Supplementary information

Structure-based Design of Potent Human Dihydroorotate Dehydrogenase Inhibitors as Anticancer Agents

Wenlin Song [a, #], Shiliang Li [a, #], Yi Tong [a, #], Jiawei Wang [a], Lina Quan [a], Zhuo Chen [a], Zhenjiang Zhao [a], Yufang Xu [a],Lili Zhu [a,*], Xuhong Qian [a,*], Honglin Li [a,*]

[a] *State Key Laboratory of Bioreactor Engineering, Shanghai Key Laboratory of New Drug Design, School of Pharmacy, East China University of Science and Technology, Shanghai 200237, China*
[*] *To whom correspondence should be addressed. Tel: +86-21-64250213; Fax: +86-21-64250213; Email: zhulfl@ecust.edu.cn, xhqian@ecust.edu.cn, hlli@ecust.edu.cn*
[#] *Authors contributed equally to this work.*

Content

Chemistry

All chemical reagents and solvents were obtained from commercial sources and used without further purification. Thin-layer chromatography (TLC) was performed to monitor the process of reactions. Purification of compounds was achieved by column chromatography with silica gel (Haiyang, Qingdao), 200−300 mesh. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM-400 and a Bruker AM-500 spectrometer with chemical shifts expressed as ppm (in $CDCl_3$ or DMSO-d_6, Me_4Si as internal standard). Melting points were analyzed on a WRS-1B digital melting point apparatus. The mass spectra were measured at the Institute of Fine Chemistry of ECUST.

General procedure for 5a-9a. A mixture of thiosemicarbazide (0.01 mol) and acetone (0.01 mol) in ethanol (40 mL) was refluxed for about 7 h in the presence of five drops of glacial acetic acid as catalyst. The progress of reaction was monitored by TLC. After completion of the reaction, the solution was cooled. The resulting precipitate was filtered and washed with ethanol. White crystal was obtained in good yield without further purification.

General procedure for 5b-9b, 18b-23b. Substituted acetophenone (10 mmol) was solved in 20 mL anhydrous ether in ice bath. One drop of glacial acetic acid was added as catalyst, and then bromine (10 mmol) was added gradually through a separatory funnel. After the addition of bromine, the ice bath was removed and the solution was stirred at ambient temperature overnight. After completion of the reaction, the mixture was washed with saturated aqueous sodium bicarbonate and brine, dried over anhydrous sodium sulfate and concentrated in reduced pressure. The obtained crude product was further purified by column chromatography, eluting with PE/DCM (PE/DCM = 4:1, v/v) to give the colorless oil product.

General procedure for 5c-9c, 11c-17c. The corresponding intermediates from **5a-9a** and **5b-9b** or **18b-23b** were dissolved in ethanol, and then the mixture was heated to reflux until the TLC indicated the reaction was complete. After being cooled down to room temperature, the resulting yellow solid was filtrated off, washed with ethanol and dried. The product can be used directly without further purification.

General procedure for 5d-9d. 80% Hydrazine hydrate (30 mmol) was added into the solution of corresponding compound from **5c-9c** (10 mmol) in ethanol, and the mixture was heated to reflux for about 24 h. Then the solution was cooled to ambient temprature, then to ice bath for a while, finally the resulting precipitate was filtrated off and washed with ethanol. The obtained crude product was further purified through recrystallization using ethanol, and the product with about 30% yield as needle crystal

was obtained.

General procedure for 5-9. To a solution of the corresponding thiazolyl hydrazines (1 mmol) in ethanol, 2-carboxybenzaldehyde (1 mmol) was added. The mixture was stired at room temperature and yellow precipitate formed very quickly. After about 20 min, the target product was filtered, and washed with ethanol without any further purification.

General procedure for 11e-17e. To a solution of compounds **11c** (5 mmol) and Cs_2CO_3 (25 mmol) dissolved in DMF, the corresponding alkyl halides were added gradually through a separatory funnel in ice bath. After addition of alkyl halides, the ice bath was removed and the mixture was heated to 80 ^oC. TLC monitored the reaction until the starting material was consumed totally. Then cooled down the solution to room temperature, extracted with ethyl acetate, collected the organic layer, and concentrated in reduced pressure. The crude product can be directly used for the next step without further purification.

General procedure for 11d-17d. To a solution of compound **11e** (1 mmol) in 10 mL THF and 5 mL H_2O, ten drops of concentrate hydrochloride acid were added to provide an acidic environment. Then the solution was heated to 50 ^oC for about 3 h until TLC indicated the reaction was complete. After cooling down the mixture to room temperature, the mixture was extracted with ethyl acetate, neutralized with saturated aqueous sodium bicarbonate. The organic layer was washed with brine, dried over anhydrous sodium sulphate, and concentrated with reduced pressure. The crude product was purified by column chromatography, eluting with DCM/MeOH (DCM/MeOH = 100:1, v/v) to give brown needle solid in good yield.

General procedure for 11-17. To a solution of the corresponding thiazolyl hydrazines (1 mmol) in ethanol, 2-carboxybenzaldehyde (1 mmol) was added and the mixture was heated to reflux until TLC indicated the reaction was complete. Cooling down the solution to room temperature, removed the solvent under reduced pressure. The crude product was purified by column chromatography with DCM/MeOH (DCM/MeOH = 50:1, v/v) to get yellow solid in good yield.

General procedure for 10f, 18f-23f. To a solution of n-butyl lithium (25 mmol, 2.5 mol/L in hexane) in 10 mL THF at -78 °C, was added a solution of substituted 2-bromobenzoic acid (10 mmol) in THF over 1 h. After stirring at -78°C for 1 h, anhydrous N, N-dimethylformamide (25 mmol) was added dropwise and the mixture was allowed to warm to room temperature slowly. After stirring for around 6 h, a solution of 2 N sodium hydroxide was added, and then the mixture was extracted with

ethyl eater. The aqueous layer was separated and concentrated hydrochloric acid was added until the pH was adjusted to 4-5. The mixture was extracted with ethyl acetate and the organic layer was combined, washed with brine, dried with sodium sulfate, filtered and concentrated. The residue was purified by column chromatography with PE/EA/MeOH (PE/EA/MeOH = 9:1:0.1, v/v/v) to achieve the target product in medium yield.

General procedure for 10g, 18g-23g. Methylhydrazine sulfate (0.1 mol) and ammonium thiocyanate (0.12 mol) were dissolved in 250 ml of ethanol, and the mixture was heated to reflux. After refluxing for 72 h, cooled down the mixture to room temperature, filtrated off the precipitate, concentrated the filtrate under reduced pressure and purified the residue by column chromatography with DCM/MeOH (DCM/MeOH = 100:1, v/v) to get the white power.

General procedure for 10h, 18h-23h. 2-Methyl thiosemicarbazide (1 mmol) and corresponding 2-carboxybenzaldehyde (1 mmol) were dissolved in ethanol and the mixture was heated to reflux until the starting material was consumed totally. After cooling down the mixture to room temperature, removed the solvent under reduced pressure and the residue was purified by column chromatography with DCM/MeOH (DCM/MeOH = 50:1, v/v) to obtain the white solid as the product in good yield.

General procedure for 10, 18-23. To a solution of the corresponding (E)-2-((2-carbamothioyl-2-methylhydrazono)methyl)benzoic acid (1 mmol) in ethanol, substituted 2-bromoacetophenone or 2-bromopropiophenone (1 mmol) was added. Then the mixture was heated to reflux and the process of reaction was monitored by TLC. After completion of the reaction, cooled down the solution to room temperature, removed the solvent under reduced pressure and purified the residue by column chromatography with DCM/MeOH (DCM/MeOH = 50:1, v/v) to obtain the yellow solid as the product in good yield.

(E)-4-phenyl-2-(((2-carboxyl)benzylidene)hydrazinyl)thiazole 5. Mp 196.8-197.5 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 13.45 (br, 1H), 12.49 (br, 1H), 8.81 (s, 1H), 7.99 (d, J = 7.6 Hz, 1H), 7.87 (m, 3H), 7.63 (t, J = 7.4 Hz, 1H), 7.47 (t, J = 7.2 Hz, 1H), 7.41 (t, J = 7.6 Hz, 2H), 7.33 (s, 1H), 7.30 (t, J = 7.4 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 168.53, 168.53, 150.97, 140.58, 135.02, 134.99, 132.31, 130.74, 130.15, 129.15, 128.96, 128.96, 127.90, 126.34, 125.90, 125.90, 104.20. HRMS (ESI) calcd for $C_{18}H_{16}N_3O_2S$ $[M-H]^-$ 322.0650, found 322.0656.

(E)-5-methyl-4-phenyl-2-(((2-carboxyl)benzylidene)hydrazinyl)thiazole 6. Mp 215.9-216.0 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 12.7 (br, 2H), 8.77 (s, 1H),

7.99 (d, J = 7.6 Hz, 1H), 7.87 (d, J = 8.0Hz, 1H), 7.63-7.59 (m, 3H), 7.48-7.43(m, 3H), 7.33 (t, J = 7.2 Hz, 1H), 2.43 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 168.66, 164.72, 146.00, 140.05, 135.64, 135.24, 132.36, 130.83, 130.12, 129.08, 128.73, 128.73, 128.35, 128.35, 127.51, 126.33, 117.61, 12.74. HRMS (ESI) calcd for $C_{18}H_{16}N_3O_2S$ $[M+H]^+$ 338.0963, found 338.0954.

(E)-4-(2-chlorophenyl)-2-(((2-carboxyl)benzylidene)hydrazinyl)thiazole 7. Mp 195.2-195.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 13.2 (br, 1H), 12.4 (br, 1H), 8.82 (s, 1H), 8.00 (d, J = 7.6 Hz, 1H), 7.88 (t, J = 7.0 Hz, 2H), 7.64 (t, J = 7.4 Hz, 1H), 7.6 (d, J = 7.6 Hz, 1H), 7.48 (t, J = 7.6 Hz, 1H), 7.42 (t, J = 7.8 Hz, 1H), 7.36 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 168.63, 167.77, 147.63, 140.76, 135.08, 133.71, 132.43, 131.54, 131.23, 130.83, 130.83, 130.26, 129.47, 129.28, 127.69, 126.47, 109.26. HRMS (ESI) calcd for $C_{17}H_{13}N_3O_2SCl$ $[M+H]^+$ 358.0417, found 358.0417.

(E)-5-methyl-4-(2-chlorophenyl)-2-(((2-carboxyl)benzylidene)hydrazinyl)thiazole 8. Mp 220.0-220.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 12.7 (br, 2H), 8.77 (s,1H), 7.99 (d, J = 7.6 Hz, 1H), 7.87 (d, J = 7.2 Hz, 1H), 7.62 (t, J = 7.4 Hz, 1H), 7.562-7.543 (m, 1H), 7.481-7.397 (m, 4H), 2.14 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 168.65, 165.18, 144.49, 140.16, 135.25, 134.56, 133.21, 132.46, 130.83, 130.14, 130.03, 129.11, 127.45, 126.37, 119.49, 12.15. HRMS (ESI) calcd for $C_{18}H_{15}N_3O_2SCl$ $[M+H]^+$ 372.0574, found 372.0569.

(E)-5-ethyl-4-(2-chlorophenyl)-2-(((2-carboxyl)benzylidene)hydrazinyl)thiazole 9. Mp 223.9-223.9 °C. ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 12.57 (br, 2H), 8.79 (s, 1H), 8.01 (d, J = 8.0 Hz, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.62 (t, J = 7.5 Hz, 1H), 7.56-7.51 (m, 1H), 7.46 (t, J = 7.5 Hz, 1H), 7.44-7.36 (m, 3H), 2.49 (q, J = 7.5 Hz, 1H), 1.13 (t, J = 7.5 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 169.28, 165.92, 144.14, 140.84, 135.89, 135.46, 133.99, 133.03, 133.02, 131.46, 130.81, 130.73, 130.60, 129.73, 128.09, 127.73, 127.01, 20.99, 17.25. HRMS (ESI) calcd for $C_{19}H_{17}N_3O_2SCl$ $[M+H]^+$ 386.0730, found 386.0729.

(E)-4-(2-chlorophenyl)-2-(1-methyl-2-((2-carboxyl)benzylidene)hydrazinyl)thiazole 10. Mp 210.4-212.0 °C. ^{1}H NMR (400 MHz, DMSO-d6, ppm): δ 13.30 (s, 1H), 8.62 (s, 1H), 8.02 (d, J = 7.6 Hz, 1H), 7.98-7.90 (m, 2H), 7.66 (t, J = 7.6 Hz, 1H), 7.56-7.49 (m, 2H), 7.47 (s, 1H), 7.43 (td, J_1 = 7.4 Hz, J_2 = 1.2 Hz, 1H), 7.36 (td, J_1 = 7.6 Hz, J_2 =1.6 Hz, 1H), 3.68 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 168.98, 168.57, 147.11, 136.94, 135.17, 133.42, 132.48, 131.51, 131.09, 130.98, 130.75, 130.16, 129.45, 129.19, 127.63, 126.51,

111.41, 32.88. HRMS (ESI) calcd for $C_{18}H_{15}N_3O_2SCl$ $[M+H]^+$ 372.0574, found 372.0575.

(E)-4-(2-chlorophenyl)-2-(1-ethyl-2-((2-carboxyl)benzylidene)hydrazinyl)thiazole 11. Mp 203.3-204.1 °C. ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 13.32 (br, 1H), 8.64 (s, 1H), 7.98-7.91 (m, 3H), 7.66 (t, J = 7.5 Hz, 1H), 7.56-7.46 (m, 2H), 7.45-7.39 (m, 2H), 7.34 (t, J = 6.8 Hz,1H), 4.32 (q, J = 6.8 Hz, 2H), 1.27 (t, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 168.56, 168.36, 147.34, 137.00, 135.42, 133.53, 132.50, 131.53, 131.12, 130.92, 130.72, 130.22, 129.42, 129.19, 127.62, 126.62, 111.22, 40.38, 10.33. HRMS (ESI) calcd for $C_{19}H_{17}N_3O_2SCl$ $[M+H]^+$ 386.0730, found 386.0728.

(E)-4-(2-chlorophenyl)-2-(1-propyl-2-((2-carboxyl)benzylidene)hydrazinyl)thiazole 12. Mp 172.7-173.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 13.35 (br, 1H), 8.65 (s, 1H), 7.98 (d, J = 8.0 Hz, 1H), 7.95-7.92 (m, 2H), 7.67 (t, J = 7.2 Hz, 1H), 7.54 (d, J = 8.0 Hz, 1H), 7.5 (t, J = 7.6 Hz, 1H), 7.45-7.42 (m, 2H), 7.36 (t, J_1 = 7.6 Hz, J_2 = 1.6 Hz, 1H), 4.25 (t, J = 7.2 Hz, 2H), 1.82-1.73 (m, 2H), 0.97 (t, J = 7.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 168.54, 168.22 146.95, 136.63, 134.96, 133.17, 132.11, 131.10, 130.74, 130.55, 130.34, 129.82, 129.04, 128.80, 127.26, 126.09, 110.77, 46.31, 17.98,11.17. HRMS (ESI) calcd for $C_{20}H_{19}N_3O_2SCl$ $[M+H]^+$ 400.0887, found 400.0879.

(E)-4-(2-chlorophenyl)-2-(1-isopropyl-2-((2-carboxyl)benzylidene)hydrazinyl)thiazole 13. Mp 185.4-187.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 13.35 (br, 1H), 8.91 (s, 1H), 7.99 (d, J = 8.0 Hz, 1H), 7.95-7.92 (m, 2H), 7.67 (t, J = 7.6 Hz, 1H), 7.54 (d, J = 8.0 Hz, 1H), 7.52 (t, J = 7.6 Hz, 1H), 7.46-7.42 (m, 2H), 7.36 (td, J_1 = 7.6 Hz, J_2 = 1.6 Hz, 1H), 5.22-5.11 (m, 1H), 1.57 (d, J = 6.8 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 168.22, 168.21, 146.90, 137.32, 135.23, 133.14, 132.07, 131.06, 130.64, 130.58, 130.39, 129.88, 128.97, 128.78, 127.28, 125.91, 111.12, 49.61, 18.07, 18.07. HRMS (ESI) calcd for $C_{20}H_{19}N_3O_2SCl$ $[M+H]^+$ 400.0887, found 400.0885.

(E)-4-(2-chlorophenyl)-2-(1-hydroxyethyl-2-((2-carboxyl)benzylidene)hydrazinyl)thiazole 14. Mp 187.9-188.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 13.24 (br, 1H), 8.75 (s, 1H), 7.98-7.01 (m, 3H), 7.66 (t, J = 7.6 Hz, 1H), 7.54 (d, J = 8.0 Hz, 1H), 7.49 (t, J = 7.6 Hz, 1H), 7.45-7.41 (m, 2H), 7.36 (td, J_1 = 7.6 Hz, J_2 = 1.6 Hz, 1H), 4.35 (t, J = 6.4 Hz, 2H), 3.76 (t, J = 6.4 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 168.64, 168.23, 146.87, 136.89, 134.91, 133.15, 131.99, 131.19, 130.71, 130.46, 130.32, 130.16, 129.04, 128.79, 127.22,

126.28, 110.78, 56.24, 47.46. HRMS (ESI) calcd for $C_{19}H_{17}N_3O_3SCl$ $[M+H]^+$ 402.0679, found 402.0678.

(E)-4-(2-chlorophenyl)-2-(1-(2-butyl)-2-((2-carboxyl)benzylidene)hydrazinyl)thiazole 15. Mp 172.7-173.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 13.32 (br, 1H), 8.91 (s, 1H), 7.99 (d, J = 8.0 Hz, 1H), 7.95-7.90 (m, 2H), 7.67 (t, J = 7.6 Hz, 1H), 7.55-7.48 (m, 2H), 7.46-7.42 (m, 2H), 7.36 (td, J_1 = 7.6 Hz, J_2 = 1.6 Hz, 1H), 5.00-4.93 (m, 1H), 2.35-2.24 (m, 1H), 1.92-1.81 (m, 1H), 1.54 (d, J = 6.8 Hz, 3H), 0.88 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 168.66, 168.19, 146.97, 137.03, 135.24, 133.19, 132.13, 131.04, 130.67, 130.58, 130.39, 129.70, 128.98, 128.77, 127.29, 125.91, 111.04, 55.67, 25.26, 16.33, 11.14. HRMS (ESI) calcd for $C_{21}H_{21}N_3O_2SCl$ $[M+H]^+$ 414.1043, found 414.1029.

(E)-4-(2-chlorophenyl)-2-(1-(pentan-2-yl)-2-((2-carboxyl)benzylidene)hydrazinyl)thiazole 16. Mp 146.7-148.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 13.35 (br, 1H), 8.91 (s, 1H), 7.98 (d, J = 7.6 Hz, 1H), 7.94 (d, J = 8.0 Hz, 1H), 7.91 (dd, J_1 = 7.6 Hz, J_2 = 1.6 Hz, 1H), 7.67 (t, J = 7.6 Hz, 1H), 7.53 (d, J = 8.0 Hz, 1H), 7.49 (t, J = 7.6 Hz, 1H), 7.46-7.42 (m, 2H), 7.36 (td, J_1 = 7.6 Hz, J_2 = 1.6 Hz, 1H), 5.14-5.06 (m, 1H), 2.34-2.25 (m, 1H), 1.82-1.73 (m, 1H), 1.53 (d, J = 6.8 Hz, 3H), 1.34-1.24 (m, 2H), 0.89 (t, J = 7.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 168.68, 168.20, 146.98, 137.04, 135.30, 133.20, 132.14, 131.00, 130.68, 130.59, 130.40, 129.68, 128.99, 127.30, 125.90, 111.09, 53.81, 34.20, 19.46, 16.46, 13.60. HRMS (ESI) calcd for $C_{22}H_{23}N_3O_2SCl$ $[M+H]^+$ 428.1200, found 428.1193.

(E)-5-methyl-4-(2-chlorophenyl)-2-(1-(pentan-2-yl)-2-((2-carboxyl)benzylidene)hydrazinyl)thiazole 17. Mp. 89.9-90.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 13.29 (br, 1H), 8.85 (s, 1H), 7.98 (d, J = 8.0 Hz, 1H), 7.92 (d, J = 7.6 Hz, 1H), 7.65 (t, J = 7.6 Hz, 1H), 7.56-7.54 (m, 1H), 7.51-7.41 (m, 4H), 5.05-4.96 (m, 1H), 2.29-2.22 (m, 1H), 2.16 (s, 3H), 1.75-1.67 (m, 1H), 1.46 (d, J = 7.2 Hz, 3H), 1.28-1.23 (m, 2H), 0.87 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 168.23, 166.06, 144.08, 136.14, 135.46, 134.09, 132.79, 132.09, 131.93, 130.58, 129.64, 129.60, 129.48, 128.56, 126.95, 125.73, 121.62, 53.39, 34.01, 19.41, 16.35, 13.58, 11.52. HRMS (ESI) calcd for $C_{23}H_{25}N_3O_2SCl$ $[M+H]^+$ 442.1356, found 442.1354.

(E)-4-(2,5-dichlorophenyl)-2-(1-methyl-2-((2-carboxyl)benzylidene)hydrazinyl)thiazole 18. Mp. 268.4-269.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 13.31 (br, 1H), 8.63 (s, 1H), 8.02-8.00 (m, 2H), 7.93 (d, J =

7.2 Hz, 1H), 7.67 (t, J = 7.6 Hz, 1H), 7.63 (s, 1H), 7.58 (d, J = 7.6 Hz, 1H), 7.50 (t, J = 7.2 Hz, 1H), 7.43 (dd, J_1 = 7.2 Hz, J_2 = 2.8 Hz, 1H), 3.68 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 168.68, 168.15, 145.13, 136.79, 134.70, 134.21, 132.14, 132.06, 131.85, 130.59, 130.17, 129.76, 129.20, 128.84, 128.58, 126.13, 112.31, 32.49. HRMS (ESI) calcd for $C_{18}H_{14}N_3O_2SCl2$ $[M+H]^+$ 406.0184, found 406.0187.

(E)-4-(3-chlorophenyl)-2-(1-methyl-2-((2-carboxyl)benzylidene)hydrazinyl)thiazole 19. Mp. 244.7-245.4 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d6, ppm): δ 13.32 (br, 1H), 8.63 (s, 1H), 8.01 (d, J = 7.6 Hz, 1H), 7.97 (s, 1H), 7.93 (d, J = 7.6 Hz, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.66 (t, J = 7.6 Hz, 1H), 7.61 (s, 1H), 7.50 (t, J = 7.6 Hz, 1H), 7.45 (t, J = 7.6 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H), 3.71 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 169.57, 168.18, 148.56, 136.64, 136.50, 134.72, 133.48, 132.06, 130.59, 130.44, 129.76, 128.81, 127.28, 126.12, 125.16, 124.05, 107.56, 32.52. HRMS (ESI) calcd for $C_{18}H_{15}N_3O_2SCl$ $[M+H]^+$ 372.0574, found 372.0574.

(E)-4-(2-chlorophenyl)-2-(1-methyl-2-((2-carboxyl)-(4-trifluoromethyl)benzylidene)hydrazin-yl)thiazole 20. Mp 209.3-210.7 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 14.02 (br, 1H), 8.66 (s, 1H), 8.21 (d, J = 8.0 Hz, 1H), 8.17 (s, 1H), 8.02 (d, J = 8.0 Hz, 1H), 7.95 (d, J = 7.6 Hz, 1H), 7.56-7.53 (m, 2H), 7.43 (t, J = 7.6 Hz, 1H), 7.37 (t, J = 7.6 Hz, 1H), 3.70 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 168.33, 167.19, 146.79, 138.35, 135.04, 132.91, 131.12, 130.70, 130.37, 129.14, 128.57, 128.24, 128.18, 127.26, 127.02, 125.10, 122.40, 111.51, 32.68. HRMS (ESI) calcd for $C_{19}H_{14}N_3O_2SClF_3$ $[M+H]^+$ 440.0447, found 440.0433.

(E)-4-(2-chlorophenyl)-2-(1-methyl-2-((2-carboxyl)-(4-methyl)benzylidene)hydrazinyl)thiazole 21. Mp 231.3-232.5 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 13.30 (br, 1H), 8.59 (s, 1H), 7.96 (d, J = 7.6 Hz, 1H), 7.92 (d, J = 8.0 Hz, 1H), 7.75 (s, 1H), 7.54 (d, J = 7.6 Hz, 1H), 7.49-7.46 (m, 2H), 7.43 (t, J = 7.6 Hz, 1H), 7.36 (t, J = 7.6 Hz, 1H), 3.66 (s, 3H), 2.38 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 168.60, 168.35, 146.68, 138.54, 136.64, 133.03, 132.70, 132.07, 131.11, 130.86, 130.67, 130.34, 129.83, 129.00, 127.21, 126.04, 110.84, 32.39, 20.69. HRMS (ESI) calcd for $C_{19}H_{17}N_3O_2SCl$ $[M+H]^+$ 386.0730, found 386.0738.

(E)-4-(2-chlorophenyl)-2-(1-methyl-2-((2-carboxyl)-(4-fluoro)benzylidene)hydrazinyl)thiazole 22. Mp 236.2-237.1 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 8.61 (s, 1H), 8.06-8.02 (dd, J_1 = 5.6 Hz, J_2 = 8.4 Hz, 1H), 7.96 (d, J = 7.2 Hz, 1H), 7.67-7.64 (dd, J_1 = 9.6 Hz, J_2 = 2.4 Hz, 1H), 7.55-7.51 (m, 2H), 7.47

(s, 1H), 7.43 (t, J = 7.2 Hz, 1H), 7.36 (t, J = 7.2 Hz, 1H), 3.68 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 168.55, 168.01, 159.74 (d, ^1J = 249 Hz), 146.67, 133.68, 133.10, 131.50, 131.12, 130.72, 130.32, 130.10 (d, ^3J = 8.8 Hz), 129.06, 127.23, 125.53 (d, ^4J = 3.1 Hz), 121.75 (d, ^2J = 11.8 Hz), 118.76 (d, ^2J = 22 Hz), 111.13, 32.28. HRMS (ESI) calcd for $C_{18}H_{14}N_3O_2FSCl$ $[M+H]^+$ 390.0479, found 390.0475.

(E)-5-methyl-4-phenyl-2-(1-methyl-2-((4-methyl)-(2-carboxyl)benzylidene)hydrazinyl)thiazole 23. Mp 243.2-245.2 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d6, ppm): δ 13.24 (br, 1H), 8.54 (s, 1H), 7.91(d, J = 8.0 Hz, 1H), 7.73 (s, 1H), 7.64-7.53 (m, 2H), 7.46-7.42 (m, 3H), 7.33 (t, J = 7.4 Hz, 1H), 3.60 (s, 3H), 2.44 (s, 3H), 2.38 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 168.35, 165.40, 145.43, 138.33, 135.76, 135.16, 132.68, 132.22, 130.86, 129.62, 128.24, 128.24, 127.85, 127.85, 127.06, 125.89, 119.27, 31.85, 20.68, 12.24. HRMS (ESI) calcd for $C_{20}H_{20}N_3O_2S$ $[M+H]^+$ 366.1276, found 366.1273.

***In vitro* enzyme assay**

Recombined *h*DHODH was expressed and purified as described in our previous works.[1-3] The *h*DHODH inhibition assays were performed using the DCIP-based assay. For each inhibitor, eight different concentrations were used to determine the IC_{50} value. Brequinar was measured as the positive control. Each inhibitor concentration point was tested in triplicate and IC_{50} values were determined using origin 8.0 and summarized in Table 1.

Cell proliferation assay

Human colon cancer cell line HCT-116 and human pancreatic cancer cell line BxPC-3 were cultured in RPMI-1640 (Hyclone) and DMEM (Hyclone), respectively, and human lung fibroblast cell line Wi-38 was cultured in MEM (Hyclone), all containing 10% fetal bovine serum (FBS). Cell proliferation assay was evaluated by the MTT reduction assay.[4] Firstly, 5000 cells were placed in each well of a 96-well plate. After 24 h incubation, the cells attached to the plate. 10 μL compounds diluted to seven different concentrations (ranging from 0.016 μM to 10 μM for cancer cell lines and ranging from 0.082 μM to 100 μM for normal cell line) were added to each well and cell cultures were incubated for another 72 h. The concentration of DMSO in each well was 0.5% (*v/v*). Then 10 μL MTT solutions (5 mg/mL) were added to each well. Subsequently, the plate was incubated for 4 h at 37 ^{o}C in a cell culture incubator. Afterwards, the culture medium was removed and aliquots (100 μL) of DMSO were added to each well to dissolve the formazan crystals. Finally, the absorbance was measured at 570 nm. The final results were recorded by averaging at least three

independent determinations. The IC_{50} values were analyzed using Origin 8.0.

Analysis of apoptosis

HCT-116 and BxPC-3 cell lines were also used for the cell apoptosis assay by flow cytometry. The cells were incubated with 200 nM compound **22** for 24 h, 36 h and 48 h, respectively. Then the cells were collected and washed twice with cold PBS and resuspended in the binding buffer. The suspension was stained with 5 μL propidium iodide (PI) and 5 μL Annexin V-FITC and then incubated in the dark for 15 min at RT.

Analysis of cell cycle

HCT-116 and BxPC-3 cell lines were used for the cell cycle analysis. The cells were harvested after incubation with compound **22** (5 nM, 10 nM, 20 nM) for 12 h and washed twice in ice-cold PBS buffer before being fixed with cold ethanol (70%) for 12 h at 4 ^{o}C. Fixed cells were rinsed twice with cold PBS and resuspended in PBS. The suspension was incubated with RNase and propidium iodides (PI) for 15 min at RT. The distribution of the cell cycle was analyzed by flow cytometry.

Molecular docking simulation

To thoroughly elucidate the binding modes of this series of derivatives, molecular docking method was employed. The X-ray crystal structure of *h*DHODH in complex with compound **7** (PDB ID: 4LS1) was used for protein preparation. Hydrogen atoms and charges were added to the receptor using the Protein Preparation workflow in Maestro (Schrödinger Inc, version 9.0). The grid-enclosing box was centered on the centroid of ligand (compound **7**) and defined so as to enclose residues located within 20 Å around the ubiquinone binding site, and a scaling factor of 1.0 was set to van der Waals (VDW) radii of those receptor atoms with the partial atomic charge less than 0.25. Then compounds **17** and **22** were prepared by the LigPrep module in Maestro, and Epik method was selected to predict the ionization states of the compounds within the default pH range of 7.0 ± 2.0. Finally, Extra-Precision (XP) mode in Glide was used to perform the molecular docking simulations, and the top 10 docked poses of each compound ranked by GlideScore were remained for further analysis.

References

1 J. Zhu, L. Han, Y. Diao, X. Ren, M. Xu, L. Xu, S. Li, Q. Li, D. Dong and J. Huang, *J. Med. Chem.*, 2015, **58**, 1123-1139.

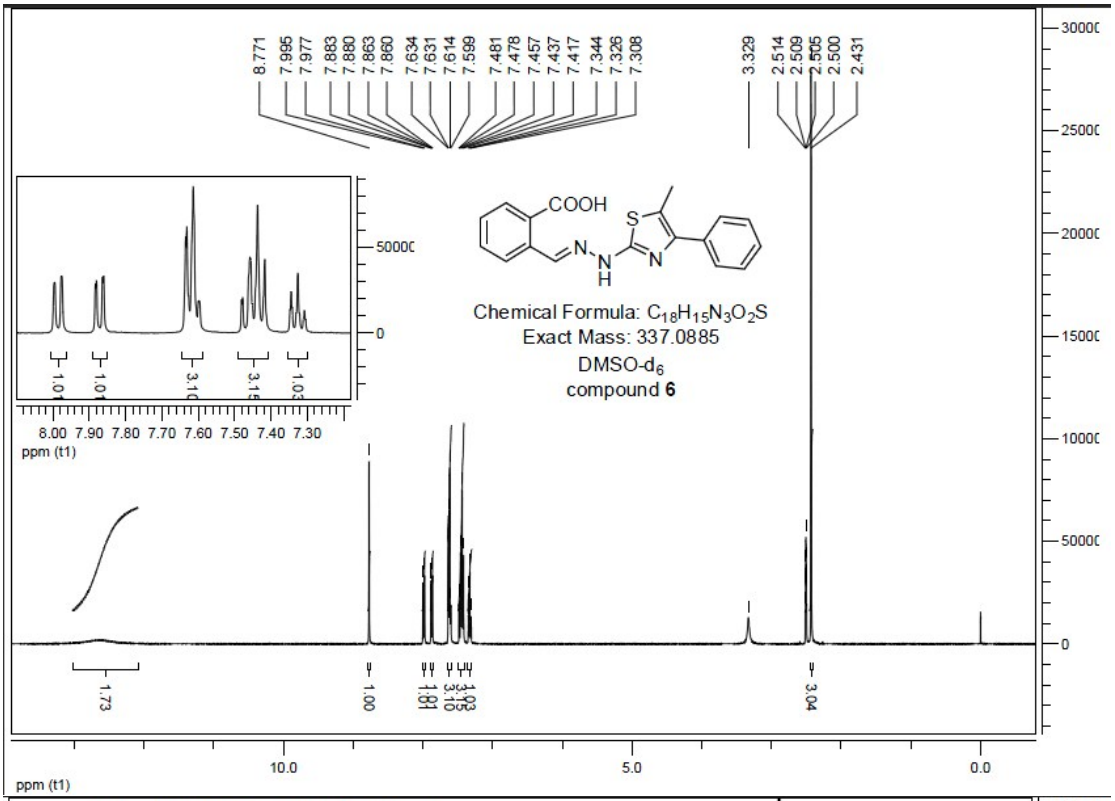
2 S. Li, G. Luan, X. Ren, W. Song, L. Xu, M. Xu, J. Zhu, D. Dong, Y. Diao and X. Liu, *Sci. Rep.*, 2015, **5**, 14836.

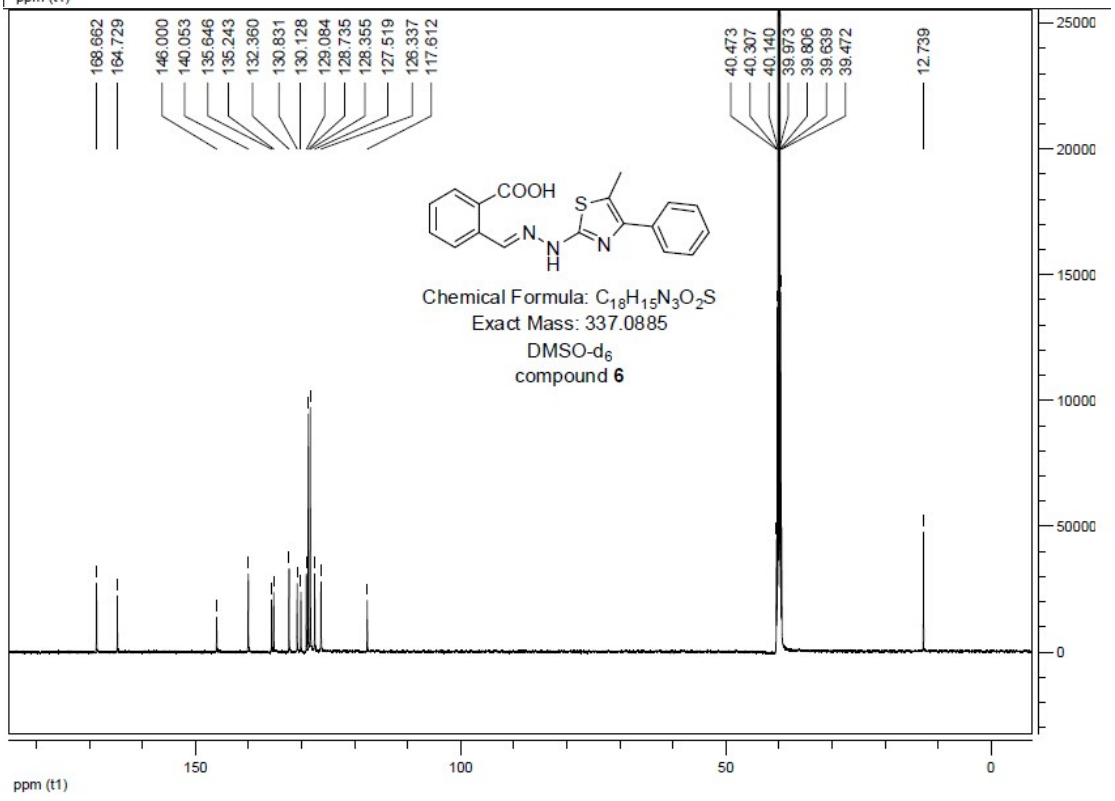
3 Y. Diao, W. Lu, H. Jin, J. Zhu, L. Han, M. Xu, R. Gao, X. Shen, Z. Zhao and X. Liu, *J. Med. Chem.*, 2012, **55**, 8341-8349.

4 J. Carmichael, W. G. DeGraff, A. F. Gazdar, J. D. Minna and J. B. Mitchell, *Cancer Res.*, 1987, **47**, 936-942.

The spectra of ^{1}H NMR, ^{13}C NMR and HRMS (ESI) of representative compounds.

Compound **6**





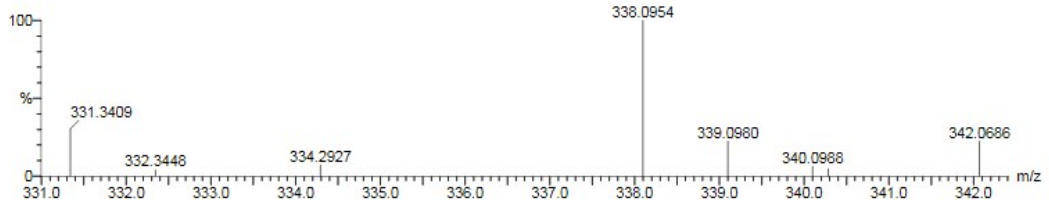
Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
104 formula(e) evaluated with 4 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
C: 0-30 H: 0-50 N: 0-3 O: 0-2 S: 0-1

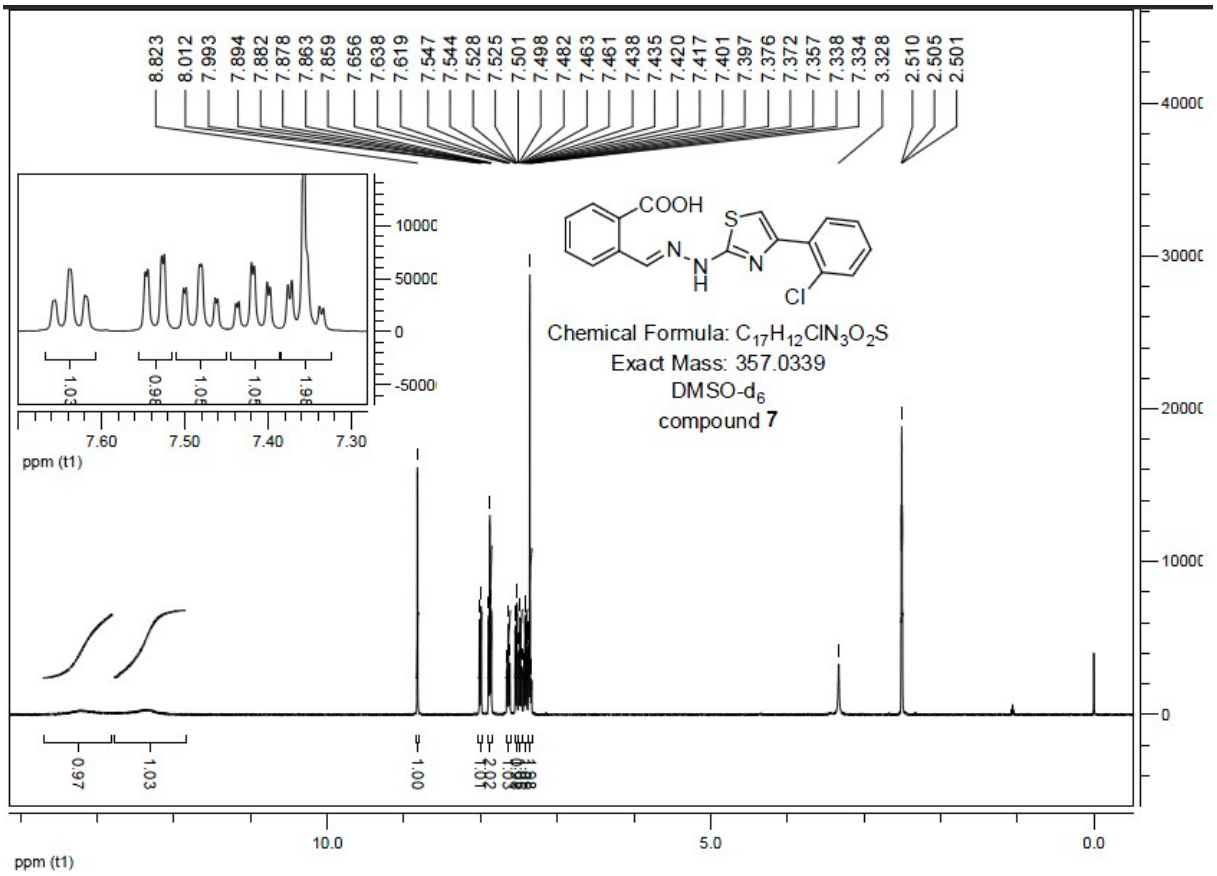
WP-ZHU ECUST institute of Fine Chem 30-Mar-2015 21:39:45

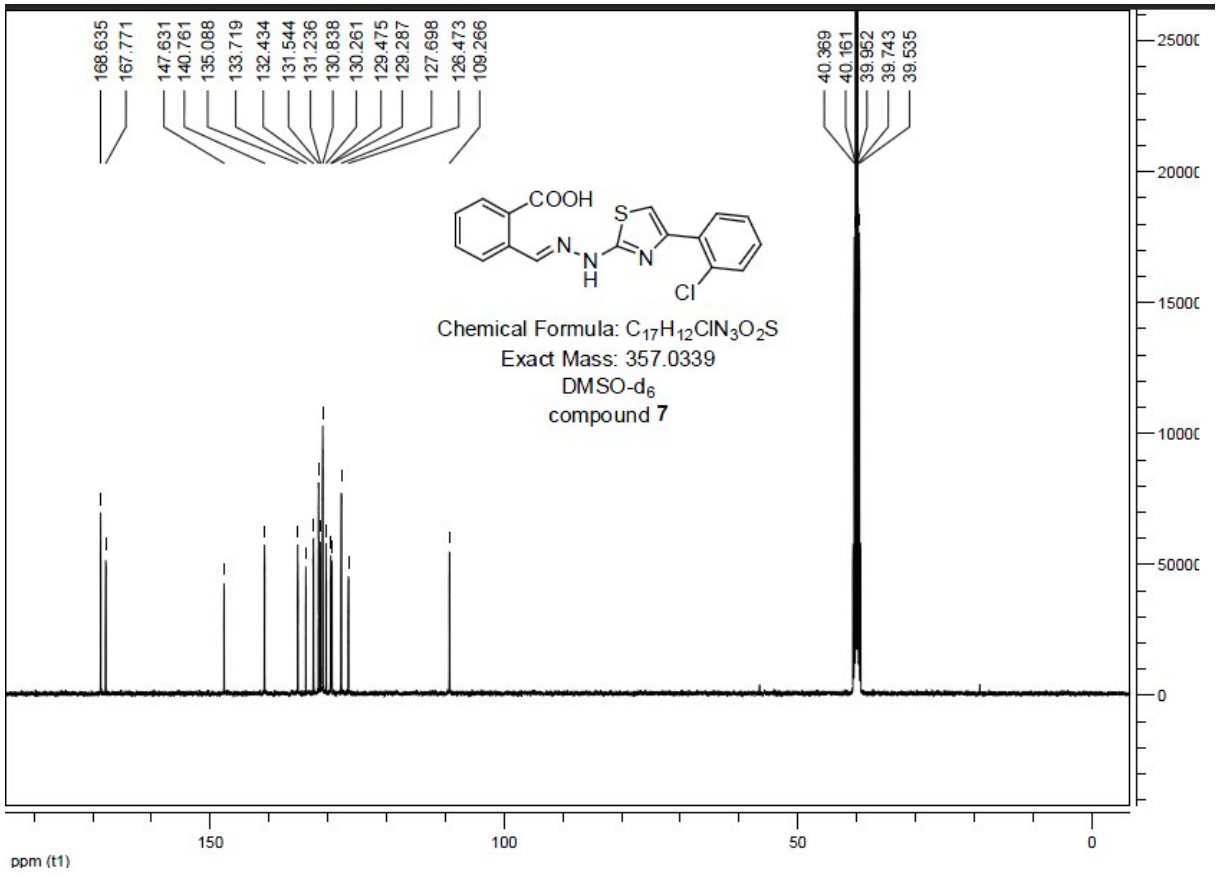
ZWP-SWL-084 4 (0.200) Cm (1:6) 1: TOF MS ES+ 1.55e+003



Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
338.0954	338.0963	-0.9	-2.7	12.5	6.0	0.0	C18 H16 N3 O2 S

Compound **7**



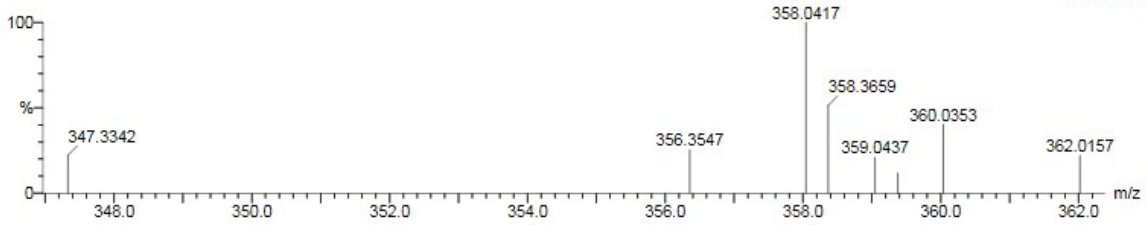


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

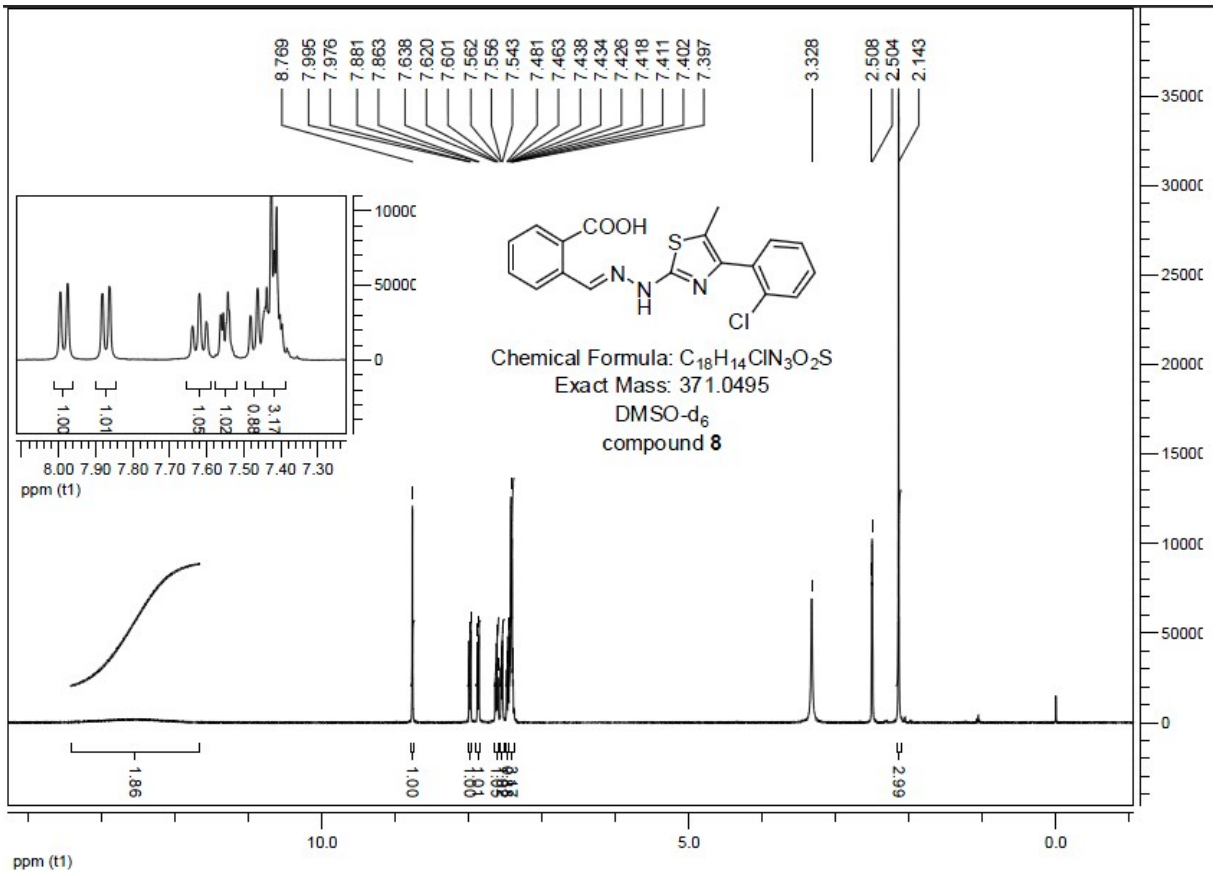
Monoisotopic Mass, Even Electron Ions
86 formula(e) evaluated with 2 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
C: 0-20 H: 0-50 N: 0-3 O: 0-2 S: 0-1 Cl: 0-1

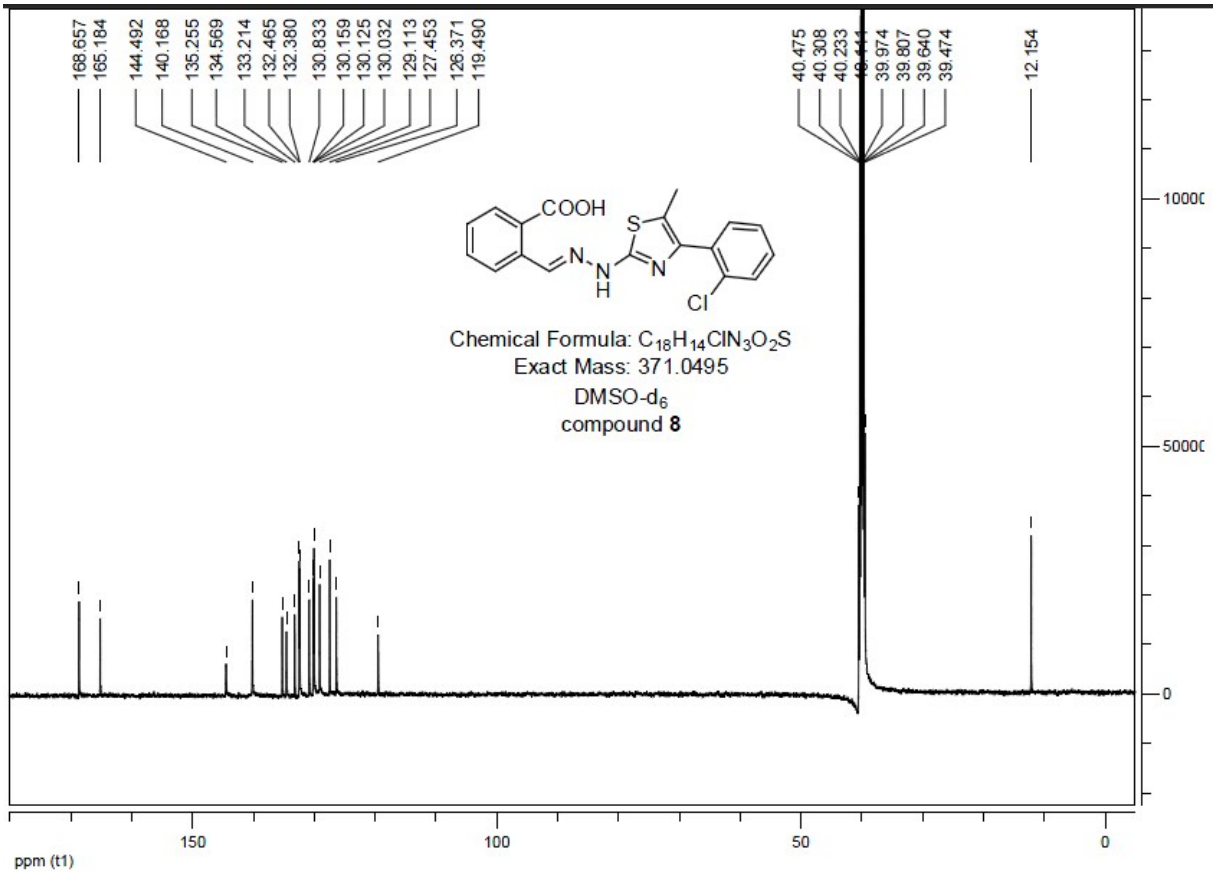
WP-ZHU ECUST institute of Fine Chem 30-Mar-2015 22:05:45
ZWP-SWL-112 13 (0.497) Cm (13:14) 1: TOF MS ES+ 2.87e+002



Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
358.0417	358.0417	0.0	0.0	12.5	23.1	0.0	C17 H13 N3 O2 S Cl

Compound **8**


8.769
7.995
7.976
7.881
7.863
7.638
7.620
7.601
7.562
7.556
7.543
7.481
7.463
7.438
7.434
7.426
7.418
7.411
7.402
7.397
3.328
2.508
2.504
2.143
COOH
S
N
N
H
N
Cl
Chemical Formula: $C_{18}H_{14}ClN_3O_2S$
Exact Mass: 371.0495
DMSO-d_6
compound **8**
1.00
1.01
1.05
1.02
0.88
3.17
8.00 7.90 7.80 7.70 7.60 7.50 7.40 7.30
ppm (t1)
1.86
1.00
2.99
10.0
5.0
0.0
ppm (t1)

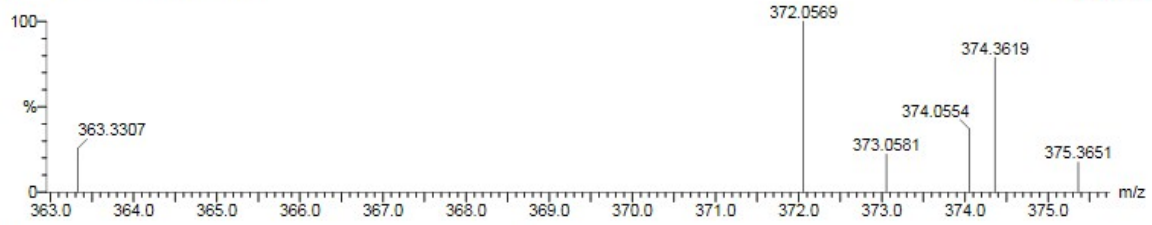

168.657
165.184
144.492
140.168
135.255
134.569
133.214
132.465
132.380
130.833
130.159
130.125
130.032
129.113
127.453
126.371
119.490
40.475
40.308
40.233
39.974
39.807
39.640
39.474
12.154
COOH
S
N
N
H
N
Cl
Chemical Formula: $C_{18}H_{14}ClN_3O_2S$
Exact Mass: 371.0495
DMSO-d_6
compound **8**
150
100
50
0
ppm (t1)

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

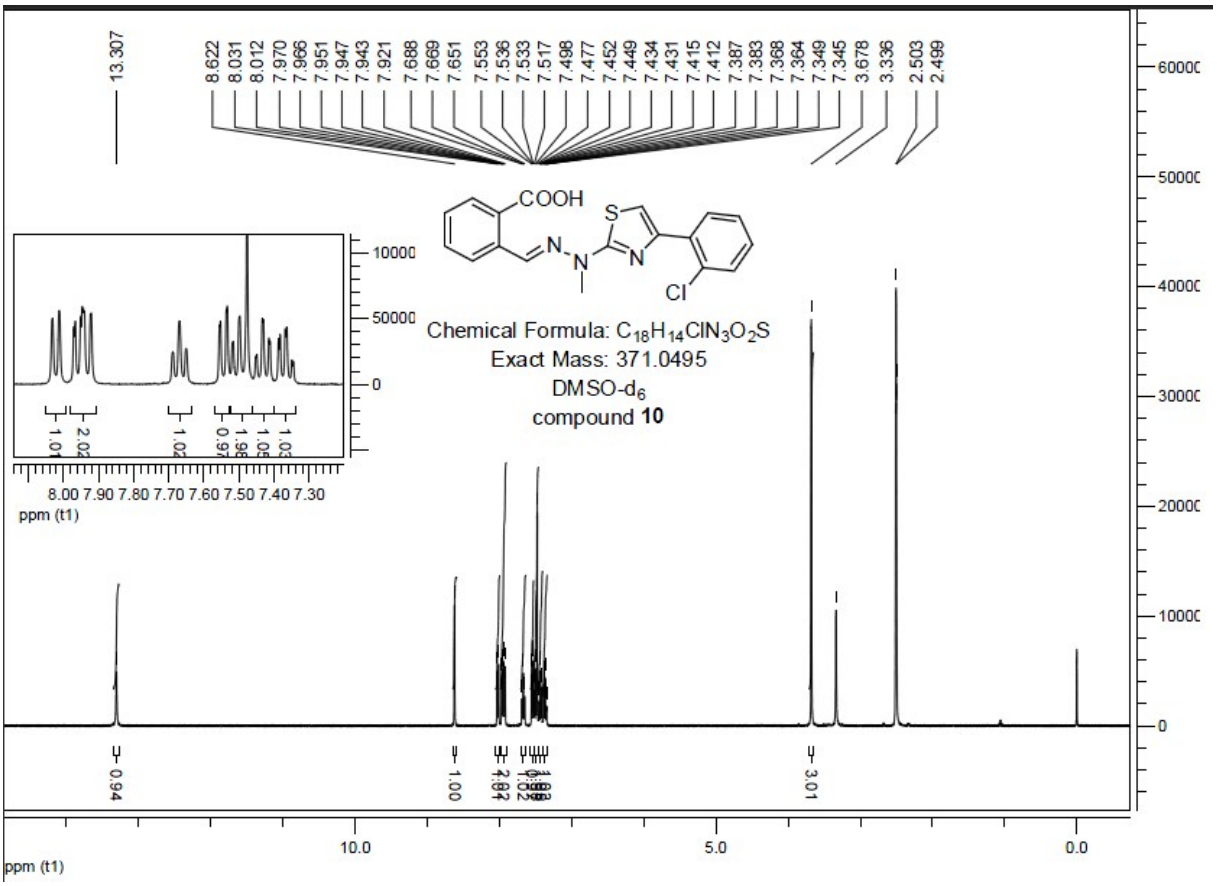
Monoisotopic Mass, Even Electron Ions
68 formula(e) evaluated with 1 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
C: 0-20 H: 0-50 N: 0-3 O: 0-2 S: 0-1 Cl: 0-1

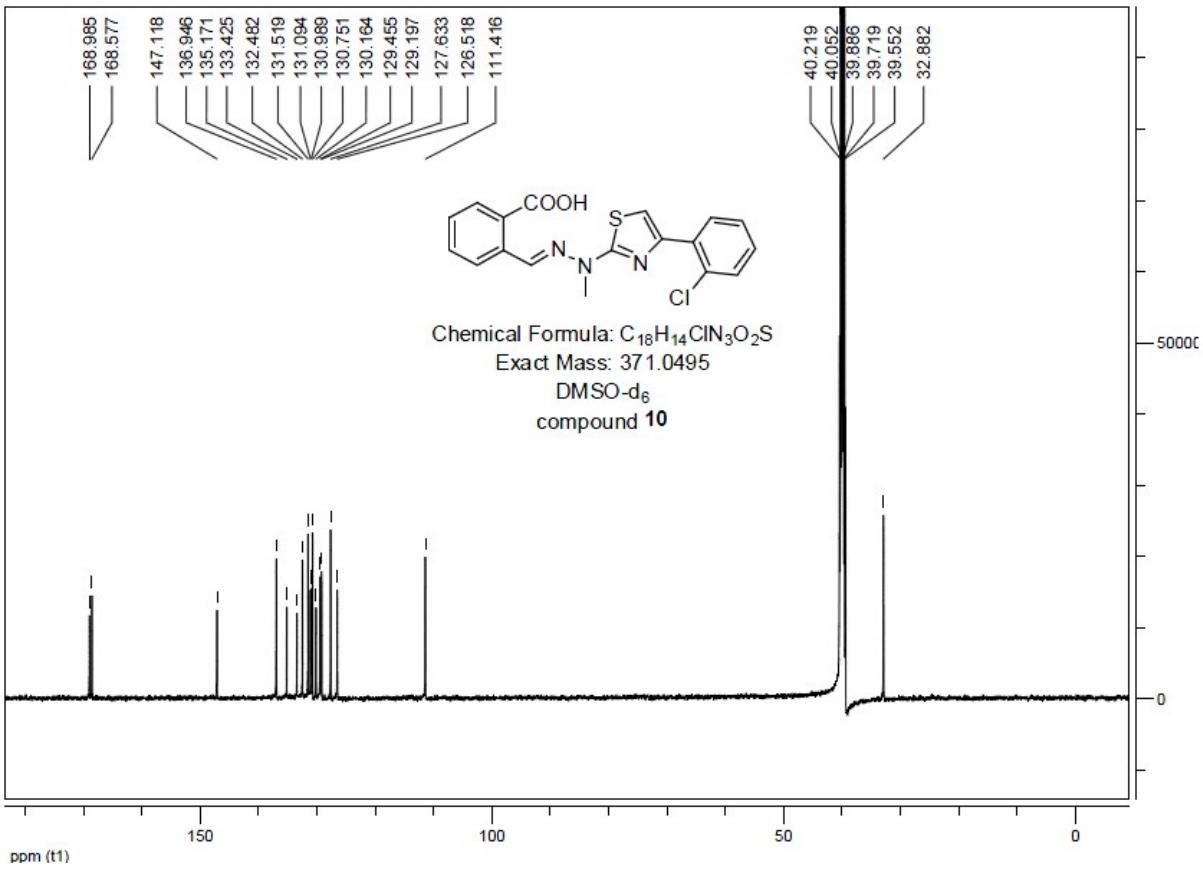
WP-ZHU ECUST institute of Fine Chem 30-Mar-2015 22:08:23
ZWP-SWL-111 4 (0.200) Cm (4:12) 1: TOF MS ES+ 1.60e+003



Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
372.0569	372.0574	-0.5	-1.3	12.5	7.3	0.0	C18 H15 N3 O2 S Cl

Compound **10**





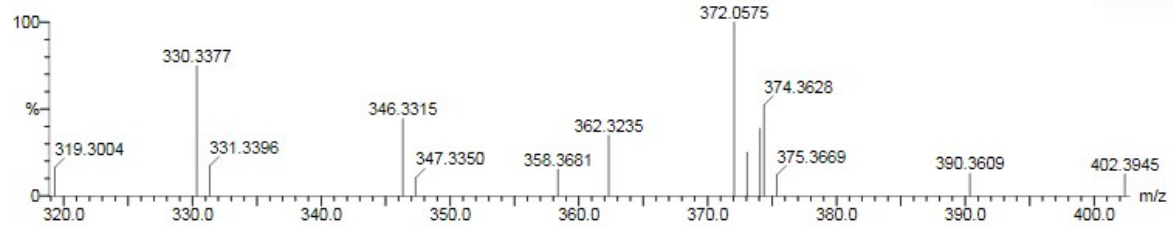
Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
68 formula(e) evaluated with 1 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
C: 0-20 H: 0-50 N: 0-3 O: 0-2 S: 0-1 Cl: 0-1

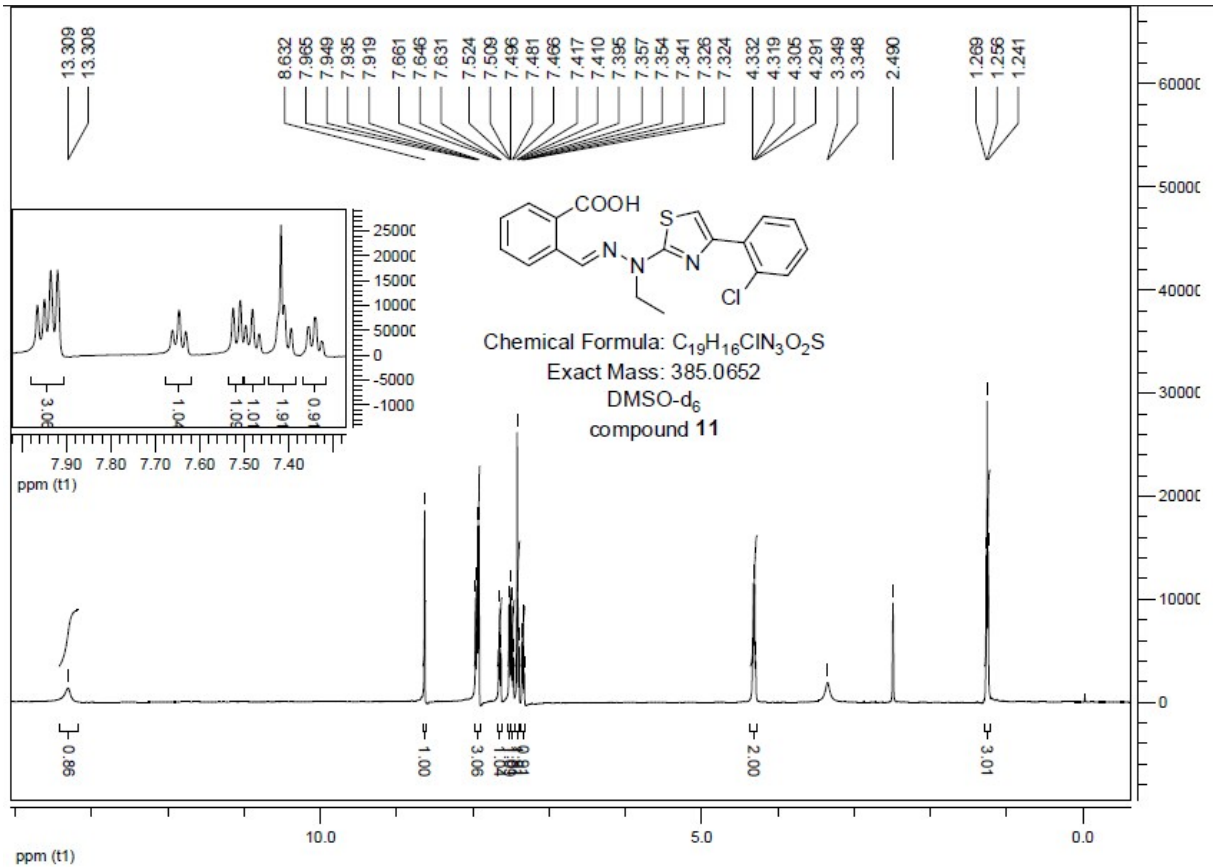
WP-ZHU ECUST institute of Fine Chem 30-Mar-2015 22:02:44

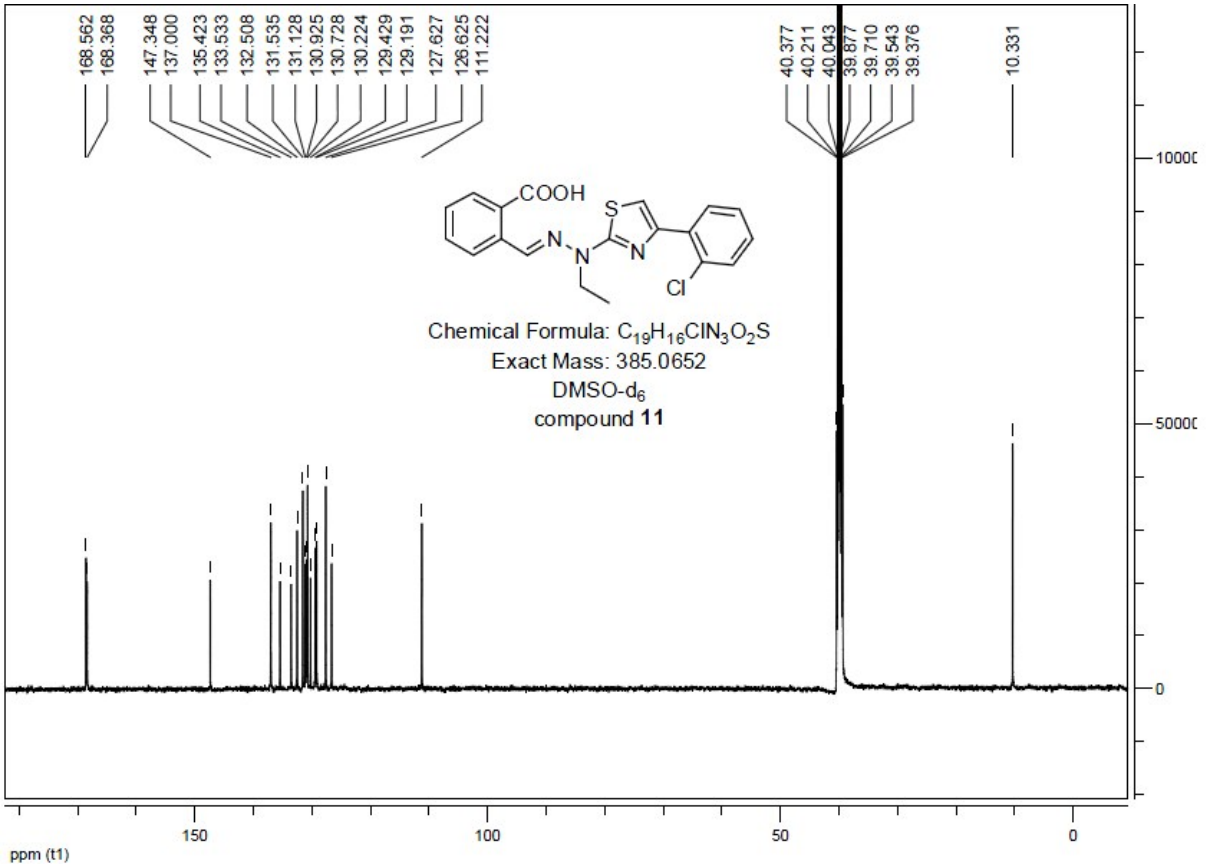
ZWP-SWL-127 12 (0.445) Cm (9:12) 1: TOF MS ES+ 1.29e+003



Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
372.0575	372.0574	0.1	0.3	12.5	7.1	0.0	C18 H15 N3 O2 S Cl

Compound **11**


13.309
13.308
8.632
7.965
7.949
7.935
7.919
7.661
7.646
7.631
7.524
7.509
7.496
7.481
7.466
7.417
7.410
7.395
7.357
7.354
7.341
7.326
7.324
4.332
4.319
4.305
4.291
3.349
3.348
2.490
1.269
1.256
1.241
COOH
S
N
N
N
Cl
Chemical Formula: $C_{19}H_{16}ClN_3O_2S$
Exact Mass: 385.0652
DMSO-d_6
compound **11**
ppm (t1)
10.0
5.0
0.0


168.562
168.368
147.348
137.000
135.423
133.533
132.508
131.535
131.128
130.925
130.728
130.224
129.429
129.191
127.627
126.625
111.222
40.377
40.211
40.043
39.877
39.710
39.543
39.376
10.331
COOH
S
N
N
N
Cl
Chemical Formula: $C_{19}H_{16}ClN_3O_2S$
Exact Mass: 385.0652
DMSO-d_6
compound **11**
ppm (t1)
150
100
50
0

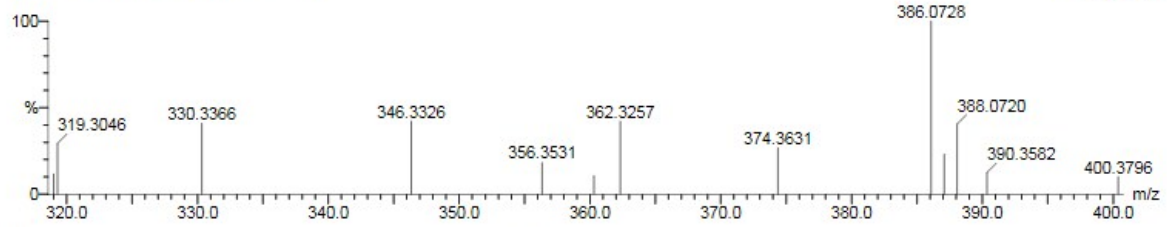
Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
41 formula(e) evaluated with 1 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
C: 0-19 H: 0-17 N: 0-3 O: 0-2 S: 0-1 Cl: 0-1

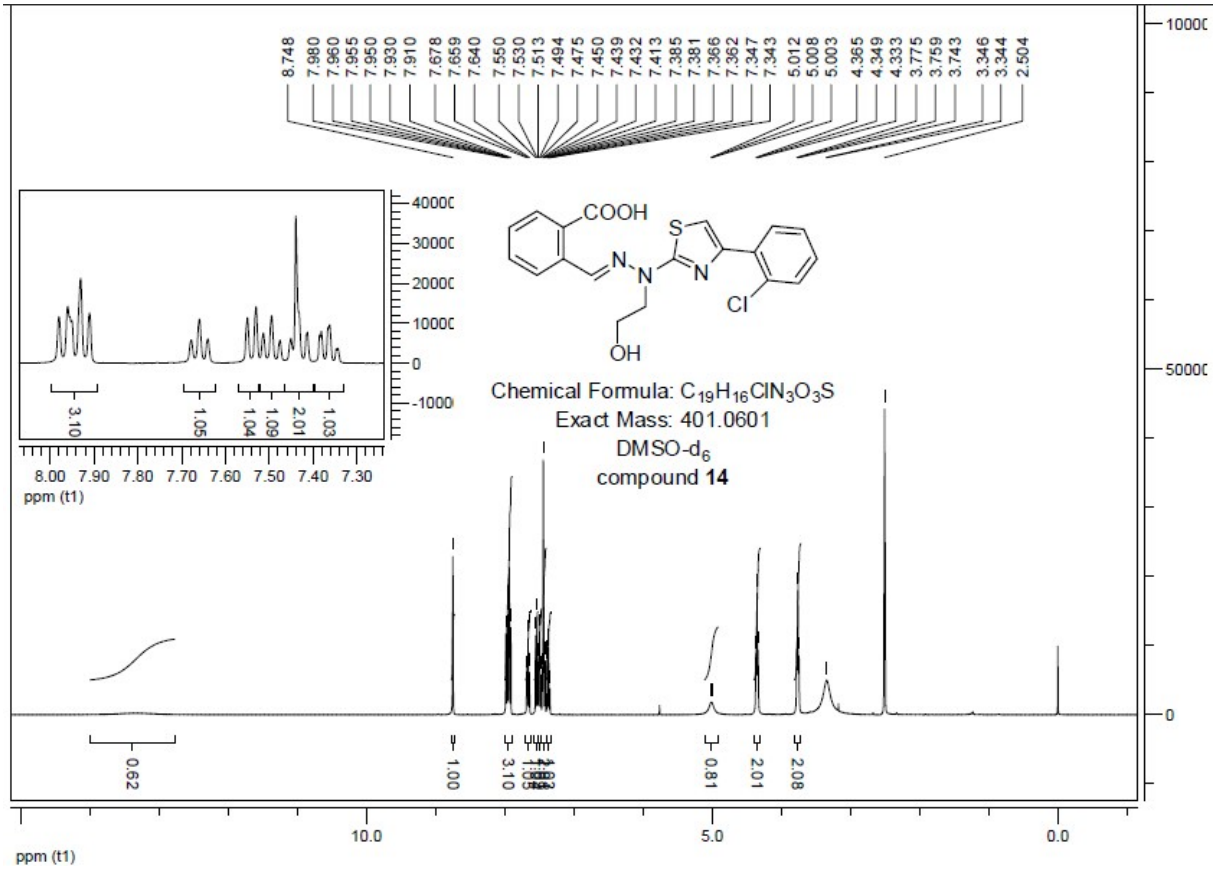
XH-QIAN ECUST institute of Fine Chem 19-Apr-2015 10:12:15

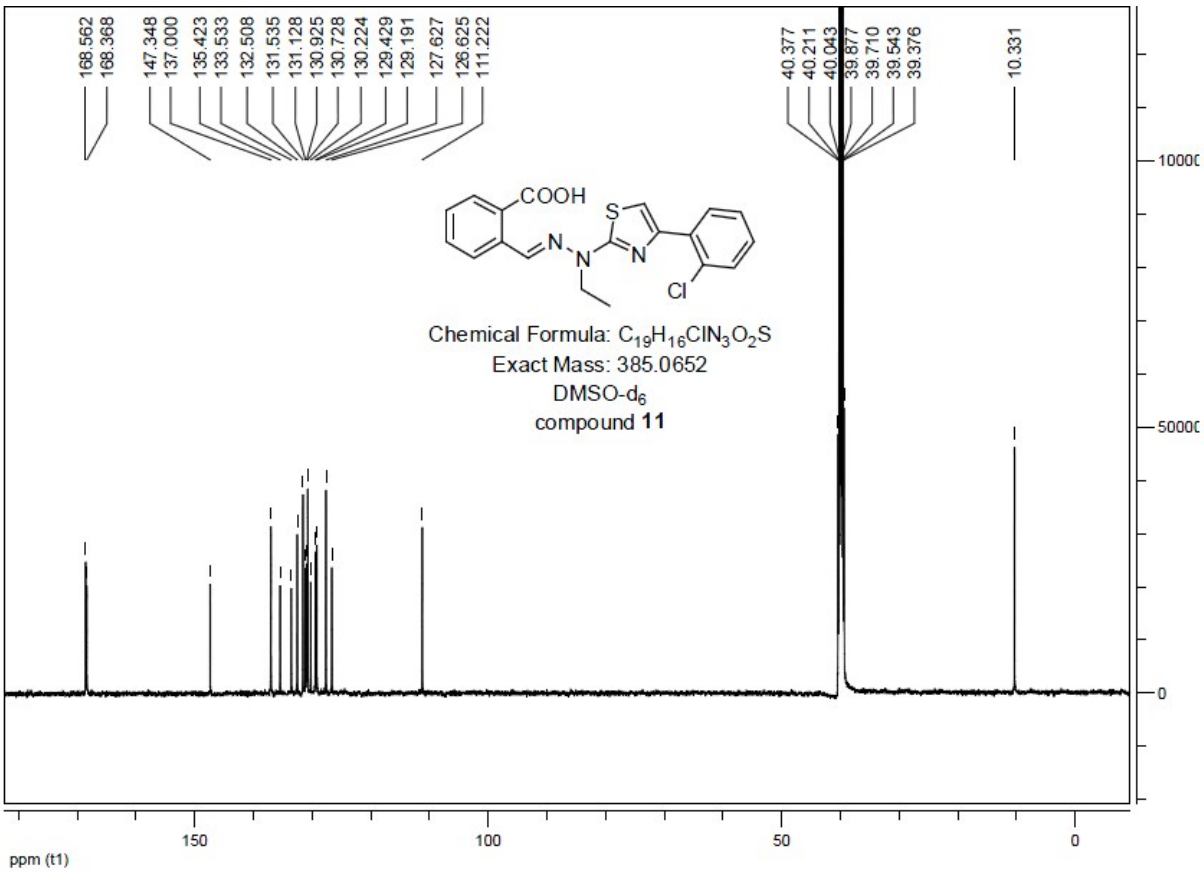
ZWP-SWL-149 185 (1.235) Cm (182:187) 1: TOF MS ES+ 7.45e+003



Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
386.0728	386.0730	-0.2	-0.5	12.5	6.6	0.0	C19 H17 N3 O2 S Cl

Compound **14**





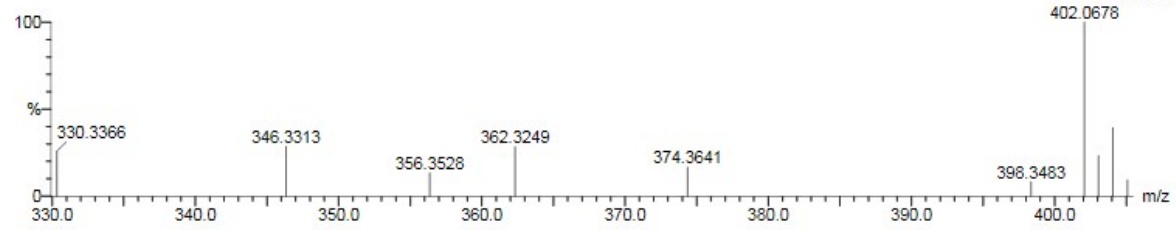
Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
57 formula(e) evaluated with 1 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
C: 0-19 H: 0-17 N: 0-3 O: 0-3 S: 0-1 Cl: 0-1

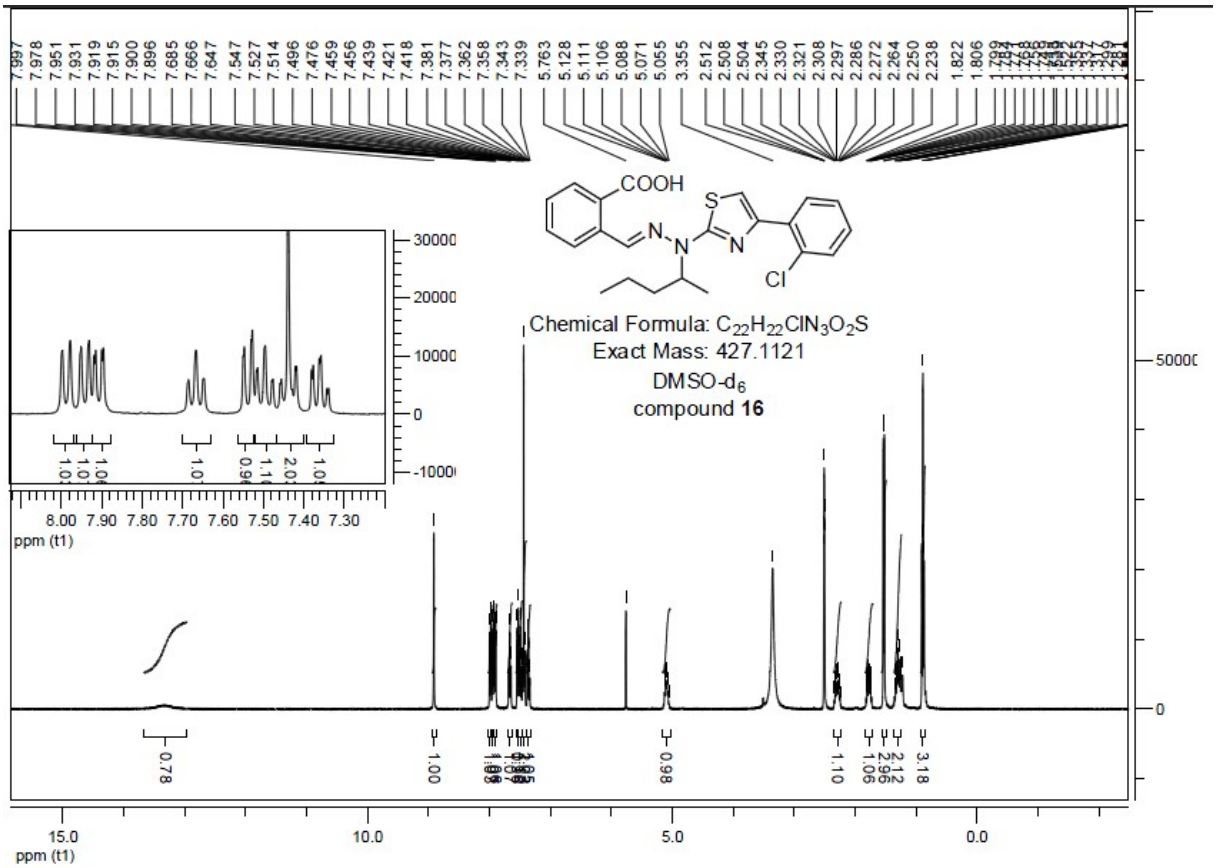
XH-QIAN ECUST institute of Fine Chem 19-Apr-2015 10:28:28

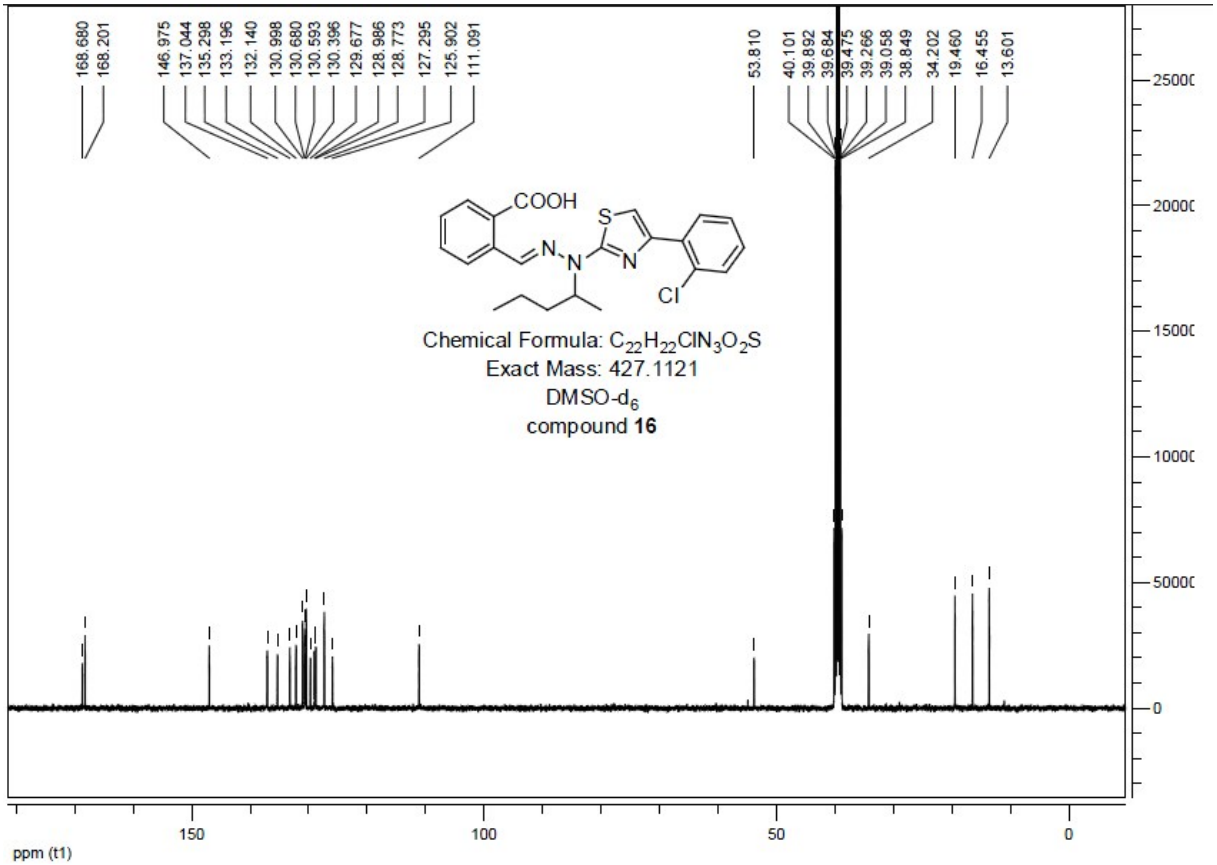
ZWP-SWL-158 23 (0.234) Cm (20:27) 1: TOF MS ES+ 6.89e+003



Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
402.0678	402.0679	-0.1	-0.2	12.5	6.7	0.0	C19 H17 N3 O3 S Cl

Compound 16


Chemical Formula: $C_{22}H_{22}ClN_3O_2S$
Exact Mass: 427.1121
DMSO-d_6
compound **16**
ppm (t1)


Chemical Formula: $C_{22}H_{22}ClN_3O_2S$
Exact Mass: 427.1121
DMSO-d_6
compound **16**
ppm (t1)

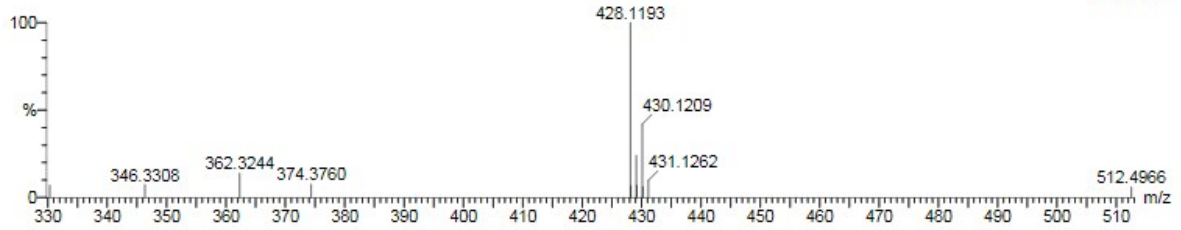
Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
41 formula(e) evaluated with 1 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-22 H: 0-23 N: 0-3 O: 0-2 S: 0-1 Cl: 0-1

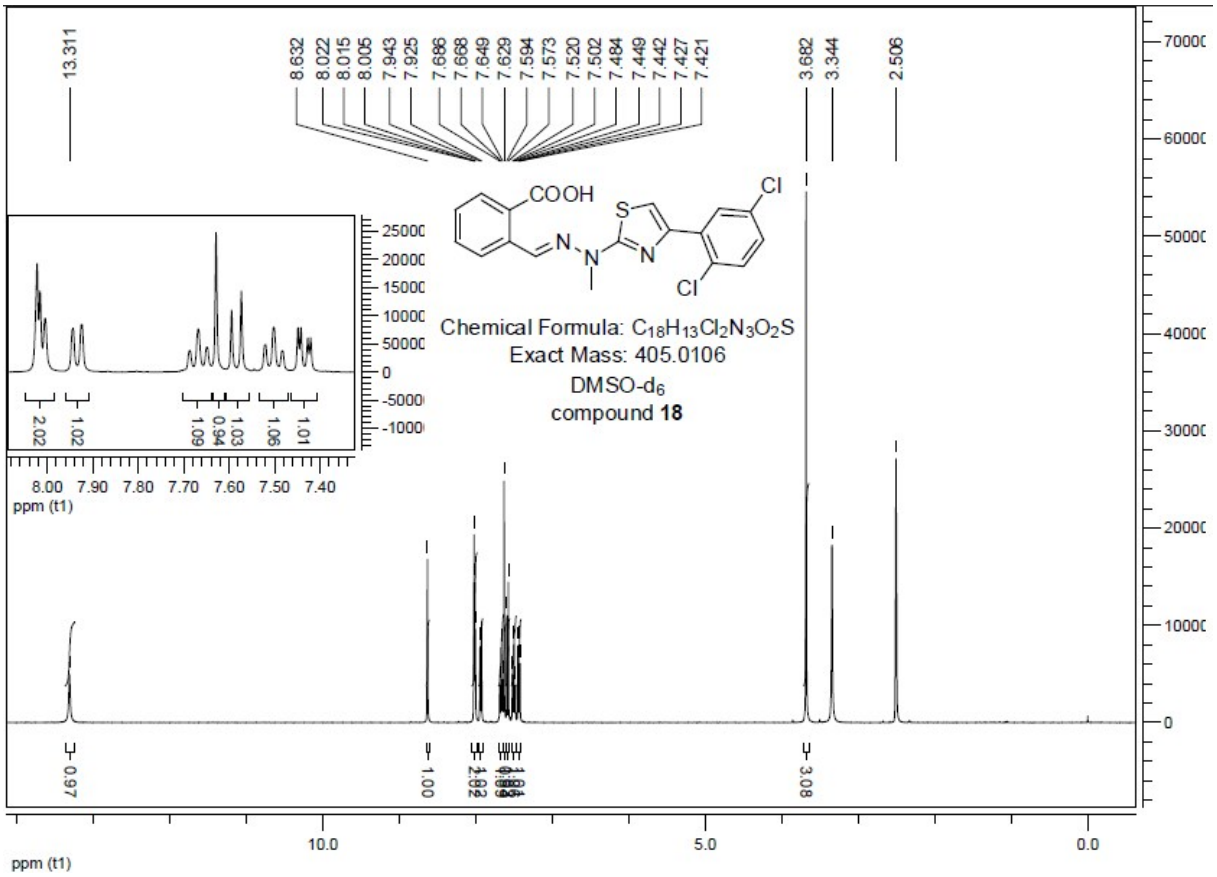
XH-QIAN ECUST institute of Fine Chem 20-Jul-2015 14:36:40

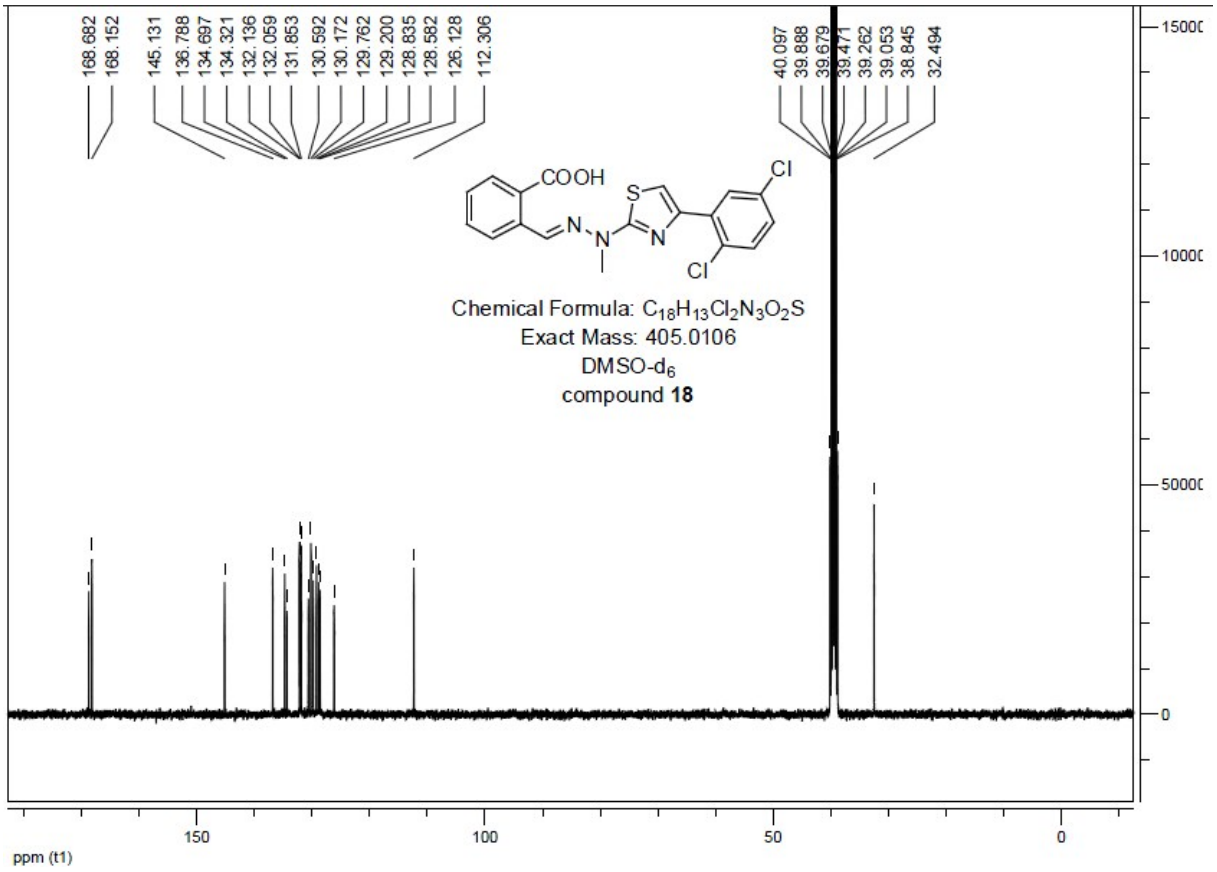
ZWP-SWL-192 160 (1.079) Cm (159:160) 1: TOF MS ES+ 2.44e+003



Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
428.1193	428.1200	-0.7	-1.6	12.5	32.0	0.0	C22 H23 N3 O2 S Cl

Compound **18**



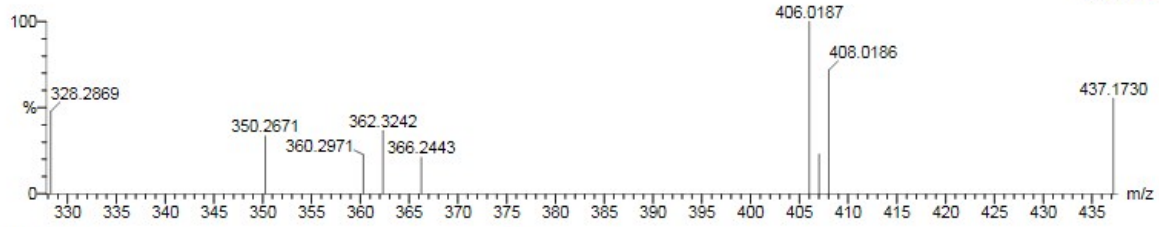


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

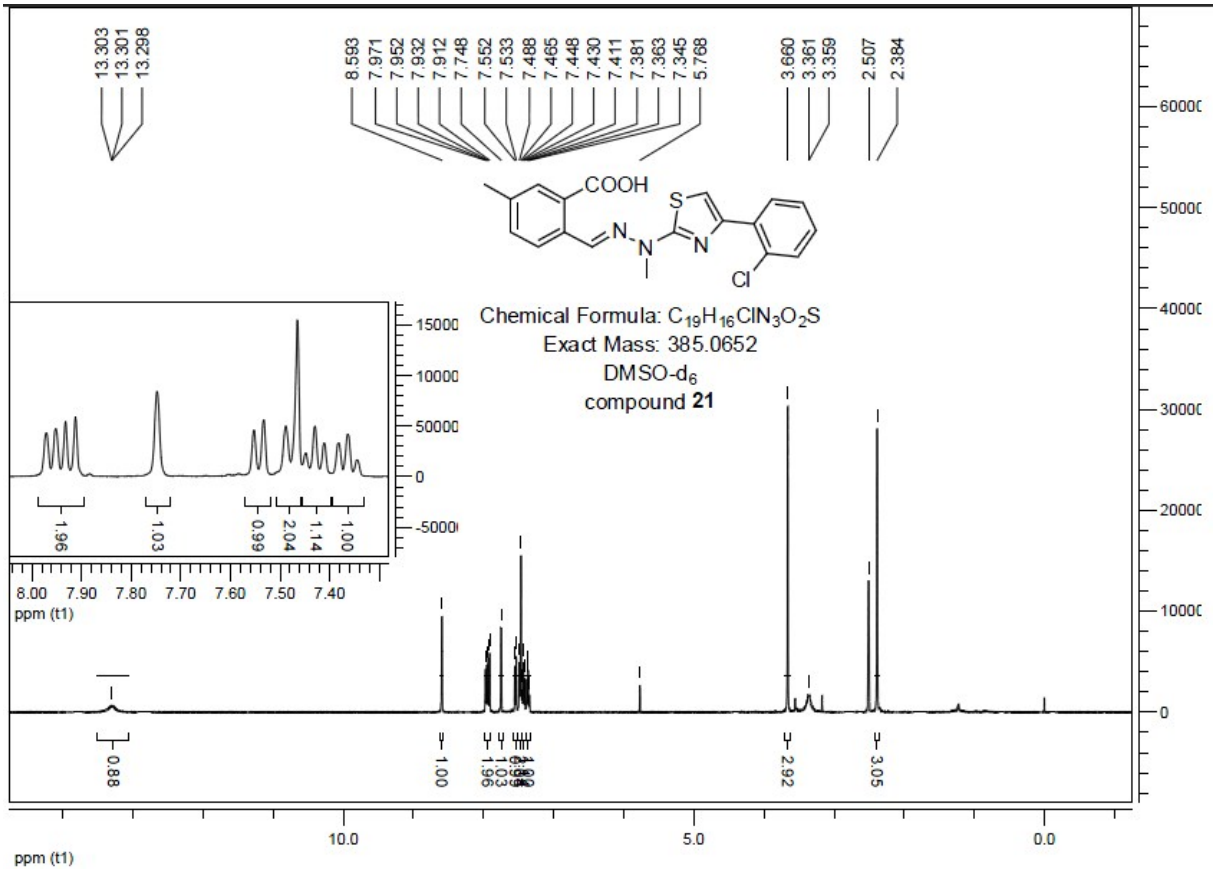
Monoisotopic Mass, Even Electron Ions
83 formula(e) evaluated with 1 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-20 H: 0-43 N: 0-3 O: 0-2 S: 0-1 Cl: 0-2

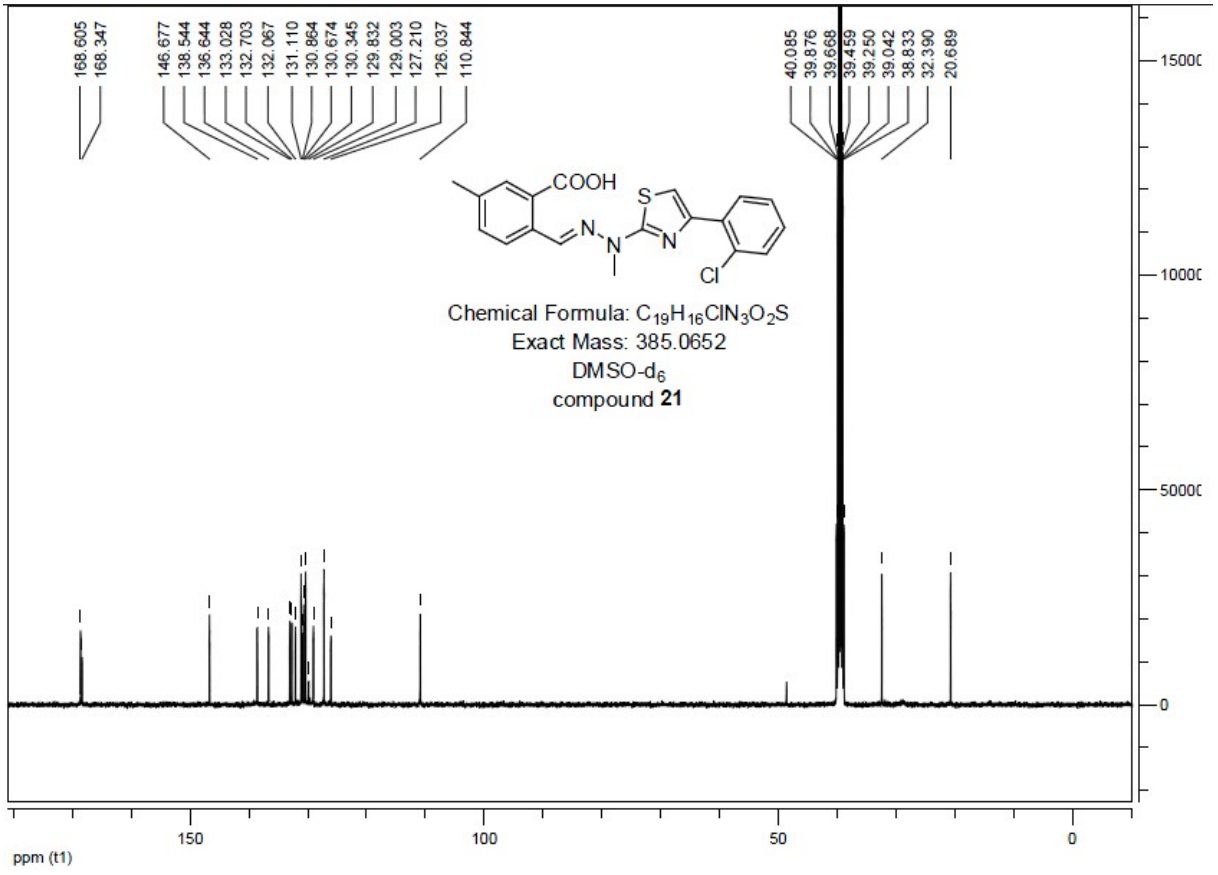
WP-ZHU ECUST institute of Fine Chem 21-Sep-2015 19:50:30
ZWP-SWL-206 51 (1.650) Cm (51:56) 1: TOF MS ES+ 1.76e+003



Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
406.0187	406.0184	0.3	0.7	12.5	7.3	0.0	C18 H14 N3 O2 S Cl2

Compound **21**


13.303
13.301
13.298
8.593
7.971
7.952
7.932
7.912
7.748
7.552
7.533
7.488
7.465
7.448
7.430
7.411
7.381
7.363
7.345
5.768
3.660
3.361
3.359
2.507
2.384
COOH
S
N
N
N
Cl
Chemical Formula: C19H16ClN3O2S
Exact Mass: 385.0652
DMSO-d6
compound 21
ppm (t1)


168.605
168.347
146.677
138.544
136.644
133.028
132.703
132.067
131.110
130.864
130.674
130.345
129.832
129.003
127.210
126.037
110.844
40.085
39.876
39.668
39.459
39.250
39.042
38.833
32.390
20.689
COOH
S
N
N
N
Cl
Chemical Formula: C19H16ClN3O2S
Exact Mass: 385.0652
DMSO-d6
compound 21
ppm (t1)

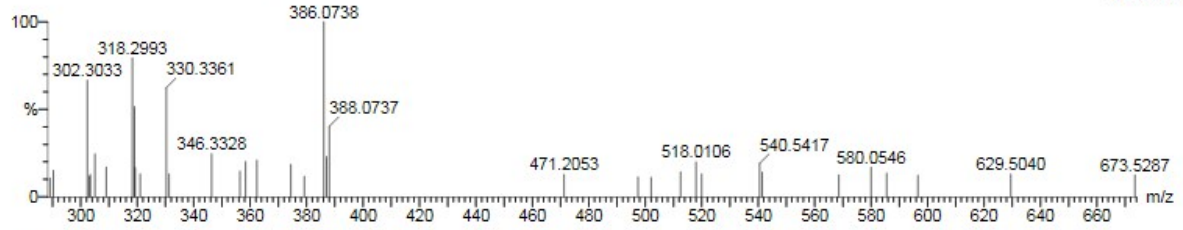
Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
41 formula(e) evaluated with 1 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-19 H: 0-17 N: 0-3 O: 0-2 S: 0-1 Cl: 0-1

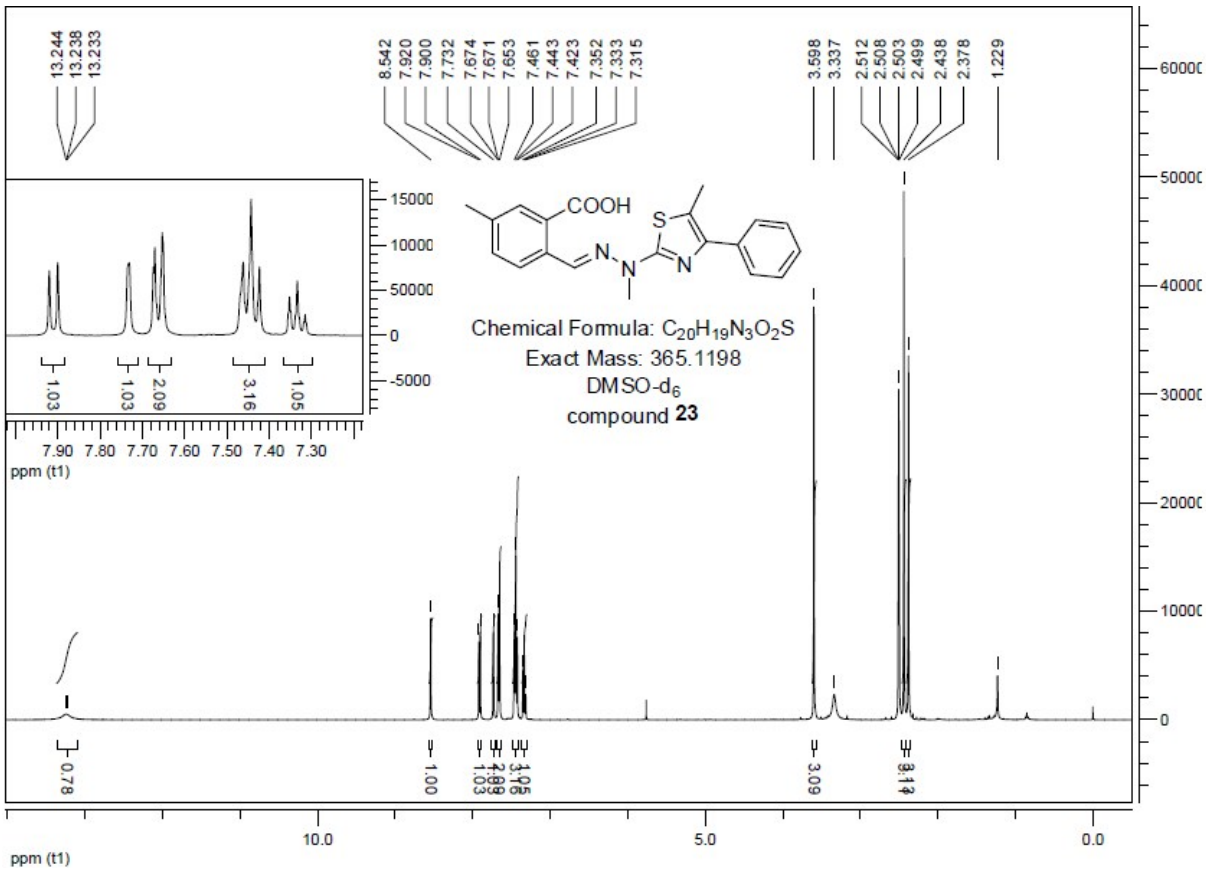
WP-ZHU ECUST institute of Fine Chem 03-Jun-2015 20:45:37

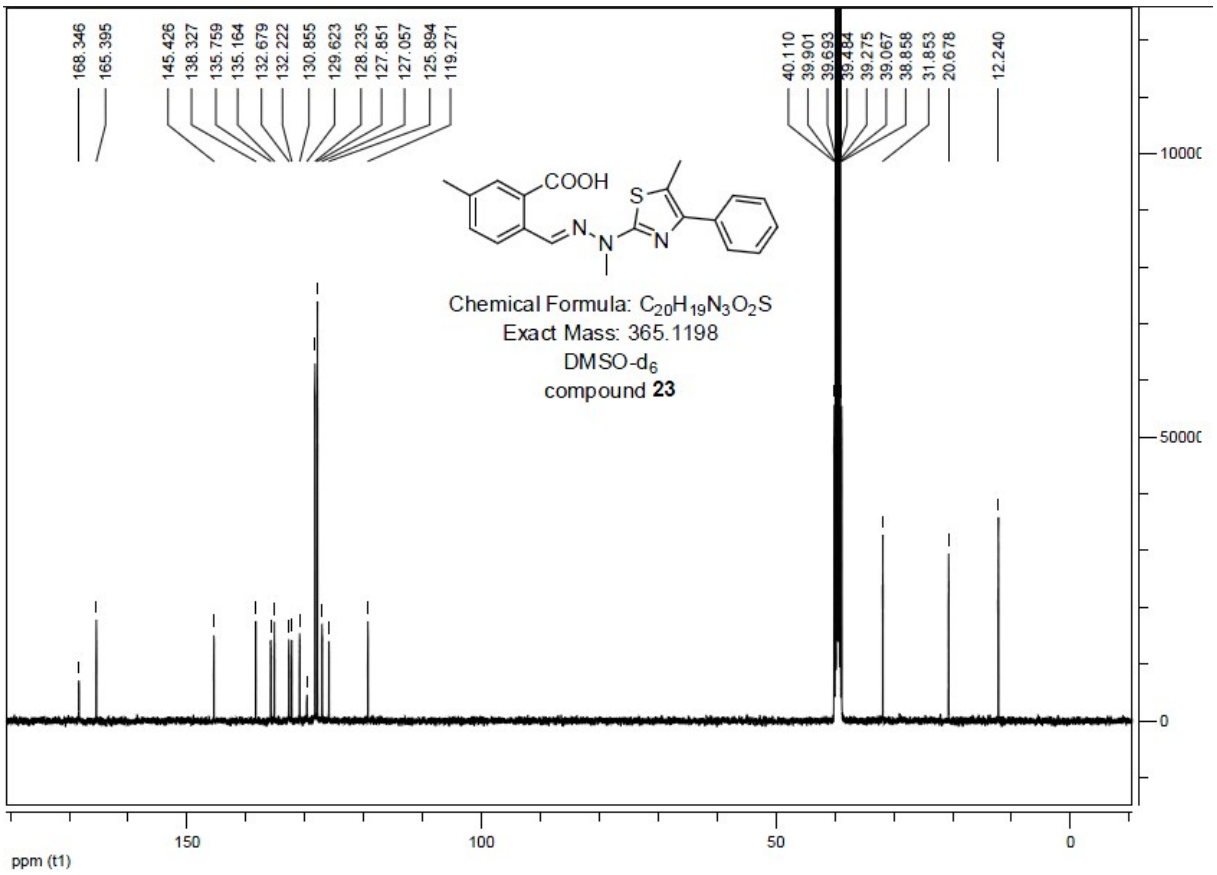
ZWP-SWL-179 83 (0.608) Cm (80:83) 1: TOF MS ES+ 1.63e+003



Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
386.0738	386.0730	0.8	2.1	12.5	6.6	0.0	C19 H17 N3 O2 S Cl

Compound **23**



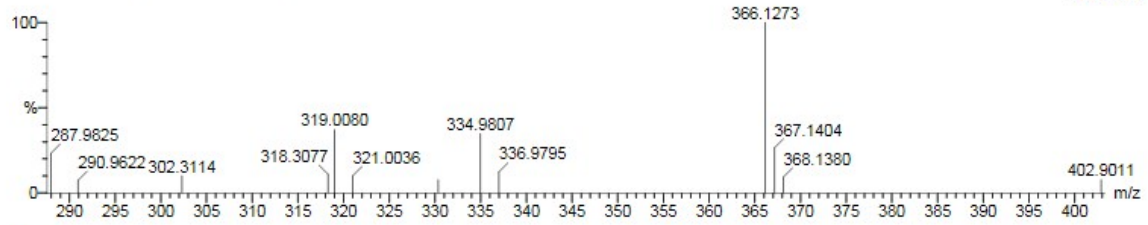


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
28 formula(e) evaluated with 1 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-20 H: 0-100 N: 0-3 O: 0-2 S: 0-1

WP-ZHU ECUST institute of Fine Chem 23-Jun-2015 22:09:01
ZWP-SWL-187 54 (0.422) Cm (54:56) 1: TOF MS ES+ 1.38e+004



Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
366.1273	366.1276	-0.3	-0.8	12.5	17.1	0.0	C20 H20 N3 O2 S