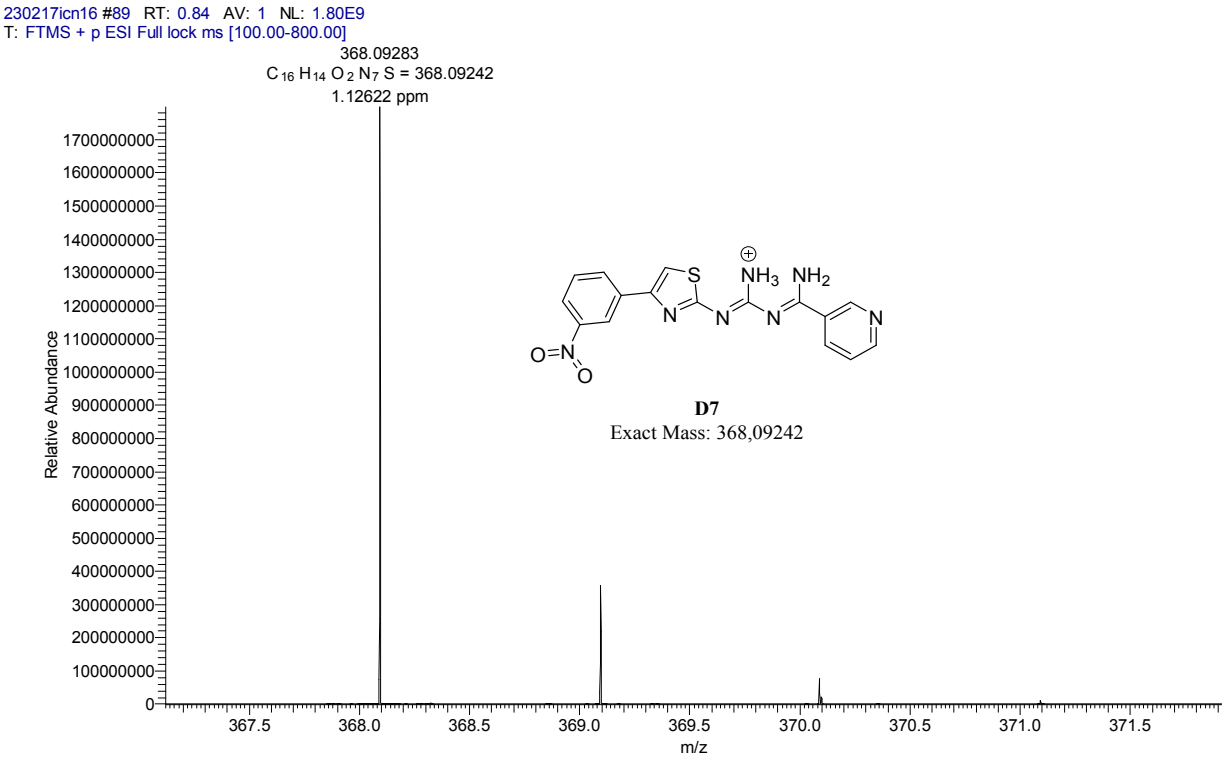
^{1}H NMR (DMSO-*d6*, 500 MHz)

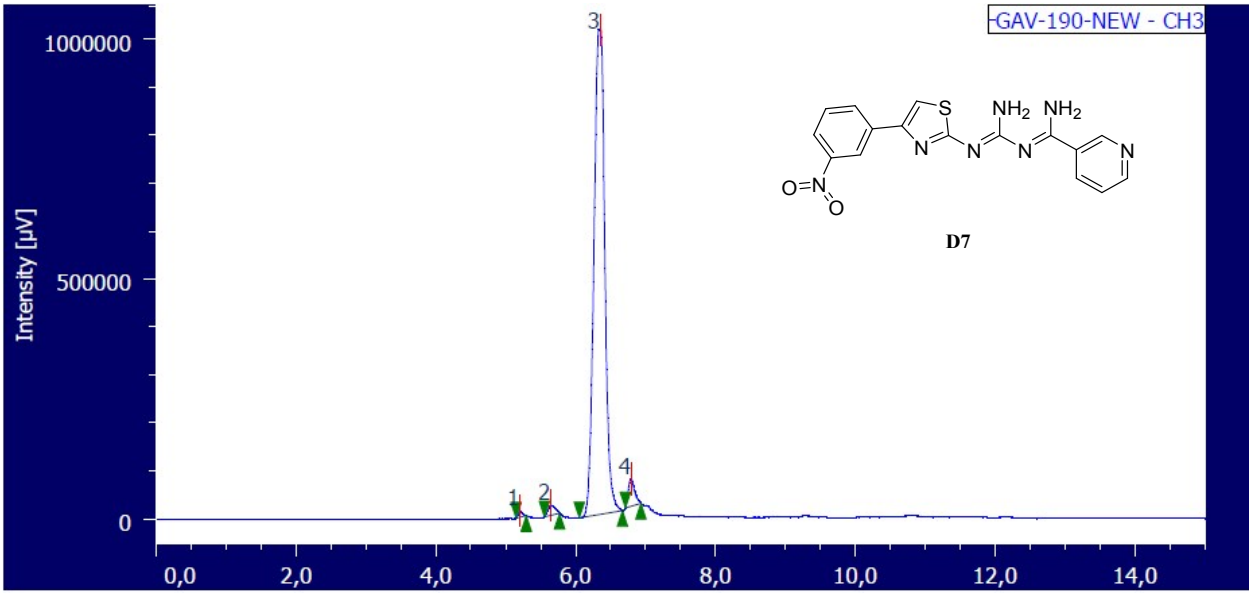




D7

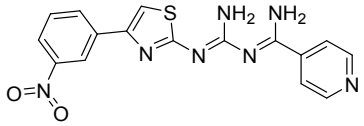
^{13}C NMR (DMSO-*d6*, 50 MHz)





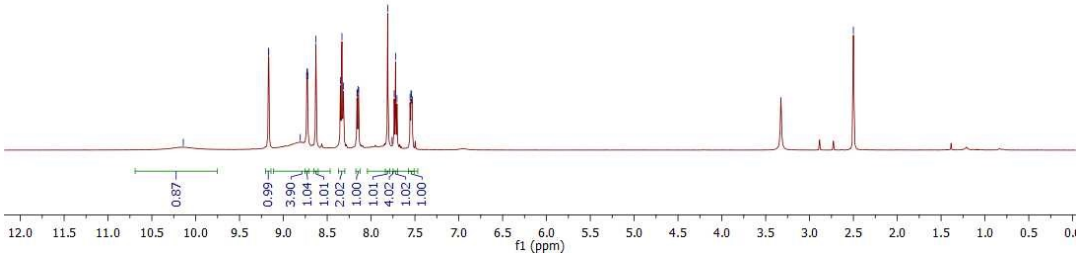
#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,200	55610	13914	0,485	1,263	N/A	42098	2,766	1,359	
2	Unknown	3	5,642	152461	20416	1,330	1,854	N/A	10630	2,845	1,190	
3	Unknown	3	6,350	10897156	1009735	95,054	91,690	N/A	8209	1,989	0,970	
4	Unknown	3	6,792	359000	57181	3,131	5,192	N/A	27113	N/A	1,332	

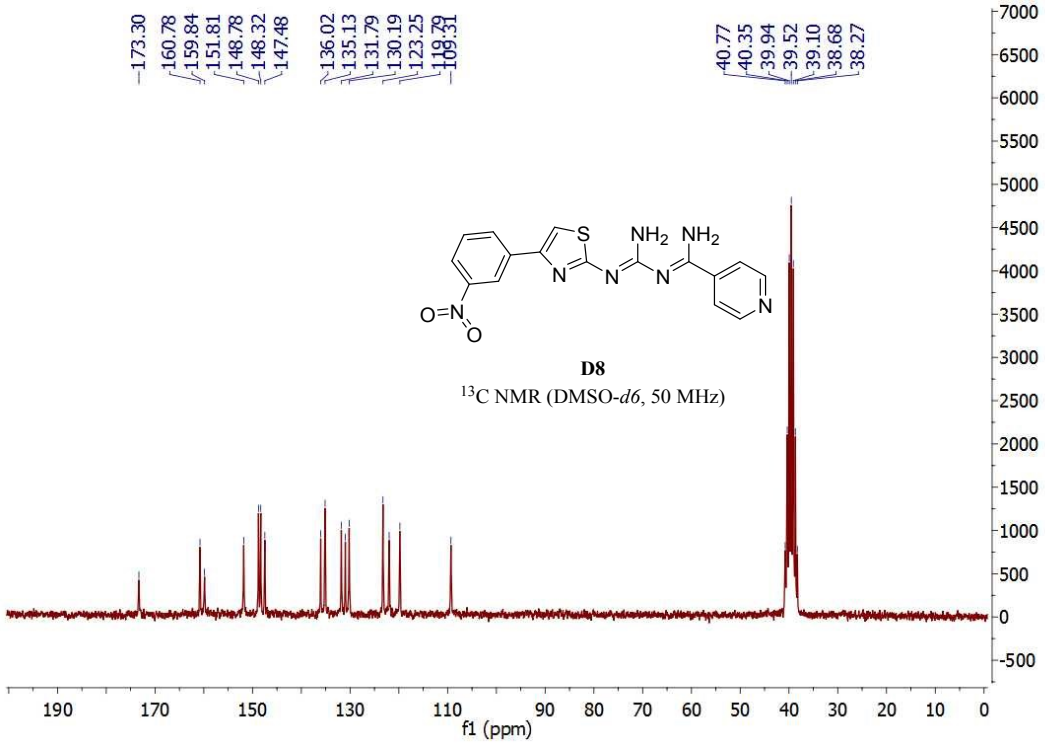
N-(N-(4-(3-nitrophenyl)thiazol-2-yl)carbamimidoyl)isonicotinimidamide (**D8**)



D8

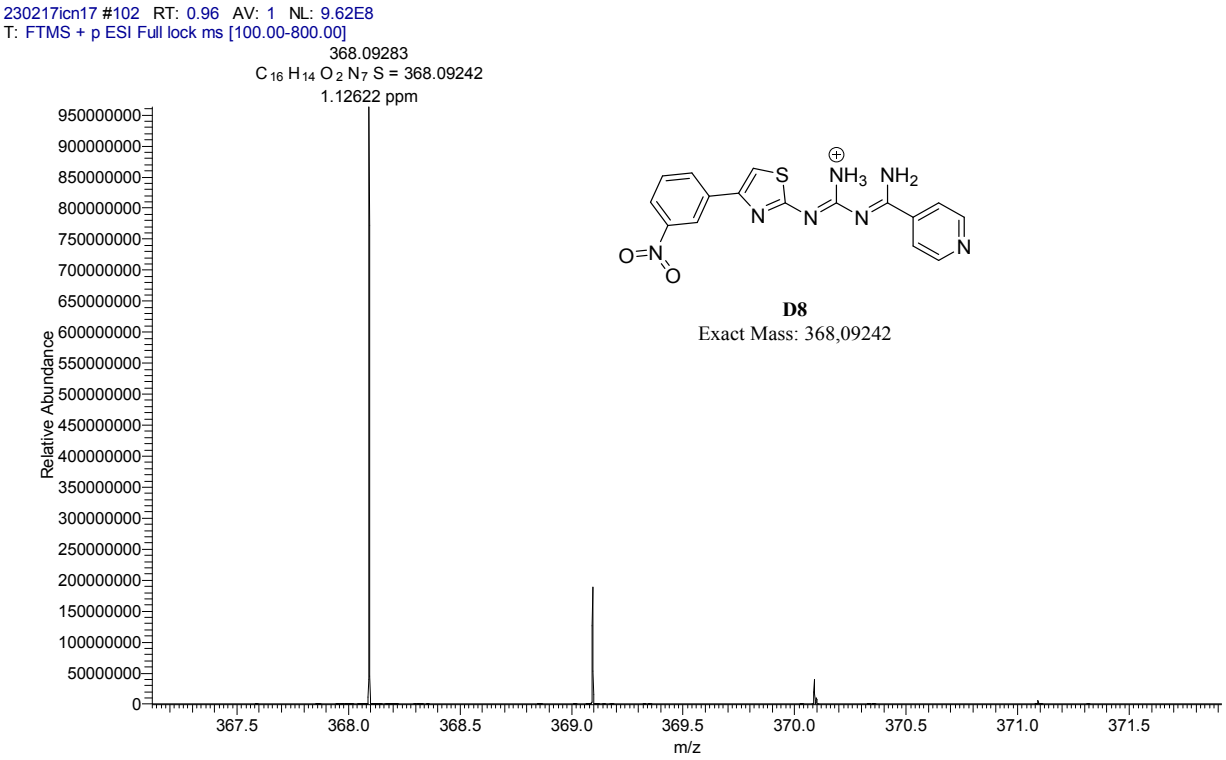
^{1}H NMR (DMSO-*d6*, 500 MHz)

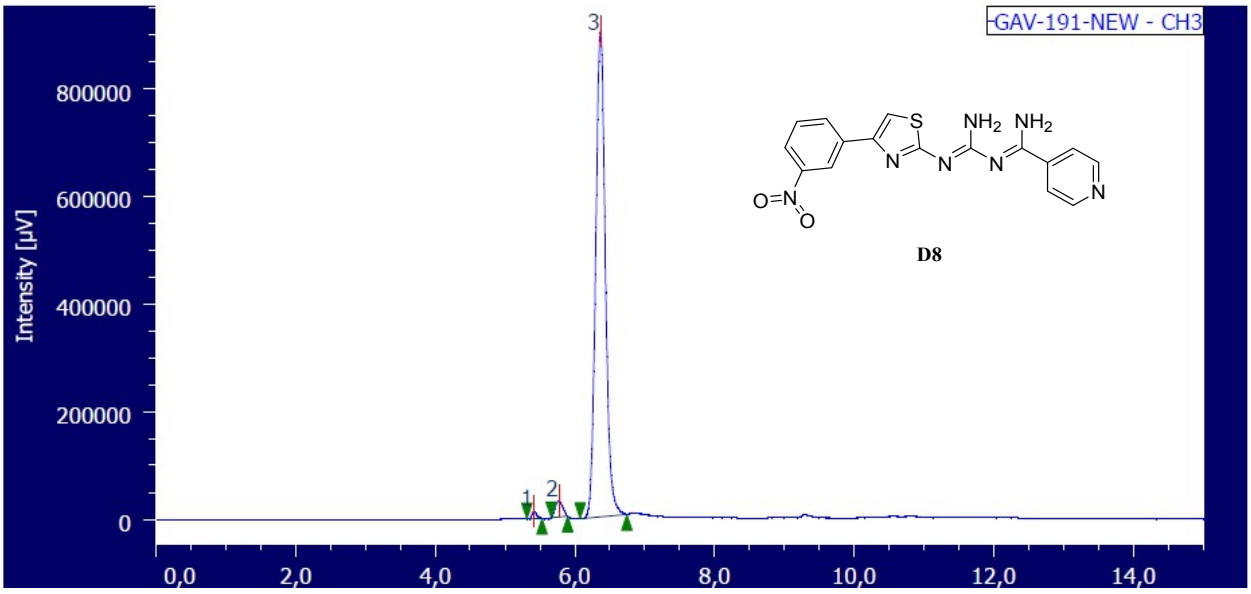




D8

^{13}C NMR (DMSO-*d6*, 50 MHz)

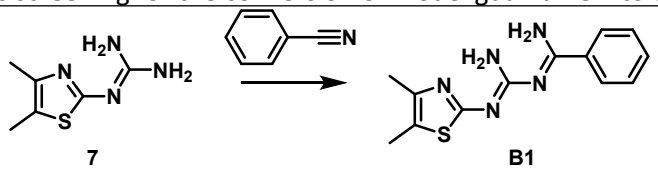




#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	5,408	63617	14342	0,708	1,516	N/A	37622	2,239	1,542	
2	Unknown	3	5,767	222053	29743	2,470	3,144	N/A	12127	2,641	1,073	
3	Unknown	3	6,367	8704059	901977	96,822	95,340	N/A	10701	N/A	1,072	

III. Condition screening

Conditions screening for the conversion of model guanidine **7** to the arylbiamidine **B1.**



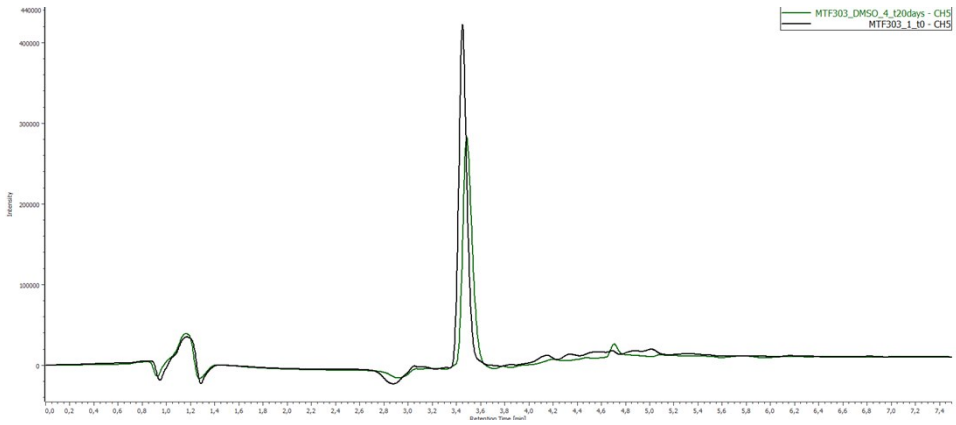
7 B1

Entry	Conditions [a]	Conversion (%)[b] 2 h	20 h
1	Ethanol, 70°C	0	0
2	12M aq. HCl/Ethanol (1/1), 70°C	0	0
3	Acetic acid, 70°C	0	0
4	Acetic acid, 90°C	0	1
5	TFA /1,2-propanediol (1:1), 70°C	0	0
6	THF/aq. 5M KOH/MeOH (0.5/0.2/0.3)	0	1
7	**NaH, DMF, argon, 0°C to rt**	**92**	**92**
8	$AlCl_3$ (1.2 equiv.), DCE, 80°C	0.5	0.5
9	$FeCl_3$ (1.2 equiv.), DCE, 80°C	0	0
10	$SnCl_4$ (1.2 equiv.), DCE, 80°C	0	0
11	$ZnCl_2$ (1.2 equiv.), DCE, 80°C	0	0
12	$POCl_3$ (1.2 equiv.), DCE, 80°C	14	12

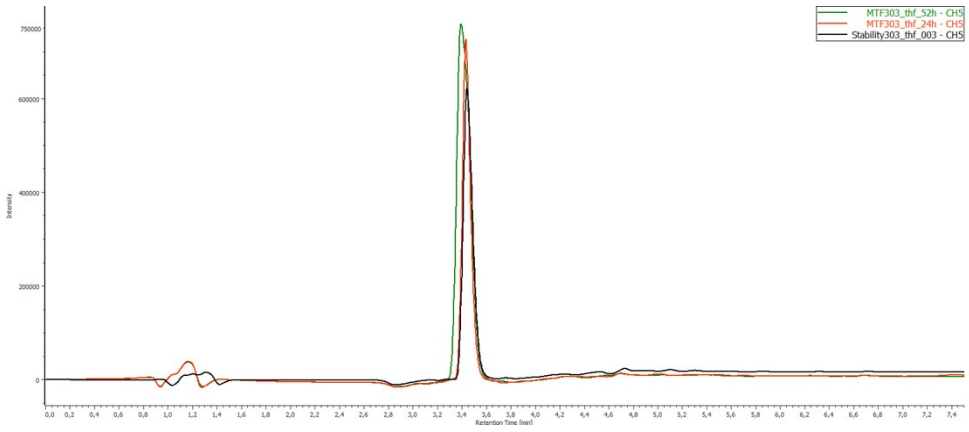
[a] Reactions were performed using 1.0 equiv. of benzonitrile. [b] Conversions are reported in terms of product formation observed by UHPLC-DAD at 254 nm. DCE: 1,2-dichloroethane.

IV. Stability of compound B1

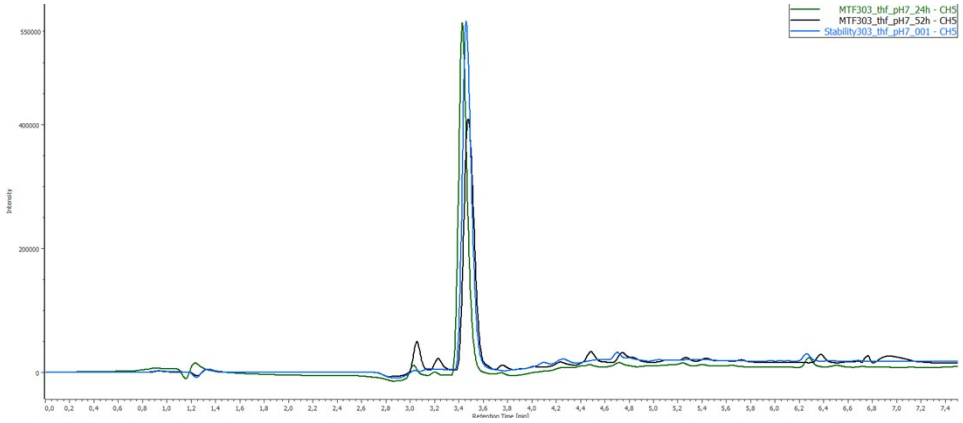
Stability in DMSO. A 10 mg/mL stock solution of **B1** was prepared in DMSO and incubated at 20°C. Aliquots were taken and in injected for 20 days in methanol to the UPLC (*Method B, 254 nm*).



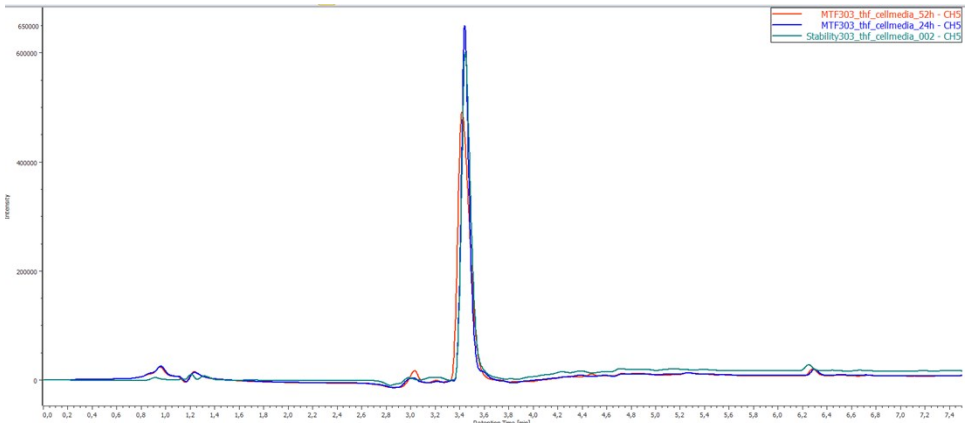
Stability in THF. A 10 mg/mL stock solution of **B1** was prepared in THF and incubated at 20°C. Aliquots were taken and in injected for 96 h in methanol to the UPLC (*Method B, 254 nm*).



Stability in 0.1 M phosphate buffer pH 7.0. An aliquot of the 10 mg/mL stock solution of **B1** prepared in THF was taken and incubated in 0.1 M phosphate buffer pH 7.0 containing 33% THF to improve solubility. Aliquots were taken and in injected for 96 h to the UPLC (*Method B, 254 nm*).

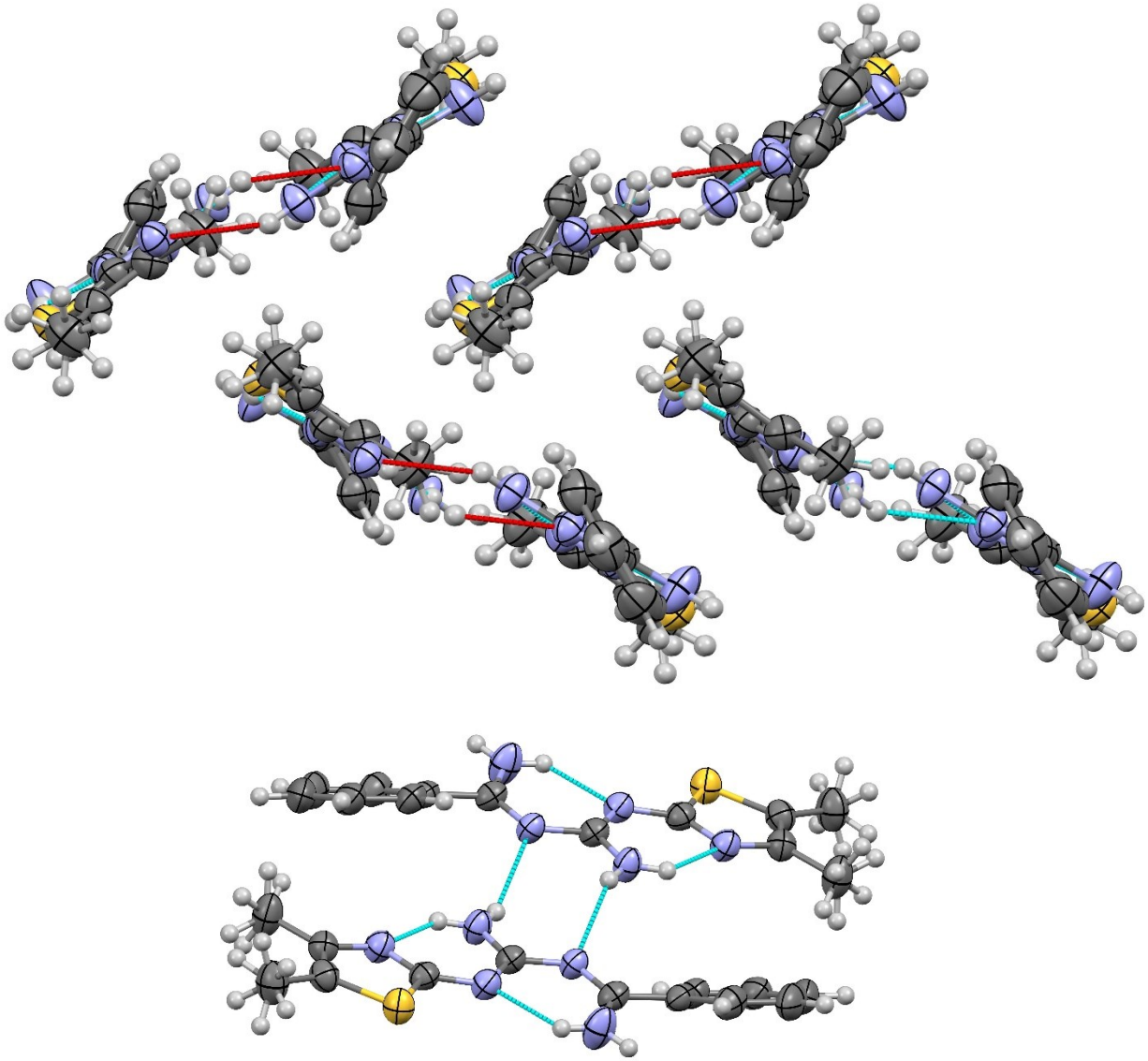


Stability in cell media (DMEM-PS + FBS). An aliquot of the 10 mg/mL stock solution of **B1** prepared in THF was taken and incubated in the cell media used in the experiments of cell viability (DMEM-PS + FBS) containing 33% THF to improve solubility. Aliquots were taken and in injected for 96 h to the UPLC (*Method B, 254 nm*).

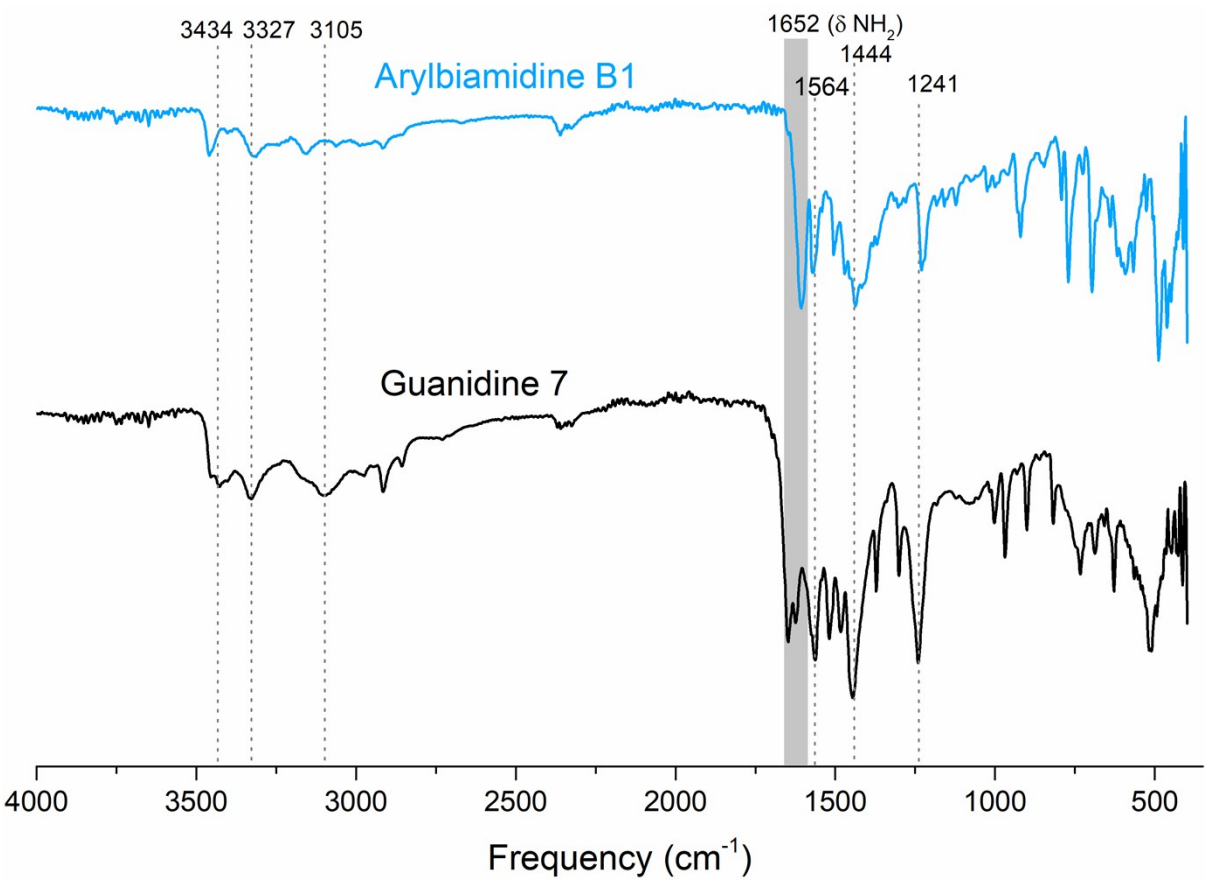


V. Crystallographic data for compound B1

Compound **B1** was crystallized from a dichloromethane saturated solution. The packing of this compound is shown below. Compound **B1** is observed in solid-state as head-to-tail dimers, formed by two intermolecular hydrogen bonds N–H···N, as well as hydrophobic dimethylthiazole-phenyl short contacts. Interestingly, the crystal structure analysis also showed a 3D structure in which the phenyl substituent is twisted by 31° relative to the amidine fragment N1–C7–N2. This can be explained by the low intermolecular aromatic interaction with the thiazole moiety.



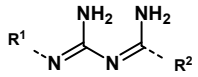
VI. IR comparison between guanidine 7 and arylbiamidine B1



VII. Determination of pK_{aH}

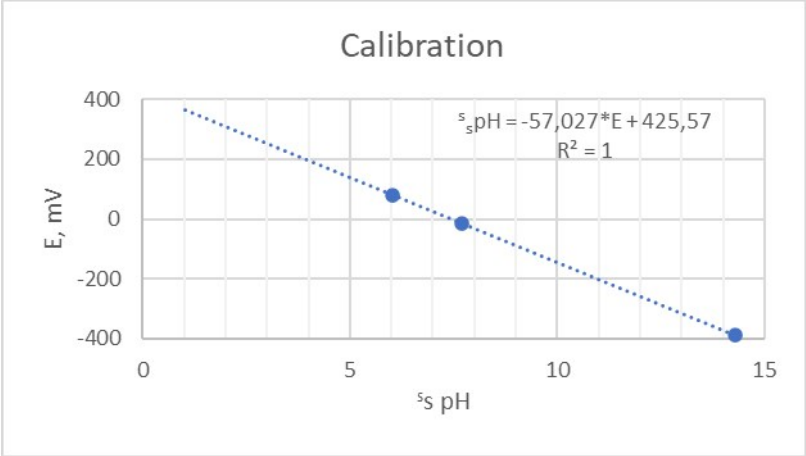
Compound	pK_{aH} (predicted) [a]	pK_{aH} (experimental)		log D[b]	log S[b]
		$^{s}_{s}$pK_{aH}	$^{s}_{w}$pK_{aH}	(pH 7)	(pH 7)
GUANIDINES					
Guanidine	11.40	13.09 ± 0.11	12.56 ± 0.10	-3.65	2.42
Phenyl	9.70			-1.45	0.37
Methylthiazolyl	7.20			-1.3	0.63
Dimethylthiazolyl	7.30	7.30 ± 0.02	6.89 ± 0.01	-0.7	0.25
Phenylthiazolyl	6.10			1.02	-2.27
Nitrophenylthiazolyl	5.60			1.42	-3.52
BIAMIDINES [c]					
Biamidine	9.00			-5.57	1.63
R^1-Phenyl	8.30			-3.22	0.00
R^1,R^2-Diphenyl	6.85			0.45	-1.32
Methylthiazolyl	6.35			0.09	-1.42
Dimethylthiazolyl	6.40	7.00 ± 0.02	6.61 ± 0.01	0.73	-1.92
Phenylthiazoyl	6.45			2.00	-3.50
m-Nitrophenylthiazolyl	6.30			1.94	-4.14
BIGUANIDES					
Biguanide	11.00			-4.17	2.15
o-Tolyl	10.20	10.94 ± 0.02	10.44 ± 0.01	-1.58	0.00
N_1,N_5-Diphenyl	6.77			0.49	-1.54
N_1,N_5-Thiazolephenyl	5.95			0.58	-1.85

[a]Values refer to pK_{aH} (couples amidine/amidine-H^+) and were predicted with the ChemAxon online calculator (https://disco.chemaxon.com/calculators/demo/plugins). [b]log D and log S were estimated from ChemAxon calculators. Log D values presented refer to log P (water:octanol partition coefficient) estimations at a pH 7. Log S is the 10-based logarithm of the solubility measured in mol/L, log S = log (solubility in water measured in mol/L).The ChemAxon model is based on the VG method (derived from Viswanadhan et al.[6]). The Chemaxon Plugin Calculator was accessed as freeware online: https://disco.chemaxon.com/calculators/demo/plugins. [C] Values estimated for the major tautomer:



pK_{aH} determination procedure

The p*K* values were determined with two different potentiometric procedures which lead to two different p*K* values: the $^{s}_{s}$pK_{aH} value in the pH scale relative to each acetonitrile–water mixture ($^{s}_{s}$pH) and the $^{s}_{w}$pK_{aH} value in the absolute pH scale ($^{s}_{w}$pH) referring to water as standard state. The system was calibrated with buffer solutions in the corresponding solvent (acetonitrile/water, 1/1, v/v).[7, 8]



$$pK_a = pH + \log\left(\frac{c_a}{c_b}\right) + \log \gamma_{BH^+}$$

$$\log \gamma_{BH^+} = -\frac{AI^{1/2}}{(1 + a_0 BI^{1/2})}$$

Macroscopic properties of relevant interest for pH measurement in acetonitrile-water mixtures at 25°C (50% mixture): A = 0.791; a_oB = 1.74.

VIII. Biological evaluation

Reagents. Trypan blue, DMEM, RPMI, penicillin/streptomycin, trypsin, were purchased from Life Technologies (Waltham, MA, USA), fetal calf serum (FCS) from Hyclone.

Cell culture. The cell culture of melanoma cell line A375 was done as previously described.[9] A375 melanoma cell line was purchased from the American Tissue Culture Collection. Cells were grown in DMEM (Thermofisher) supplemented with 10% FCS and penicillin/streptomycin (100 U/mL / 50 mg/mL) at 37°C and 5% CO_2. PLX-4032 (vemurafenib) was purchased from Selleckchem and resuspended in DMSO (Sigma Aldrich).

Trypan blue exclusion assays for viability test. For trypan blue staining, 200 µL of cells was aseptically transferred to a 1.5mL clear Eppendorf tube and incubated for 3 minutes at room temperature with an equal volume of 0.4% trypan blue solution. Viable cells were counted with a Malassez chamber and the results are expressed as the percentage of the value of control cells. All the experiments are performed in triplicate.

Western Blot Assays. Western blot analyses were performed as described previously[10]. Proteins were extracted in buffer containing 50 mmoL/L Tris-HCl (pH 7.5), 15 mmoL/L NaCl, 1% Triton X-100, and 1X protease and phosphatase inhibitors. Briefly, cell lysates (30 µg) were separated by SDS-PAGE, transferred to a polyvinylidene fluoride membrane (Millipore), and then exposed to the PARP (Cell Signaling Technologies #9542) or Actin (Cell Signaling Technologies #3700) antibodies. Proteins were visualized with the ECL System from Amersham.

Crystal violet staining. A375 Cells were plated in 12 well-plate and treated with the indicated compounds for 24 h. Cells were washed twice with PBS, then stained with crystal violet solution (2 g crystal violet in 100 mL ethanol, V_f = 500 mL) for 1 h at room temperature. Cells were washed 5 times with PBS then decolorated with 60% acetic acid for 1 h. Optical density of each sample was measured at 561 nm.

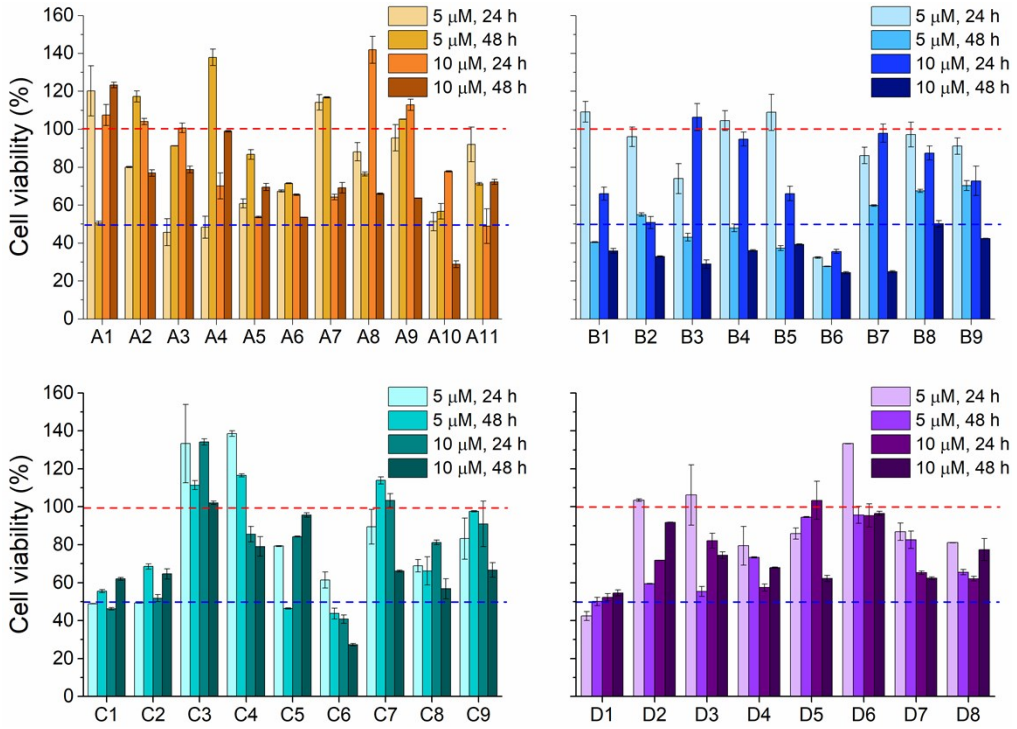
1. Viability test – Melanoma cell line A375

Activity against A375 melanoma cells after 24 h and 48 h incubation at 5 µM and 10 µM concentrations. Values are shown in terms of cell viability (%).

Biamidine derivatives	5 µM[a]		10 µM[a]	
	24 h (%)	48 h (%)	24 h (%)	48 h (%)
A1	120.2 ± 13.2	50.5 ± 1.0	107.5 ± 5.6	123.3 ± 1.5
A2	80.1 ±0.3	117.3 ± 3.0	104.1 ± 1.6	76.9 ± 1.7
A3	45.7 ± 7.1	91.3 ± 0.9	100.7 ± 2.5	78.7 ± 1.8

A4	48.4 ± 5.8	137.9 ± 4.3	70.1 ± 6.9	99.0 ± 0.4
A5	60.9 ± 2.4	86.7 ± 2.6	53.7 ± 0.4	69.5 ± 1.9
A6	67.4 ± 0.6	71.5 ± 0.3	65.5 ± 0.4	53.6 ± 0.1
A7	114.2 ± 4.01	116.8 ± 0.3	64.3 ± 1.5	69.1 ± 2.8
A8	88.1 ± 4.8	76.4 ± 1.0	141.9 ± 7.2	66.0 ± 0.4
A9	95.5 ± 6.9	105.3 ± 0.1	112.9 ± 2.9	63.7 ± 0.1
A10	51.3 ± 4.8	56.8 ± 4.1	77.8 ± 0.25	28.8 ± 1.76
A11	114.9 ± 1.2	95.2 ± 1.3	128.2 ± 1.0	41.3 ± 0.1
B1	109.2 ± 5.4	40.5 ± 0.3	66.1 ± 3.4	35.9 ± 1.3
B2	96.0 ± 5.1	55.0 ± 0.9	50.8 ± 3.2	32.9 ± 0.3
B3	74.0 ± 7.9	43.1 ± 2.1	106.4 ± 7.1	29.0 ± 2.2
B4	104.6 ± 5.2	48.0 ± 2.1	94.8 ± 3.7	36.1 ± 0.4
B5	108.9 ± 9.6	37.3 ± 1.3	66.1 ± 3.8	39.3 ± 0.3
B6	32.4 ± 0.4	27.7 ± 0.1	35.6 ± 1.1	24.4 ± 0.6
B7	86.2 ± 4.3	59.8 ± 0.4	97.9 ± 4.8	24.9 ± 0.5
B8	97.2 ± 6.5	67.5 ± 0.8	87.5 ± 3.7	50.2 ± 1.7
B9	91.1 ± 4.3	70.3 ± 2.6	72.8 ± 7.7	42.4 ± 0.2
C1	48.9 ± 0.1	55.5 ± 0.8	46.2 ± 0.7	62.0 ± 0.7
C2	49.3 ± 0.1	68.5 ± 1.4	51.8 ± 1.9	64.6 ± 2.7
C3	133.3 ± 20.6	111.4 ± 2.3	134.2 ± 1.6	102.1 ± 0.9
C4	138.6 ± 1.5	116.5 ± 0.8	85.5 ± 4.1	79.1 ± 5.1
C5	79.3 ±0.2	46.4 ± 0.3	84.3 ± 0.3	95.7 ± 1.1
C6	61.4 ± 4.2	43.7 ± 2.9	40.8 ± 2.2	27.3 ± 0.7
C7	89.5 ± 9.1	113.9 ± 1.9	103.4 ± 3.6	66.1 ± 0.5
C8	122.1 ± 3.5	95.3 ± 1.3	135.2 ± 1.0	114.2 ± 0.2
C9	83.2 ± 10.9	97.6 ± 0.3	91.0 ± 12.0	66.7 ± 3.9
D1	42.4 ± 2.3	50.1 ± 2.2	52.3 ± 1.9	54.6 ± 1.6
D2	103.5 ± 0.6	59.4 ± 0.2	71.8 ± 0.1	91.8 ± 0.1
D3	106.2 ± 15.9	55.3 ± 2.6	82.1 ± 3.9	74.4 ± 1.8
D4	79.2 ± 9.1	73.3 ± 0.3	57.4 ± 1.8	67.9 ± 0.3
D5	85.9 ± 2.9	94.6 ± 0.3	103.4 ± 10.0	62.2 ± 1.7
D6	133.3 ± 0.1	95.7 ± 4.5	95.4 ± 6.1	96.5 ± 1.1
D7	86.9 ± 4.6	82.6 ± 4.6	65.3 ± 0.9	62.4 ± 0.6
D8	81.1 ± 0.1	65.5 ± 1.4	62.1 ± 1.2	77.4 ± 5.8

[a] Cell viability on A375 melanoma cells relative to a DMSO control ± SD from three independent experiments, as determined by trypan blue exclusion assay. The colors are defined as follows: most promising compounds with cell viabilities lower than 30% in green and between 30-50% light gray. N=3 ± SD.



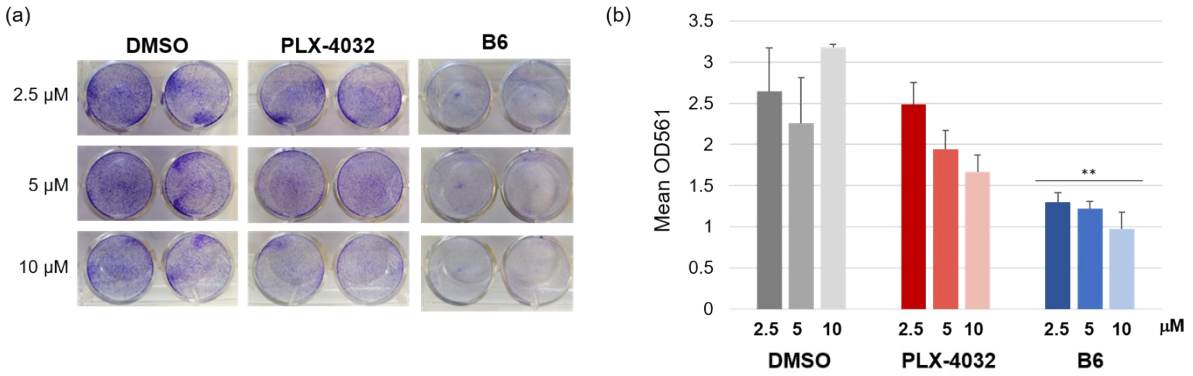
Cell viability of A375 melanoma cells relative to DMSO upon incubation of two doses (5 and 10 μM) of the aryl-biamidine derivatives of groups (a) **A**, (b) **B**, (c) **C** and (d) **D** at 24 h and 48 h.

2. National Cancer Institute (NCI) - 60 cancer cell line panel evaluation[11]

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	33.97
HL-60(TB)	12.39
K-562	15.90
MOLT-4	39.67
RPMI-8226	55.12
SR	35.58
Non-Small Cell Lung Cancer	
A549/ATCC	56.83
EKVX	60.24
HOP-62	50.03
HOP-92	88.03
NCI-H226	73.15
NCI-H23	59.69
NCI-H322M	86.20
NCI-H460	30.09
NCI-H522	45.74
Colon Cancer	
COLO 205	55.60
HCC-2998	64.86
HCT-116	25.44
HCT-15	25.08
HT29	17.83
KM12	37.39
SW-620	33.03
CNS Cancer	
SF-268	80.04
SF-295	8.89
SF-539	37.81
SNB-19	42.92
SNB-75	62.92
U251	46.97
Melanoma	
LOX IMVI	43.48

MALME-3M	62.01
M14	47.14
MDA-MB-435	10.80
SK-MEL-2	64.01
SK-MEL-28	72.76
SK-MEL-5	69.78
UACC-257	88.33
UACC-62	28.13
Ovarian Cancer	
IGROV1	52.30
OVCAR-3	47.21
OVCAR-4	87.30
OVCAR-5	71.05
OVCAR-8	58.24
NCI/ADR-RES	36.13
SK-OV-3	67.83
Renal Cancer	
786-0	75.19
A498	43.52
ACHN	49.87
CAKI-1	48.63
RXF 393	56.89
SN12C	47.33
TK-10	83.30
UO-31	52.53
Prostate Cancer	
PC-3	59.77
DU-145	57.86
Breast Cancer	
MCF7	13.20
MDA-MB-231/ATCC	76.45
HS 578T	73.11
BT-549	84.67
T-47D	56.39
MDA-MB-468	32.48
Mean	51.65
Delta	42.76
Range	79.44

3. Comparison of compound B6 to the BRAF-inhibitor PLX-4032 (vemurafenib)

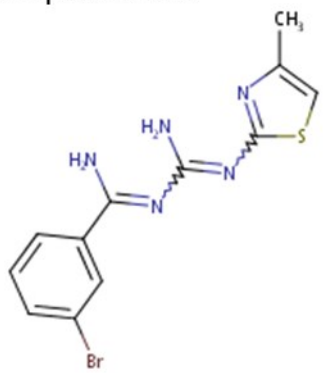


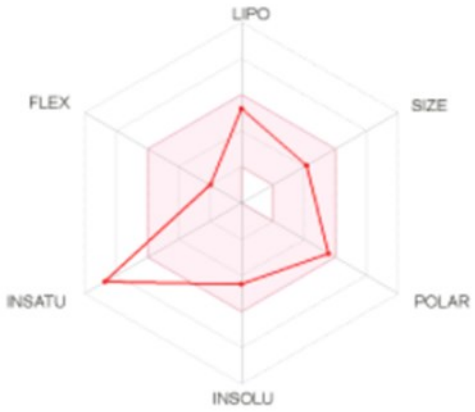
(a) Cell viability assessed with crystal violet staining of A375 melanoma cells incubated for 24 h with DMSO (negative control condition), PLX-4032 or **B6** (both at 2.5 µM 5 µM or 10 µM). (b) Quantification of crystal violet staining (optic density at 561 nm) after discoloration with acetic acid. Values are mean of 2 independent series of experiments in triplicate. Data represented as the mean ± standard deviation ** $P < 0.05$ between DMSO condition and PLX-4032 or **B6** conditions.

IX. Physical-chemical properties of compound B6

The druglikeness of compound **B6** was assessed by calculation from the freeware http://www.swissadme.ch/. In particular, no violation was observed for all criteria assayed.

Compound **B6**





SMILES Brc1cccc(c1)/C(=N/C(=N/c1scc(n1)C)/N)/N

Physicochemical Properties	
Formula	C12H12BrN5S
Molecular weight	338.23 g/mol
Num. heavy atoms	19
Num. arom. heavy atoms	11
Fraction Csp3	0.08
Num. rotatable bonds	3
Num. H-bond acceptors	3
Num. H-bond donors	2
Molar Refractivity	82.38
TPSA	117.89 Å²
Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability Score	0.55

X. Supporting references

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