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Synthesis of new compounds in the series of aryl-substituted ureas with cytotoxic and antioxidant activity

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Synthesis and the target compound characterization

Structures of all synthesized compounds were confirmed by ^{1}H and ^{13}C NMR, mass-spectrometry and elemental analysis data. ^{1}H and ^{13}C NMR-spectra were recorded with «Bruker DRX-400» spectrometer operating at 400.13 MHz frequency, using DMSO-d_6 as solvent and TMS as an internal standard. Chemical shifts were measured with 0.01 ppm accuracy, coupling constants are reported in Hz. HPLC-MS was recorded on an inductively coupled plasma mass spectrometer XSeries II ICP-MS (Thermo Scientific Inc., USA). The melting point was determined by using the melting point apparatus Stuart SMP20 (UK).

For a qualitative analysis of reaction mixtures compositions, aluminum TLC plates with silica gel (0.015-0.040 mm) with a fluorescent indicator F254 (20 x 20 cm) (Merck Millipore, Germany) were used. For preparative chromatographic separation of the substances mixtures, «Kieselgel 60» silica gel (0.015-0.040 mm, Merck Millipore, Germany) was used.

List of used reagents: toluene, acetonitrile, isopropanol, carbon tetrachloride, diethylene triamine, urea, 2 commercially available as a 75% aqueous solution, triethylamine, p-chlorophenyl isocyanate, 3,4-dichlorophenyl isocyanate.

Cultural media and cell culture additives: DMEM, MEM, fetal bovine serum (FBS), L-glutamine, a mixture of penicillin and streptomycin, amphotericin B, trypsin solution, Versen's solution, MTT, D-glucose, Hanks's solution, solution of essential amino acids, sodium pyruvate solution was purchased from Paneco, Russia. Isopropanol, DMSO, H_2O_2 and hydrochloric acid were purchased from Himmed, Russia. Triton X-100, $CoCl_2*6H_2O$, Hoechst 33258 were purchased from Sigma-Aldrich, USA. All reagents used in the work were of the highest purity available.

1-(2-Aminoethyl)-2-imidazolidinone (1). In a three-neck flask equipped with a Dean-Stark apparatus, a reflux condenser, a thermometer and a magnetic stirrer, 38.6 g (0.375 mol) of diethylene triamine was placed, then 40% aqueous urea solution was added containing 15.0 g (0.25 mol) of urea. The mixture was heated for 1 hour at a temperature of 100-125 °C with a gradual distillation of water. Then a gradual increase in temperature was made: another 90 minutes at 140 °C and another 90 minutes at 150 °C. Then, temperature was maintained at 160 °C for 4 hours until cessation of ammonia emission. The product was purified by vacuum distillation. As a result, 20.95 g (65% yield) of a viscous yellowish liquid were obtained, with a crystallization temperature close to room temperature and with a boiling point T_{bp} = 166-170 ° C / 1 mm Hg. Literature data: T_{bp} = 138-140 ° C / 0.15 mm Hg [S1]. ^{1}H NMR (DMSO-d6, δ, ppm, *J*, Hz): 2.62 (**t**, 2H, $-N-CH_2-C\underline{H}_2-NH_2$, *J* = 6.6). 3.01 (**t**, 2H, $-N-CH_2-C\underline{H}_2-_{cycle}$, *J* = 6.6), 3.25-3.17 (**m**, 2H, $-N-C\underline{H}_2-CH_2-NH_2$), 3.37-3.31 (**m**, 2H $-N-C\underline{H}_2-CH_2-_{cycle}$), 6.09 (**s**, 1H, -NH-C(O)-). ^{13}C NMR (DMSO-d$_6$, δ, ppm): 37.5, 39.8, 44.9, 46.5, 162.6. HPLC-MS: $[M + 1]^+$ 130.08; calculated value is 130.09.

1-(2-Hydroxyethyl)-2-imidazolidinone (2). Compound **2** is commercially available as a 75% aqueous solution. This initial solution of compound 2 was evaporated on a rotary evaporator until a very viscous liquid appeared that solidifies when cooled (the melting point is close to room temperature). Additional drying was carried out by azeotropic distillation of water with CCl_4. The substance was yellow-brown in color with a melting point of 45°C. Literature data: T_{mp} = 46-49°C [S2]. ^{1}H NMR (DMSO-d6, δ, ppm, *J*, Hz): 3.07 (**t**, 2H, $-N-CH_2-C\underline{H}_2-_{cycle}$, *J* = 5.9). 3.23-3.16 (**m**, 2H, $-N-C\underline{H}_2-CH_2-OH$), 3.41-3.34 (**m**, 2H, $-N-C\underline{H}_2-CH_2-_{cycle}$), 3.45 (**t**, 2H, $-N-CH_2-C\underline{H}_2-OH$, *J* = 5.9), 6.09 (**br. s**, 1H, -NH-C(O)-). ^{13}C NMR (DMSO-d$_6$, δ, ppm): 37.6, 45.5, 45.9, 59.4, 162.6. HPLC-MS: $[M + 1]^+$ 131.10; calculated value is 131.07.

*General procedure of arylcarbamates (**4**, **6**) synthesis.*

0.851 g (6.55 mmol) of compound **2** in a dry acetonitrile (20 ml) was placed in a round bottom flask equipped with a calcium chloride tube and magnetic stirrer. Then solution with 1.01 g (6.58 mmol) of the relevant phenylisocyanate in dry acetonitrile (30 ml) and 2-3 drops of triethylamine were added. The reaction mixture was stirred at room temperature for 24 hours. The solution was evaporated to dryness, the residue was recrystallized from methanol and a second time from isopropanol. The precipitate was filtered and washed with a small amount of cold isopropanol.

2-(2-Oxoimidazolidin-1-yl)ethyl *N*-(*p*-chlorophenyl)carbamate (4) 54% yield. T_{mp} = 172-175 ° C. ^{1}H NMR (DMSO-d$_6$, δ, ppm, *J*, Hz): 3.22 (**dt**, 2H, $-N-C\underline{H}_2-CH_2-_{cycle}$, *J* = 9.0, 6.6); 3.31 (**t**, 2H, $-N-CH_2-\underline{C}H_2-_{cycle}$, *J* = 5.5); 3.45-3.36 (**m**, 2H, $-N-C\underline{H}_2-CH_2-O-$); 4.16 (**t**, 2H, $-N-C\underline{H}_2-CH_2-O-$, *J* = 5.6); 6.21 (**s**, 1H, -NH-C(O)-N-); 7.35-7.24 (**m**, 2H, CH_{aryl}); 7.53-7.41 (**m**, 2H, CH_{aryl}); 9.67 (**s**, 1H, -NH-C(O)-O-). ^{13}C NMR (DMSO-d$_6$, δ, ppm): 37.9, 43.1, 45.6, 62.7, 120.4, 126.6, 129.0, 138.5, 153.8, 162.6. HPLC-MS: $[M + 1]^+$ 284.11; calculated value is 284.07.

2-(2-Oxoimidazolidin-1-yl)ethyl *N*-(3,4-dichlorophenyl)carbamate (6) 39% yield. T_{mp} = 148-149 ° C. ^{1}H NMR (DMSO-d$_6$, δ, ppm, *J*, Hz): 3.25-3.17 (**m**, 2H),3.34-3.29 (**m**, 2H), 3.44-

3.37 (**m**, 2H), 4.22-4.14 (**m**, 2H) (-CH_2-); 6.21 (**s**,1H, -NH-C(O)-N-);7.39 (**ddd**, 1H, *J* = 8.8, 2.4, 0.9), 7.48(**d**, 1H, *J* = 8.8), 7.74 (**d**, 1H, *J* = 2.4) (CH_{aryl}); 9.86 (**s**, 1H, -C(O)-NH-Ar). ^{13}C NMR (DMSO-d_6, δ, ppm): 37.4, 42.5, 45.0, 62.4; 118.3, 119.4, 123.9, 131.0, 130.5, 139.1, 153.1, 162.0. HPLC-MS: $[M + 1]^+$ 318.29; calculated value is 318.03.

*General procedure for the synthesis of arylureas (**3**, **5**, **7**).*

In a three-neck flask with a thermometer, a dropping funnel and a magnetic stirrer, 4.00 g (31 mmol) of compound 1 in 50 ml of dry toluene was placed. The mixture was cooled in an ice bath to a temperature no higher than 5 °C. Then solution with 3.69 g (31 mmol) of the relevant phenylisocyanate in 50 ml of dry toluene was added dropwise with stirring, keeping a temperature no higher than 5 °C. The reaction mixture was stirred at room temperature for 24 hours. The precipitate was filtered and recrystallized from acetone.

***N*-[2-(2-Oxoimidazolidin-1-yl)ethyl]-*N'*-phenylurea (3)** 35% yield, T_{mp} = 163-164 ° C (Lit .: T_{mp} = 162-164 ° C [S3]). ^{1}H NMR (DMSO-d_6, δ, ppm, *J*, Hz): 3.16-3.10 (**m**, 2H), 3.25-3.18 (**m**, 4H), 3.41-3.35 (**m**, 2H) (-CH_2-); 6.07 (**t**, 1H, -CH_2-N$\underline{H}$-C(O)-NH-, *J* = 5.5); 6.18 (**s**, 1H, -NH-C(O)-N-); 6.86 (**tt**, 1H, *J* = 7.3, 1.0), 7.24-7.15 (**m**, 2H), 7.39-7.34 (**m**, 2H) (CH_{aryl}); 8.43 (**s**, 1H, -C(O)-NH-Ar). HPLC-MS: $[M + 1]^+$ 249.23; calculated value is 249.29.

***N*-[2-(2-Oxoimidazolidin-1-yl)ethyl]-*N'*-(*p*-chlorophenyl)urea (5)** 68% yield. T_{mp} = 165-166 ° C. ^{1}H NMR (DMSO-d6, δ, ppm, *J*, Hz): 3.15-3.09 (**m**, 2H),3.24-3.17 (**m**, 4H), 3.39-3.33 (**m**,2H) (-CH_2-); 6.10 (**t**, 1H, -CH_2-N$\underline{H}$-C(O)-NH-, *J* = 5.4); 6.16 (**s**,1H, -NH-C(O)-N-);7.27-7.16 (**m**, 2H), 7.43-7.33 (**m**, 2H)(CH_{aryl}); 8.58 (**s**, 1H, -C(O)-NH-Ar). ^{13}C NMR (DMSO-d_6, δ, ppm) 37.9, 38.0, 43.8; 45.3, 120.4, 126.6, 129.0, 138.5, 153.8, 162.6. HPLC-MS: $[M + 1]^+$ found 283.27; calculated value is 283.09.

***N*-[2-(2-Oxoimidazolidin-1-yl)ethyl]-*N'*-(3,4-dichlorophenyl)urea (7).** 53% yield. T_{mp} = 184-186°C. ^{1}H NMR (DMSO-d_6, δ, ppm, *J*, Hz): 3.16-3.09 (**m**, 2H),3.24-3.17 (**m**, 4H), 3.39-3.33 (**m**,2H) (-CH_2-); 6.17 (**s**,1H, -NH-C(O)-N-); 6.19 (**t**, 1H, -CH_2-N$\underline{H}$-C(O)-NH-, *J* = 5.3); 7.22 (**dd**, 1H, *J* = 8.8, 2.5), 7.40 (**d**, 1H, *J* = 8.8), 7.79 (**d**, 1H, *J* = 2.5) (CH_{aryl}); 8.78 (**s**, 1H, -C(O)-NH-Ar). ^{13}C NMR (DMSO-d_6, δ, ppm): 37.9, 38.0, 43.7, 45.2 (-CH2-); 118.2, 119.3, 122.7, 130.8, 131.4, 141.2, 155.3, 162.8. HPLC-MS: $[M + 1]^+$ found 317.32; calculated value is 317.05.

Cultivation of cells.

All used cell lines were cultured in atmosphere with 95% humidity, 5% CO_2 and 37 °C. Human glioblastoma cells U-87 MG (HTB-14) were purchased from ATCC (American Type Culture Collection), Manassas, Virginia. Cultivation was carried out in MEM medium containing 10% FBS, 2 mM L-glutamine, 1% non-essential amino acids, 1 mM sodium pyruvate. Neuroblastoma cells SH-SY5Y (CRL-2266) were purchased from ATCC (American Type Culture Collection), Manassas, Virginia. Cultivation was performed in DMEM medium containing 10% FBS, 4 mM L-glutamine, 1% non-essential amino acids. Human melanoma cells A-375 (CRL-1619) and human breast cancer MDA-MB-231 (HTB-26) were purchased from ATCC (American Type Culture Collection), Manassas, Virginia. The cultivation was carried out in DMEM medium containing 10% FBS and 4 mm L-glutamine. All cultural media contained 100 units / ml of penicillin, 100 μg / ml of streptomycin, 2.5 μg / ml of amphotericin B. Adherent cells were removed by treatment with 0.25% trypsin solution in 0.53 mM EDTA with Hanks salts (Paneco, Russia). Cells were counted using a Goryaev chamber. The cell cultures were regularly tested for the absence of mycoplasma infection by Hoechst 33258 staining following microscopy.

Assessment of cytotoxicity.

For toxicity analysis cell cultures were seeded into 96-well plates and grown to a density of 3x104 cells / cm2. Serial dilutions of the test substances in the range of concentration 0.1–50 μM (final after adding to the cells) were prepared in DMSO and dissolved in the culture medium, after that solutions were added to the cell culture (each concentration was repeated in triplicate) and incubated for 18 hours. The incubation time was chosen based on the most significant differences+.363* between the substances. The final concentration of DMSO was 0.5%. To the control wells only DMSO was added, and the final concentration of it was 0.5%. Another variant of control was without DMSO (no difference with control with 0.5% DMSO). MTT assay (assessment of MTT reduction by living cell mitochondria) was used to assess cell viability under the action of the studied substances [S4]. Briefly, after removing the medium with the studied substances, the cells were incubated for 1.5 h with 0.5 mg / ml MTT solution (Paneco, Russia) in the Hanks solution (Paneco, Russia). After this, the solution was removed, and cells were dissolved in DMSO. Assessment of MTT reduction was evaluated photometrically at wavelengths of 594 and 620 nm using EFOS 9505 ("Moskovskij Zavod" Sapphir, Russia). Additionally, before the MTT assay, cells and cultural behavior were examined microscopically. Statistical analysis and plotting of the curves for calculating the LC50 were performed using GraphPad Prism 6.0. Each experiment was repeated three times. While processing statistical data, differences at $p < 0.05$ were considered significant.

References

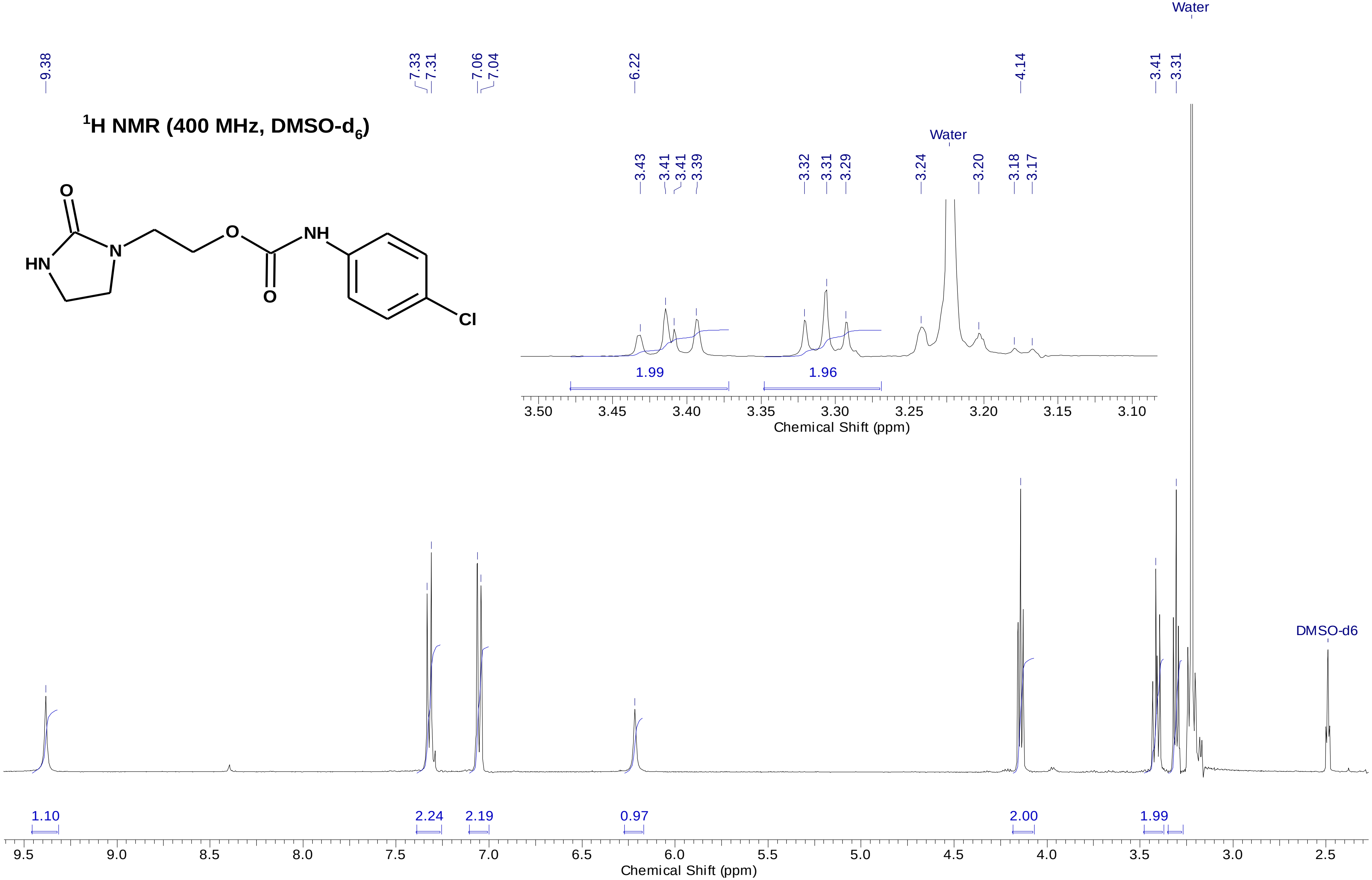
[S1] Y. He, J. Cai and Y. Zhang, *J. Mater. Sci. Chem. Eng.*, 2017, **5**, 155.

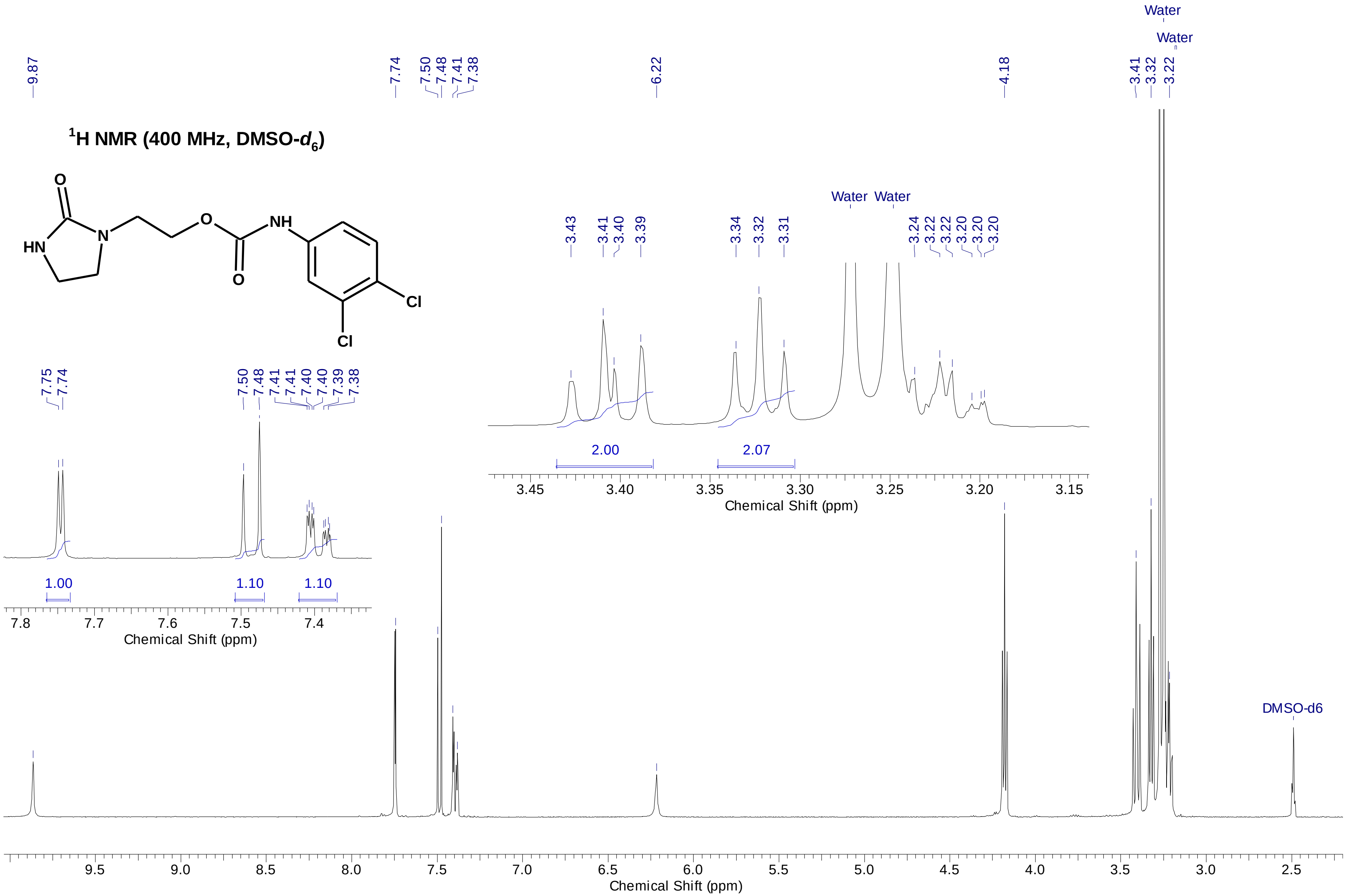
[S2] E. S. Streng, D. S. Lee, M. W. George and M. Poliakoff, *Beilstein J. Org. Chem.*, 2017, **13**, 329.

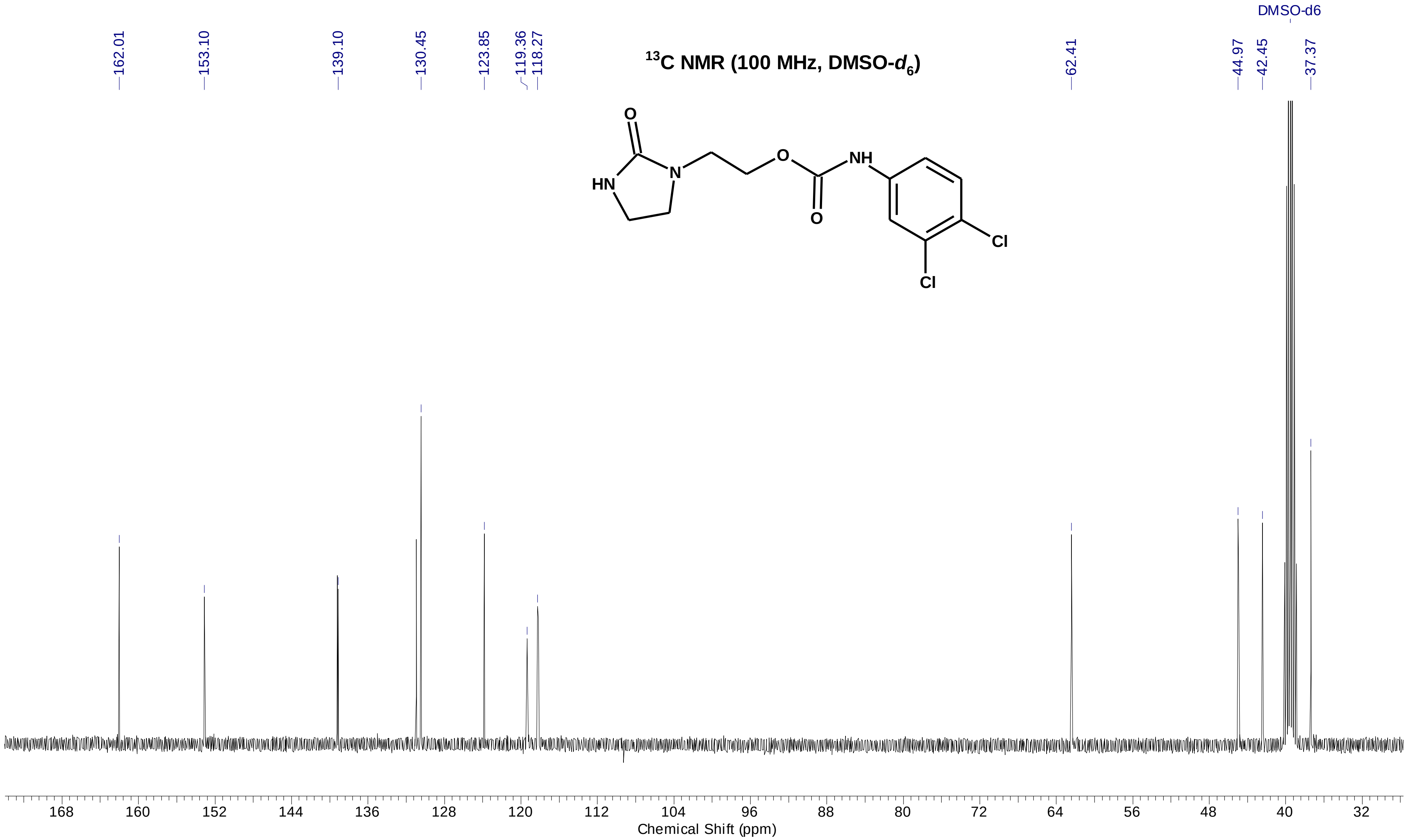
[S3] E. K. W. Wat, *US Patent 4,032,638*, 1977.

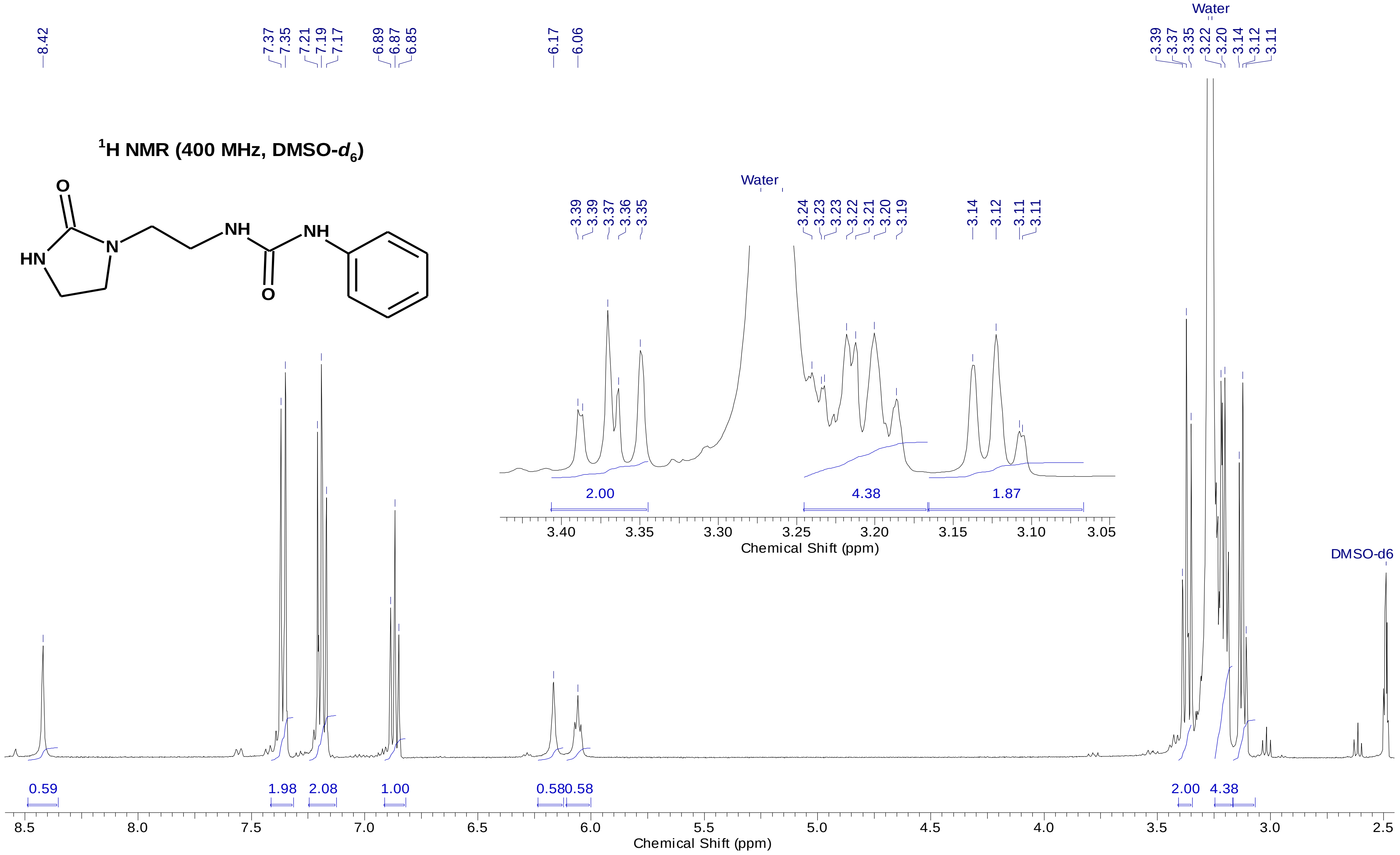
[S4] L. M. Green, J. L. Reade and C. F. Ware, *J. Immunol. Methods*, 1984, **70**, 257.

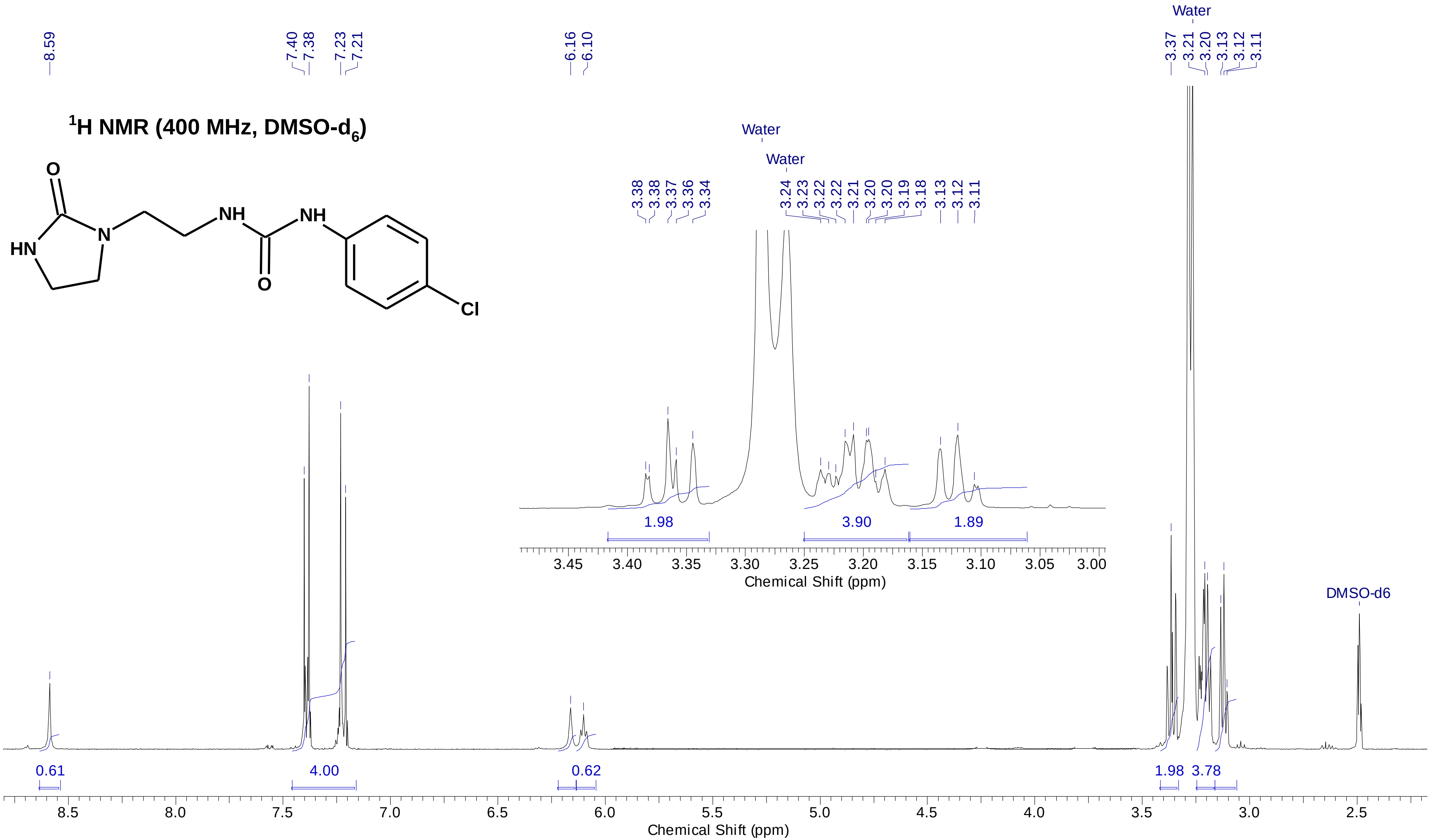
^{1}H NMR (400 MHz, DMSO-d_6)



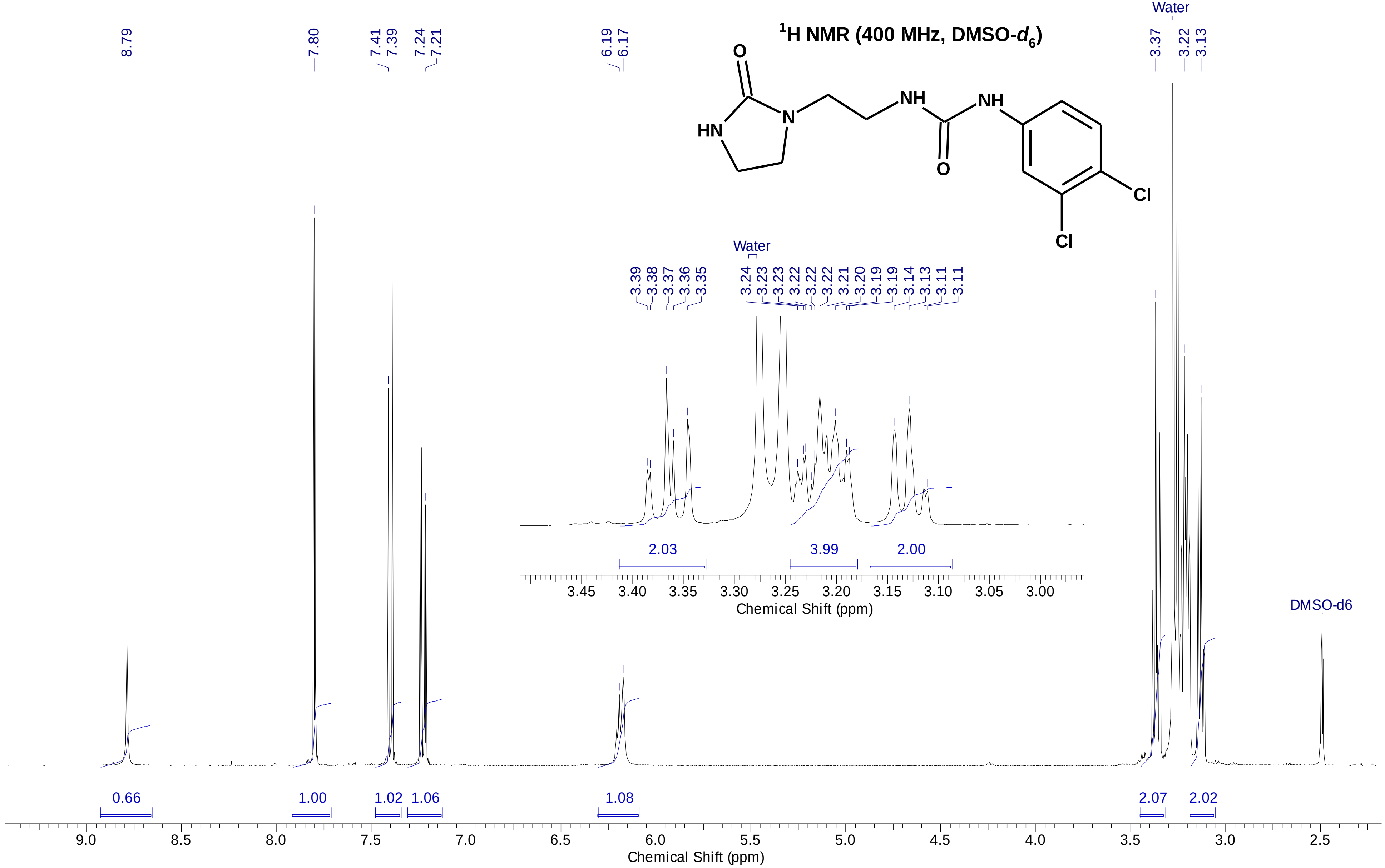

^{1}H NMR (400 MHz, DMSO-d_6)
O
HN
N
O
NH
O
Cl
Cl
9.87
7.74
7.50
7.48
7.41
7.38
6.22
4.18
3.41
3.32
3.22
Water
Water
DMSO-d6
7.75
7.74
7.50
7.48
7.41
7.41
7.40
7.40
7.39
7.38
1.00
1.10
1.10
7.8
7.7
7.6
7.5
7.4
Chemical Shift (ppm)
3.43
3.41
3.40
3.39
3.34
3.32
3.31
Water
Water
3.24
3.22
3.22
3.20
3.20
3.20
2.00
2.07
3.45
3.40
3.35
3.30
3.25
3.20
3.15
Chemical Shift (ppm)
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
Chemical Shift (ppm)


^{13}C NMR (100 MHz, DMSO-d_6)


^{1}H NMR (400 MHz, DMSO-d_6)
O
HN
N
NH
NH
O
8.42
7.37
7.35
7.21
7.19
7.17
6.89
6.87
6.85
6.17
6.06
Water
3.39
3.37
3.35
3.22
3.20
3.14
3.12
3.11
DMSO-d6
0.59
1.98
2.08
1.00
0.58
0.58
2.00
4.38
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
Chemical Shift (ppm)
Water
3.39
3.39
3.37
3.36
3.35
3.24
3.23
3.23
3.22
3.21
3.20
3.19
3.14
3.12
3.11
3.11
2.00
4.38
1.87
3.40
3.35
3.30
3.25
3.20
3.15
3.10
3.05
Chemical Shift (ppm)



^{1}H NMR (400 MHz, DMSO-d_6)



¹H NMR (400 MHz, DMSO-d₆)
O
HN
N
NH
NH
O
Cl
Cl
Water
8.79
7.80
7.41
7.39
7.24
7.21
6.19
6.17
3.37
3.22
3.13
Water
3.39
3.38
3.37
3.36
3.35
3.24
3.23
3.23
3.22
3.22
3.22
3.21
3.20
3.19
3.19
3.14
3.13
3.11
3.11
2.03
3.99
2.00
3.45
3.40
3.35
3.30
3.25
3.20
3.15
3.10
3.05
3.00
Chemical Shift (ppm)
DMSO-d6
0.66
1.00
1.02
1.06
1.08
2.07
2.02
9.0
8.5
8.0
7.5
7.0
6.5
6.0
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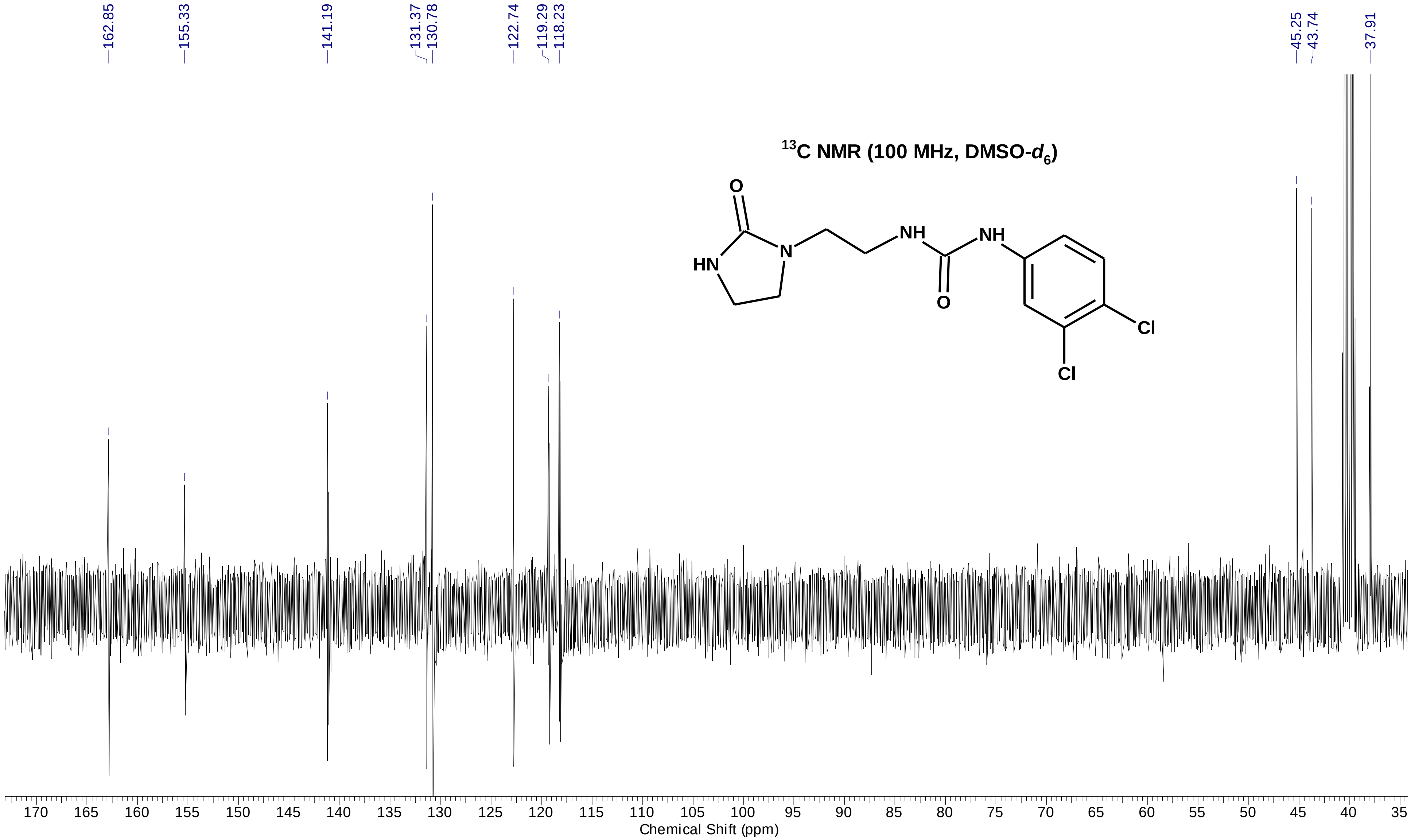
3.0
2.5
Chemical Shift (ppm)



162.85
155.33
141.19
131.37
130.78
122.74
119.29
118.23
45.25
43.74
37.91
^{13}C NMR (100 MHz, DMSO-d_6)
O
HN
N
NH
NH
O
Cl
Cl
170
165
160
155
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
Chemical Shift (ppm)