Electronic Supplementary Material (ESI) for RSC Advances

Efficient microwave-assisted preparation of squaric acid monoamides in water

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Supporting Information

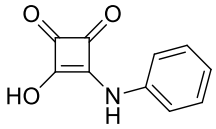
General information. All reagents were purchased commercially and used without further isolation. Mass spectra were measured on a MICROMASS Autospec3000 spectrometer equipped with an electrospray module. ^{1}H and ^{13}C spectra were recorded on Bruker AVANCE 300 (^{1}H at 300 MHz and ^{13}C at 75 MHz) in DMSO-*d6* using the residual proton signal as reference. Chemical shifts (δ) are in ppm and coupling constants (J) in Hz. The microwave-assisted reactions were carried out in a Biotage Initiator Microwave Synthesizer (microwave power supply 0-400 W, 1 W increments, IR temperature sensor, open or closed vessel mode, pressure range 0-20 bar, 0.5-2 mL vial). pH values were measured with a Knick 766 Calimatic pH-meter. UV-Vis spectra were acquired on a Varian Cary 300Bio spectrophotometer. IR spectra were recorded on (FT-IR) Bruker IFS66 spectrophotometer.

General procedure for the microwave-assisted synthesis of squaric acid monoamides.

A mixture of squaric acid (0.5 mmol) and the amine (the molar ratio depends on the amine used) in 1.5 mL of water (milliQ) was placed, with a magnetic stirring bar, into 0.5-2 mL vial and sealed. The reaction mixture was heated in the microwave reactor. The time and the temperature reaction vary depending on the amine used. After cooling down to 50 ºC via gas jet cooling (compressed air), the vial was removed from the microwave reactor and the product was isolated and purified as described below.

3-phenylamino-4-hydroxycyclobut-3-ene-1,2-dione (5a).[1]
Reaction conditions for microwave irradiation: squaric acid (57 mg, 0.5 mmol) and aniline (91 µL, 1.0 mmol) at 120 ºC for 15 min

Product isolation: the reaction mixture was transferred from the vial to an Erlenmeyer flask and diluted with 25 mL water. 1M NaOH was added dropwise until pH 10. The basic aqueous solution extracted three times with Et_2O discarding the organic extracts. After acidification to pH 1 with 1M HCl, crystallization took place after hours, sometimes being necessary to concentrate the solution. Finally, the solid was filtered off and dried affording **5a** (79 mg, 83%) as a pale yellow solid.

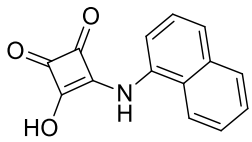


5a

Spectroscopic characterization of this product has already been reported.[1]

3-(1-naphthylamine)-4-hydroxycyclobut-3-ene-1,2-dione (5b).[2]
Reaction conditions for microwave irradiation: squaric acid (57 mg, 0.5 mmol) and 1-naphthylamine (24 mg, 0.25 mmol) at 120 ºC for 30 min.

Product isolation: the reaction mixture was diluted with 0.5 M NaOH (20 mL) and washed four times with 10 mL of CH_2Cl_2, discarding the organic extracts. The aqueous solution was filtered and then, acidified with 3M HCl (8mL). The resulting aqueous suspension was extracted with ethyl acetate (5×15 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to dryness. The product **5b** (41 mg, 64%) was obtained as ochre solid.

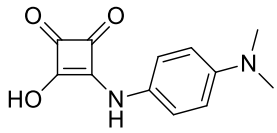


5b

Mp 214-217 ºC dec. λ_{max} (H_2O)/nm 216, 273 and 324 (ε/dm^{3}mol^{-1}cm^{-1} 28 000, 11 700 and 11 000). λ_{max} (MeCN)/nm 220, 260 and 355 (ε/dm^3 mol^{-1} cm^{-1} 44 100, 15 000 and 15 100). IR: ν_{max}/cm^{-1} 3266 (NH), 2684br (OH), 1829 and 1808 (CO). ^{1}H NMR: δ_H(300 MHz; DMSO-*d6*) 10.58 (1H, s, NH), 8.14 (1H, br m, Ar), 7.96 (1H, br m, Ar), 7.77 (1H, d, Ar), 7.57 (2H, m, Ar), 7.50 (1H, t, Ar), 7.36 (1H, d, Ar). ^{13}C NMR[2]: δ_C (75 MHz; DMSO-*d6*,): 190.4, 187.6, 184.8, 172.3, 133.6, 133.2, 128.1, 127.1, 126.4, 126.0, 125.5, 125.4, 122.8, and 120.3. HRMS ESI(+), calc. for $C_{28}H_{18}N_2O_6Na$ $[2M+Na]^+$ 501.1080; found 501.1063.

3-(4-(N,N-dimethylamino)phenylamino)-4-hydroxycyclobut-3-ene-1,2-dione (5c).[3]
Reaction conditions for microwave irradiation: squaric acid (57 mg, 0.5 mmol) and N,N-dimethylamino-1,4-phenylendiamine (136 mg, 1.0 mmol) at 150 ºC for 20 min.

Product isolation: the reaction mixture was diluted with 10 mL of water and filtered. The resulting greenish solid was washed with water (3×5 mL) and dried. The product **5c** (77 mg, 66%) was obtained as a pale green solid.

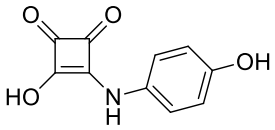


5c

Characterization have been reported previously.[3]

3-(*p*-hydroxyphenylamino)-4-hydroxycyclobut-3-ene-1,2-dione (5d).[4]
Reaction conditions for microwave irradiation: squaric acid (57 mg, 0.5 mmol) and 4-aminophenol (11 mg, 0.1 mmol) at 130 ºC for 15 min.

Product isolation: the reaction mixture was diluted with 15 mL of 5% $NaHCO_3$ and extracted with Et_2O (3×10 mL) discarding the organic extracts. The aqueous solution was acidified with 3N HCl (5 mL), saturated with NaCl and extracted with AcOEt (5×15 mL). The combined organic layers were dried with anhydrous Na_2SO_4 and concentrated to dryness. The product **5d** (17 mg, 82 %) was obtained as a brown solid.

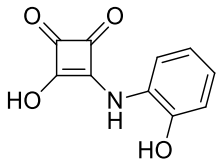


5d

Mp 255-260 ºC dec. λ_{max} (H_2O)/nm 217, 274 and 304 (ε/dm^3 mol^{-1} cm^{-1} 10 300, 15 500 and 21 600). λ_{max} (MeCN)/nm 267, and 316 (ε/dm^3 mol^{-1} cm^{-1} 13 100, and 24 200). IR: ν_{max}/cm^{-1} 3341 (NH), 2652br (ArOH), 2652br (OH), 1818 (CO). ^{1}H NMR: δ_H(300 MHz; DMSO-*d6*) 10.24 (1H, br s, NH), 7.20 (2H, d, J = 4.5, Ar(o)H), 6.71 (2H, d, J = 4.5, Ar(m)H). ^{13}C NMR: δ_C (75 MHz; DMSO-*d6*,): 190.4, 186.0, 184.0, 170.3, 154.0, 130.1, 120.9 and 115.4. HRMS ESI(+), calc. for $C_{20}H_{14}N_2O_8Na$ $[2M+Na]^+$ 433.0649; found 433.0648.

3-(*o*-hydroxyphenylamino)-4-hydroxycyclobut-3-ene-1,2-dione (5e).[4]
Reaction conditions for microwave irradiation: squaric acid (57 mg, 0.5 mmol) and 2-aminophenol (27 mg, 0.25 mmol) at 130 ºC for 15 min.

Product isolation: the reaction mixture was diluted with 15 mL of 5% $NaHCO_3$ and extracted with Et_2O (5×10 mL) discarding the organic extracts. The aqueous solution was acidified with 3N HCl (5 mL), saturated with NaCl and extracted with AcOEt (5×15 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to dryness. The product **5e** (40 mg, 78 %) was obtained as a brown solid.

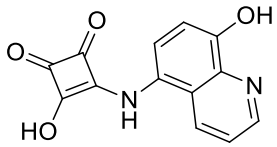


5e

Mp >225 ºC dec. λ_{max} (H_2O)/nm 275 and 313 (ε/dm^3 mol^{-1} cm^{-1}, 16 800 and 20 400). IR: ν_{max}/cm^{-1} 3300 (NH), 3200-2800br (ArOH), 2800-2550br (OH), 1813 (CO). ^{1}H NMR: δ_H (300 MHz; DMSO-*d6*) 9.90 (1H, br s, NH), 9.31 (1H, s, OH), 7.48 (1H, d, J = 8.1, Ar(o)H), 6.94+6.87+6.78 (3H, m, ArH). ^{13}C NMR: δ_C (75 MHz; DMSO-*d6*,): 186.6, 184.3, 171.4, 148.2, 125.9, 125.0, 121.6, 119.1, and 115.5. HRMS ESI(+), calc. for $C_{20}H_{14}N_2O_8Na$ $[2M+Na]^+$ 433.0648; found 433.0656.

3-hydroxy-4-((8-hydroxyquinolin-5-yl)amino)cyclobut-3-ene-1,2-dione (5f).
Reaction conditions for microwave irradiation: squaric acid (57 mg, 0.5 mmol) and 5-amino-8-hydroxyquinoline dihydrochloride (117 mg, 0.5 mmol) at 120 ºC for 15 min.

Product isolation: the reaction mixture was diluted in 10 mL of water and filtered. The resulting solid was washed three times with 10 mL of water and dried. The product **5f** (122 mg, 95%) was obtained as a dark red solid.

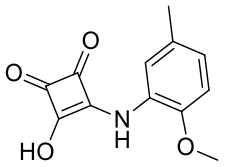


5f

Mp >210 ºC dec. IR: ν_{max}/cm^{-1} 3419 (NH), 2635br (OH), 1800 (CO). ^{1}H NMR: δ_H(300 MHz; DMSO-*d6*) 10.36 (1 H, s, NH), 8.92 (1 H, d, J = 3.9, CH[2]), 8.64 (1 H, d, J = 8.7, CH[4]), 7.26 (2 H, m, CH[3]), 7.38 (1 H, d, J = 8.1, CH[6]), 7.14 (1 H, d, J = 8.1, CH[7]). ^{13}C NMR: δ_C (75 MHz; DMSO-*d6*,): 189.8, 185.2, 173.4, 149.8, 147.4, 136.0, 134.3, 124.9, 123.5, 122.3, 121.6 and 111.7. HRMS ESI(+), calc. for $C_{13}H_9N_2O_4$ $[MH]^+$ 257.0562; found 257.0570.

3-(2-methoxy-5-methylphenylamino)-4-hydroxycyclobut-3-ene-1,2-dione (5g).
Reaction conditions for microwave irradiation: squaric acid (57 mg, 0.5 mmol) and 2-methoxy-5-methylaniline (137 mg, 1.0 mmol) at 120 ºC for 30 min.

Product isolation: the reaction mixture was diluted with 20 mL of 5% $NaHCO_3$ and extracted with Et_2O (5×10 mL) discarding the organic extracts. The aqueous solution was acidified with 3N HCl (10mL) and extracted with AcOEt (4×20 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to dryness. The product **5g** (73 mg, 63 %) was obtained as a white solid.

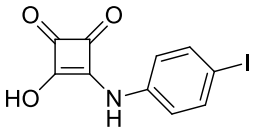


5g

Mp >220 ºC dec. λ_{max} (H_2O)/nm 205, 285 and 322 (ε/dm^3 mol^{-1} cm^{-1} 20 000, 10 600 and 14 900). λ_{max} (MeCN)/nm 205, 251, 265, 293, and 338 (ε/dm^3 mol^{-1} cm^{-1} 19 400, 8 100, 8 500, 7 600, and 22 500). IR: ν_{max}/cm^{-1} 3234 (NH), 2360br (OH), 1804 (CO). ^{1}H NMR: δ_H(300 MHz; DMSO-*d6*) 9.44 (1H, s, NH), 7.28 (1H, s, Ar(o)H), 6.92 (2H, dd, J = 8.4, Ar(m,p)H), 3.78 (3H, s, OCH_3), 2.22 (3H, s, $ArCH_3$). ^{13}C NMR: δ_C (75 MHz; DMSO-*d6*,): 190.1, 186.8, 184.3, 171.4, 148.4, 129.3, 126.4, 125.5, 122.4, 111.4, 55.8, and 20.3. HRMS ESI(+), calc. for $C_{24}H_{23}N_2O_8$ $[2M+H]^+$ 467.1454; found 467.1446.

3-(p-Iodophenylamino)-3-hydroxycyclobut-3-ene-1,2-dione (5i).
Reaction conditions for microwave irradiation: squaric acid (57 mg, 0.5 mmol) and 4-iodoaniline (219 mg, 1.0 mmol) at 120 ºC for 20 min.

Product isolation: the reaction mixture was diluted with 30 mL of 5% $NaHCO_3$, filtering and wasting the remaining solid. The aqueous solution was extracted with Et_2O (4×10 mL) discarding the organic extracts. The aqueous solution was acidified with 3N HCl (15 mL), the precipitate formed was filtered, washed with 10 mL of water, dried over anhydrous Na_2SO_4 and concentrated to dryness. The product **5i** (92 mg, 58 %) was obtained as a white solid.

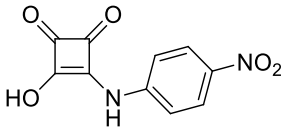


5i

Mp >280 ºC dec. λ_{max} (H_2O)/nm 233, 268, and 320 (ε/dm^3 mol^{-1} cm^{-1} 12 700, 13 700, and 34 800). IR: ν_{max}/cm^{-1} 3239 br (NH), 2641 br (OH), 1817 (CO). ^{1}H NMR: δ_H(300 MHz; DMSO-*d6*) 10.44 (1 H, s, NH), 7.64 (1 H, d, J = 8.7, Ar(o)H), 7.25 (2 H, d, J = 8.7, Ar(m)H). ^{13}C NMR: δ_C (75 MHz; DMSO-*d6*,): 188.3, 184.8, 170.9, 138.7, 137.6, 121.1, and 86.8. HRMS ESI(+), calc. for $C_{10}H_6INO_3Na$ $[MNa]^+$ 337.9290; found 337.9279.

3-(p-nitrophenylamino)-4-hydroxycyclobut-3-ene-1,2-dione (5h).
Reaction conditions for microwave irradiation: squaric acid (57 mg, 0.5 mmol) and 4-nitroaniline (14 mg, 0.1 mmol) at 120 ºC for 30 min.

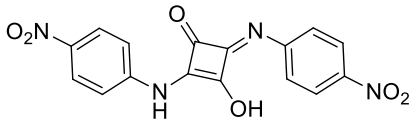
Product isolation: the reaction mixture was diluted with 10 mL of 5% $NaHCO_3$. The remaining solid was filtered and washed ten times with 3 mL of water. The resultant solid is squaraine **3h**. Pooled aqueous fractions were extracted with Et_2O (5×20 mL) discarding the organic extracts. After acidification with 3N HCl (10 mL), the aqueous layer was extracted with AcOEt (5×15 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to dryness. The product **5h** (21 mg, 90%) was obtained as a yellow solid.



5h

Mp >260 ºC dec. λ_{max} (H_2O)/nm 262 and 393 (ε/dm^3 mol^{-1} cm^{-1} 19 600 and 21 400). λ_{max} (MeCN)/nm 258, 291, and 428 (ε/dm^3 mol^{-1} cm^{-1} 15 800, 10 700, and 17 900). IR: ν_{max}/cm^{-1} 3222 (NH), 2634br (OH), 1825 (CO), 1696 (CO). ^{1}H NMR: δ_H(300 MHz; DMSO-*d6*) 10.44 (1 H, br s, NH), 8.14 (2 H, d, J = 7.8, Ar(o)H), 7.74 (2 H, d, J = 9, Ar(m)H). ^{13}C NMR: δ_C (75 MHz; DMSO-*d6*,): 192.4, 189.9, 185.9, 171.7, 145.6, 141.3, 125.4, and 117.9. HRMS ESI(+), calc. for $C_{20}H_{12}N_4O_{10}Na$ $[2M+Na]^+$ 491.0451; found 491.0455.

3-hydroxy-2-((4-nitrophenyl)amino)-4-((4-nitrophenyl)imino)cyclobut-2-enone (3h).
This product is obtained as a yellow solid by-product in the synthesis of amidosquaric acid **5h**.

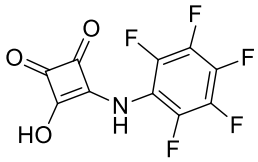


3h

IR: ν_{max}/cm^{-1} 3417 (NH), 1586 (CO). ^{1}H NMR: δ_H(300 MHz; H_2SO_4; DMSO-*d6*) 9.39 (1 H, br s, NH), 9.25 (1 H, br s, NH), 7.78 (2 H, br s, Ar(o)H), 7.09 (2 H, br s, Ar(m)H). ^{13}C NMR: δ_C (75 MHz; H_2SO_4; DMSO-*d6*): 166.2, 165.3, 164.8, 164.4, 144.2, 139.5, 125.3, 121.2. HRMS MALDI-TOF(+), calc. for $C_{16}H_{11}N_4O_6^+$ $[MH]^+$ 355.06786; found 355.06683.

3-(2,3,4,5,6-pentafluorofenylamine)-4-hydroxycyclobut-3-ene-1,2-dione (5j).
Reaction conditions for microwave irradiation: squaric acid (57 mg, 0.5 mmol) and pentafluoroaniline (183 mg, 1.0 mmol) at 120 ºC for 40 min.

Product isolation: the reaction mixture was diluted with 15 mL of 5% $NaHCO_3$, and extracted with CH_2Cl_2 (5×10 mL) discarding the organic extracts. The aqueous solution was acidified with 3N HCl and saturated with NaCl. Then the aqueous suspension was extracted with AcOEt (5×10 mL). The organic layers were dried over anhydrous Na_2SO_4 and concentrated to dryness. The product **5j** (52 mg, 37%) was obtained as a light brown solid.



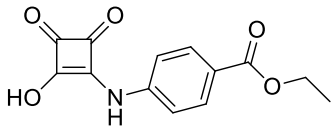
5j

Mp 209-211 ºC. λ_{max} (H_2O)/nm 258 and 296 (ε/dm^3 mol^{-1} cm^{-1} 20 500 and 23 100). λ_{max} (MeCN)/nm 253 and 312 (ε/dm^3 mol^{-1} cm^{-1} 17 600 and 16 700). IR: ν_{max}/cm^{-1} 3226 (NH), 2648br (OH), 1815 (CO). ^{1}H NMR: δ_H (300 MHz; DMSO-*d6*) 10.66 (1 H, s, NH), 8.68 (1 H, br, OH). ^{13}C NMR: δ_C (75 MHz; DMSO-*d6*): 190.0, 189.1, 185.2, 172.4, 143.9 (m), 140.6 (m), 138.9 (m), 137.2 (m), 135.6 (m), 113.8 (m). HRMS ESI(+), calc. for $C_{20}H_4N_2O_6F_{10}Na$ $[2M+Na]^+$ 580.9803; found 580.9807.

3-(4-carboxyethylphenylamino)-4-hydroxycyclobut-3-ene-1,2-dione (5k).
Reaction conditions for microwave irradiation: squaric acid (57 mg, 0.5 mmol) and ethyl-4-aminobenzoate (25 mg, 0.15 mmol) at 120 ºC for 20 min.

Product isolation: the reaction mixture was diluted in 20 mL of 5% $NaHCO_3$, filtering and wasting the remaining insoluble solid. The aqueous layer was extracted with Et_2O (4×20 mL) discarding the organic extracts. After acidification with 3N HCl (10 mL) the aqueous fraction was extracted Et_2O (10×20 mL). The combined organic layers were dried over anhydrous

Na_2SO_4 and concentrated to dryness. The product **5k** (33 mg, 83%) was obtained as a white solid.

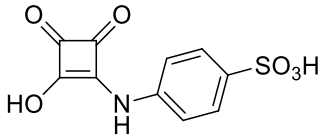


5k

Mp >230 ºC dec. λ_{max} (H_2O)/nm 241, 268 and 337 (ε/dm^3 mol^{-1} cm^{-1} 13 500, 12 900 and 33 000). λ_{max} (MeCN)/nm 249, 268 and 353 (ε/dm^3 mol^{-1} cm^{-1} 15 100, 10 000 and 30 600). IR: ν_{max}/cm^{-1} 3233 (NH), 2673br (OH), 1804 (CO), 1699 (CO). ^{1}H NMR: δ_H(300 MHz; DMSO-*d6*) 10.60 (1 H, s, NH), 7.89 (2 H, d, J = 8.7, Ar(o)H), 7.56 (2 H, d, J = 8.7, Ar(m)H), 4.27 (2 H, q, J = 6.8, CH_2), 1.29 (3 H, t, J = 6.9, CH_3). ^{13}C NMR: δ_C (75 MHz; DMSO-*d6*,): 189.9, 185.2, 171.2, 165.3, 143.3, 130.6, 130.4, 123.5, 118.0, 60.3, and 14.2. HRMS ESI(+), calc. for $C_{26}H_{23}N_2O_{10}$ $[2M+H]^+$ 523.1353; found 523.1346.

4-((2-hydroxy-3,4-dioxocyclobut-1-en-1-yl)amino)benzenesulfonic acid (5l).
Reaction conditions for microwave irradiation: squaric acid (57 mg, 0.5 mmol) and 4-aminobenzenesulfonic acid (43 mg, 0.25 mmol) at 120 ºC for 30 min.

Product isolation: the reaction mixture was diluted in 5 mL of 1M HCl and concentrated to dryness. The resulting solid was washed with ethyl acetate (10x10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to dryness. The resulting solid was washed with boiling isopropyl ether (3x5 mL). The solid was dried under vacuum. The product **5l** (47 mg, 70 %) was obtained as a white powder.

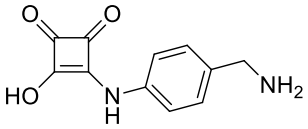


5l

Mp >200 ºC dec. IR: ν_{max}/cm^{-1} 3418br (NH), 2635br (OH), 1800 (CO). ^{1}H NMR: δ_H(300 MHz; DMSO-*d6*) 10.52 (1 H, s, NH), 7.52 (2 H, d, J = 8.1, Ar(*o*)H), 7.32 (2 H, d, J = 8.4, Ar(*p*)H). ^{13}C NMR: δ_C (75 MHz; DMSO-*d6*,): 189.9, 187.0, 184.7, 170.5, 142.7, 138.9, 126.6, and 118.3. HRMS ESI(+), calc. for $C_{20}H_{15}N_2O_{12}S_2$ $[2M+H]^+$ 539.0066; found 539.0073.

3-(4-(ethylamine)phenylamino)-4-hydroxycyclobut-3-ene-1,2-dione (5m).[5]
Reaction conditions for microwave irradiation: squaric acid (57 mg, 0.5 mmol) and 4-amino-benzylamine (38 µl, 0.3 mmol) at 120 ºC for 20 min.

Product isolation: the reaction mixture was diluted in 10 mL of water and filtrated. The resulting yellow-orange solid was washed three times with 10 mL of water and dried. The product **5m** (60 mg, 92%) was obtained as a yellow-orange solid.

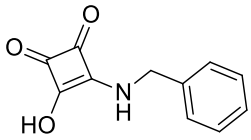


5m

Mp >270 ºC dec. IR: ν_{max}/cm^{-1} 3226 (NH), 3188 (NH_2), 2644br (OH), 1788 (CO). ^{1}H NMR: δ_H(300 MHz; DMSO-*d6*) 9.37 (1 H, s, NH), 7.98 (3 H, br s, NH_3^+), 7.67 (2 H, d, J = 8.4, Ar(o)H), 7.26 (2 H, d, J = 8.4, Ar(m)H), 3.91 (2 H, d, J = 5.4, CH_2). ^{13}C NMR: δ_C (75 MHz; DMSO-*d6*,): 202.3, 187.8, 177.4, 141.6, 129.6, 124.9, 117.2 and 42.1. HRMS ESI(+), calc. for $C_{11}H_{01}N_2O_3$ $[M+Na]^+$ 467.1454; found 467.1446.

3-benzylamino-4-hydroxycyclobut-3-ene-1,2-dione (5n).
Reaction conditions for microwave irradiation: squaric acid (57 mg, 0.5 mmol) and benzylamine (109 µl, 1.0 mmol) at 200 ºC for 20 min.

Product isolation: the reaction mixture was diluted in 20 mL of 3N HCl and extracted with Et_2O (5×20 mL). The organic layers were dried over anhydrous Na_2SO_4 and concentrated to dryness. The product **5n** (61 mg, 60%) was obtained as a pale yellow solid.

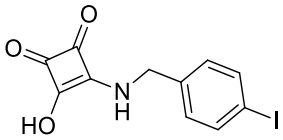


5n

Mp 200 ºC dec. λ_{max} (H_2O)/nm 283 (ε/dm^3 mol^{-1} cm^{-1} 17 100). λ_{max} (MeCN)/nm 260 and 293 (ε/dm^3 mol^{-1} cm^{-1} 16 700 and 20 800). IR: ν_{max}/cm^{-1} 3260br (NH), 2585br (OH), 1809 (CO). ^{1}H NMR: δ_H(300 MHz; DMSO-*d6*) 8.82 (1 H, s, NH), 7.33 (5 H, m, ArH), 4.59 (2 H, d, J = 6.3, CH_2). ^{13}C NMR: δ_C (75 MHz; DMSO-*d6*,): 192.6, 185.0, 184.0, 173.8, 138.7, 128.6, 127.4, and 46.9. HRMS ESI(+), calc. for $C_{22}H_{18}N_2O_6Na$ $[2M+Na]^+$ 429.1063; found 429.1097.

3-(4-iodobenzylamino)-4-hydroxycyclobut-3-ene-1,2-dione (5o).
Reaction conditions for microwave irradiation: squaric acid (29 mg, 0.25 mmol) and 4-iodo-benzylamine (117 mg, 0,5 mmol) in 1.5 mL of water at 200 ºC for 20 min.

Product isolation: the reaction mixture was diluted with 10 mL of 1N NaOH and 10 mL of water and extracted with CH_2Cl_2 (5×12 mL) discarding the organic extracts. The aqueous solution was acidified with 1N HCl (12 mL) and extracted with AcOEt (5×15 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to dryness. The product **5o** (68 mg, 41%) was obtained as a white solid.

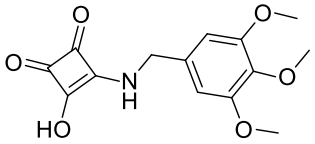


5o

Mp >205 ºC dec. λ_{max} (H_2O)/nm 234 and 285(ε/dm^3 mol^{-1} cm^{-1} 12 000 and 29 100). λ_{max} (MeCN)/nm 230, 259 and 295 (ε/dm^3 mol^{-1} cm^{-1} 15 600, 19 000 and 29 600). IR: ν_{max}/cm^{-1} 3238br (NH), 2643br (OH), 1818 (CO). ^{1}H NMR: δ_H(300 MHz; DMSO-*d6*) 8.81 (1 H, s, NH), 7.73 (2 H, d, J = 8.4, Ar(o)H), 7.11 (2 H, d, J = 8.1, Ar(m)H), 4.53 (2 H, d, J = 6, CH_2). ^{13}C NMR: δ_C (75 MHz; DMSO-*d6*,): 184.89, 184.10, 183.48, 137.29, 129.65, 93.27, and 46.32. HRMS ESI(+), calc. for $C_{11}H_8INO_3Na$ $[MNa]^+$ 351.9447; found 351.9438.

3-(3,4,5-trimethoxybenzylamino)-4-hydroxycyclobut-3-ene-1,2-dione (5p).
Reaction conditions for microwave irradiation: squaric acid (57 mg, 0.5 mmol) and 3,4,5-trimethoxybenzylamine (178 µl, 1 mmol) at 200 ºC for 50 min.

Product isolation: the reaction mixture was diluted with 10 mL of 5% $NaHCO_3$ and 10 mL of water and extracted with CH_2Cl_2 (5×15 mL) discarding these extracts. The remaining aqueous layer was acidified with 3N HCl (5 mL) and extracted with CH_2Cl_2 (5×20 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to dryness. The product **5p** (85 mg, 58%) was obtained as a white solid.

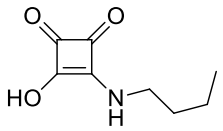


5p

Mp >205 ºC dec. λ_{max} (H_2O)/nm 202 and 284 (ε/dm^3 mol^{-1} cm^{-1} 23 600 and 16 800). λ_{max} (MeCN)/nm 204, 260 and 294 (ε/dm^3 mol^{-1} cm^{-1} 34 500, 13 900 and 18 700). IR: ν_{max}/cm^{-1} 3262br (NH), 2604br (OH), 1825 (CO). ^{1}H NMR: δ_H (300 MHz; DMSO-*d6*) 8.81 (1 H, t, J = 5.7, NH), 6.62 (2 H, s, ArH), 4.51 (2 H, d, J = 6, CH_2). ^{13}C NMR: δ_C (75 MHz; DMSO-*d6*,): 184.8, 184.4, 173.6, 152.9, 136.7, 134.1, 104.8, 60.0, 55.8, and 47.2. HRMS ESI(+), calc. for $C_{14}H_{15}NO_6Na$ $[MNa]^+$ 316.0797; found 316.0812.

3-(butylamino)-4-hydroxycyclobut-3-ene-1,2-dione (5q).
Reaction conditions for microwave irradiation: squaric acid (57 mg, 0.5 mmol) and butylamine (110 mg, 1.5 mmol) at 200 ºC for 60 min.

Product isolation: the reaction mixture was diluted with 10 mL of 1M NaOH and 10 mL of water. The solution was extracted with Et_2O (3×15 mL) discarding the organic layers. The aqueous layer was acidified with 3N HCl (12 mL), saturated with NaCl and extracted with AcOEt (6×20 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to dryness. The product **5q** (25 mg, 30%) was obtained as a white solid.



5q

Mp >205 ºC dec. λ_{max} (H_2O)/nm 276 (ε/dm^3 mol^{-1} cm^{-1} 32 700). λ_{max} (MeCN)/nm 260 and 292 (ε/dm^3 mol^{-1} cm^{-1} 13 800 and 16 700). IR: ν_{max}/cm^{-1} 3210br (NH), 2587br (OH), 1817 (CO). ^{1}H NMR: δ_H (300 MHz; DMSO-*d6*) 8.40 (1 H, br s, NH), 3.38 (2 H, q, *J* = 6.6, But(C1-H)), 1.49 (2 H, m, But(C2-H)), 1.289 (2 H, m, But(C3-H)), 0.87 (3 H, t, *J* = 7.0, But(C4-H)). ^{13}C NMR: δ_C (75 MHz; DMSO-*d6*,): 192.10, 184.87, 174.37, 43.72, 32.91, 19.52, and 13.98. HRMS EI(+), calc. for $C_8H_{11}NO_3$ [M] 169.0739; found 169.0731.

Spectrophotometric titrations.

The apparent pK_a values of representative amidosquaric acids were measured by registering the UV/vis absorption spectra in the range 200-600 nm against pH calculated from pH 13 to 1 in two steps. First, a 5.0×10^{-5} M aqueous solution of the amidosquaric acid (2.0 mL) containing ~ 0.01 M NaCl was adjusted to pH = 4.5, and the UV/vis were recorded. The pH was lowered in steps of ΔpH = 0.1 with the addition of appropriate amounts of standardized 1.0 M HCl, recording the absorption spectra at each pH interval. Second step as above, starting from pH = 9 and adding standardized 1.0 M NaOH up to pH = 13. Data sets were evaluated with HypSpec[6] (Protonic Software). For **5c** (R = *p*-$Me_2NC_6H_4$), the titration with HCl was carried out starting from pH = 6.3. Similarly, titration of **5e** (R = *o*-OHC_6H_4) with NaOH was performed from pH = 6.7. Each titration was repeated a minimum of two times.

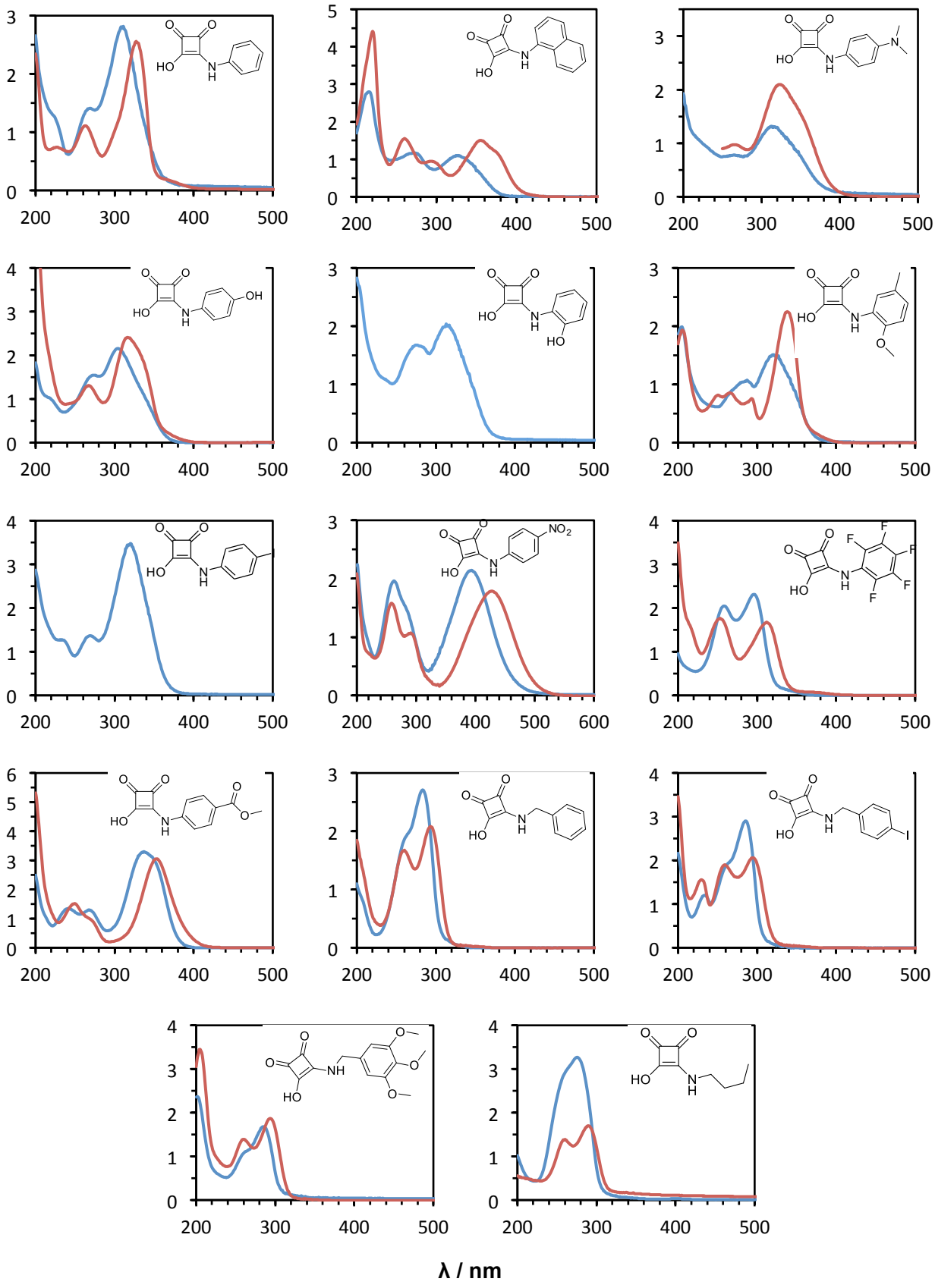
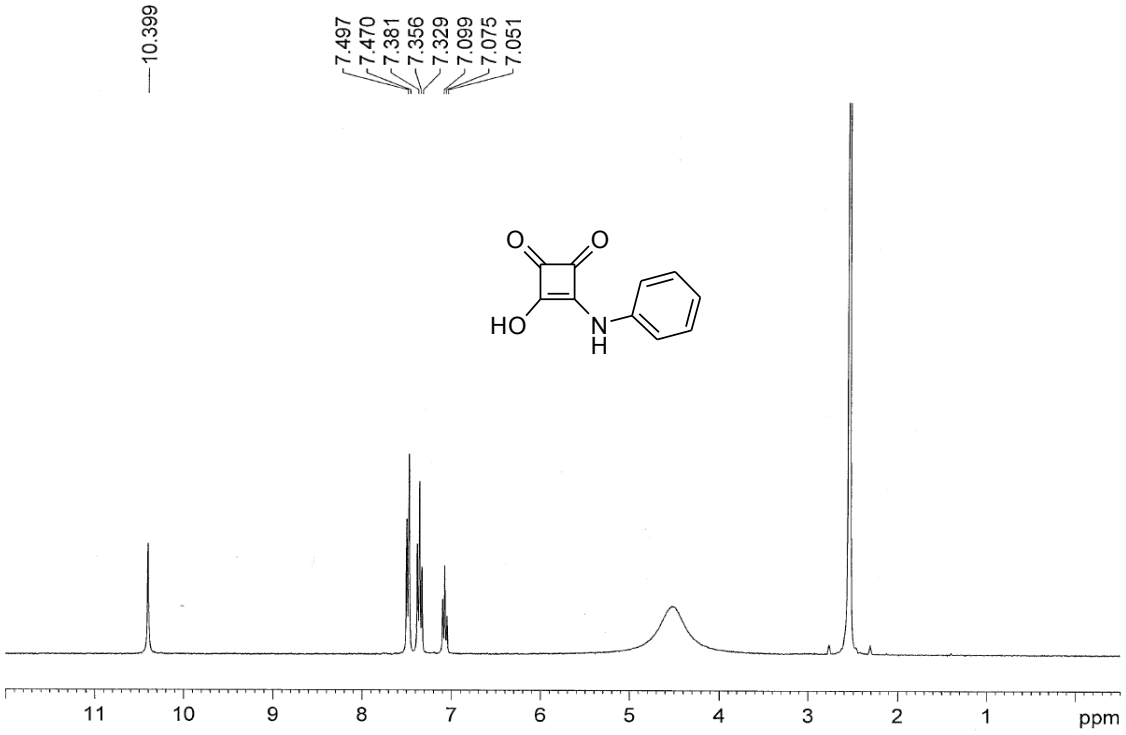


Figure S1. UV-Vis spectra of representative amidosquaric acids in water (blue line) and MeCN (red line).

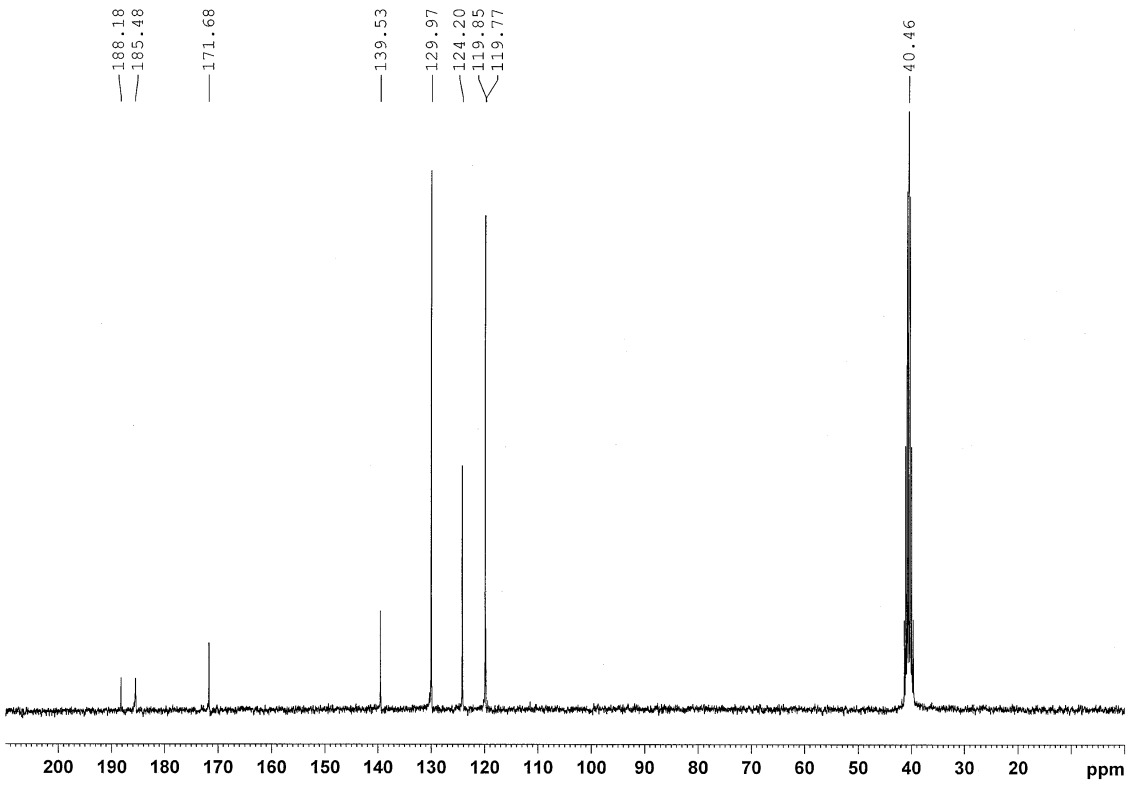
^{1}H and ^{13}C NMR spectra.

3-phenylamino-4-hydroxycyclobut-3-ene-1,2-dione (5a).

^{1}H NMR, DMSO, 300 MHz, 298 K

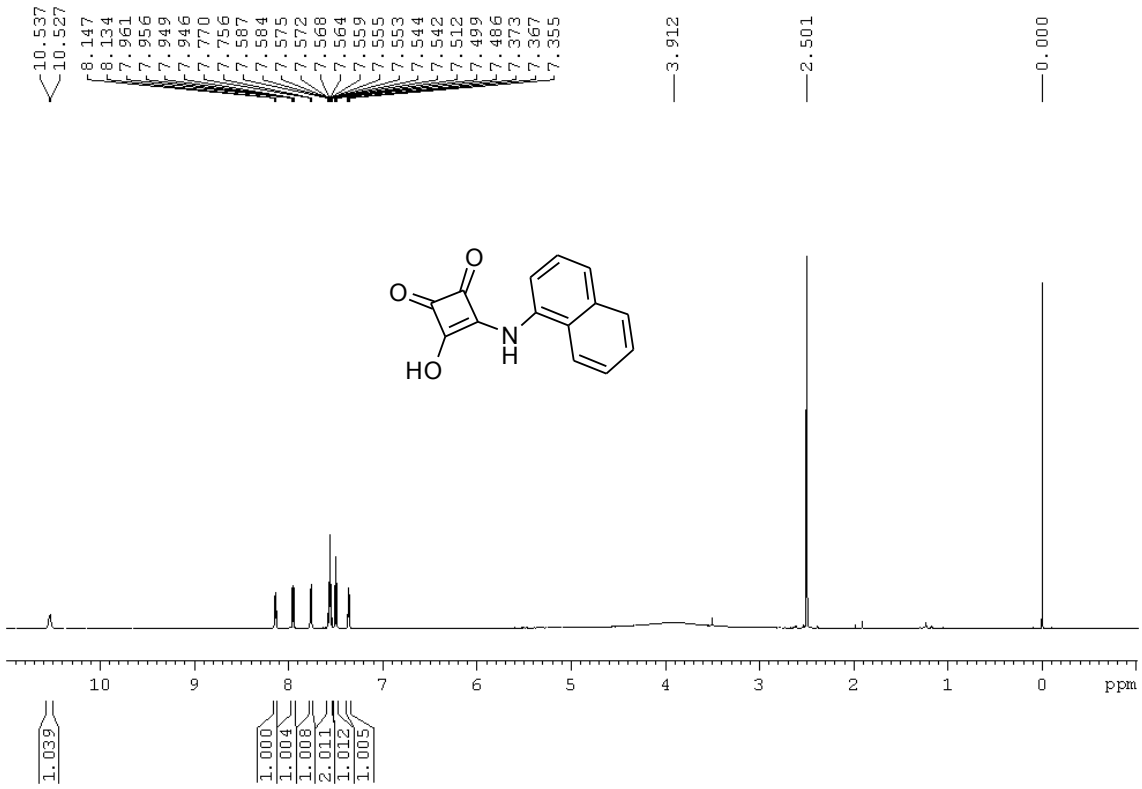


^{13}C NMR, DMSO, 75 MHz, 298 K

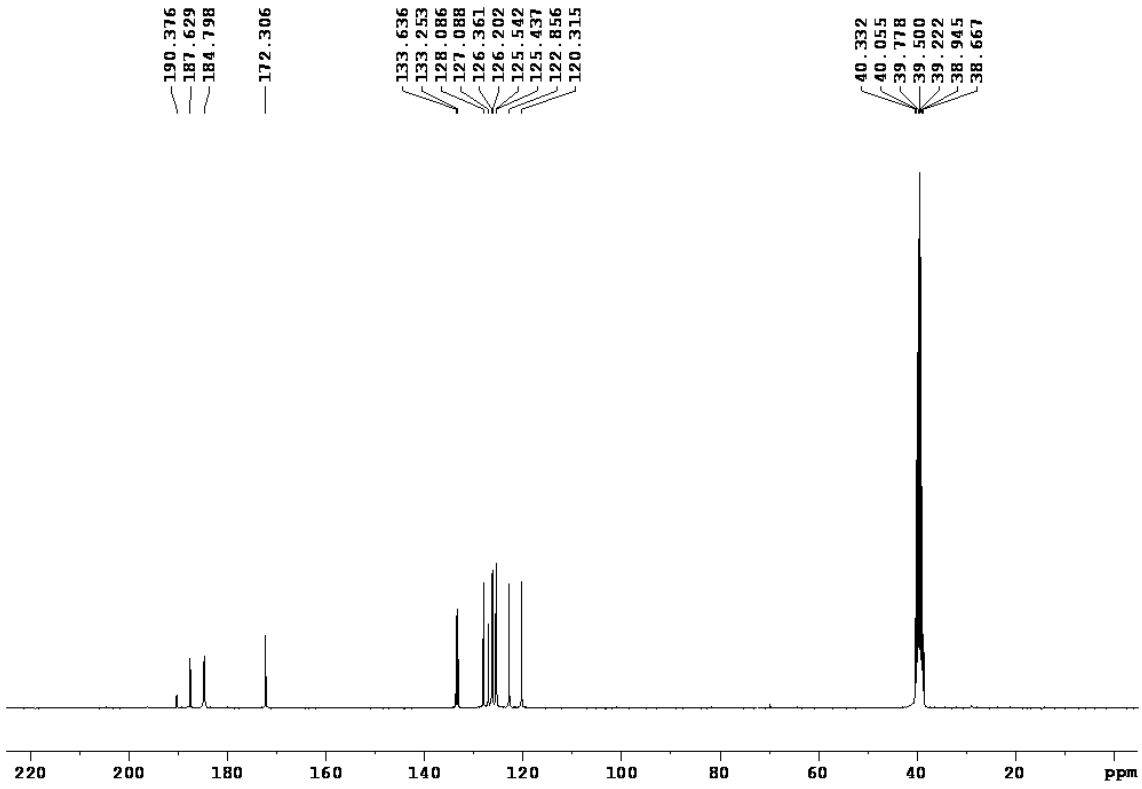


3-(1-naphthylamine)-4-hydroxycyclobut-3-ene-1,2-dione (5b).

^{1}H NMR, DMSO, 300 MHz, 298 K

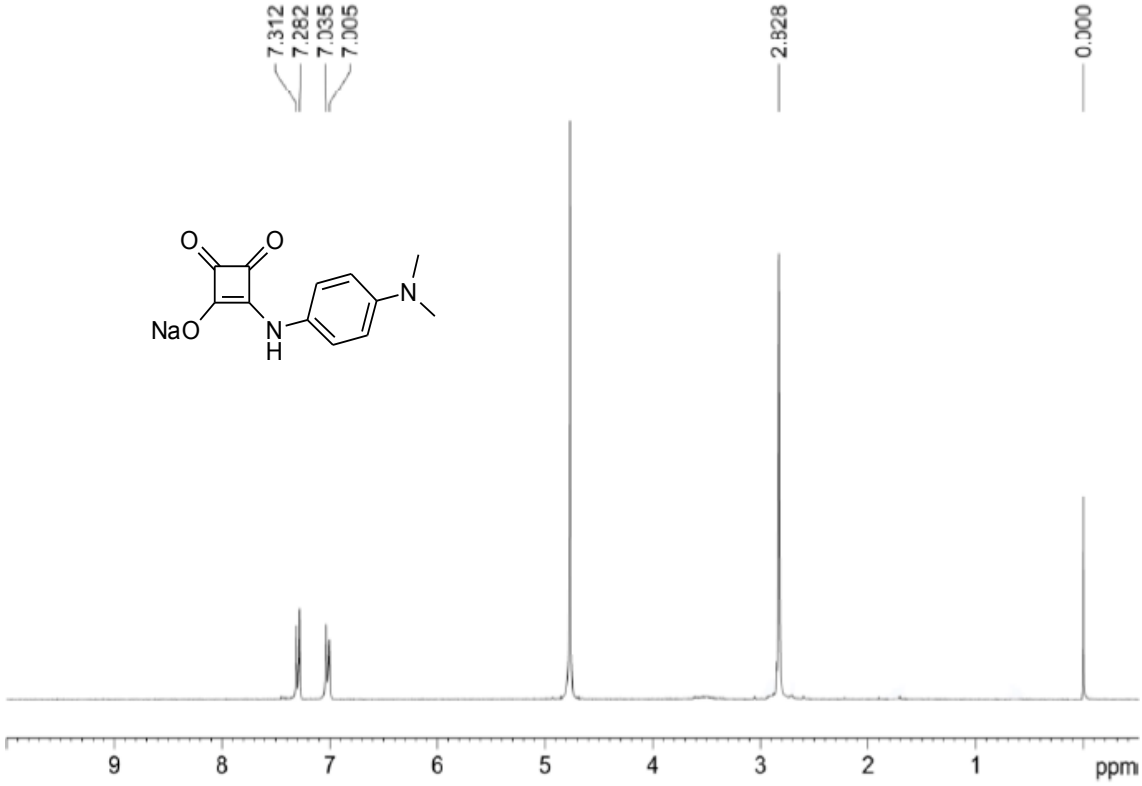


^{13}C NMR, DMSO, 75 MHz, 298 K

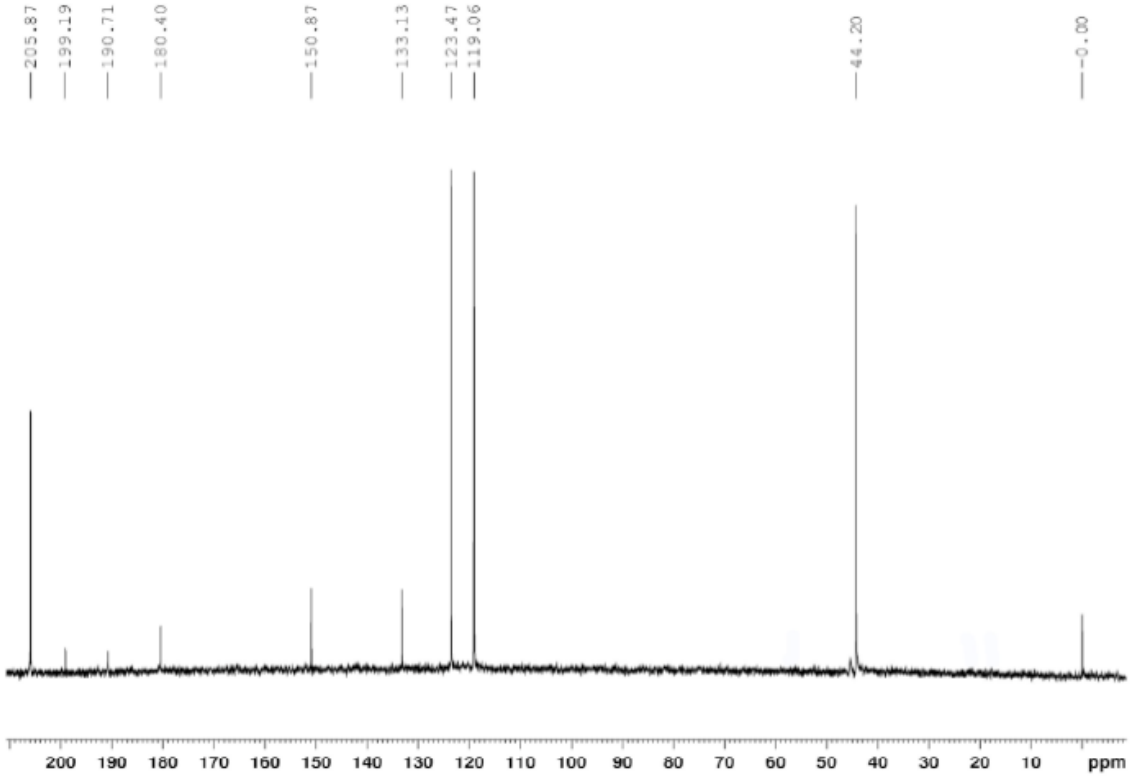


3-(4-(N,N-dimethylamino)phenylamino)-4-hydroxycyclobut-3-ene-1,2-dione (5c).

^{1}H NMR, D_2O, 300 MHz, 298 K

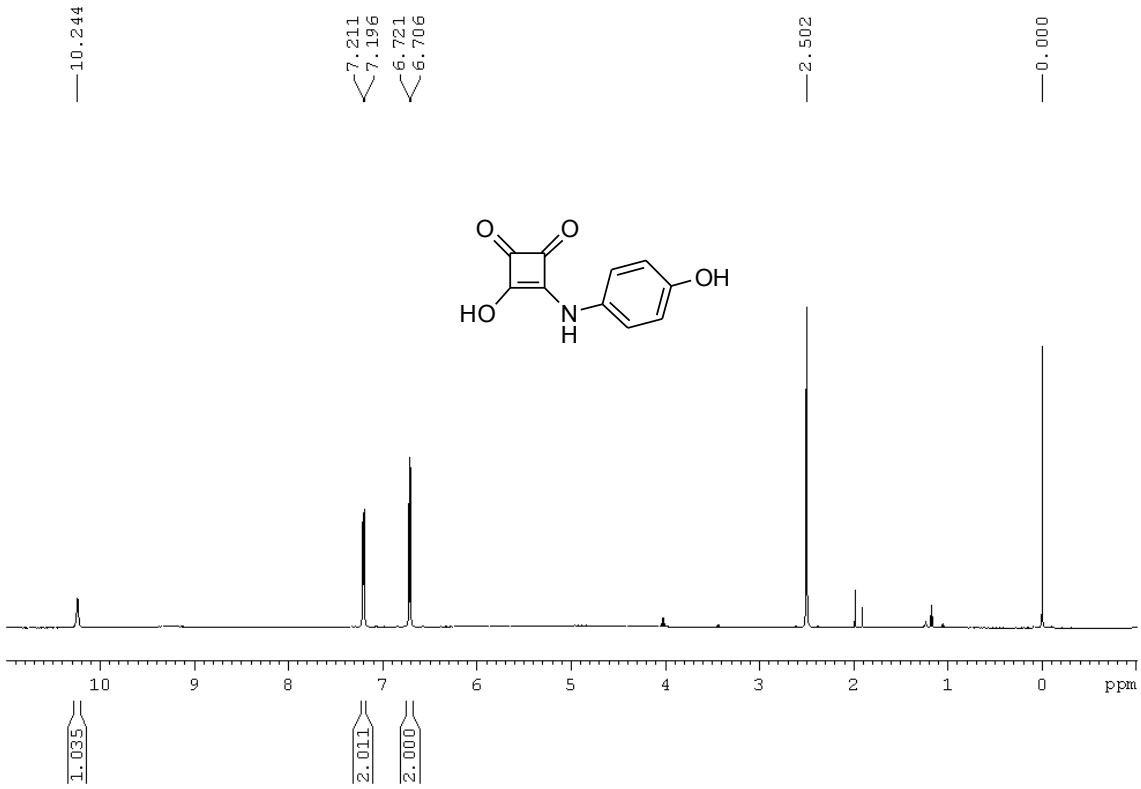


^{13}C NMR, D_2O, 75 MHz, 298 K

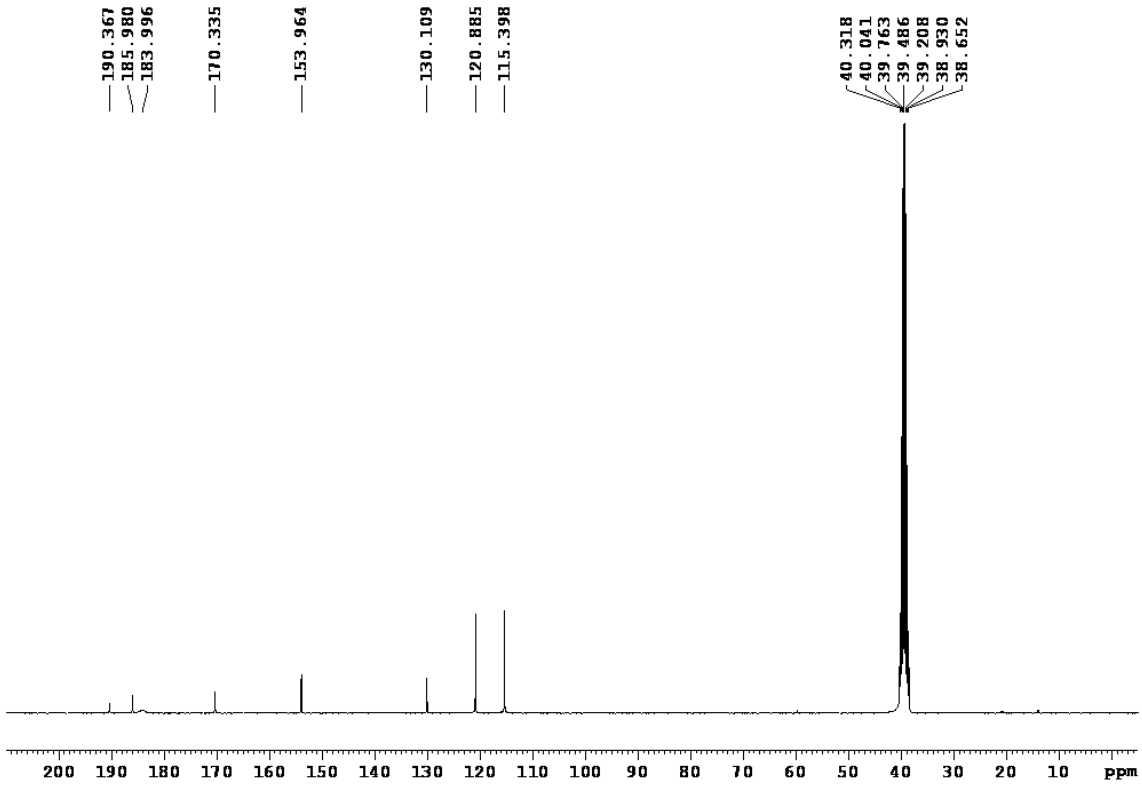


3-(*p*-hydroxyphenylamino)-4-hydroxycyclobut-3-ene-1,2-dione (5d).

^{1}H NMR, DMSO, 300 MHz, 298 K

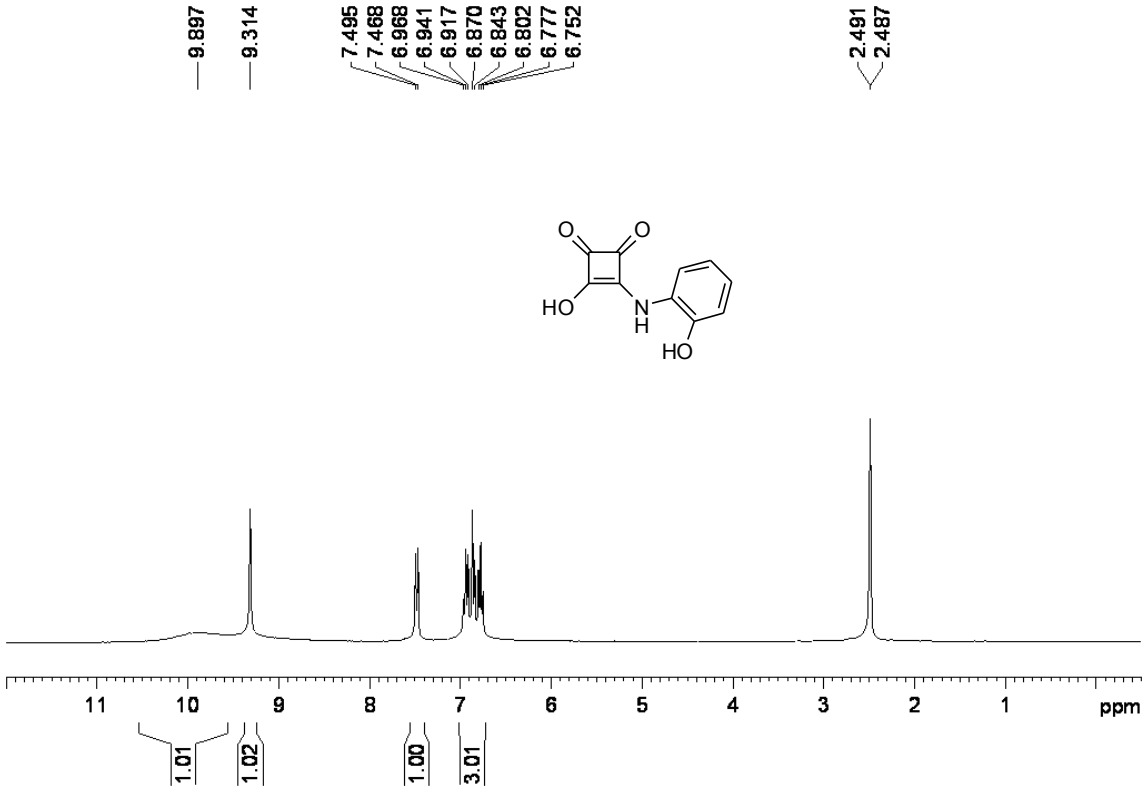


^{13}C NMR, DMSO, 75 MHz, 298 K

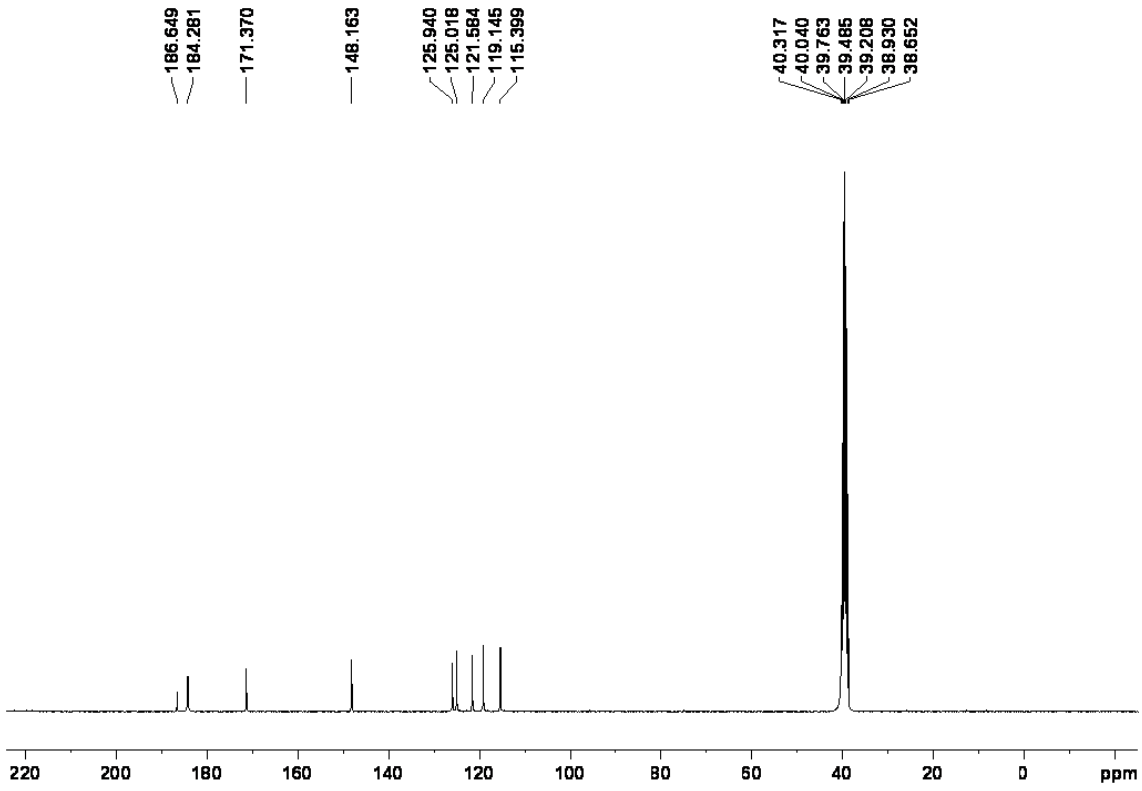


3-(*o*-hydroxyphenylamino)-4-hydroxycyclobut-3-ene-1,2-dione (5e).

^{1}H NMR, DMSO, 300 MHz, 298 K

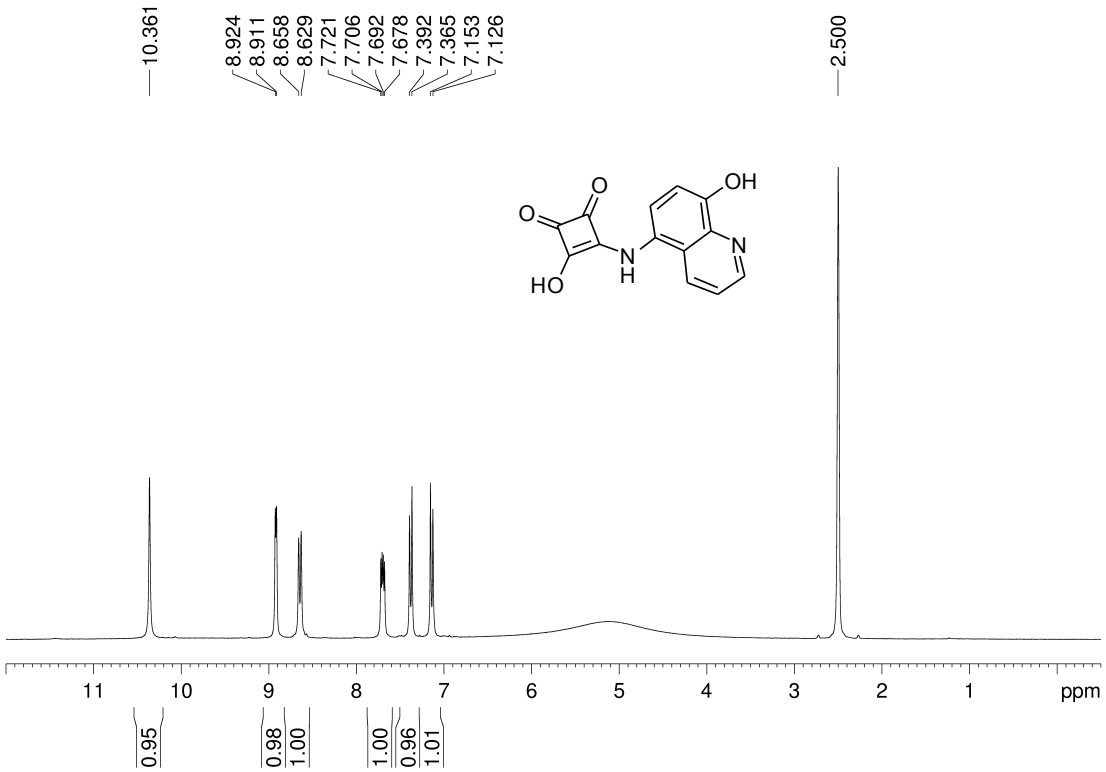


^{13}C NMR, DMSO, 75 MHz, 298 K

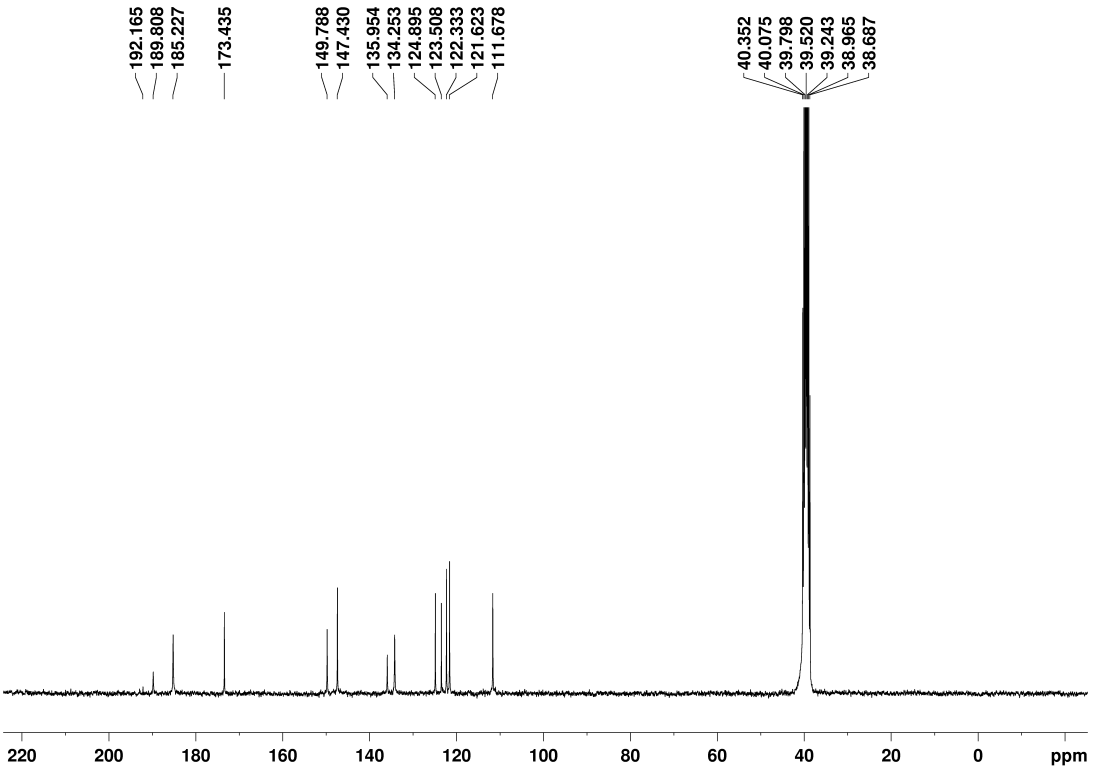


3-hydroxy-4-((8-hydroxyquinolin-5-yl)amino)cyclobut-3-ene-1,2-dione (5f).

^{1}H NMR, DMSO, 300 MHz, 298 K

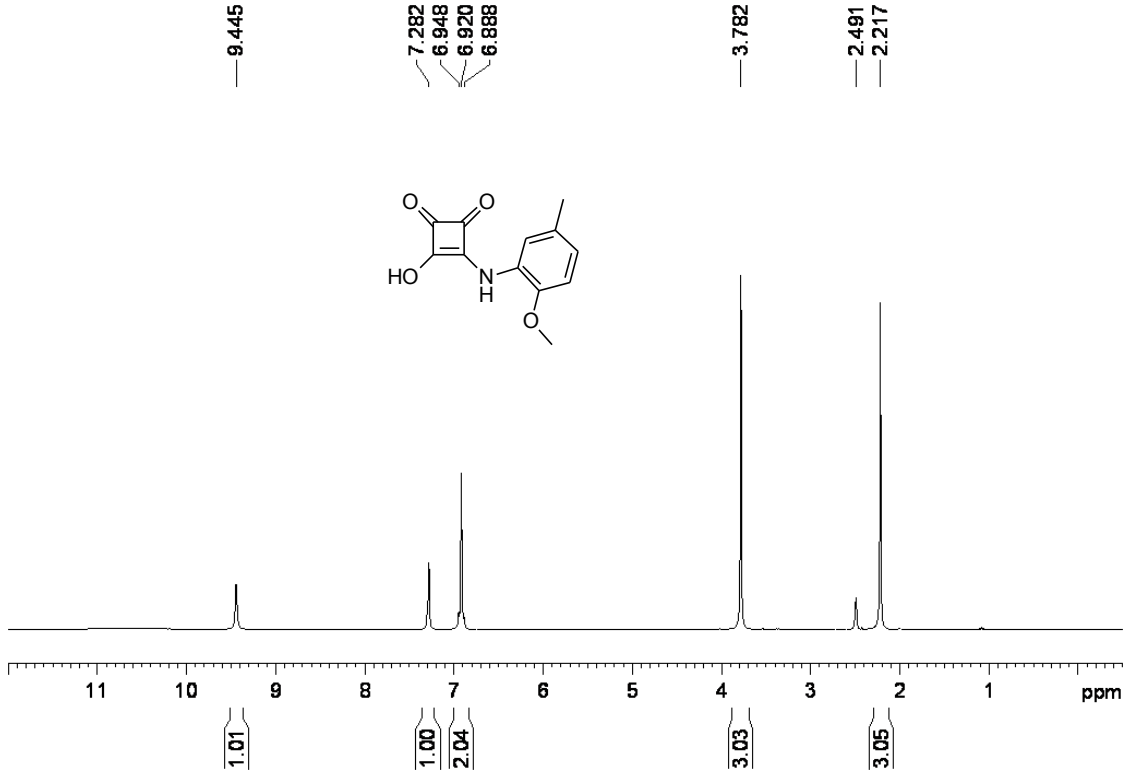


^{13}C NMR, DMSO, 75 MHz, 298 K

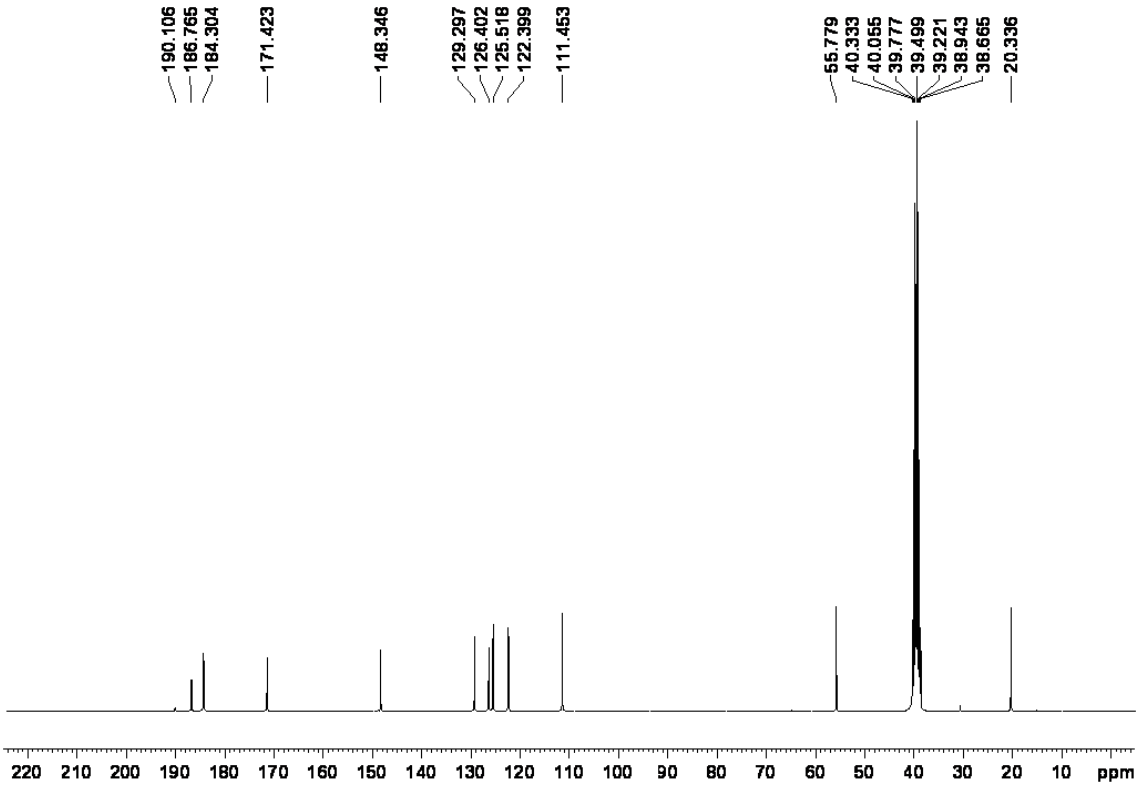


3-(2-methoxy-5-methylphenylamino)-4-hydroxycyclobut-3-ene-1,2-dione (5g).

^{1}H NMR, DMSO, 300 MHz, 298 K

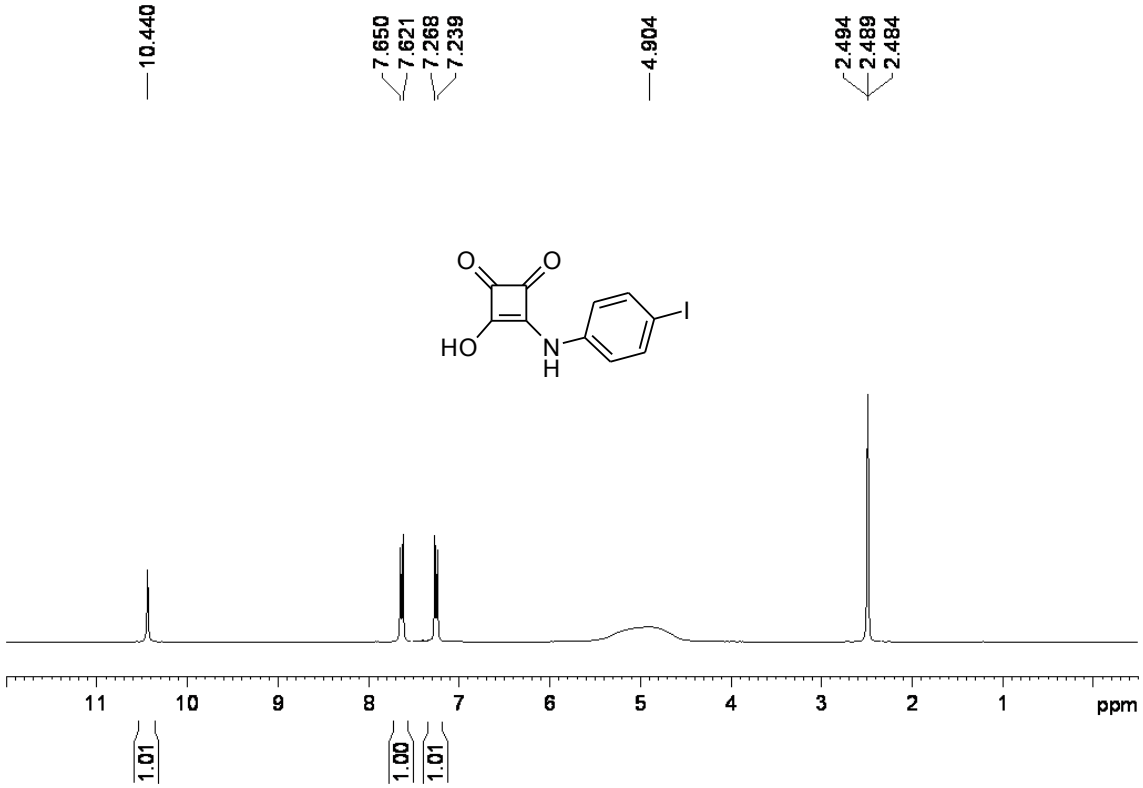


^{13}C NMR, DMSO, 75 MHz, 298 K

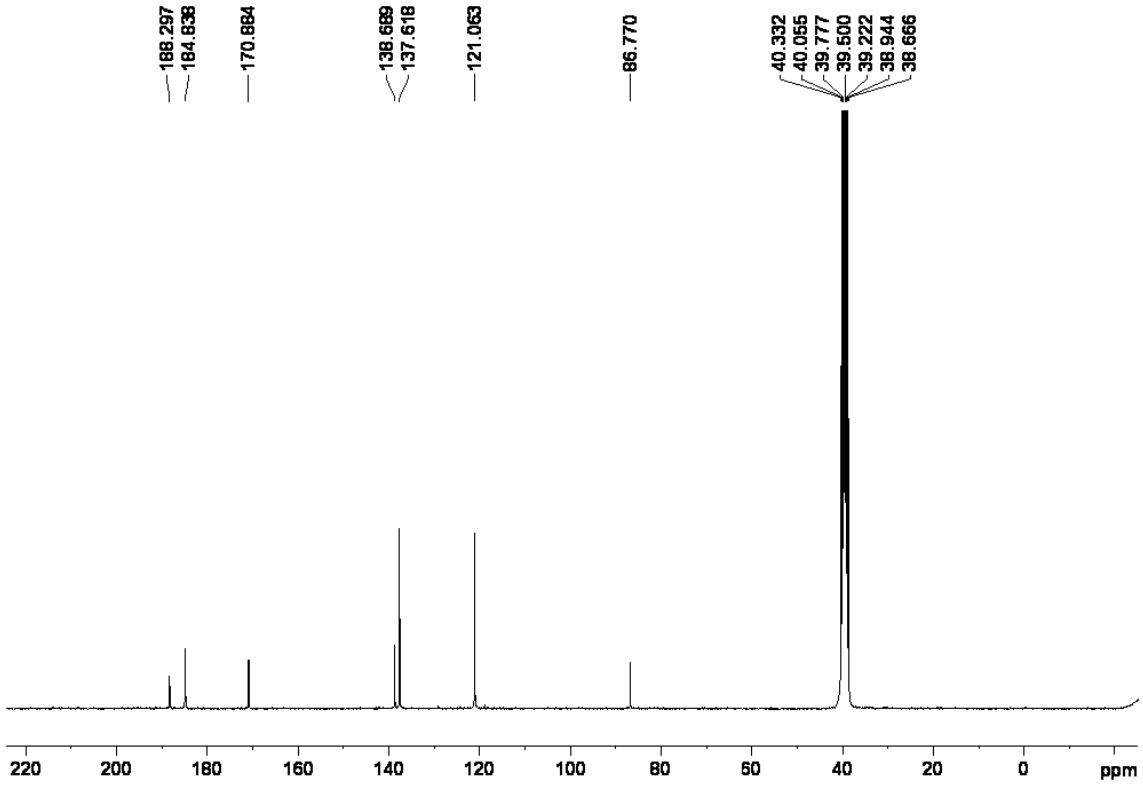


3-(p-Iodophenylamino)-3-hydroxycyclobut-3-ene-1,2-dione (5i).

^{1}H NMR, DMSO, 300 MHz, 298 K

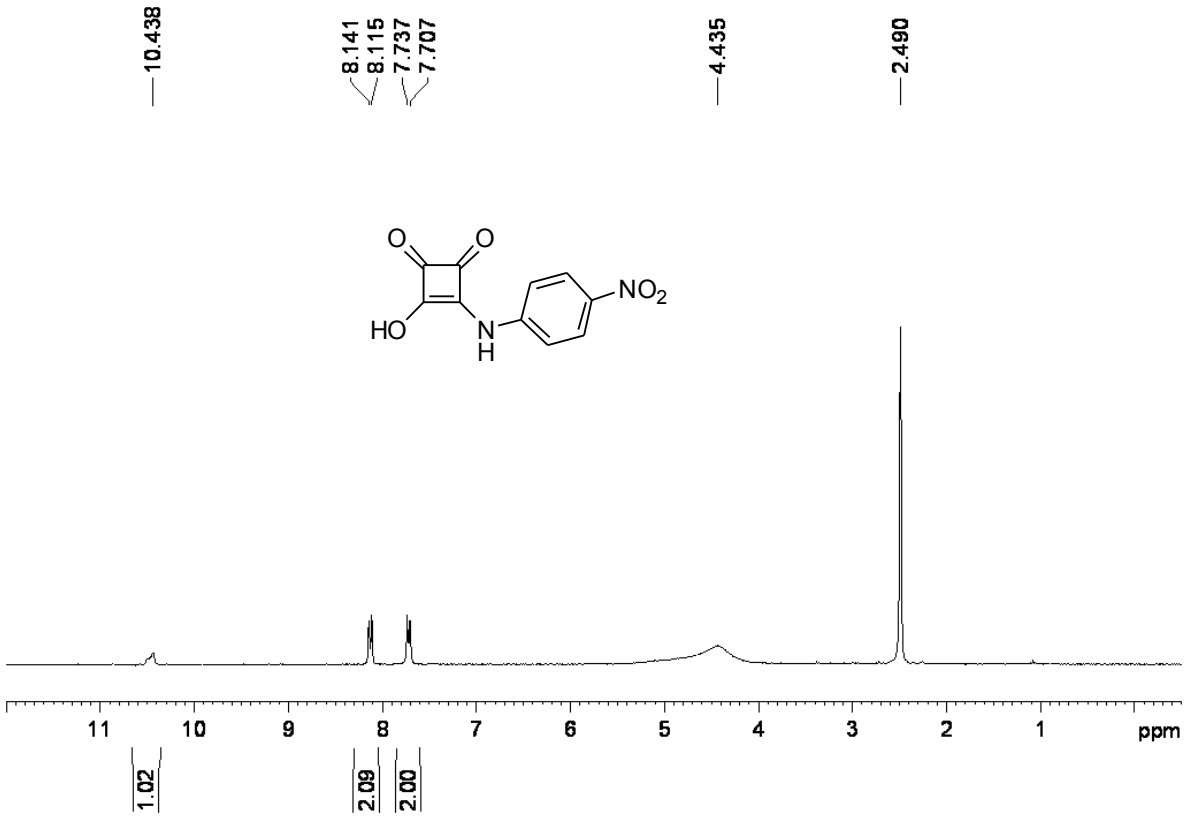


^{13}C NMR, DMSO, 75 MHz, 298 K

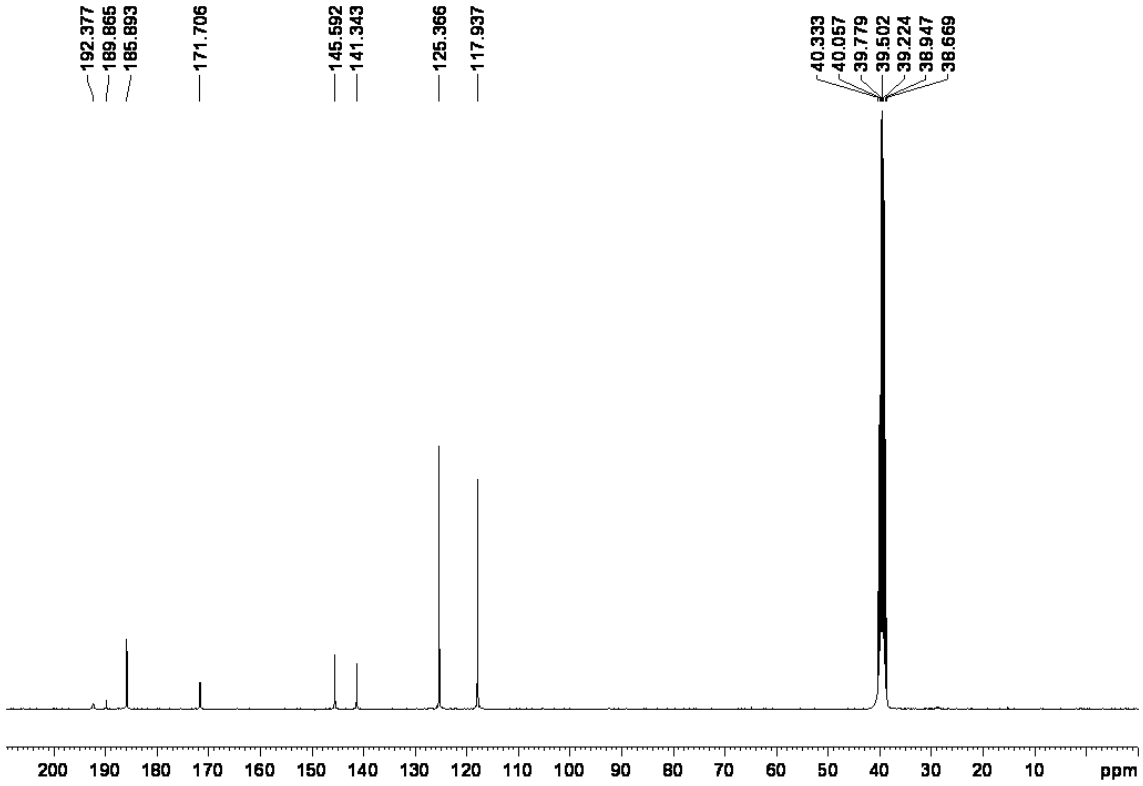


3-(p-nitrophenylamino)-4-hydroxycyclobut-3-ene-1,2-dione (5h).

^{1}H NMR, DMSO, 300 MHz, 298 K

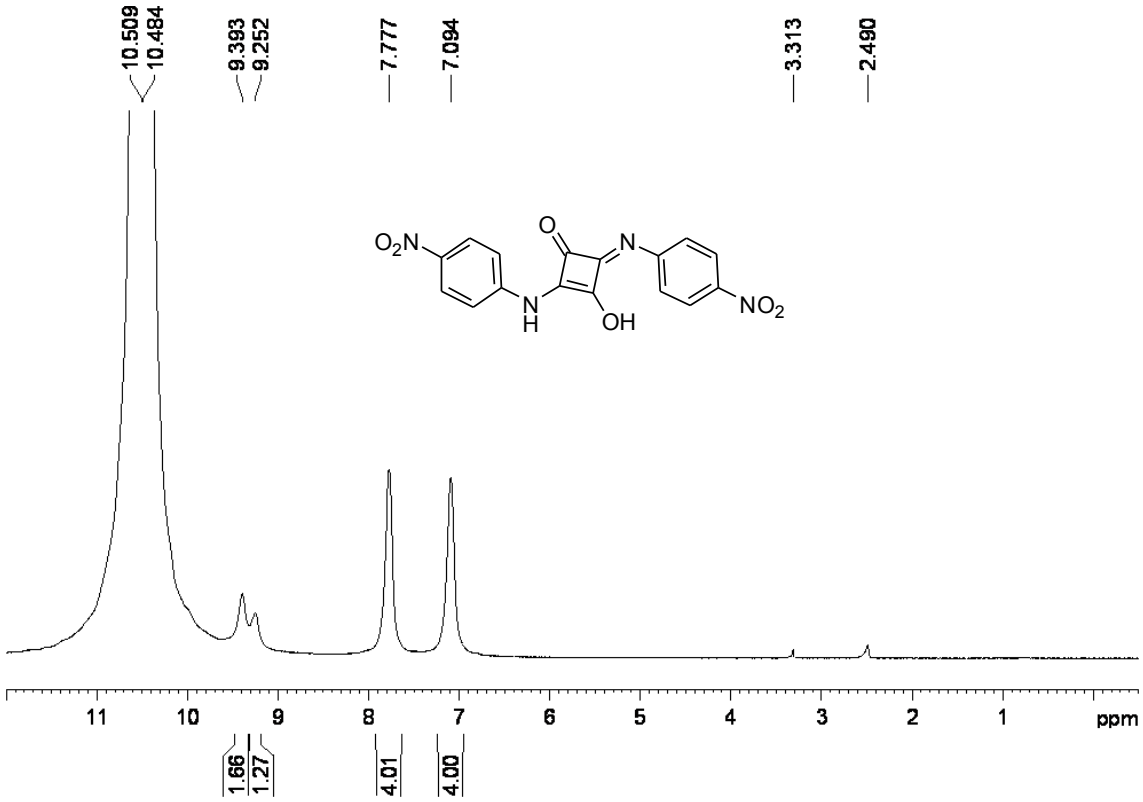


^{13}C NMR, DMSO, 75 MHz, 298 K

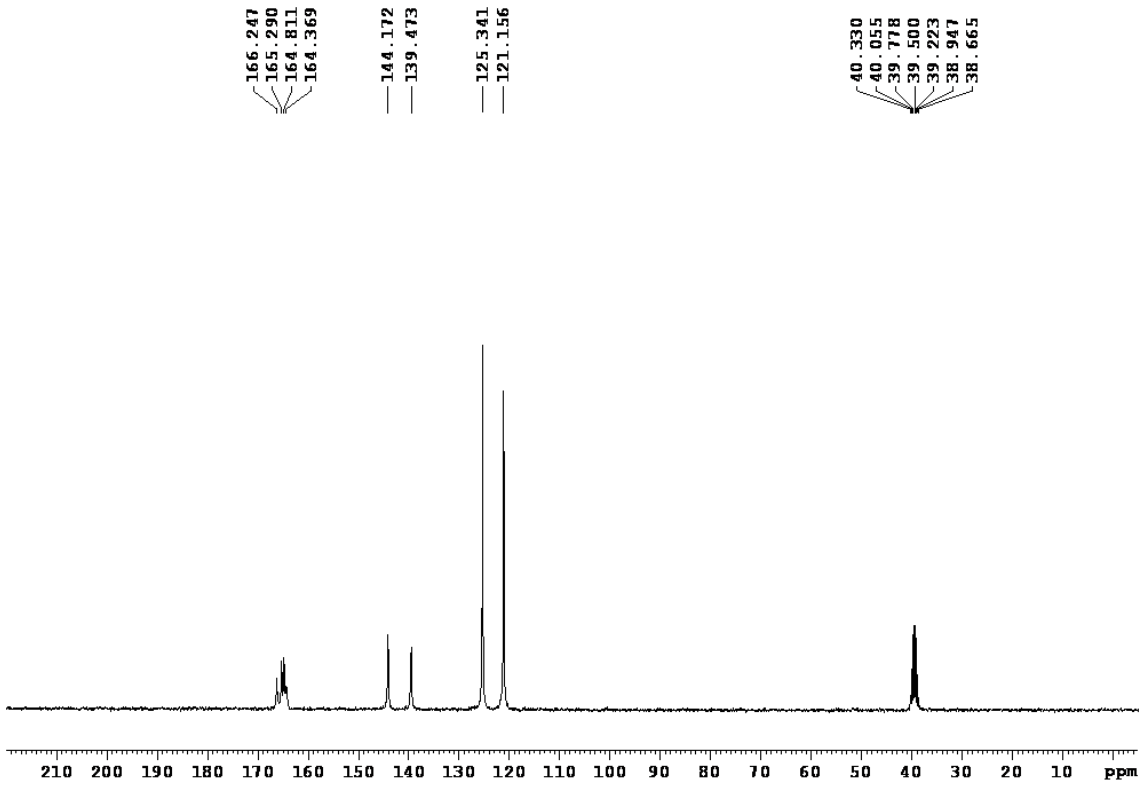


3-hydroxy-2-((4-nitrophenyl)amino)-4-((4-nitrophenyl)imino)cyclobut-2-enone (3h).

^{1}H NMR, H_2SO_4; DMSO-*d6*, 300 MHz, 298 K

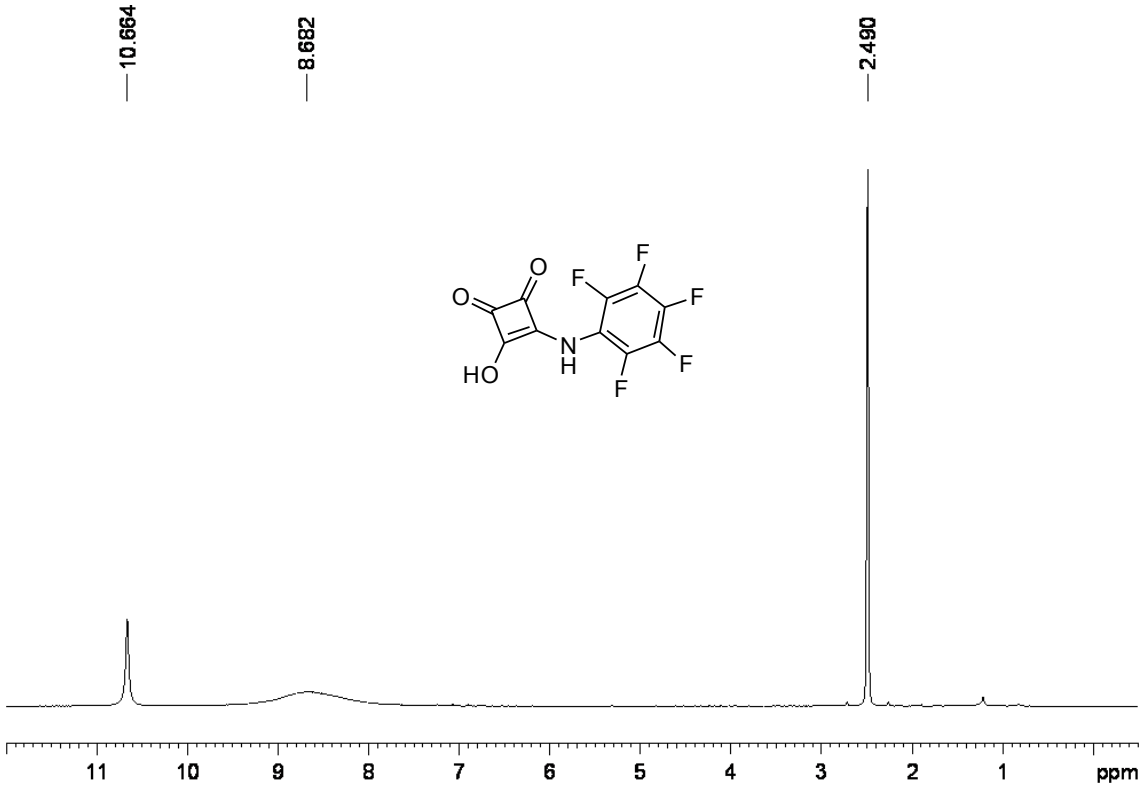


^{13}C NMR, H_2SO_4; DMSO-*d6*, 75 MHz, 298 K

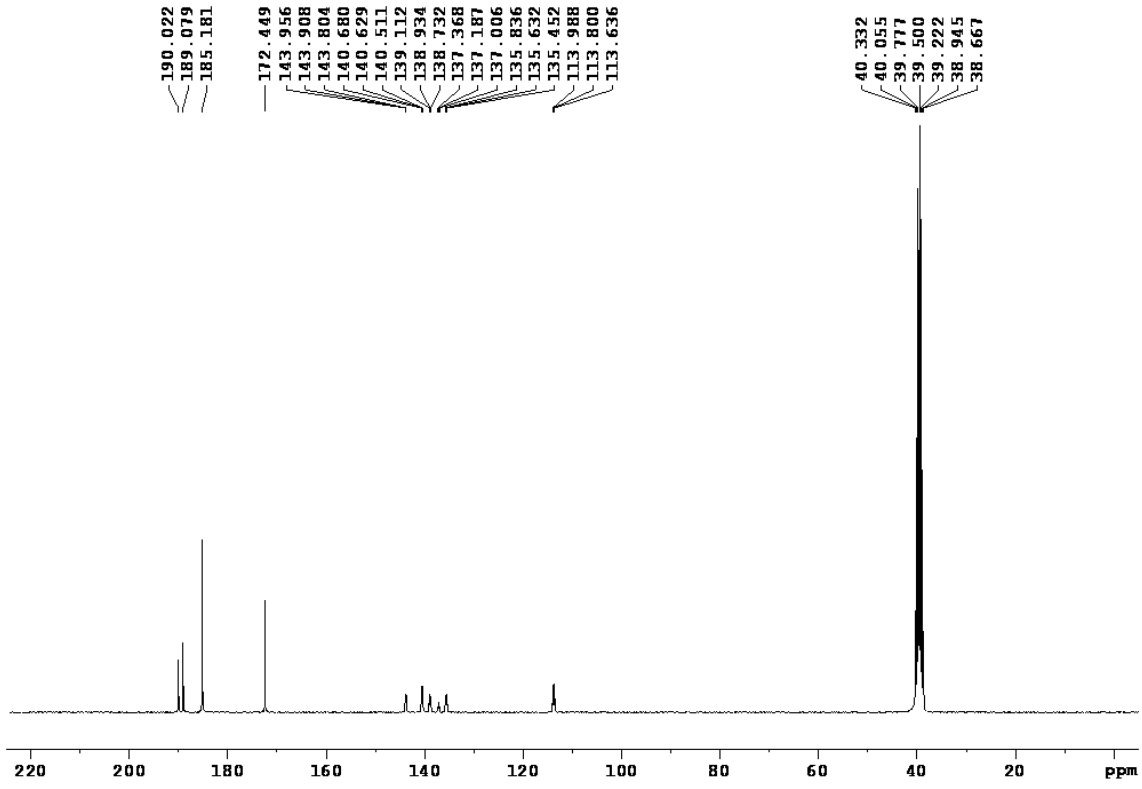


3-(2,3,4,5,6-pentafluorofenylamine)-4-hydroxycyclobut-3-ene-1,2-dione (5j).

^{1}H NMR, DMSO, 300 MHz, 298 K

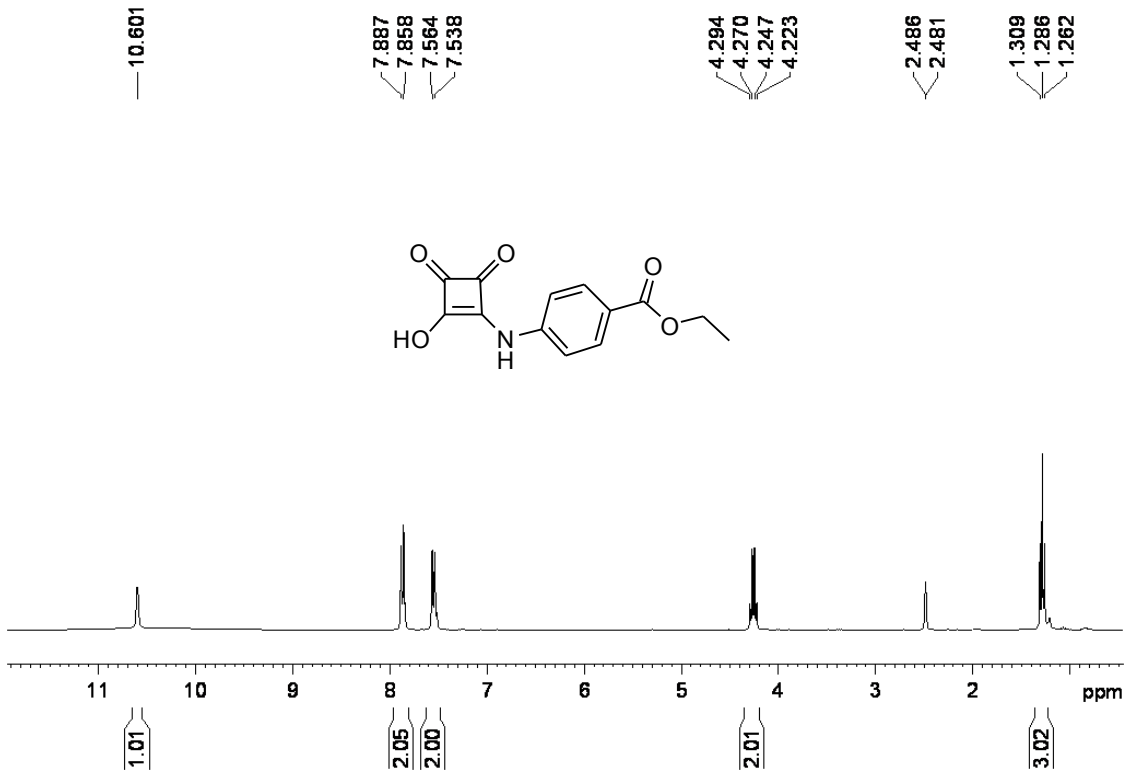


^{13}C NMR, DMSO, 75 MHz, 298 K

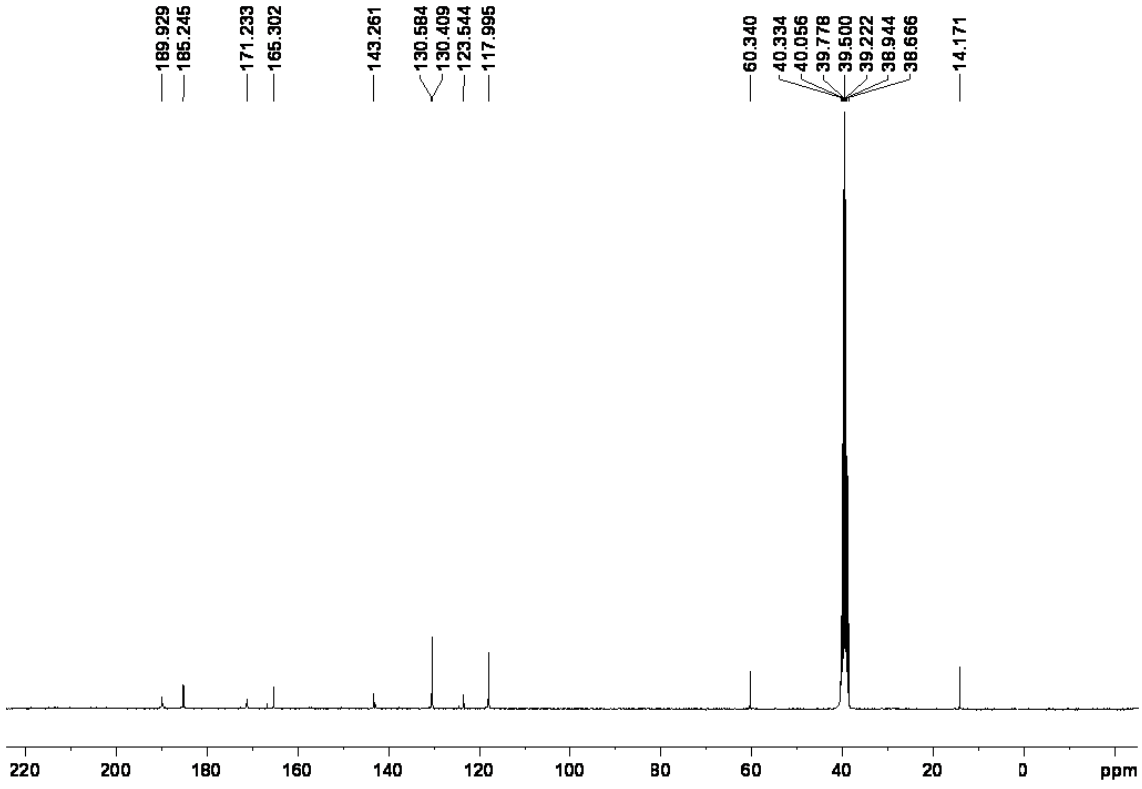


3-(4-carboxyethylphenylamino)-4-hydroxycyclobut-3-ene-1,2-dione (5k).

^{1}H NMR, DMSO, 300 MHz, 298 K

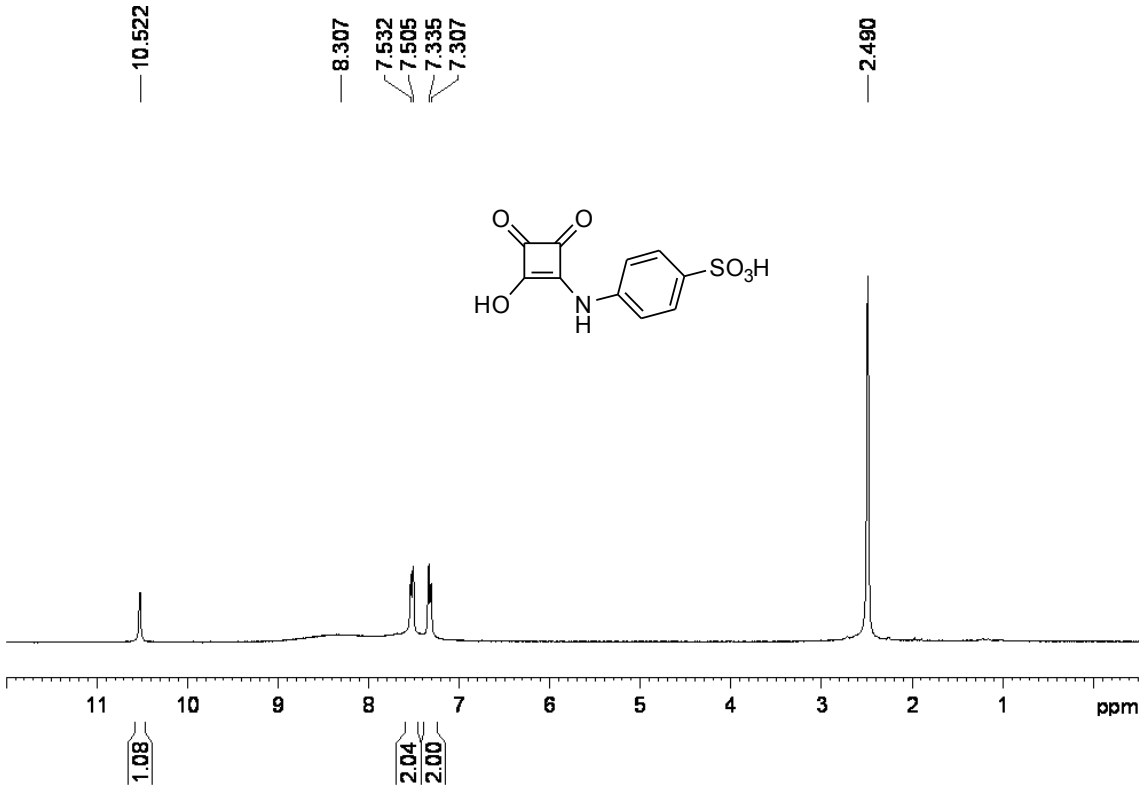


^{13}C NMR, DMSO, 75 MHz, 298 K

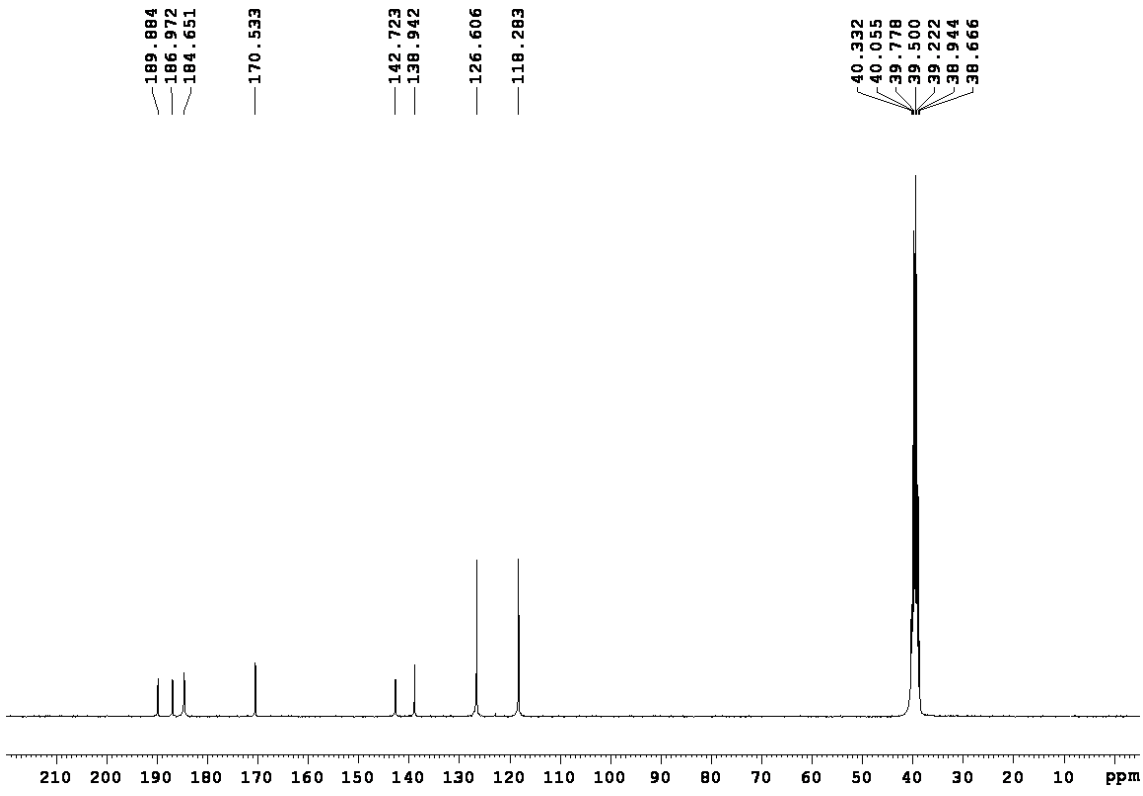


4-((2-hydroxy-3,4-dioxocyclobut-1-en-1-yl)amino)benzenesulfonic acid (5l).

^{1}H NMR, DMSO, 300 MHz, 298 K

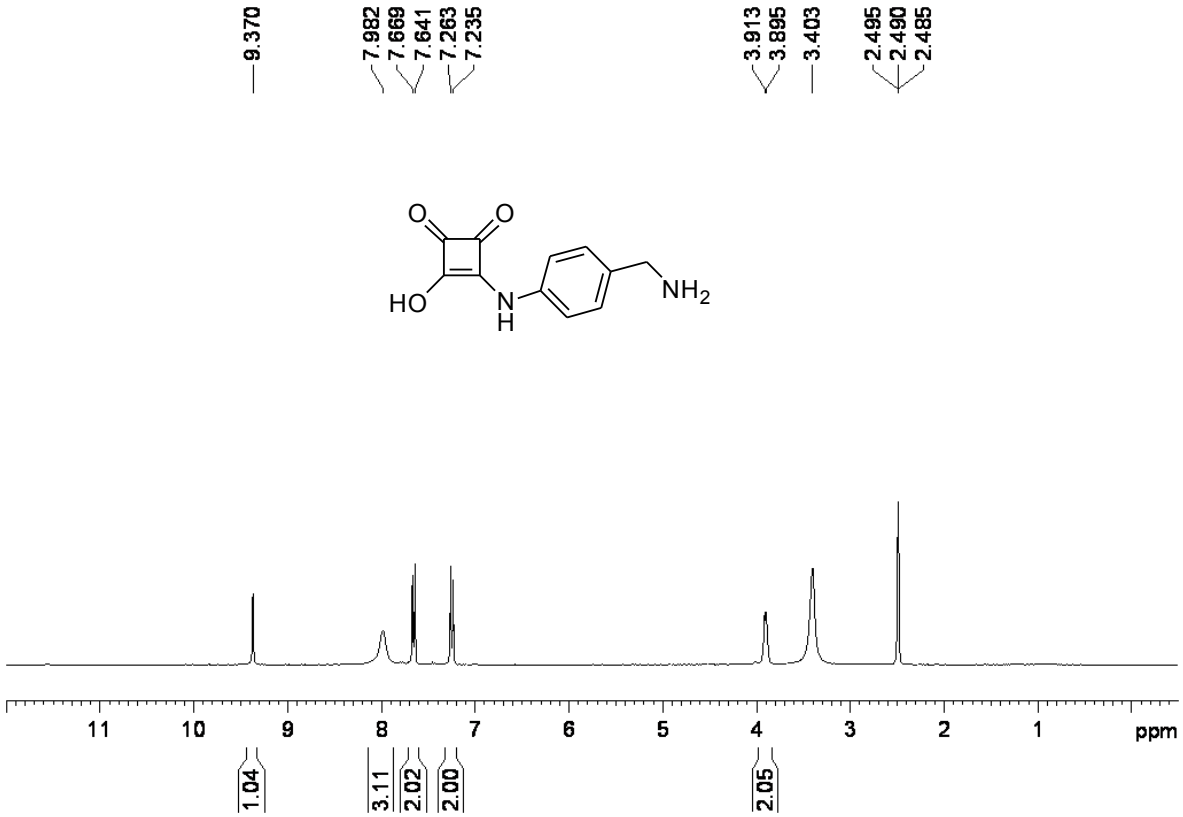


^{13}C NMR, DMSO, 75 MHz, 298 K

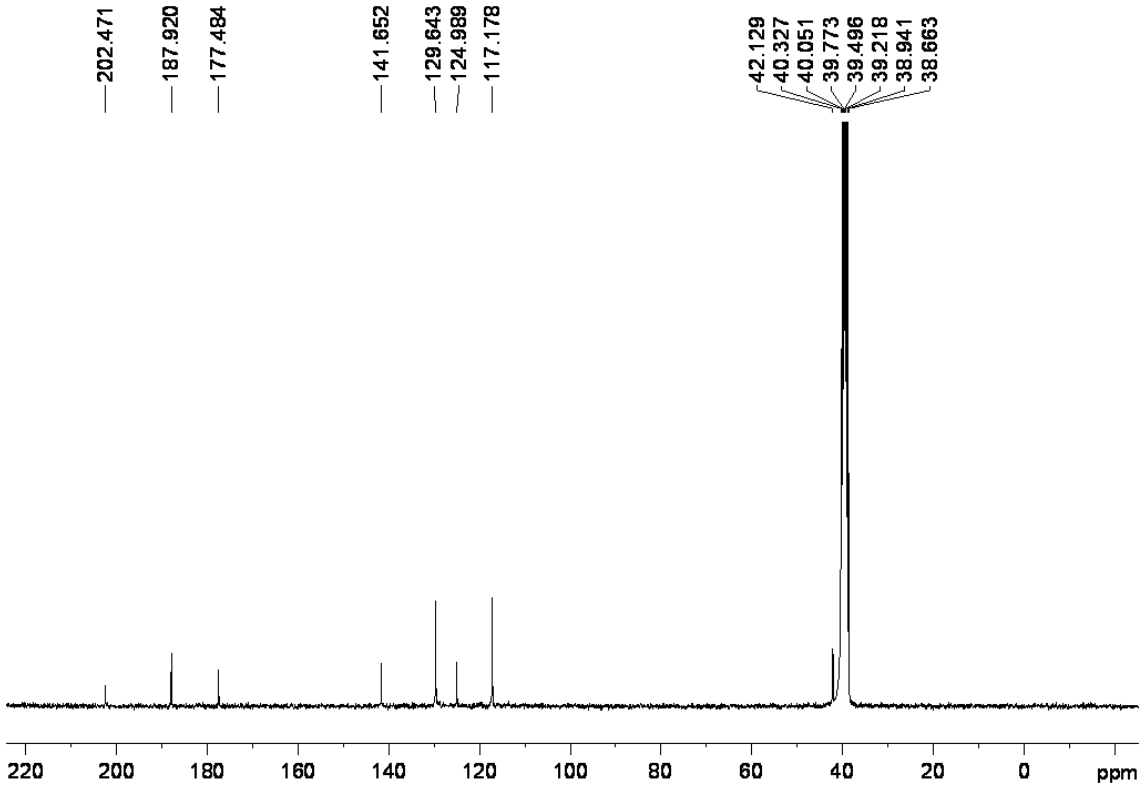


3-(4-(ethylamine)phenylamino)-4-hydroxycyclobut-3-ene-1,2-dione (5m).

^{1}H NMR, DMSO, 300 MHz, 298 K

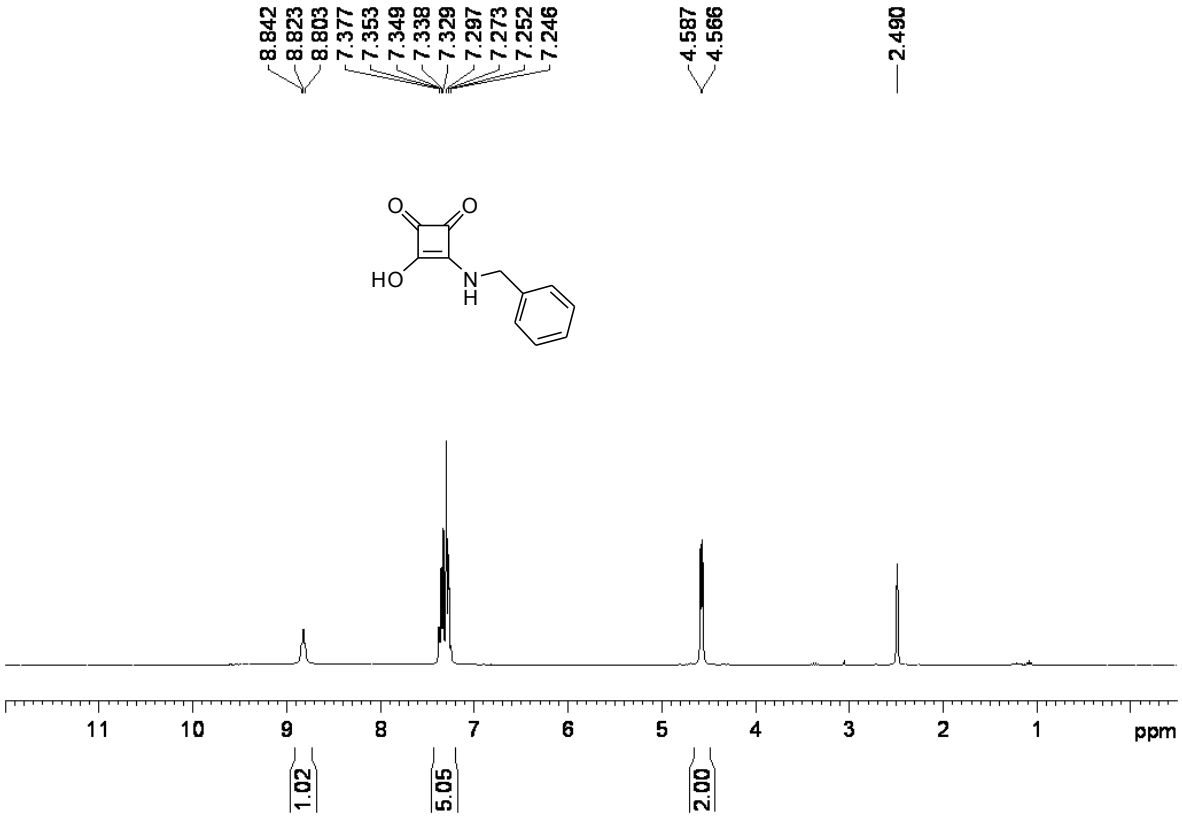


^{13}C NMR, DMSO, 75 MHz, 298 K

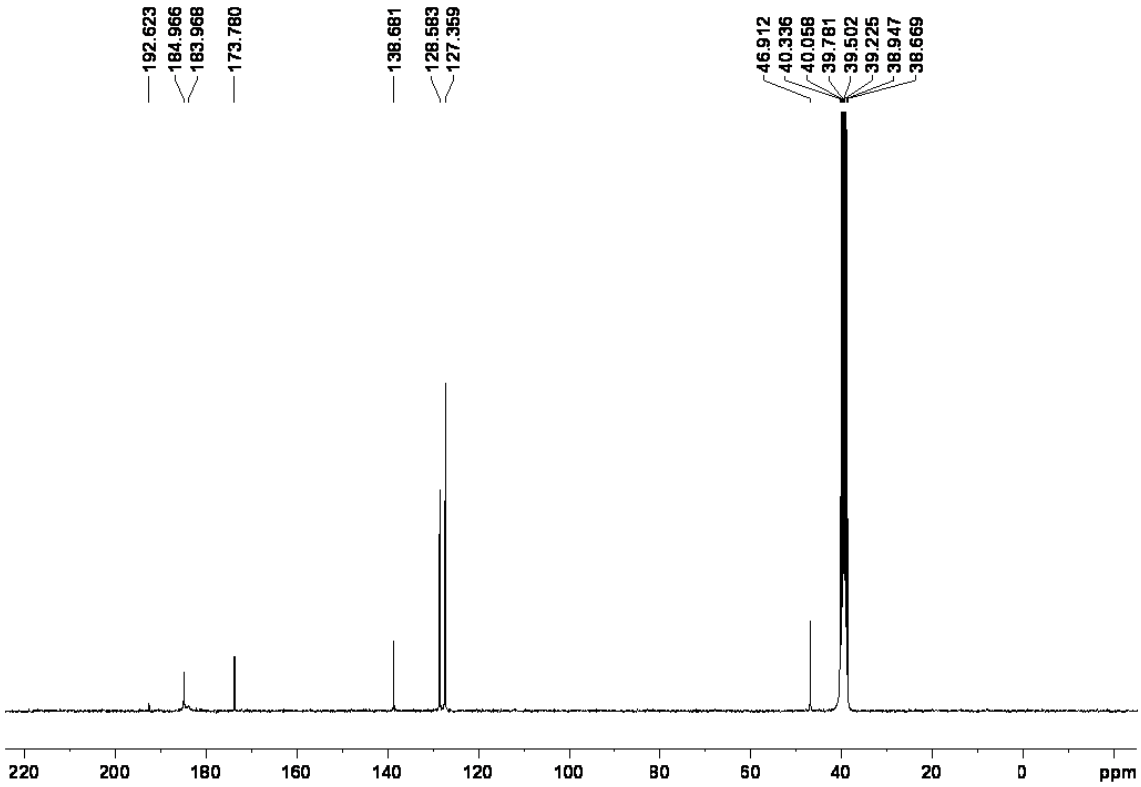


3-benzylamino-4-hydroxycyclobut-3-ene-1,2-dione (5n).

^{1}H NMR, DMSO, 300 MHz, 298 K

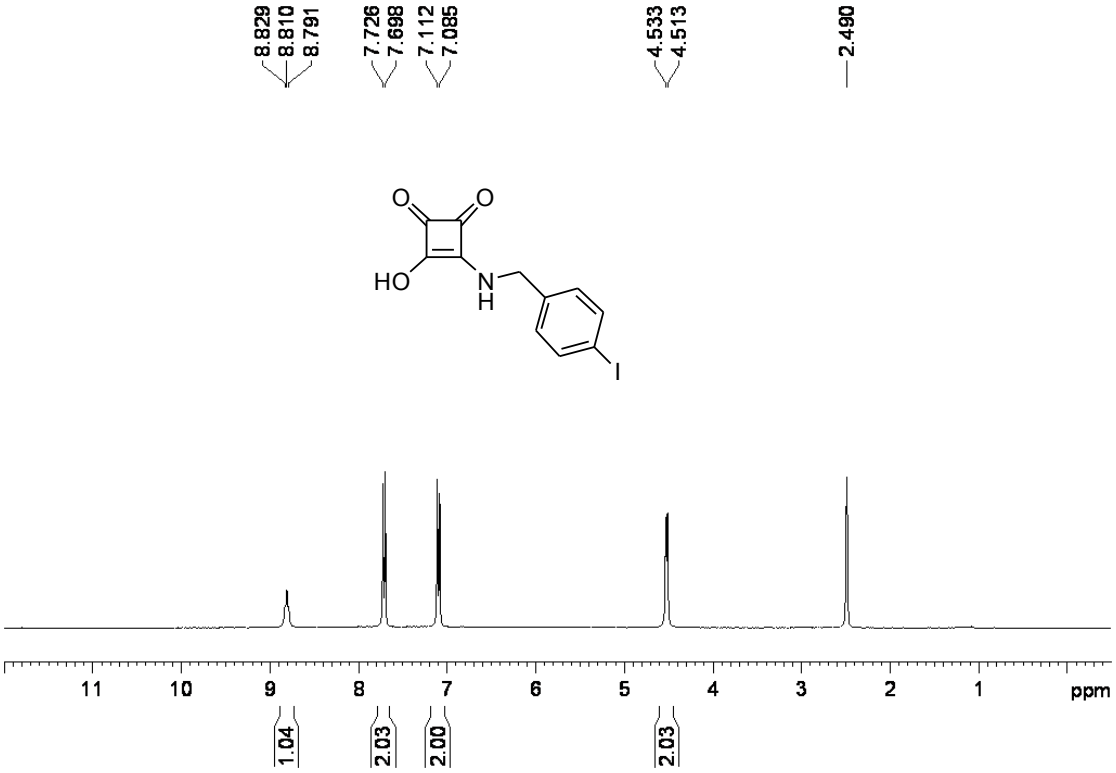


^{13}C NMR, DMSO, 75 MHz, 298 K

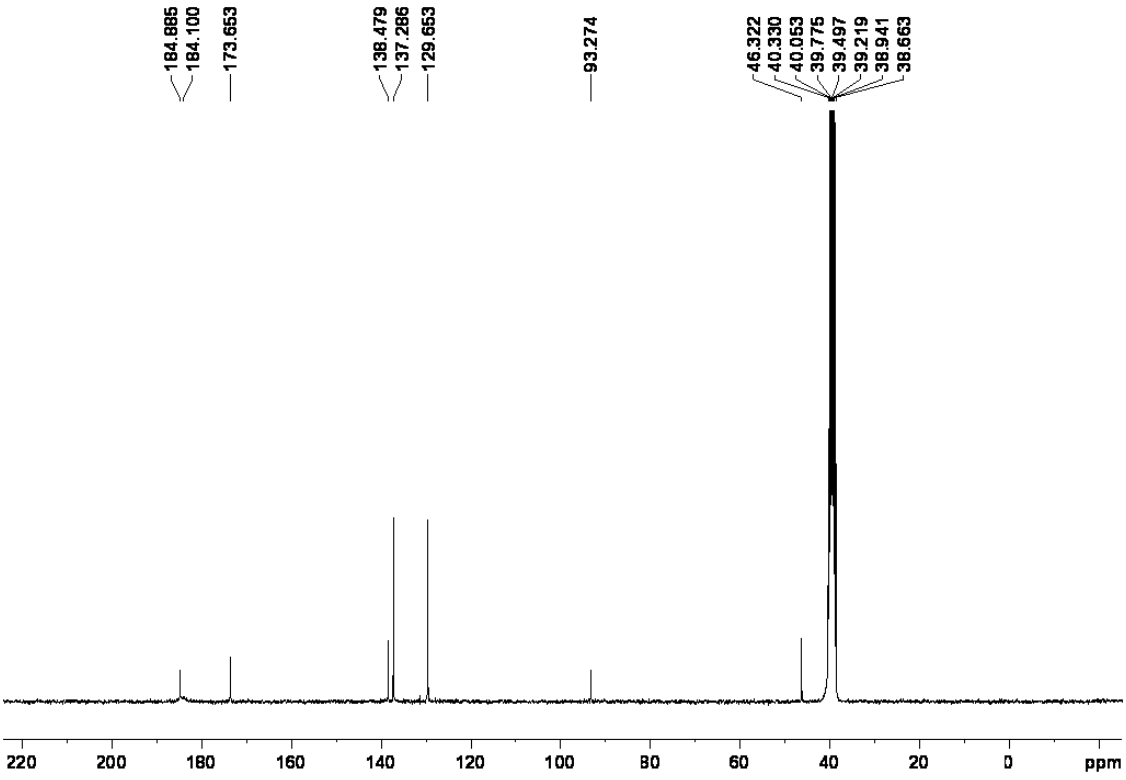


3-(4-iodobenzylamino)-4-hydroxycyclobut-3-ene-1,2-dione (5o).

^{1}H NMR, DMSO, 300 MHz, 298 K

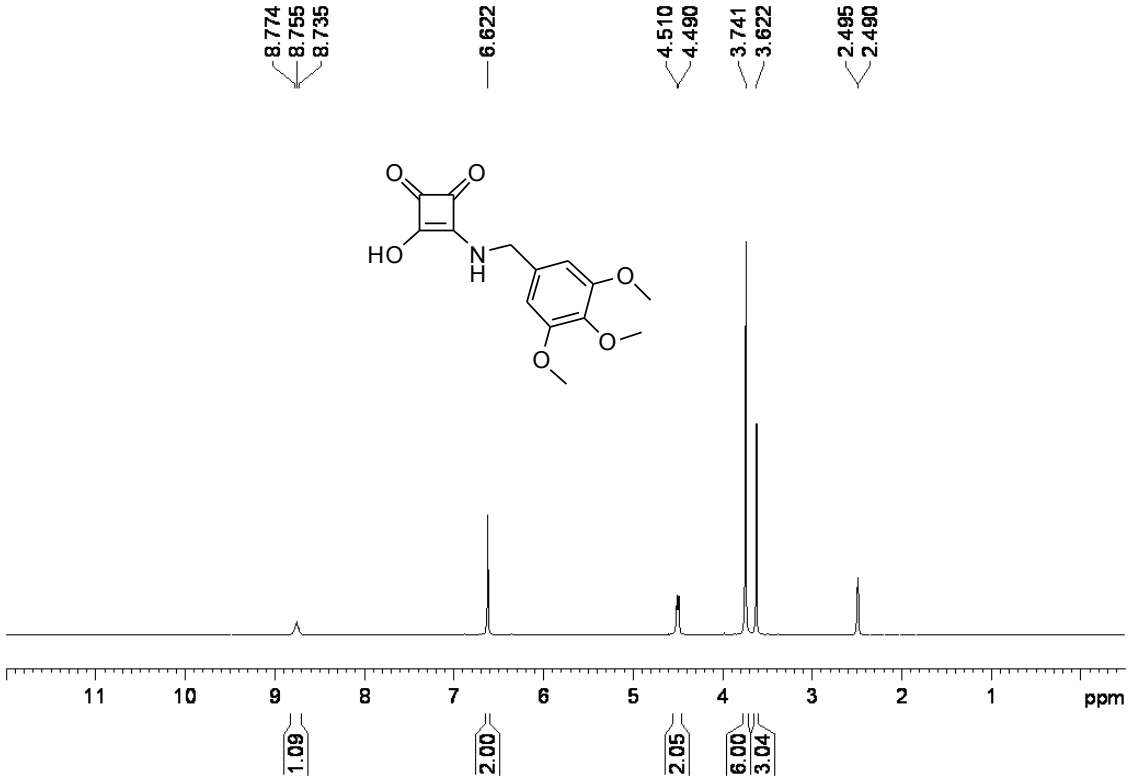


^{13}C NMR, DMSO, 75 MHz, 298 K

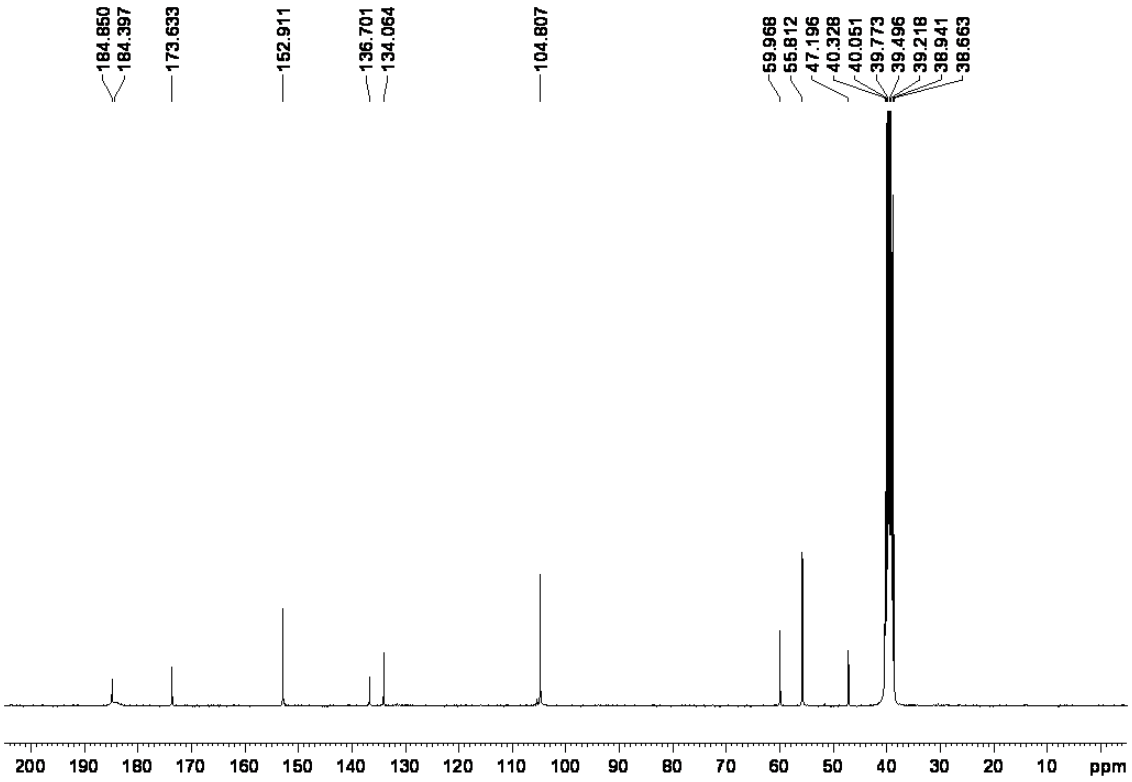


3-(3,4,5-trimethoxybenzylamino)-4-hydroxycyclobut-3-ene-1,2-dione (5p).

^{1}H NMR, DMSO, 300 MHz, 298 K

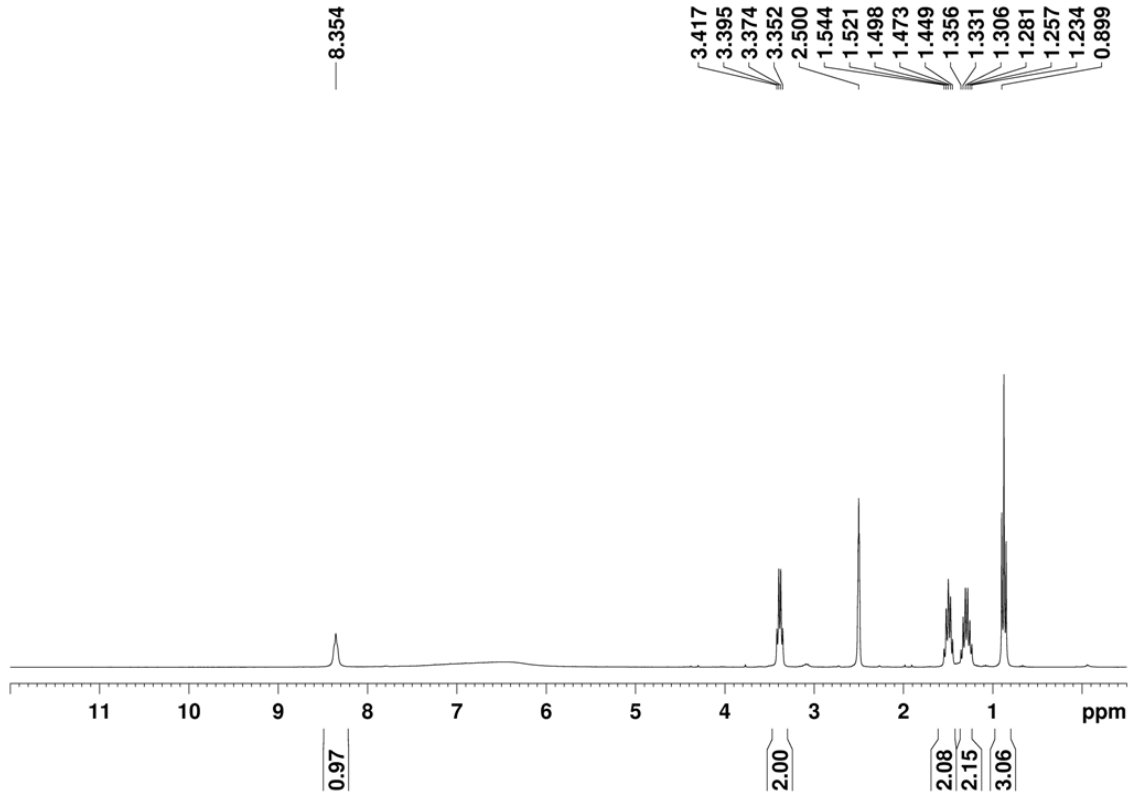


^{13}C NMR, DMSO, 75 MHz, 298 K

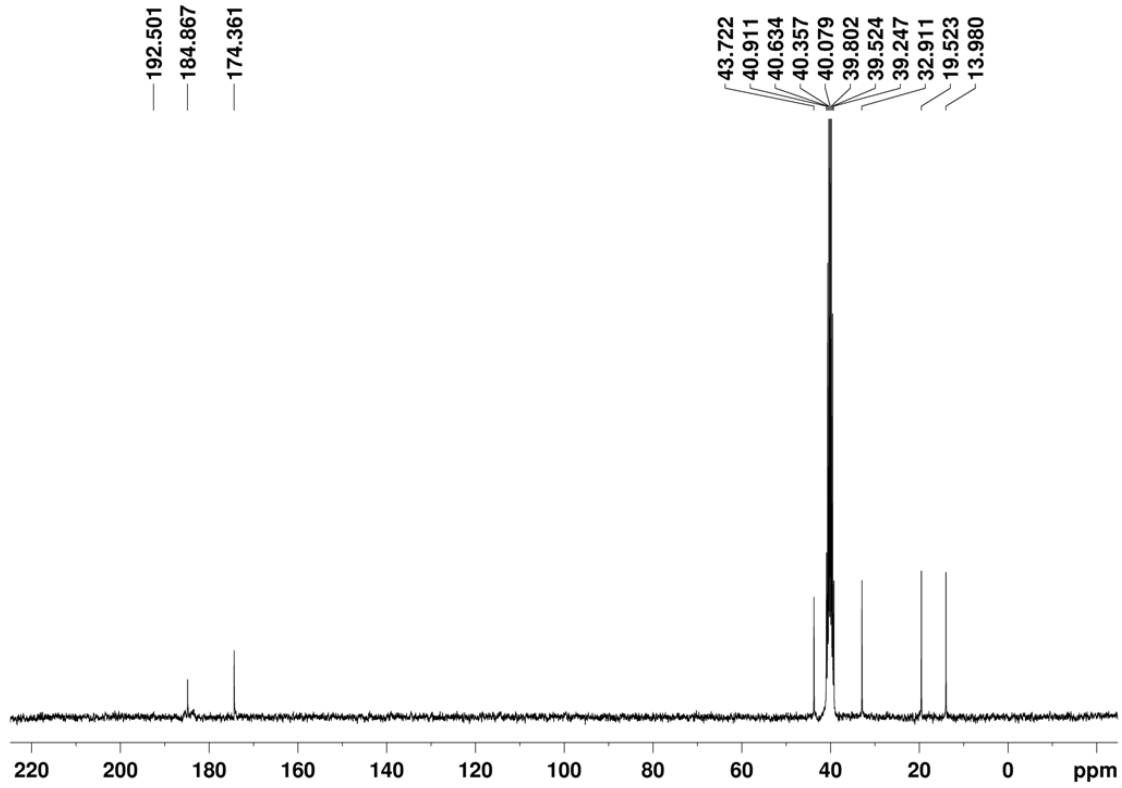


3-(butylamino)-4-hydroxycyclobut-3-ene-1,2-dione (5q).

^{1}H NMR, DMSO, 300 MHz, 298 K



^{13}C NMR, DMSO, 75 MHz, 298 K



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