

Supporting Information

Synthesis and biological evaluation of a series of aryl triazoles as firefly luciferase inhibitors

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1. Materials and instruments

Chemical synthesis materials and instruments

All reagents and solvents available from commercial sources were used as received unless otherwise noted. Twice-distilled water was used throughout all experiments. Water used for the bioluminescence studies was doubly distilled and further purified with a Mill-Q filtration system. NMR spectra were obtained in deuterated solvents on Bruker AV-300 or AV-600 spectrometers at the College of Chemistry NMR Facility, Shandong University. All chemical shifts are reported in the standard δ notation of parts per million using the peaks of residual proton and carbon signals of the solvent as internal references. NMR peaks are referred to as singlet (s), doublet (d), and doublet of doublets (dd), triplet (t), or broad singlet (br). Coupling constants (J) are reported in hertz. Mass spectra were recorded in ESI⁺ mode (70 eV).

Bioluminescence assay instrument.

Millipore water was used to prepare all aqueous solutions. Measurements for bioluminescent assays were performed in 50 mM Tris buffer, pH 7.6 with 10 mM MgCl₂ at 37°C. Luciferase was purchased from Promega (E1702, America). ATP was purchased from Aladdin. Luminescence produced by the luciferase was measured with Omega microplate reader (POLARstar Omega, Germany). The log-(inhibitor) vs normalized (control was set as 100, variable slope) response data were analyzed with the GraphPad Prism software package. In vivo bioluminescence imaging was determined with an IVIS Kinetic (Caliper Life Sciences, USA) equipped with a cooled charge coupled device (CCD) camera. The pseudo colored bioluminescent images (in photons/sec/cm²/scr) were superimposed over the gray scale photographs of the animals. Circular ROIs were drawn over the areas and quantified using Living Image software. The results were reported as total photon flux within an ROI in photons per second.

Mice model

Balb/c-nu male mice, 8 weeks of age, were purchased from Animal Center of China Academy of Medical Sciences (Beijing, China). To generate tumor xenografts in mice, ES-2-luc cells (1×10^7) were implanted subcutaneously under the right armpit region of each 4-weeks-old female nude mouse. Mice were single or group-housed on a 12:12 light–dark cycle at 22 °C with free access to food and water. Tumors were allowed to grow for two weeks before imaging. All animal studies were approved by the Ethics Committee of Qilu Health Science Center, Shandong University and conducted in compliance with European guidelines for the care and use of laboratory animals.

2. Synthesis

General Procedure for the preparation of aryl chlorides 2a-c. Procedure a (scheme 2). Take 4-chlorobenzoyl chloride 2a as an example.

To a vigorously stirred solution of 4-chlorobenzoic acid (3.03 g, 24.6 mmol) in toluene 25 ml was added dropwise sulfoxide chloride 10.5 ml, keeping the temperature at 90°C in oil bath, the mixture continue to react for 4.5 h. After cooled down to room

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temperature, the mixture was concentrated under reduced pressure at 50-60°C, yielding colorless oil, used directly for the next reaction. In the same way we got nicotinoyl chloride 2b as white crystals and 2-furoyl chloride 2c as colorless oil.

General procedure for the preparation of 8a-c. Procedure b (scheme 2). Take 4-chloro-N-(prop-2-yn-1-yl)benzamide 3a as an example.

In a two-neck bottle with 20 ml CH₂Cl₂ under ice-salt bath, 4-chlorobenzoyl chloride (1.61 g, 9.04 mmol) and Et₃N 2 ml was added. 2-Propynylamine (0.4 g, 7.26 mmol) diluted in 10 ml CH₂Cl₂ was added dropwise, keeping the temperature under 0°C. The mixture was taken to room temperature and kept stirred for another 5h. 50 ml water was added to the solution and the organic layer was separated. The aqueous layer was washed with ethyl acetate (50 mL×3). The organic layer was combined, dried over anhydrous MgSO₄ and filtered. The filtrate was evaporated in vacuum to obtain crude solid. After recrystallized in methanol, we got pale yellow solid. Yield: 60.0 %. ¹H-NMR (600 MHz, DMSO-d₆): δ 9.03 (s, 1H), 7.86 (d, J = 7.8 Hz, 2H), 7.54 (d, J = 7.2 Hz, 2H), 4.05 (d, J = 2.4 Hz, 2H), 3.14 (s, 1H), 2.32 (s, 3H). In the same way we got:

N-(prop-2-yn-1-yl)nicotinamide 3b: pale yellow solid; Yield: 60.0 %; ¹H-NMR (600 MHz, DMSO-d₆): δ 8.80 (s, 1H), 7.84 (1, 1H), 7.13 (d, J = 3.6 Hz, 1H), 6.62 (d, J = 1.2 Hz, 1H), 3.98 (m, 2H), 3.11 (s, 1H)

N-(prop-2-yn-1-yl)furan-2-carboxamide 3c: pale yellow solid; Yield: 60.2 %;

General procedure for the preparation of 5a-d. Procedure c (scheme 2). Take 2,4-dichlorophenyl azide 5a as an example.

In a 250 ml three-necked flask with 25 ml water and 25 ml hydrochloric acid, 2,4-dichloroaniline (1 eq, 10 mmol) was added followed by decreasing the temperature to -3°C. Sodium nitrite (1.5 eq, 15 mmol) dissolved in 10ml water was then added dropwise, with the solution turning dark brown. The mixture was kept stirred for another 10 minutes under 0°C. NaN₃ (1.5 eq, 15 mmol) in 15 ml water was then added dropwise, and then the mixture was stirred at room temperature for another 3h. The solution was extracted with ethyl acetate (3×30 mL), the organic is washed with brine (50 mL×3), dried over anhydrous MgSO₄ and filtered. The filtrate was evaporated in vacuum at 30°C to afford pale yellow solid 2,4-dichlorophenyl azide. Yield: 78.1%. The crude product directly went for the next procedure without further purification. In the same way we got:

2-methoxyphenyl azide (5b): brown oil; Yield: 70.7%;

2-methoxyphenyl azide (5c): brown oil; Yield: 70.0%;

2-methoxyphenyl azide (5d): brown oil; Yield: 69.7%;

General procedure for the preparation of 7a-b. Procedure d (scheme 2). Take N-benzylpropargylamine hydrochloride 7a as an example.

Benzyl bromide (0.58 mL, 4.88 mmol) was added dropwise to a suspension of K₂CO₃ (1.0 g, 7.24 mmol), 2-Propynylamine (0.40 mL, 6.25 mmol) and acetone (15 mL), over a period of 45 mins. The resulting suspension was stirred at room temperature for 24 hours. K₂CO₃ was filtered off and the organic layer was washed with dichloromethane. Filtrate was concentrated in vacuo to obtain clear oil. Purification by column chromatography on silica gel (PE/EtOAc 10:1 to 2:1) afforded pure N-benzylpropargylamine as a clear oil. The colourless oil was diluted with EtOAc (5 mL) and slowly added HCl saturated Ethanol solution (5 mL) under 0 °C. Then, the white solid was precipitated out and filtered washed with ethyl acetate (10 mL). The product was dried under vacuum to afford pure product as a white powder. Yield: 17.1 %; ¹H-NMR (600 MHz, DMSO-d₆) δ 9.81

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(s, 2H), 7.52~7.54 (m, 2H), 7.41~7.45 (m, 3H), 4.16 (s, 2H), 3.85 (d, J = 3.0 Hz, 2H), 4.23 (s, 2H), 3.74~3.76 (m, 1H). In the same way we got:

N-propargyl-2-Fluorobenzylamine hydrochloride (7b): white solid, yield 28.8 %

N-propargyl-4-Methylbenzylamine hydrochloride (4c): white solid, yield 22.4 %

General procedure for the preparation of(N-(1-substitutedphenyl-1H-1,2,3-triazol-4-yl)methyl)arylamide 8a-l. Procedure e (scheme 2).

To a mixture of N-propargyl arylamide (1.03 mmol) and aromatic azide (3.62 mmol) in water and t-BuOH (v/v = 1:1, 8 mL), sodium ascorbate (0.42 mmol) was added, followed by the addition of 0.5 M CuSO₄ (200 μ L, 0.1 mmol). The heterogeneous mixture was stirred about 18 hours in the dark at room temperature. The reaction was worked up by dilution with saturated NaHCO₃ (aq) (30 mL) and EtOAc (3 \times 40 mL). The organic layer was washed with brine and dried over MgSO₄. Evaporation of solvent afforded the crude product, which was purified by recrystallization from ethanol.

(N-[2,4-dichloro-phenyl]-1H-[1,2,3]triazol-4-ylmethyl)-4-chlorobenzylamide (8a): colorless solid; yield 33.5 %; mp: 177~180 $^{\circ}$ C; ¹H-NMR (600 MHz, DMSO-d₆) δ 9.21 (t, J = 5.6 Hz, 1H), 8.44 (s, 1H), 7.99 (d, J = 2.1 Hz, 1H), 7.91 (d, J = 8.5 Hz, 2H), 7.70 (d, J = 8.5 Hz, 1H), 7.67 (dd, J = 8.5, 2.2 Hz, 1H), 7.55 (d, J = 8.5 Hz, 2H), 4.62 (d, J = 5.7 Hz, 2H). HR-MS (ESI) found 381.0072, C₁₆H₁₂O₁N₄Cl₃.

(1-[2-methoxy-phenyl]-1H-[1,2,3]triazol-4-ylmethyl)-4-chlorobenzylamide (8b): colorless solid; yield 11.4 %; mp: 119~122 $^{\circ}$ C; ¹H-NMR (600 MHz, DMSO-d₆) δ 9.17 (t, J = 5.6 Hz, 1H), 8.73 (s, 1H), 7.92 (d, J = 8.6 Hz, 2H), 7.56 (d, J = 8.6 Hz, 2H), 7.50 – 7.45 (m, 3H), 7.06 – 7.02 (m, 1H), 4.60 (d, J = 5.6 Hz, 2H), 3.85 (s, 3H). HR-MS (ESI) found 343.0956, C₁₇H₁₆O₂N₄Cl₁.

(1-[3-methoxy-phenyl]-1H-[1,2,3]triazol-4-ylmethyl)-4-chlorobenzylamide (8c): yellow solid; yield 30.2 %; mp: 141~146 $^{\circ}$ C; ¹H-NMR (600 MHz, DMSO-d₆) δ 9.17 (s, 1H), 8.32 (s, 1H), 7.91 (d, J = 9.0 Hz, 2H), 7.60 (d, J = 7.8 Hz, 2H), 7.51~7.56 (m, 3H), 7.32 (d, J = 7.8 Hz, 1H), 7.13 (t, J = 7.8 Hz, 1H), 4.61 (d, J = 5.4 Hz, 2H), 3.85 (s, 3H); HR-MS (ESI) found 343.0956, C₁₇H₁₆O₂N₄Cl₁.

(1-[4-methoxy-phenyl]-1H-[1,2,3]triazol-4-ylmethyl)-4-chlorobenzylamide (8d): yellow solid; yield 57.1 %; mp: 119~122 $^{\circ}$ C; ¹H-NMR (600 MHz, DMSO-d₆) δ 9.17 (t, J = 5.4 Hz, 1H), 8.52 (s, 1H), 7.92 (d, J = 8.4 Hz, 2H), 7.80 (d, J = 9.0 Hz, 2H), 7.55 (d, J = 8.4 Hz, 2H), 7.11 (d, J = 9.0 Hz, 2H), 4.59 (d, J = 5.4 Hz, 2H), 3.82 (s, 3H); HR-MS (ESI) found 343.0957, C₁₇H₁₆O₂N₄Cl₁.

(1-[2,4-dichloro-phenyl]-1H-[1,2,3]triazol-4-ylmethyl)-furan-2-carboxamide (8e): light yellow solid; yield 45.2 %; mp: 116~120 $^{\circ}$ C; ¹H-NMR (600 MHz, DMSO-d₆) δ 8.97 (t, J = 5.4 Hz, 1H), 8.40 (s, 1H), 7.99 (d, J = 1.8 Hz, 1H), 7.84 (d, J = 1.2 Hz, 1H), 7.70 (d, J = 9.0 Hz, 1H), 7.66 (dd, J = 8.4 Hz, J = 1.8 Hz, 1H), 7.14 (d, J = 3.6 Hz, 1H), 7.62 (dd, J = 3.6 Hz, J = 1.8 Hz, 1H), 4.57 (d, J = 5.4 Hz, 2H). ESI-MS m/z: 337.0264 [M+H]⁺, 359.0077 [M+Na]⁺.

(1-[2-methoxy-phenyl]-1H-[1,2,3]triazol-4-ylmethyl)-furan-2-carboxamide (8f): brown solid; yield 75.2 %; mp: 115~118 $^{\circ}$ C; ¹H-NMR (600 MHz, DMSO-d₆) δ 8.92 (t, J = 5.4 Hz, 1H), 8.26 (s, 1H), 7.84 (s, 1H), 7.59 (d, J = 4.2 Hz, 1H), 7.52 (t, J = 8.4 Hz, 1H), 7.31 (d, J = 8.4 Hz, 1H), 7.11~7.16 (m, 2H), 6.62 (t, J = 1.5 Hz, 1H), 4.56 (d, J = 5.4 Hz, 2H), 3.85 (s, 3H). ESI-MS m/z: 299.1152 [M+H]⁺, 321.0972 [M+Na]⁺.

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(1-[3-methoxy-phenyl]-1H-[1,2,3]triazol-4-ylmethyl)-furan-2-carboxamide (8g): brown solid; yield 37.5 %; mp: 119~122 °C; ¹H-NMR (600 MHz, DMSO-d₆) δ 8.94 (t, J = 5.4 Hz, 1H), 8.69 (s, 1H), 7.84 (d, J = 1.2 Hz, 1H), 7.46~7.49 (m, 3H), 7.15 (d, J = 3.0 Hz, 1H), 7.02~7.05 (m, 1H), 6.63 (dd, J = 1.8 Hz, J = 3.0 Hz, 1H), 4.56 (d, J = 5.4 Hz, 2H), 3.85 (s, 3H); ESI-MS m/z: 299.1169 [M+H]⁺, 321.0987 [M+Na]⁺.

(1-[4-methoxy-phenyl]-1H-[1,2,3]triazol-4-ylmethyl)-furan-2-carboxamide (8h): brown solid; yield 46.5 %; mp: 125~129 °C; ¹H-NMR (600 MHz, DMSO-d₆) δ 8.93 (t, J = 5.4 Hz, 1H), 8.55 (s, 1H), 7.79~7.85 (m, 3H), 7.10~7.16 (m, 3H), 6.63 (dd, J = 3.6 Hz, J = 1.8 Hz, 1H), 4.54 (d, J = 5.4 Hz, 2H), 3.82 (s, 3H); ESI-MS m/z: 299.1132 [M+H]⁺, 321.0950 [M+Na]⁺.

(1-[2,4-dichloro-phenyl]-1H-[1,2,3]triazol-4-ylmethyl)-nicotinamide (8i): yellow solid; yield 36.0 %; mp: 178~180 °C; ¹H-NMR (600 MHz, DMSO-d₆) δ 9.32 (t, J = 5.4 Hz, 1H), 9.15 (s, 1H), 8.78 (s, 1H), 8.48 (s, 1H), 8.23 (d, J = 7.8 Hz, 1H), 7.99 (d, J = 1.8 Hz, 1H), 7.71 (d, J = 8.4 Hz, 1H), 7.67 (dd, J = 8.4 Hz, J = 1.8 Hz, 1H), 7.54 (s, 1H), 4.66 (d, J = 5.4 Hz, 2H); ESI-MS m/z: 348.0413 [M+H]⁺, 370.0221 [M+Na]⁺.

(1-[2-methoxy-phenyl]-1H-[1,2,3]triazol-4-ylmethyl)-nicotinamide hydrochloride (8j): brown solid; yield 64.3 %; mp: 204~207 °C; ¹H-NMR (600 MHz, DMSO-d₆) δ 9.73 (t, J = 5.4 Hz, 1H), 9.31 (s, 1H), 8.98 (s, 1H), 8.77 (d, J = 8.4 Hz, 1H), 8.40 (s, 1H), 7.98 (t, J = 6.0 Hz, 1H), 7.60 (dd, J = 8.4 Hz, J = 1.8 Hz, 1H), 7.53 (dt, J = 8.4 Hz, J = 1.8 Hz, 1H), 7.32 (d, J = 8.4 Hz, 1H), 7.14 (t, J = 1.8 Hz, 1H), 4.67 (d, J = 5.4 Hz, 2H), 3.85 (s, 3H). ESI-MS m/z: 310.1288 [M-HCl+H]⁺, 332.1097 [M-HCl+Na]⁺.

(1-[3-methoxy-phenyl]-1H-[1,2,3]triazol-4-ylmethyl)-nicotinamide hydrochloride (8k): brown solid; yield 50.2 %; mp: 206~210 °C; ¹H-NMR (600 MHz, DMSO-d₆) δ 9.73 (t, J = 6.0 Hz, 1H), 9.06 (s, 1H), 8.72 (d, J = 4.2 Hz, 1H), 8.62 (s, 1H), 8.24 (td, J = 8.4 Hz, J = 1.8 Hz, 1H), 7.81 (dd, J = 3.0 Hz, J = 5.4 Hz, 2H), 7.52 (dd, J = 7.8 Hz, J = 4.8 Hz, 1H), 7.13 (dd, J = 5.4 Hz, J = 3.0 Hz, 2H), 4.63 (d, J = 6.0 Hz, 2H), 3.82 (s, 3H); ESI-MS m/z: 310.1303 [M-HCl+H]⁺, 332.1121 [M-HCl+Na]⁺.

(1-[4-methoxy-phenyl]-1H-[1,2,3]triazol-4-ylmethyl)-nicotinamide (8l): brown solid; yield 21.9 %; mp: 180~182 °C; ¹H-NMR (600 MHz, DMSO-d₆) δ 9.73 (t, J = 6.0 Hz, 1H), 9.06 (s, 1H), 8.72 (d, J = 4.2 Hz, 1H), 8.62 (s, 1H), 8.24 (td, J = 8.4 Hz, J = 1.8 Hz, 1H), 7.81 (dd, J = 3.0 Hz, J = 5.4 Hz, 2H), 7.52 (dd, J = 7.8 Hz, J = 4.8 Hz, 1H), 7.13 (dd, J = 5.4 Hz, J = 3.0 Hz, 2H), 4.63 (d, J = 6.0 Hz, 2H), 3.82 (s, 3H); ESI-MS m/z: 310.1300 [M+H]⁺, 332.1107 [M+Na]⁺.

General Procedure for the Preparation of N-substituted-1-(1-phenyl-1H-1,2,3-triazol-4-yl)methanamine hydrochloride 9a-d. Procedure e (scheme 2).

To a mixture of N-Propargylbenzylamine hydrochloride (1.1 mmol) and aromatic azides (3.42 mmol) in water and t-BuOH (v/v = 1:1, 8 mL), sodium ascorbate (0.51 mmol) was added, followed by the addition of 0.5 M CuSO₄ (230 μL, 0.115 mmol). The heterogeneous mixture was stirred about 18 hours in the dark at room temperature. The reaction was worked up by dilution with saturated NaHCO₃ (aq) (30 mL) and EtOAc (3×40 mL). The organic layer was washed with brine and dried over MgSO₄. After most of the solvent was removed, a solution of HCl in ethanol was added dropwise to the EtOAc solution to generate the HCl salt of the product. After cooling in an ice bath, the colorless needles were collected by filtration and washed with EtOAc (2 x 10 ml). The product was dried under vacuum to afford pure product as a white powder.

(1-[2,4-dichloro-phenyl]-1H-[1,2,3]triazol-4-ylmethyl)-benzylamine hydrochloride (9a): white solid; yield 30.0 %; mp: 214~217 °C; ¹H-NMR (600 MHz, DMSO-d₆) δ 10.00 (s, 2H), 8.76 (d, J = 1.8 Hz, 1H), 8.04 (d, J = 7.8 Hz, 1H), 7.71~7.76 (m, 2H), 7.59 (d, J = 7.2 Hz, 2H), 7.42~7.47 (m, 3H), 4.36 (s, 2H), 4.23 (s, 2H). ESI-MS, m/z: 333.0653 [M+H-HCl]⁺..

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(1-[3-methoxy-phenyl]-1H-[1,2,3]triazol-4-ylmethyl)-benzylamine hydrochloride (9b): white solid; yield 50.0 %; mp: 238~240 °C; ¹H-NMR (600 MHz, DMSO-d₆) δ 10.08 (s, 2H), 9.01 (s, 1H), 7.62 (dd, J = 7.8 Hz, J = 1.8 Hz, 2H), 7.54 (t, J = 8.4 Hz, 1H), 7.40~7.47 (m, 5H), 7.11~7.12 (m, 1H), 4.32 (s, 2H), 4.25 (s, 2H), 3.87 (s, 3H). ESI-MS, m/z: 295.1563 [M-HCl+H]⁺.

(1-[2,4-dichloro-phenyl]-1H-[1,2,3]triazol-4-ylmethyl)-2-fluorobenzylamine hydrochloride (9c): white solid; yield 41.5 %; mp: 210~211 °C; ¹H-NMR (600 MHz, DMSO-d₆) δ 10.00 (s, 2H), 8.76 (d, J = 3.0 Hz, 1H), 8.04 (s, 1H), 7.70~7.75 (m, 3H), 7.47~7.52 (m, 1H), 7.28~7.32 (m, 2H), 4.44 (s, 2H), 4.29 (s, 2H). ESI-MS, m/z: 351.0561 [M-HCl+H]⁺, 373.0376 [M-HCl+Na]⁺, 389.0706 [M-HCl+K]⁺.

(1-[3-methoxy-phenyl]-1H-[1,2,3]triazol-4-ylmethyl)-2-fluorobenzylamine hydrochloride (9d): white solid; yield 59.2 %; mp: 220~224 °C; ¹H-NMR (600 MHz, DMSO-d₆) δ 10.15 (s, 2H), 9.02 (s, 1H), 7.76 (dt, J = 7.8 Hz, J = 1.8 Hz, 1H), 7.56 (t, J = 7.2 Hz, 1H), 7.45~7.54 (m, 3H), 7.27~7.32 (m, 2H), 7.10~7.12 (m, 1H), 4.39 (s, 2H), 4.30 (s, 2H), 3.87 (s, 3H). ESI-MS, m/z: 313.1464 [M-HCl+H]⁺.

General Procedure for the Preparation of 5-(2,4-dichlorophenyl)-furan-2-methanamine hydrochloride 10. Procedure f and g (scheme 2).

2,4-dichloroaniline (10.0 mmol) was dissolved in dilute hydrochloric acid (4 M, 30 mL) and heated to get a clear solution. The solution was cooled to -3 °C in an ice-water bath and then a solution of NaNO₂ (1.0 eq, 10 mmol) in 10 mL of water was added dropwise to the reaction mixture while keeping the internal temperature between 0-3 °C. The cold clear solution of the diazonium salt was collected by filtration. After stirring for 10 min at 0-5 °C, a mixture of 2-furfurylamine (1.3 eq, 13 mmol), cupric chloride (0.28 g) and 3 mL of water was added dropwise with stirring. The reaction mixture was stirred over night to form a colorless precipitation at room temperature. Precipitation of crude product were collected by filtration, and then washed with water and ethyl acetate (2 x 10 ml) and recrystallized from a mixture of ethanol and methanol. The product was dried under vacuum to afford pure product as a pale powder. Yield 40.8 %

General procedure for the preparation of N-[[5-(2,4-chlorophenyl)-2-furanyl]methyl]-4-chloro-benzamide 11. Procedure h (scheme 2).

A solution of amine (10) (0.72 mmol) in THF (10 mL) was added to a solution of 4-chlorobenzoyl chloride (1.01 mmol) and triethylamine (0.7 mL, 5.0 mmol) in THF (10 mL) after cooling to 0 °C. The resulting solution was stirred at room temperature for 4 h. The ice-water mixture was added dropwise into resulting mixture with vigor stirring. The precipitate was collected and crystallized from methanol.

N-[[5-(2,4-chlorophenyl)-2-furanyl]methyl]-4-chloro-benzamide (11): white solid; yield 72.9 %; mp: 143-147°C. ¹H-NMR (600 MHz, DMSO-d₆) δ 10.0 (s, 2H), 7.90~7.95 (m, 2H), 7.81 (d, J = 8.4 Hz, 1H), 7.71 (d, J = 2.4 Hz, 1H), 7.52~7.59 (m, 3H), 7.11 (d, J = 3.6 Hz, 1H), 6.49 (d, J = 3.6 Hz, 1H), 3.55 (d, J = 6.0 Hz, 2H). ESI-MS m/z: 379.9972 [M+H]⁺, 401.9797 [M+Na]⁺.

3. Luciferase Enzyme Inhibition Assays.

All compounds were dissolved in dimethylsulfoxide at 50 mM, then they were further diluted to an increasing concentrations ranging from 500 μ M to 100 nM in Tris buffer (PH=7.6) with 10 mM $MgCl_2$. The recombinant firefly luciferase were purchased from Promega(E1702, America). The luciferase was diluted to 20 μ g/mL in the Tris buffer prepared before. Substrates solution is also prepared in this Tris buffer, containing 20 μ M of luciferin and 2 mM of ATP. To a 96-well plates (WHB, black) each well containing 50 μ L of the luciferase solution, an amount of 50 μ L of increasing concentrations of compound was added as three replicates. After incubation at 25°C for 15 mins, an amount of 100 μ L of the substrates solution was added. Also blank group (containing 100 μ L Tris buffer instead of compounds and luciferase) and control group (vehicle, Containing equal amount of DMSO as experimental group) samples were measured. Luminescence produced by the luciferase was measured with Omega microplate reader (POLARstar Omega, Germany). The log-(inhibitor) vs normalized (control was set as 100, variable slope) response data were analyzed with the GraphPad Prism software package.

4. Cell Culture and ES-2-Luc cell bioluminescence inhibition Assays.

ES-2 cells (human ovarian cancers cell line) were purchased from the Committee on Type Culture Collection of Chinese Academy of Sciences. ES-2 cells expressing firefly luciferase (ES-2-Luc cells) were supplied by Cellcyto. The ES-2-Luc cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) at 37°C in a humidified atmosphere with 5% CO_2 incubator.

100 μ L cells were seeded into black 96-well plates (4×10^5 cells per well). After incubated for 12 hours, 100 μ L compounds of increasing concentration ranging from 500 μ M to 7.81 μ M dissolved by RPMI 1640 medium were added as three triplicates. After incubation for 12 hours, the medium was removed, and 100 μ L luciferin solution (100 μ M, dissolved in Tris buffer of PH 7.4) was added. Also blank group (added just Tris buffer instead of luciferin) and control group (vehicle, added RPMI 1640 medium containing equal amount of DMSO instead of inhibitors) were set. Immediately after the luciferin was added, the bioluminescence intensity was measured with IVIS Kinetic (Caliper Life Sciences, USA) instrument equipped with a cooled charge coupled device (CCD) camera for bioluminescent imaging. The pseudo colored bioluminescent images (in photons/sec/cm²/scr) were superimposed over the gray scale photographs of the animals. Circular ROIs were drawn over the areas and quantified using Living Image software. The results were reported as total photon flux within an ROI in photons per second. The log-(inhibitor) vs normalized (control was set as 100, variable slope) response data were analyzed with the GraphPad Prism software package.

5. Cell viability assay.

ES-2-Luc cells were seeded onto 96-well plates in 100 μ L of RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) (at density of 8000 cells/well). After incubated overnight at 37°C in a humidified atmosphere with 5% CO_2 incubator, 100 μ L of compounds diluted by RPMI 1640 medium to various concentration (1000, 500, 250 and 125 μ M) were added, for control group, 100 μ L RPMI 1640 medium was added, for blank group, 100 μ L RPMI 1640 medium containing equal amount of DMSO was added. After 8 h incubation, each well was treated with 1% of 0.5 mg/mL MTT reagent and incubated for additional 4 h. After that, the culture was removed and 100 μ L DMSO was added. Absorbance at 490 nm was measured using an Omega

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microplate reader (POLARstar Omega, Germany). The viability rate of compounds was calculated by $(OD - OD_{\text{blank}}) / (OD_{\text{control}} - OD_{\text{blank}}) \times 100\%$, where OD is the mean value of three triplicate wells.

6. In vivo imaging of bioluminescence inhibition

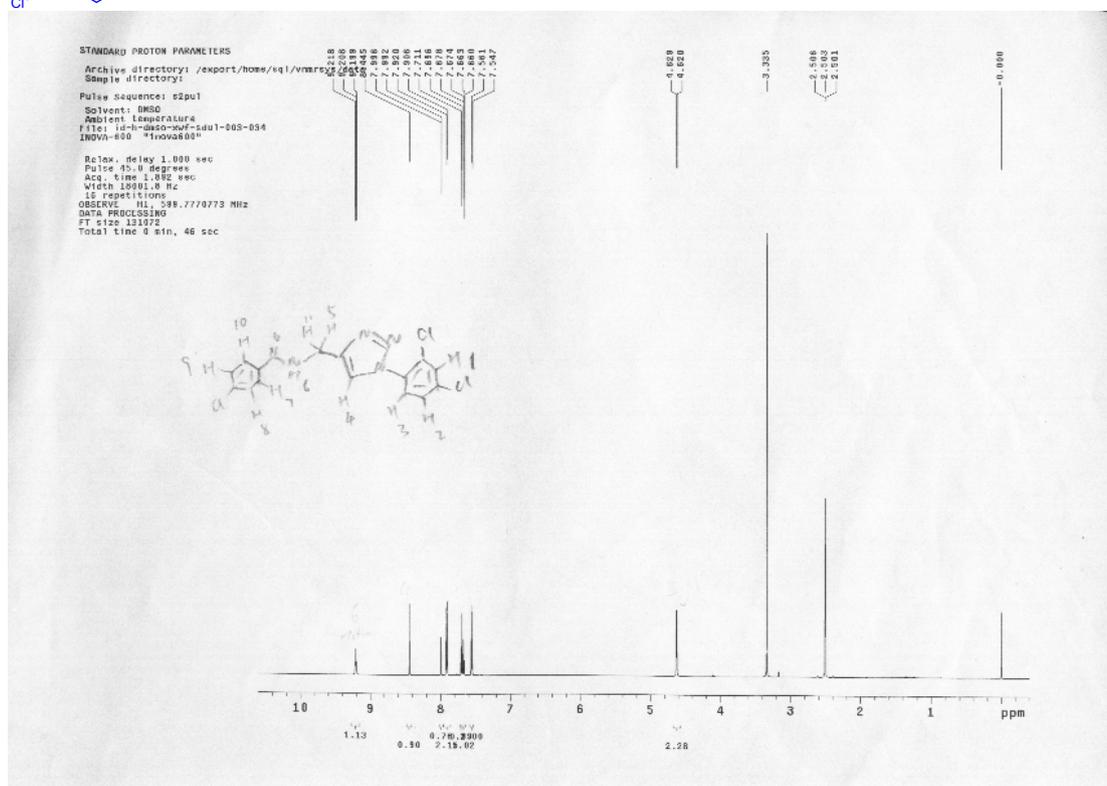
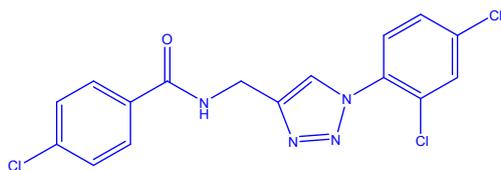
Day 1: 15 mice bearing ES-2-Luc subcutaneous tumors were divided into 3 groups (normal saline group, resveratrol group, 8c group) randomly, each group containing 5 mice. The mice were anesthetized with isoflurane and intraperitoneally injected with 100 μL luciferin (0.5 mM), followed immediately by bioluminescent imaging every 2 minutes for 30 to 40 minutes until the bioluminescence intensity went through a peak and get steady. Then give the mouse 12 hours to metabolize away the luciferin. After that, for resveratrol group and 8c group, each mouse was injected with 25 μL resveratrol or 8c (5 mM) intratumorally, for normal saline group, 25 μL normal saline containing equal amount of DMSO (15%) were injected intratumorally.

Day 2: The mice were intraperitoneally injected with 100 μL luciferin (0.5 mM, diluted by normal saline). Subsequently, bioluminescent imaging was taken every 2 minutes for 30 to 40 minutes. The relative total photon flux for each mouse was calculated by dividing the peak total photon flux of day 1 by peak total flux of day 2.

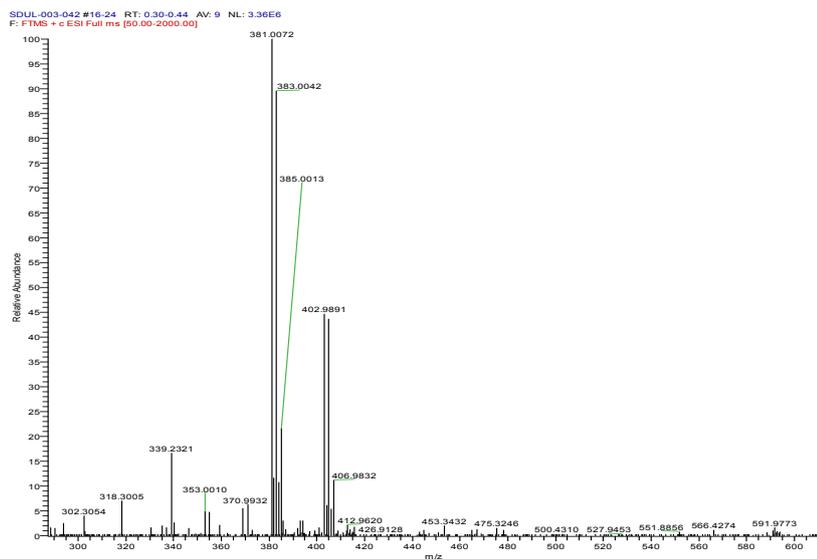
Supporting Information

7. ¹H-NMR and ESI-MS spectra of compounds

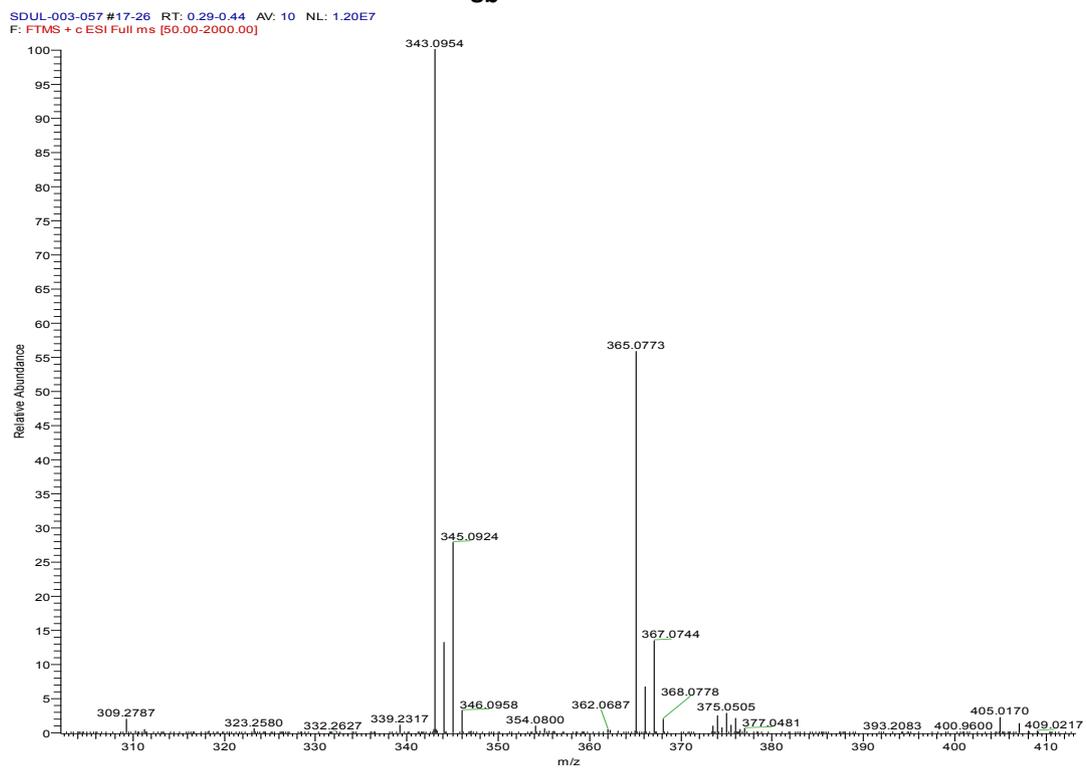
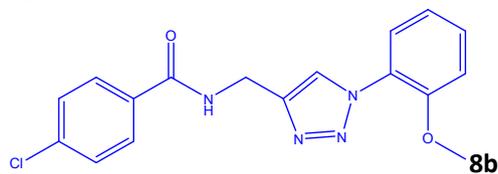
Compound 8a:



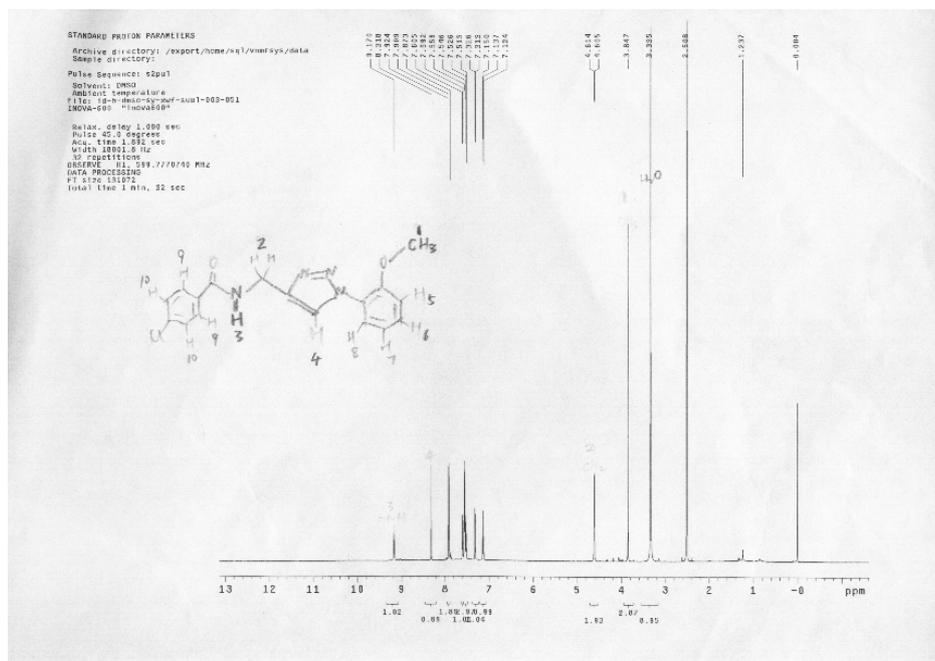
Supporting Information



8b

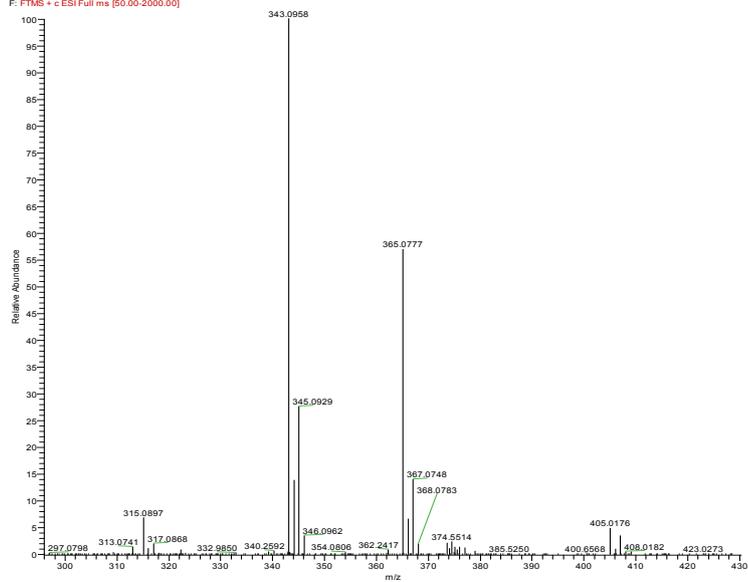


Supporting Information

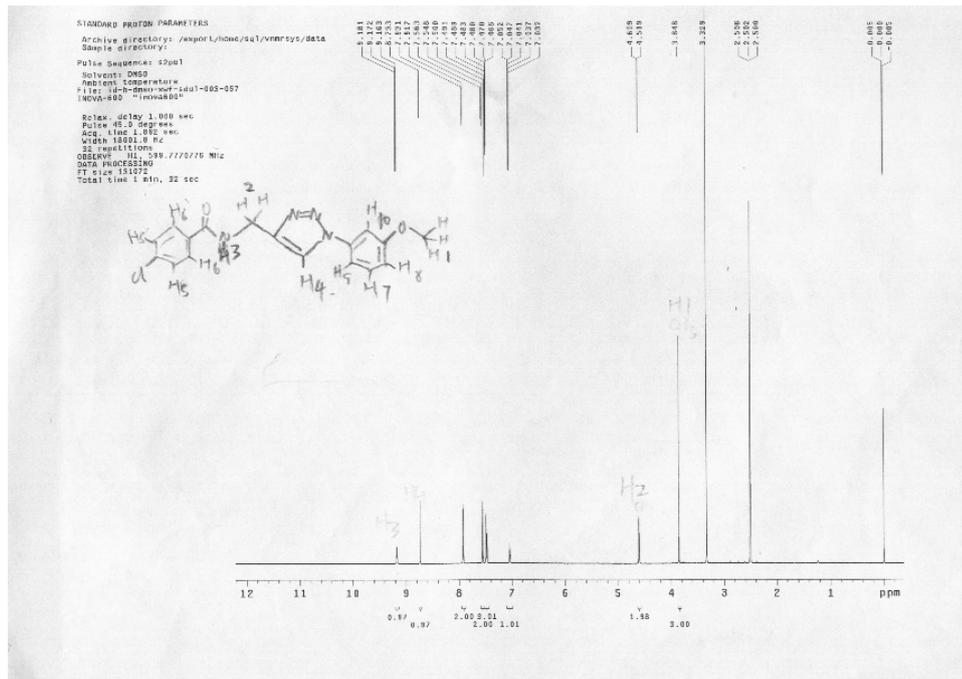
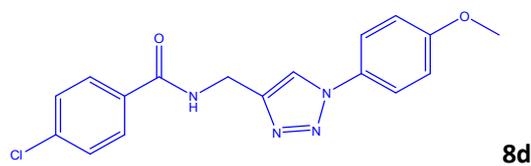
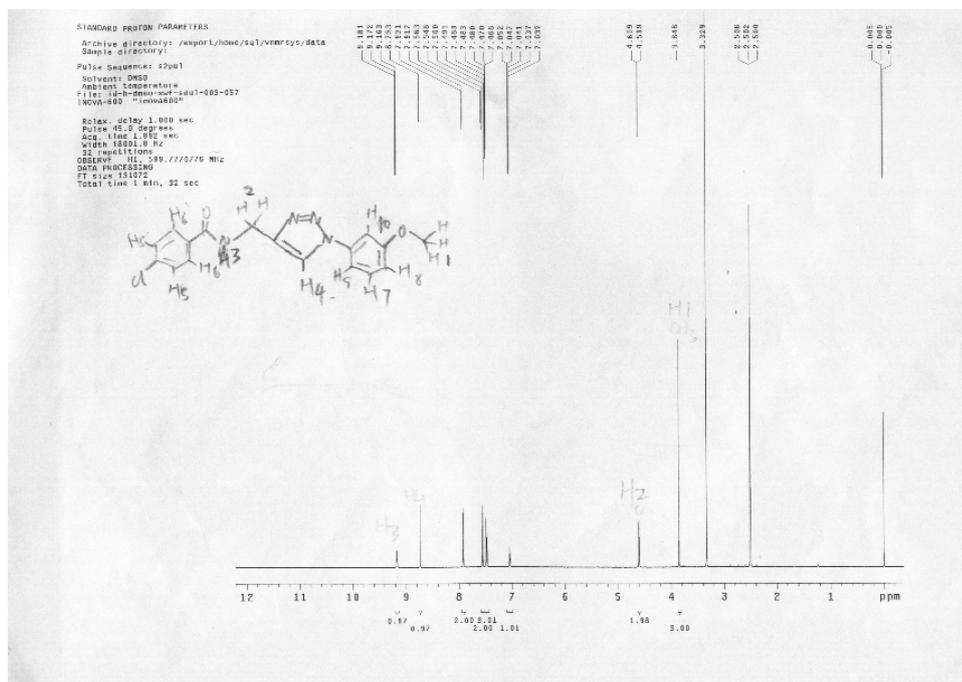


8c

SDUL-003-051 #25-34 RT: 0.44-0.58 AV: 10 NL: 1.16E7
F: FTMS + c ESI Full ms [50.00-2000.00]

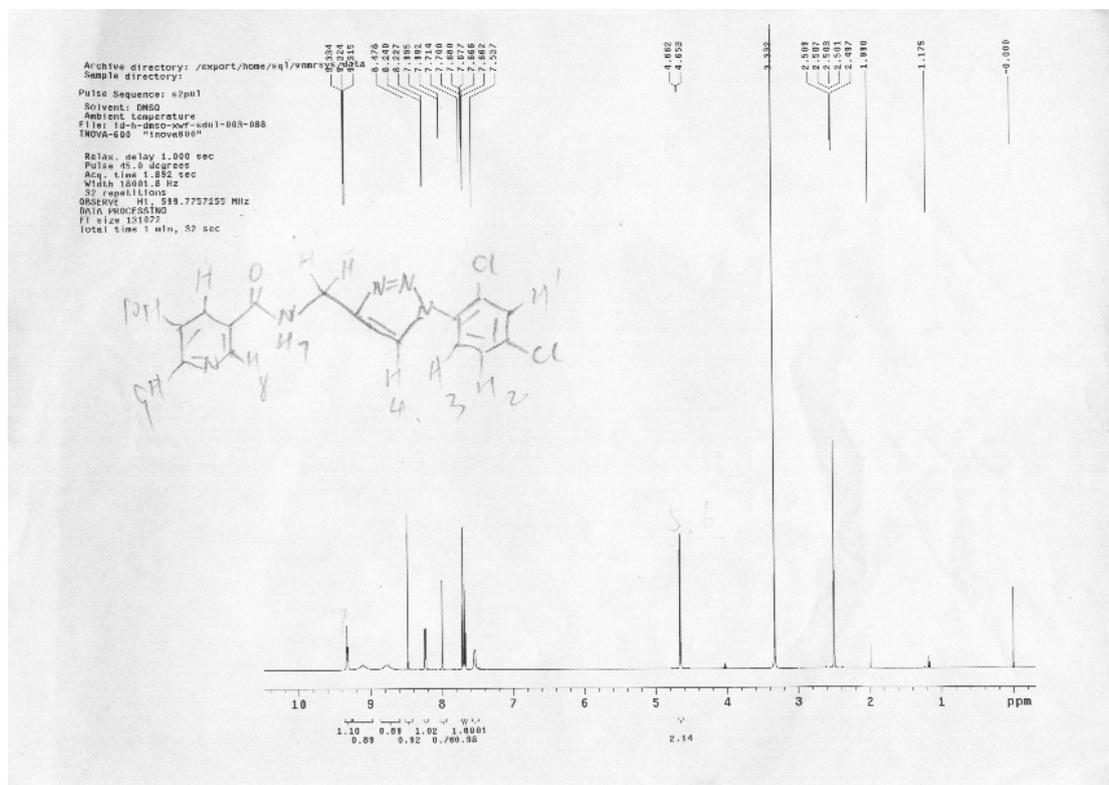
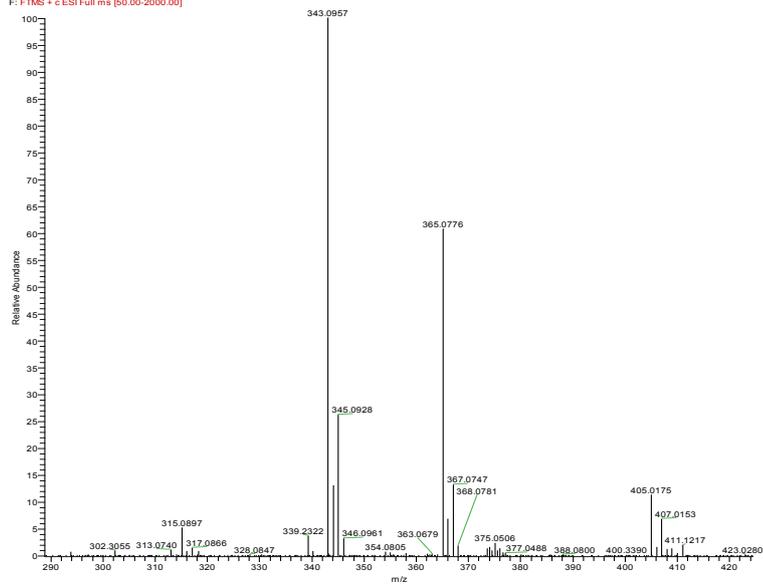


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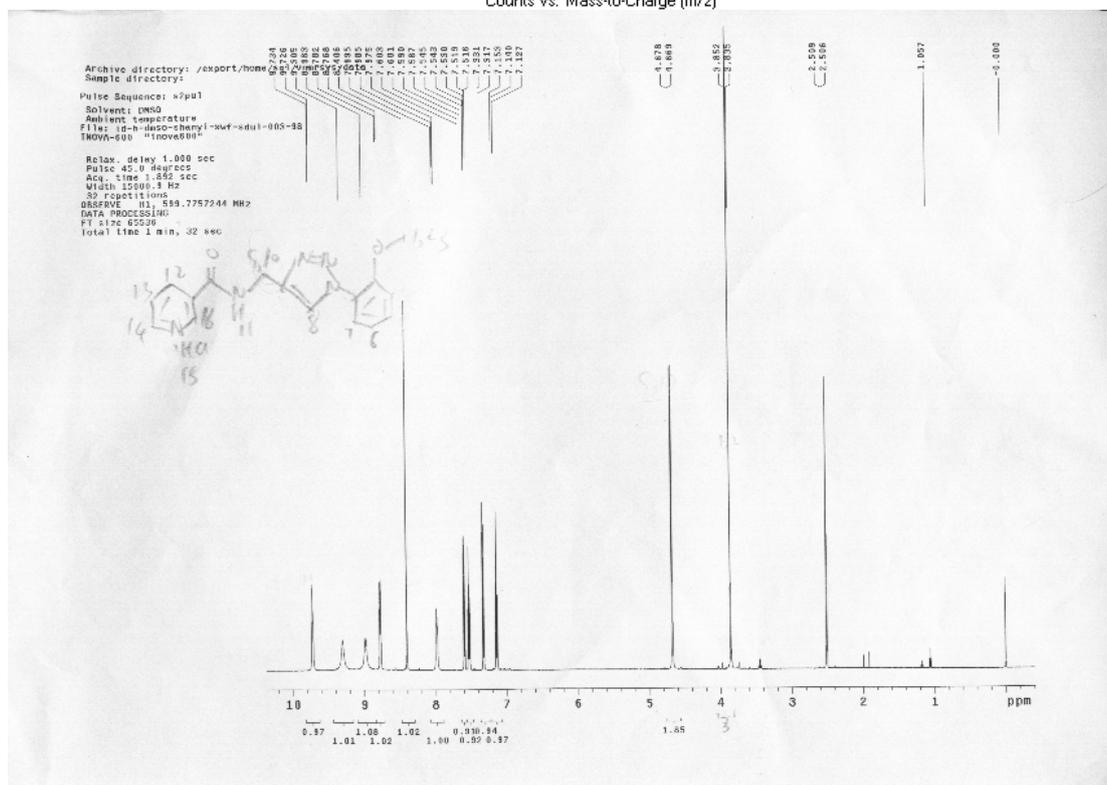
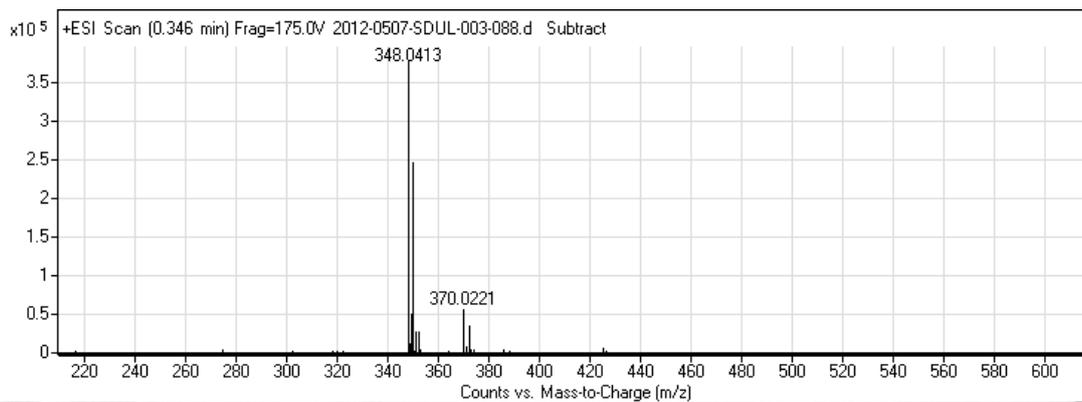
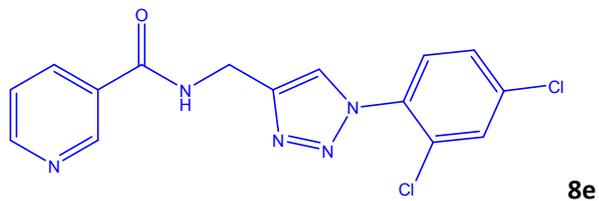


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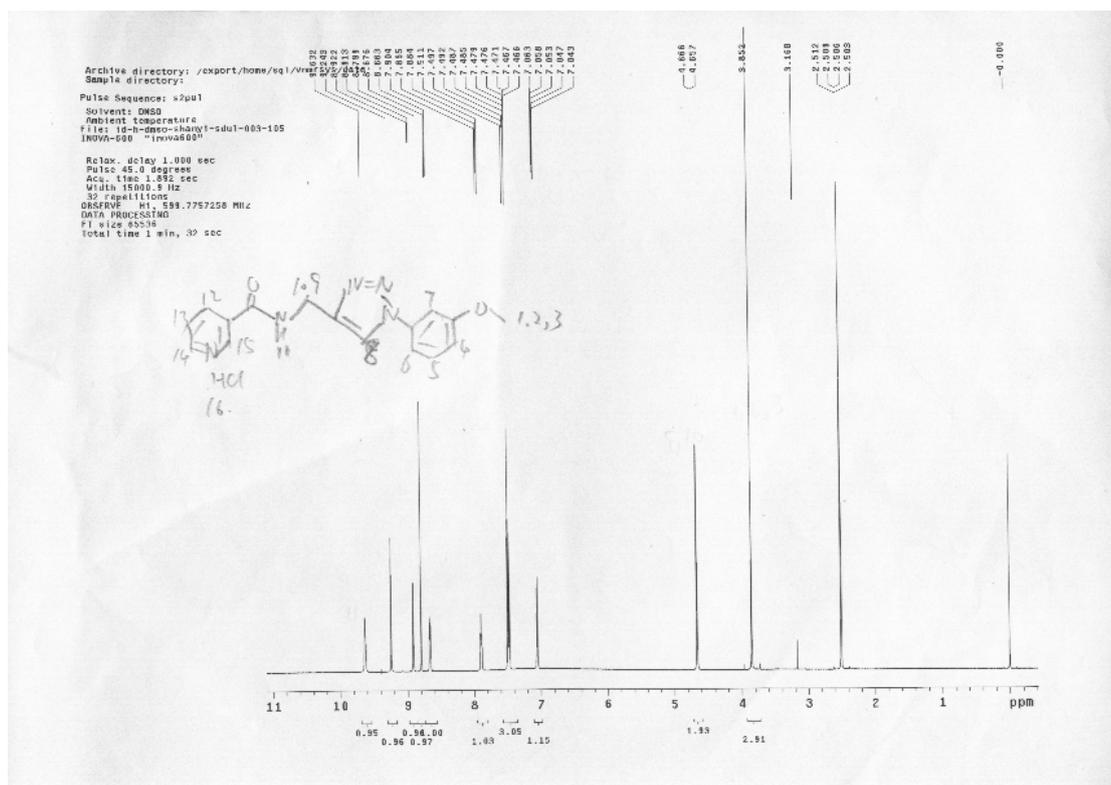
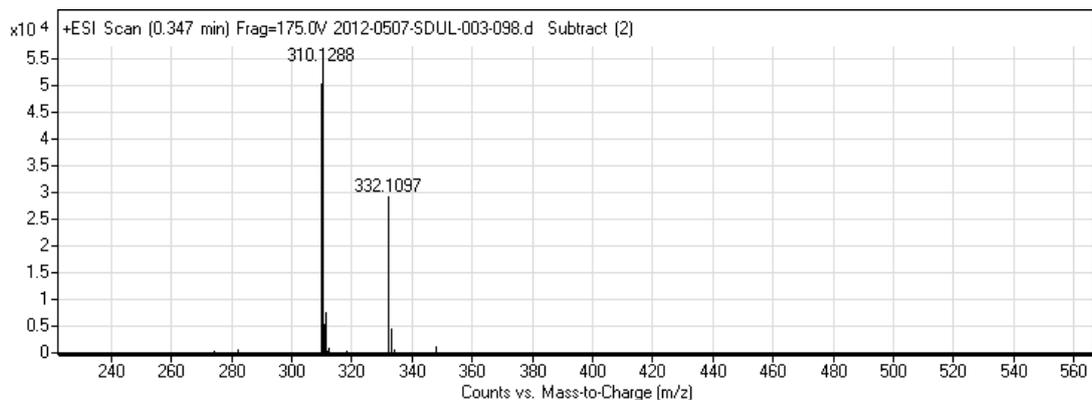
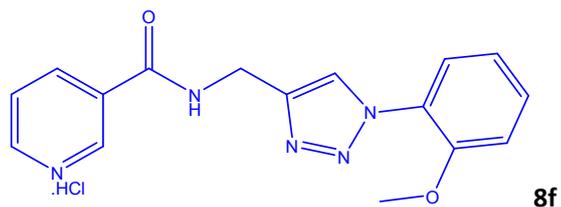
SDUL-003-048 #11-21 RT: 0.20-0.37 AV: 11 NL: 5.68E6
 F: FTMS + c ESI Full ms [50.00-2000.00]



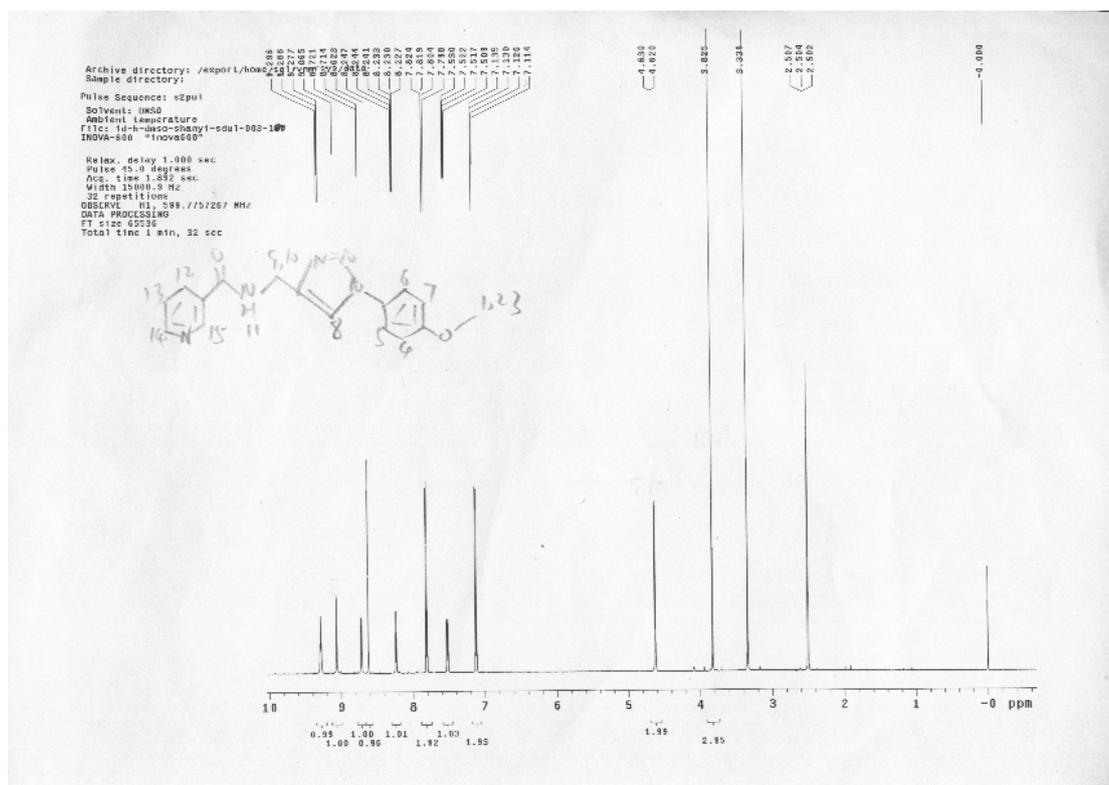
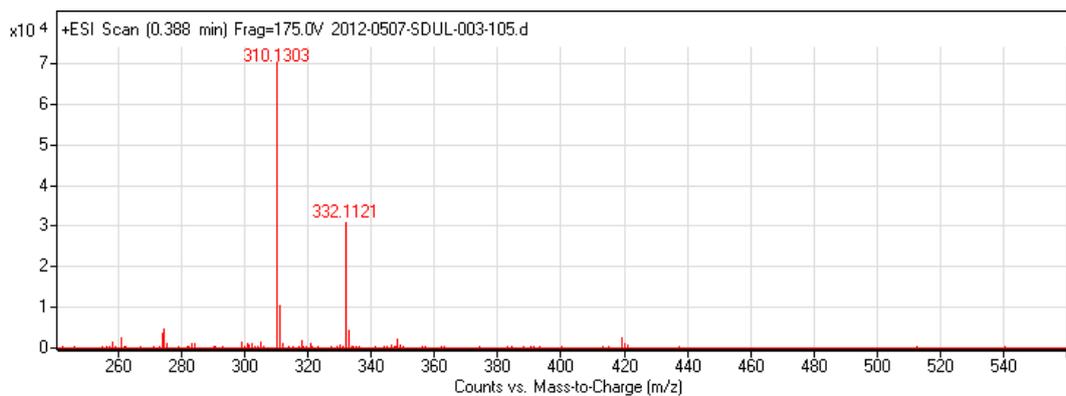
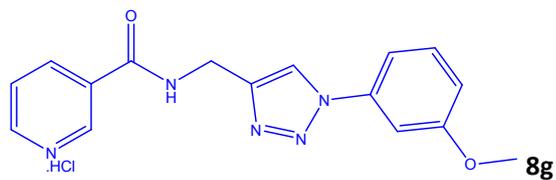
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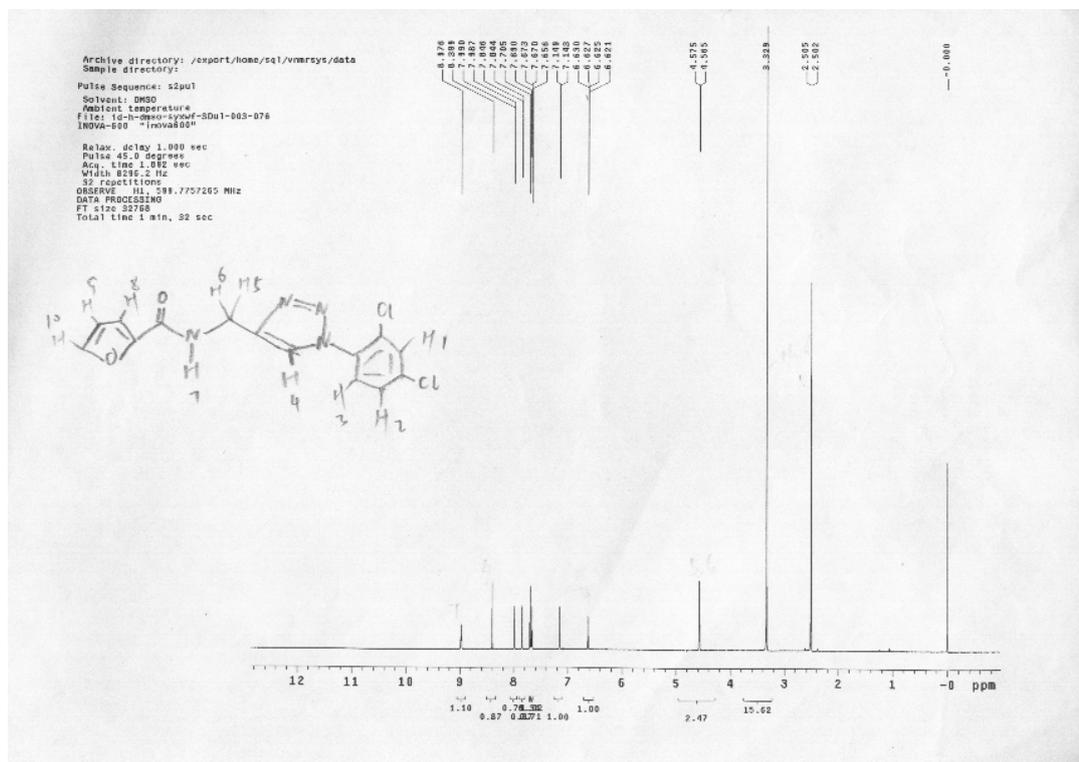
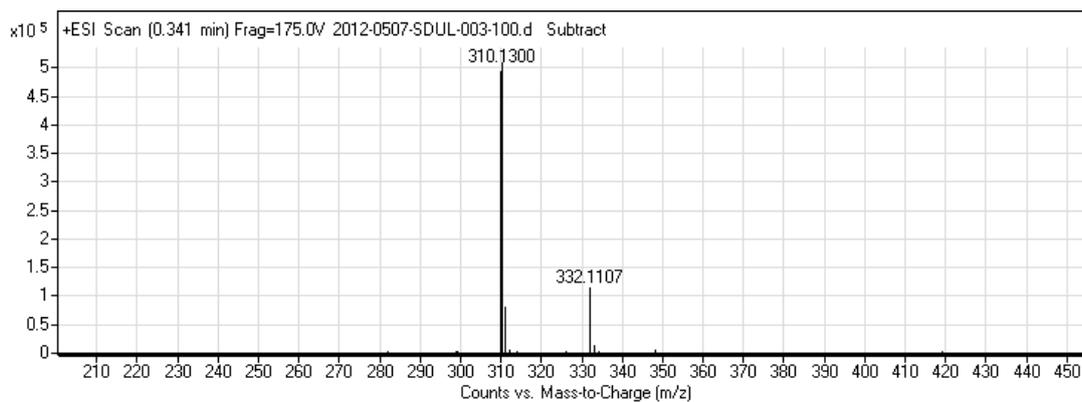
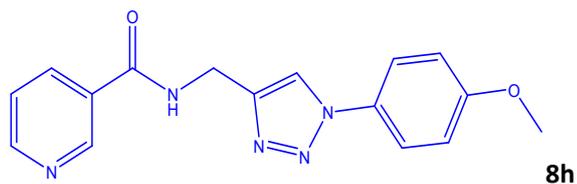
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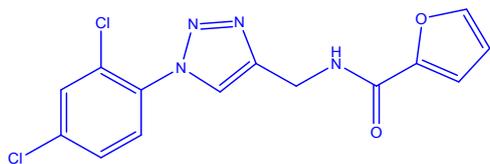
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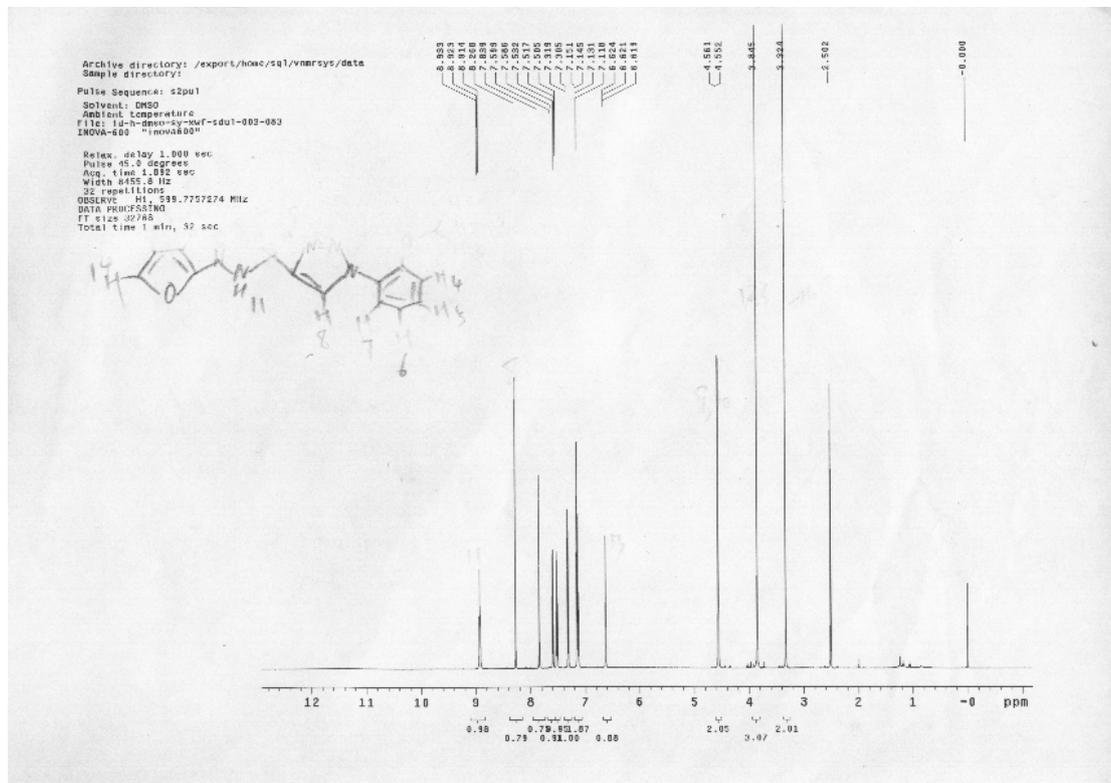
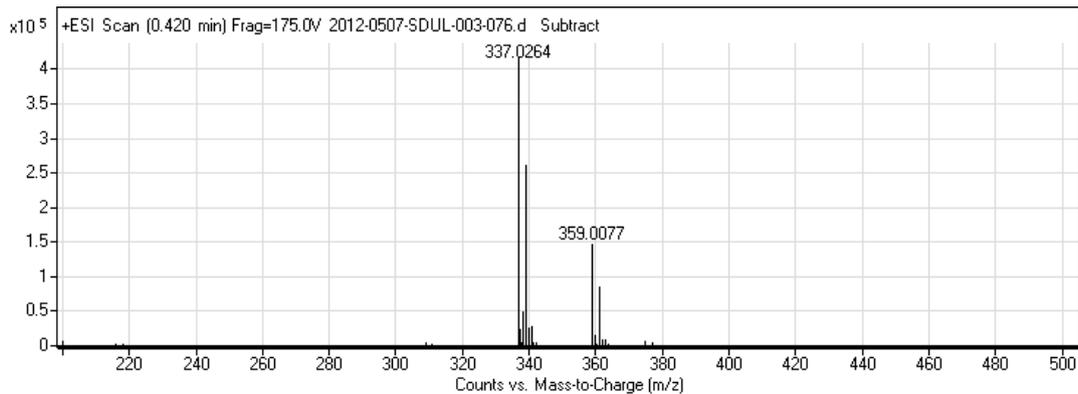
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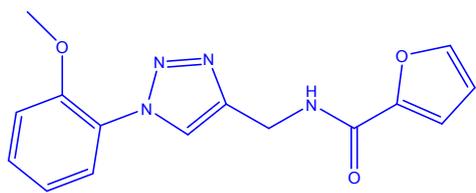
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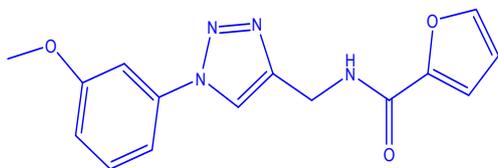
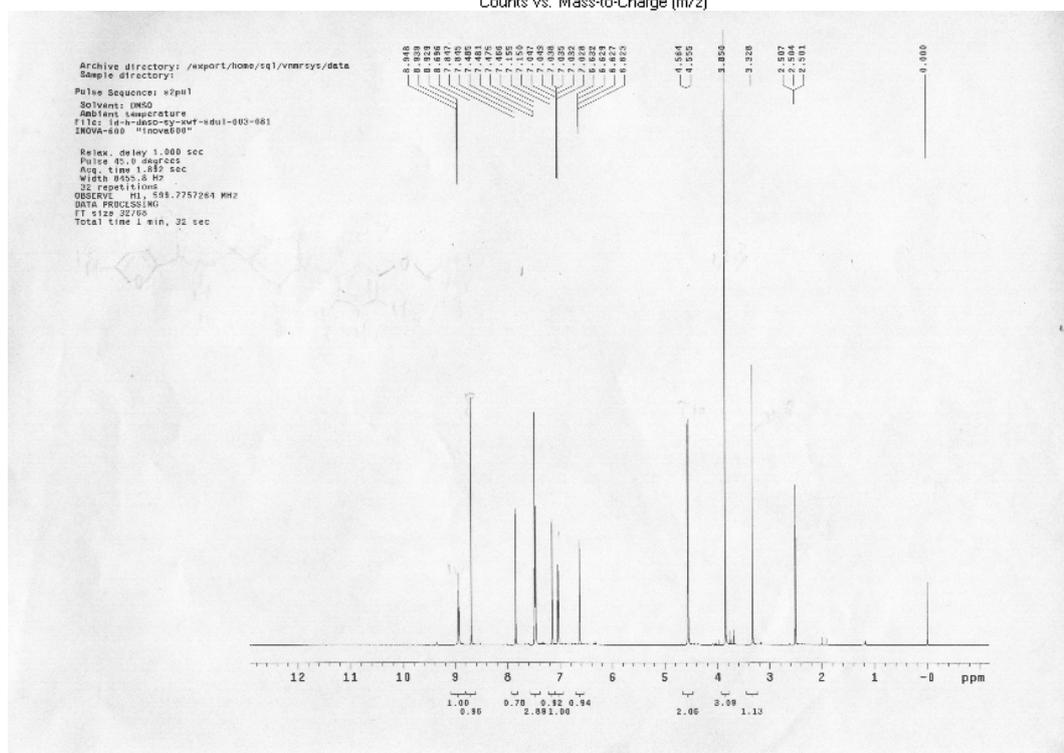
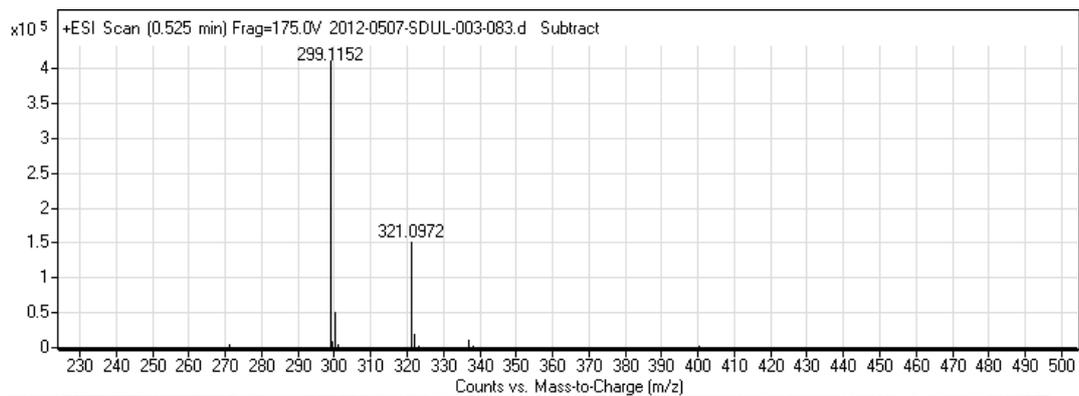
8i



Supporting Information

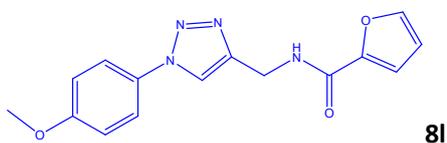
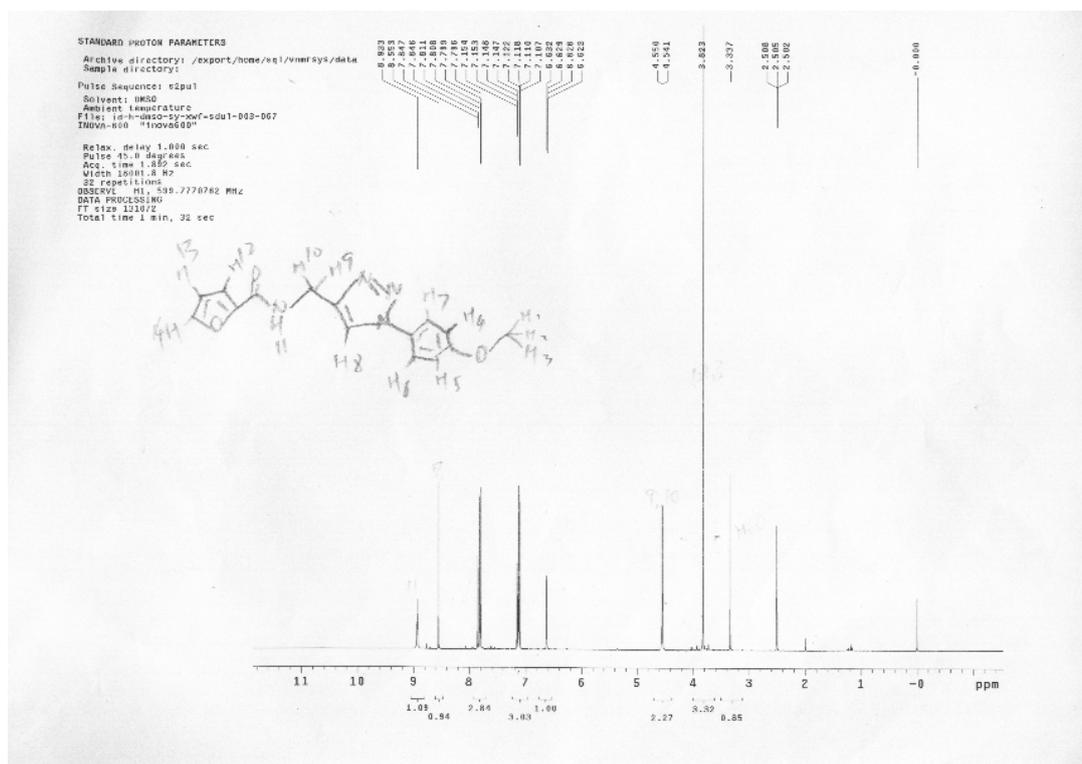
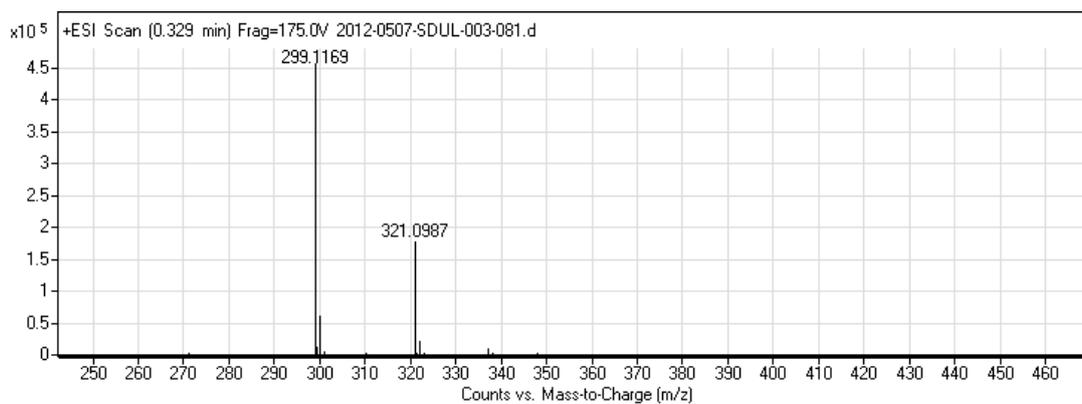


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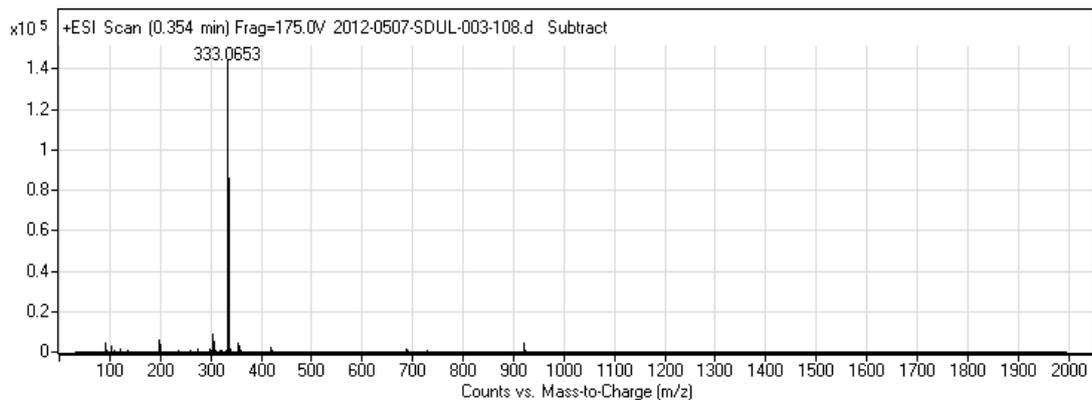
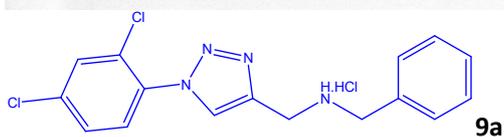
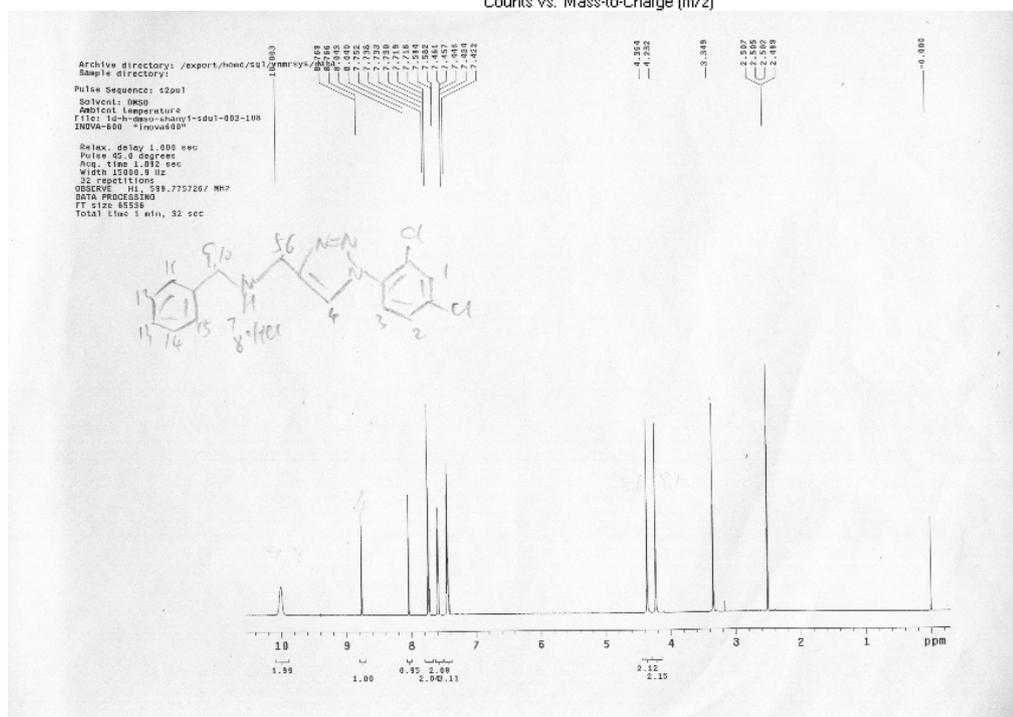
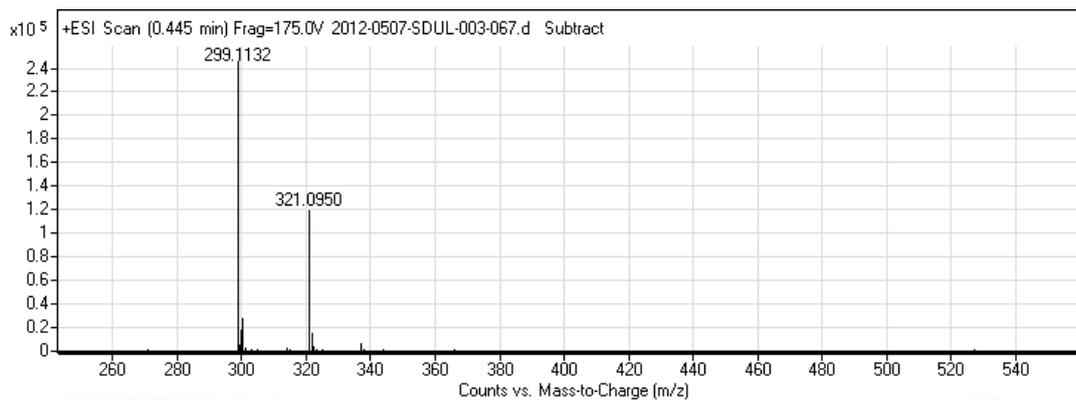


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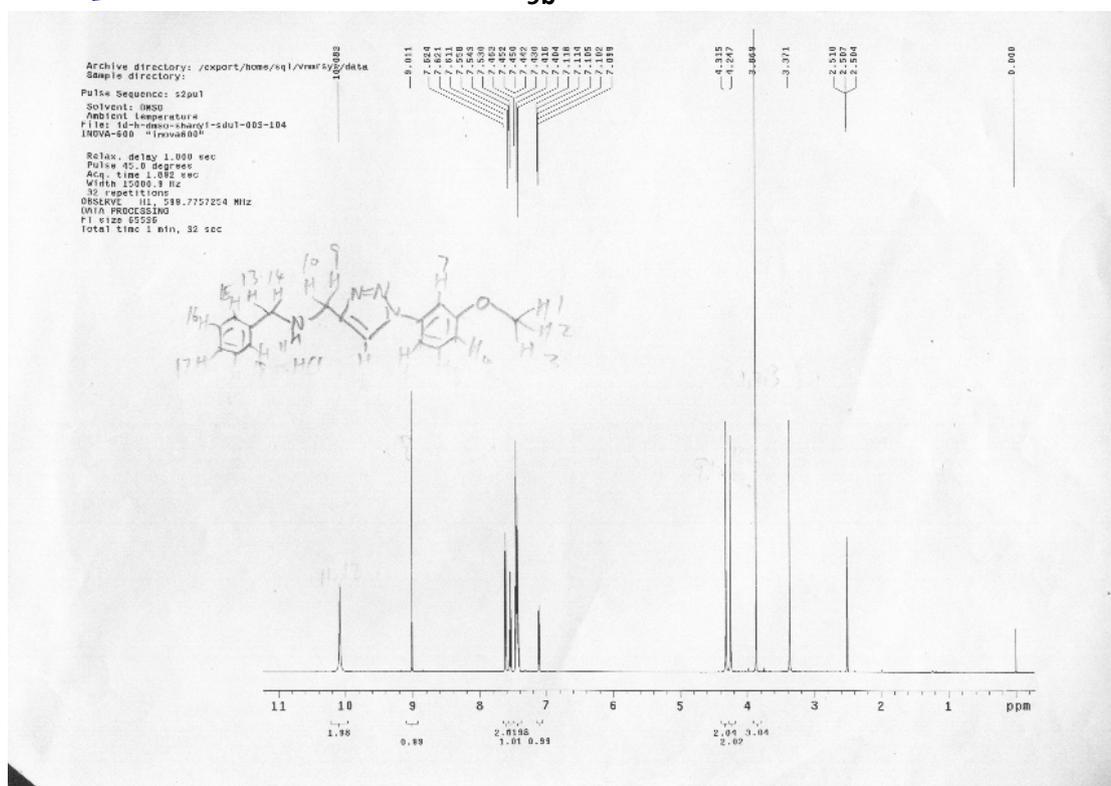
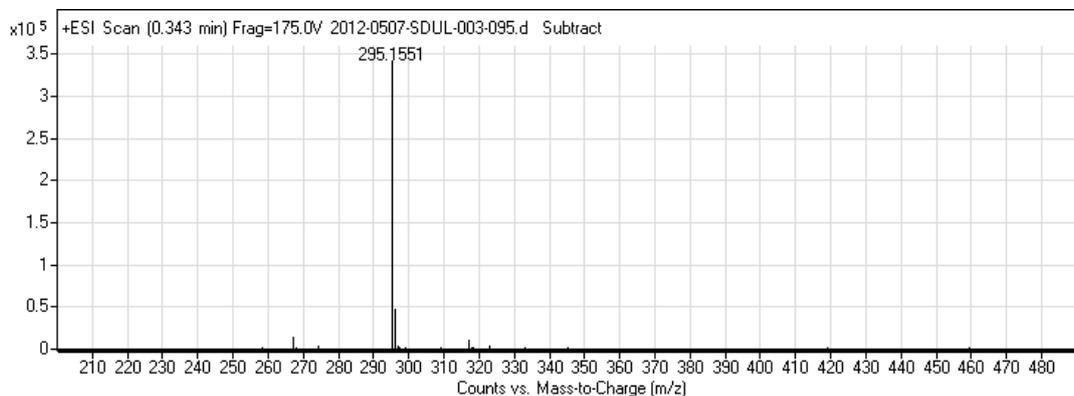
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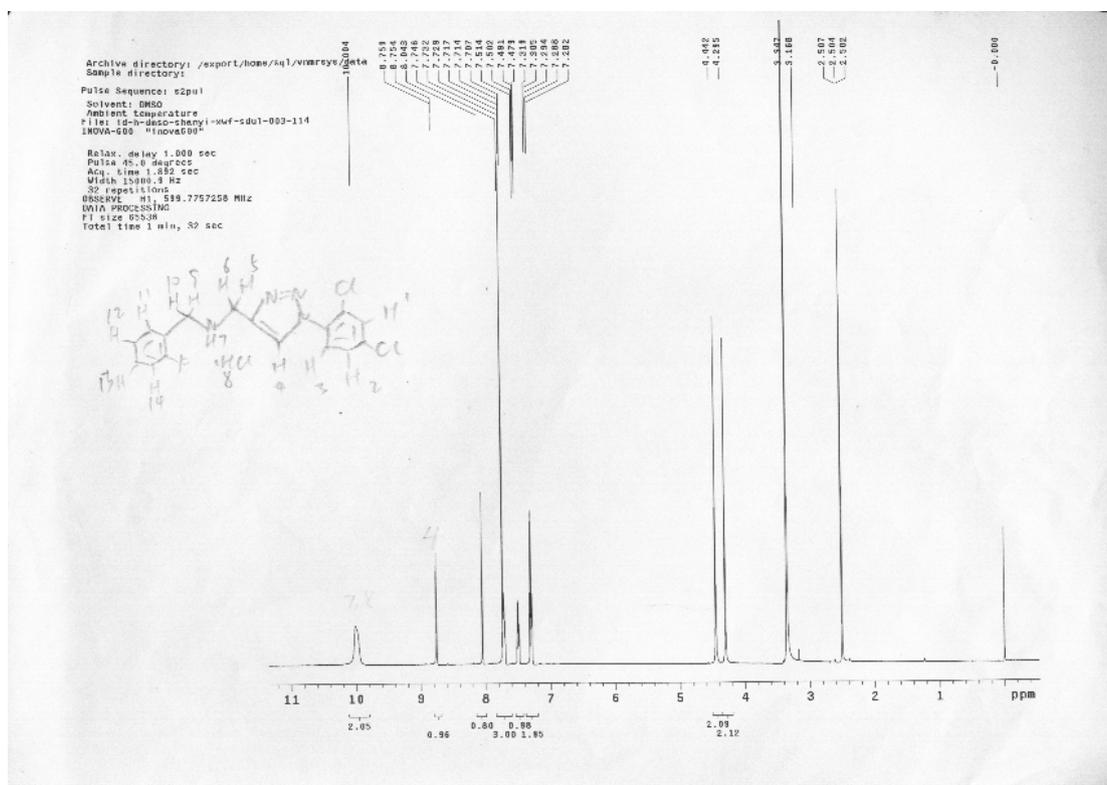
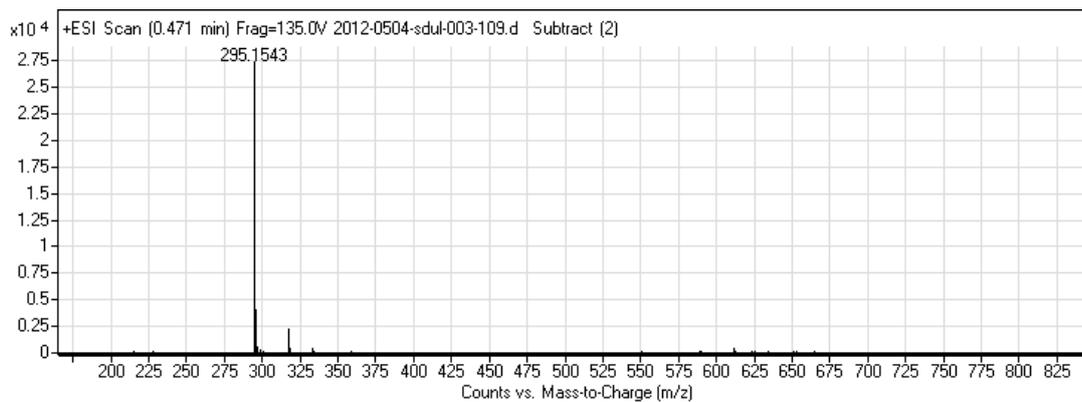
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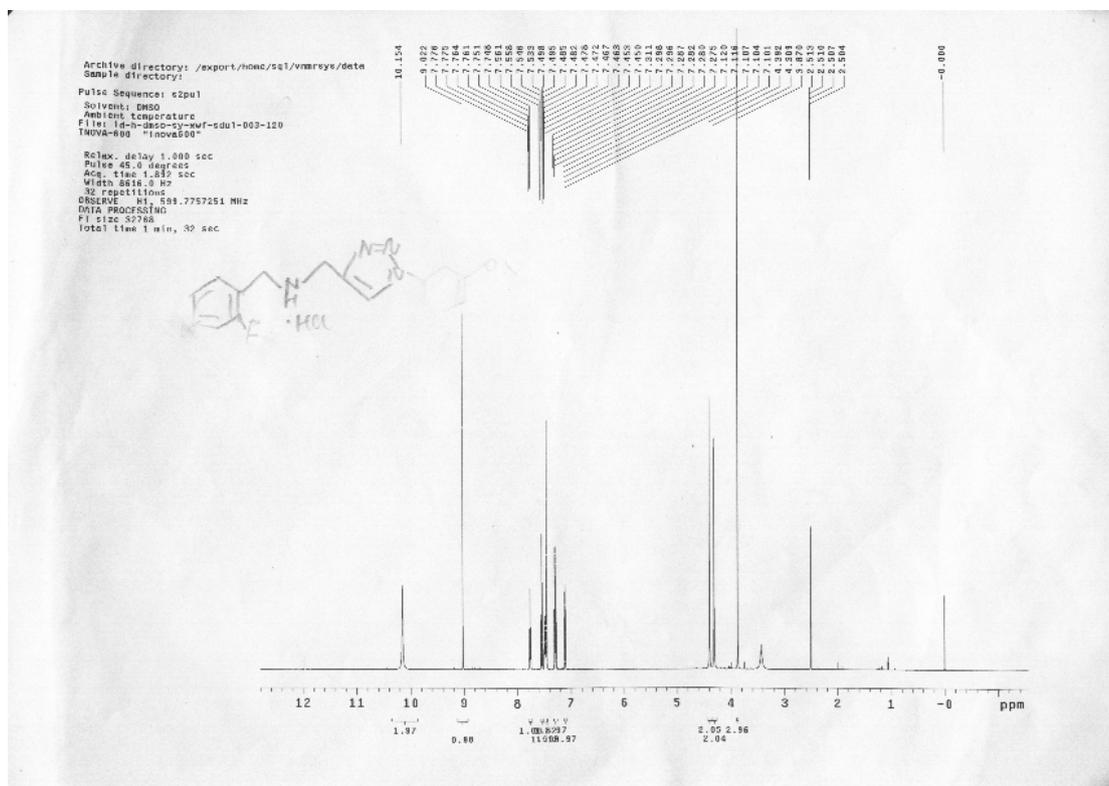
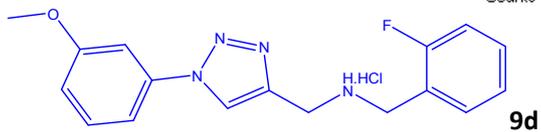
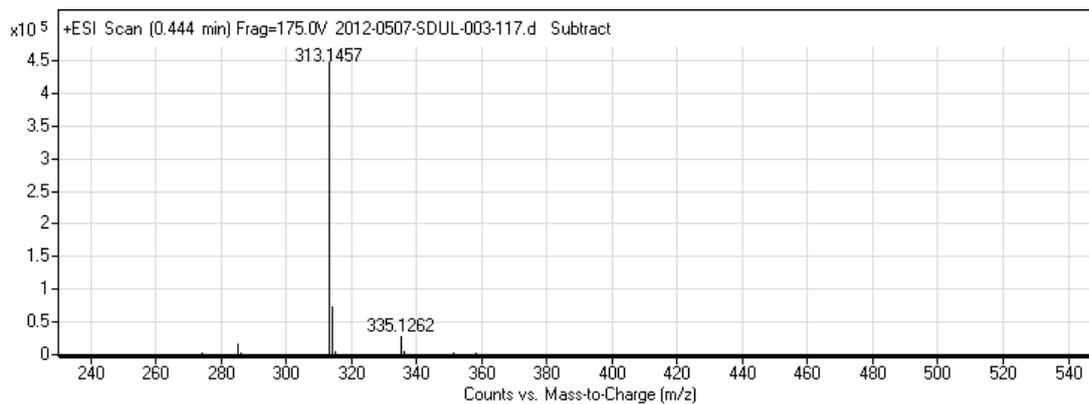
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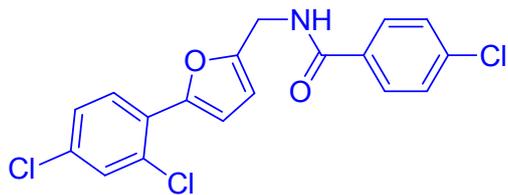
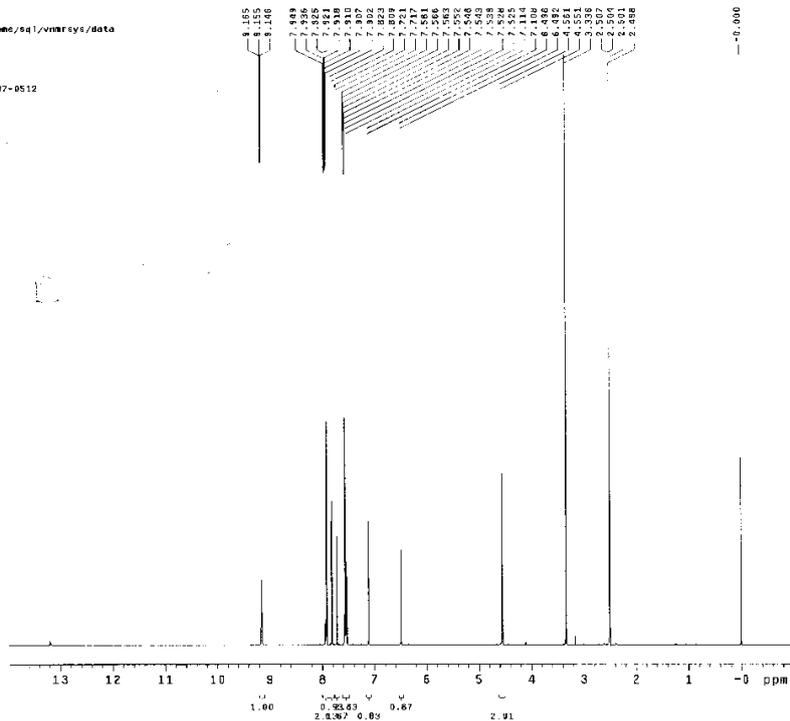


Supporting Information



Supporting Information

Archive directory: /export/home/sal/wmrrsys/data
Sample directory:
Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
Filter: f0-m-dsco-nyw-f-sdu1-007-0512
INOVA-600 "inova600"
Relax. delay: 1.000 sec
Pulse: 45.0 degrees
Acq. time: 1.892 sec
Width: 13000.9 Hz
32 repetitions
OBSERVE: 41, 519.752248 MHz
DATA PROCESSING
FT size: 95536
Total time: 1 min, 32 sec



11

