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New N^1-(4-aryloxybenzyl)uracils containing N^3-positioned 4-(trimethyleneoxy)benzoic acid moiety, and study of their antiviral activity against SARS-CoV-2 and influenza virus

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Experimental

All reagents were obtained at the highest grade available from Sigma and Acros Organics, and were used without further purification unless otherwise noted. Anhydrous DMF and isopropyl alcohol were purchased from Sigma-Aldrich Co. Anhydrous acetone, DCE, and EtOAc were obtained by distillation over P_2O_5. TLC was performed on Merck TLC Silica gel 60 F_{254} plates eluting with the specified solvents and samples were made visual with a VL-6.LC UV lamp (Vilber). Acros Organics (Belgium) silica gel (Kieselguhr 60–200 μm, 60A) was used for column chromatography. Yields refer to spectroscopically (^{1}H and ^{13}C NMR) homogeneous materials. Melting points were determined in glass capillaries on a Mel-Temp 3.0 (Laboratory Devices Inc., USA). NMR spectra were obtained using Bruker Avance 400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) and Bruker Avance 600 (600 MHz for ^{1}H and 150 MHz for ^{13}C) spectrometers in DMSO-d_6 or $CDCl_3$ with tetramethylsilane as an internal standard. High resolution mass spectra were measured on Bruker micrOTOF II instruments using electrospray ionization (HRESIMS). The measurements were done in a positive ion mode (interface capillary voltage -4500 V) in a mass range from m/z 50 to m/z 3000 Da; external or internal calibration was done with ESI Tuning MixTM (Agilent Technologies). A syringe injection was used for solutions in acetonitrile (flow rate 3 μl min^{-1}). Nitrogen was applied as a dry gas; interface temperature was set at 180 °C.

General procedure for the synthesis of 1-[4-aryloxybenzyl]uracil derivatives 3a-d

2,4-Bis(trimethylsilyloxy)pyrimidine **1**, obtained by boiling uracil (1.0 g, 8.9 mmol) in HMDS (50 ml) in the presence of NH_4Cl (0.15 g), was dissolved in anhydrous 1,2-dichloroethane (50 ml), and an equimolar amount of the corresponding 4-aryloxybenzyl bromide **2** was added. The resulting mixture was refluxed for 24 h protected from air moisture and treated with Pr^{i}OH (15 ml). The precipitate was filtered off and purified by flash chromatography eluting with $CHCl_3$-EtOH (10:1). The eluate was evaporated *in vacuo*, and the residue was crystallized from a mixture of DCM -Pr^{i}OH-H_2O (2:2:1).

1-[4-(Phenoxy)benzyl]uracil (3a).

Yield 66%, mp 147-148 °C, R_f 0.50 (ethyl acetate). ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.85 (2H, s, CH_2), 5.60 (1H, dd, *J* = 7.8 and 2.3 Hz, Ura-H-5), 6.95-7.03 (4H, m, H-2', H-6', H-2", H-6"), 7.14 (1H, t, *J* = 7.3 Hz, H-4"), 7.33-7.40 (4H, H-3', H-5', H-3", H-5"), 7.77 (1H, d, *J* = 7.8 Hz, Ura-H-6), 11.31 (1H, d, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 50.2, 101.8, 119.2, 119.5, 121.1, 124.0, 129.9, 130.5, 133.3, 146.0, 151.5, 156.7, 164.1.

1-[4-(4-*tert*-Butylphenoxy)benzyl]uracil (3b).

Yield 69%, mp 166-167.5 °C, R_f 0.53 (ethyl acetate). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.28 (9H, s, CH_3 × 3), 4.84 (2H, s, CH_2), 5.60 (1H, d, *J* = 7.8 and 2.3 Hz, Ura-H-5), 6.93 (2H, d, *J* = 8.7 Hz, H-2", H-6"), 6.97 (2H, d, *J* = 8.7 Hz, H-3", H-5"), 7.32 (2H, d, *J* = 8.5 Hz, H-2', H-6'), 7.39 (2H, d, *J* = 8.8 Hz, H-3', H-5'), 7.77 (1H, d, *J* = 7.9 Hz, Ura-H-6), 11.30 (1H, d, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 31.7, 50.2, 101.8, 118.8, 118.9, 127.2, 129.9, 132.0, 146.0, 146.4, 151.5, 154.5, 157.1, 164.1.

1-[4-(4-Fluorophenoxy)benzyl]uracil (3c).

Yield 73%, mp 167-168 °C, R_f 0.58 (ethyl acetate). ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.85 (2H, s, CH_2), 5.60 (1H, d, *J* = 7.9 and 2.3 Hz, Ura-H-5), 6.98 (2H, d, *J* = 8.7 Hz, H-2", H-6"), 7.04-7.09 (2H, m, aromatic H), 7.19-7.25 (2H, m, aromatic H), 7.33 (2H, d, *J* = 8.7 Hz, H-3', H-5'), 7.77 (1H, d, *J* = 7.8 Hz, Ura-H-6), 11.31 (1H, d, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 50.2, 101.8, 116.9, 117.2, 118.7,121.2, 121.3, 130.0, 132.2, 146.0, 151.5, 152.9, 157.2, 160.3, 164.1.

1-[4-(4-Chlorophenoxy)benzyl]uracil (3d).

Yield 74%, mp 130-132 °C, R_f 0.48 (ethyl acetate). ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.86 (2H, s, CH_2), 5.61 (1H, dd, *J* = 7.8 and 2.3 Hz, Ura-H-5), 7.01-7.04 (4H, m, H-2", H-3", H-5", H-6"), 7.36 (2H, d, *J* = 8.8 Hz, H-2', H-6'), 7.43 (2H, d, *J* = 9.0 Hz, H-3', H-5'), 7.78 (1H, d, *J* = 7.8 Hz, Ura-H-6), 11.29 (1H, d, NH); ^{13}C NMR (150 MHz, DMSO-d_6) δ 50.2, 101.8, 119.4, 120.7, 127.7, 130.0, 130.4, 132.8, 146.0, 151.5, 156.0, 156.3, 164.1.

General procedure for the synthesis of benzoic acid derivatives 5a-e.

A mixture of 1-(4-phenoxybenzyl)uracil derivative **3a-d** (3.4 mmol) and K_2CO_3 (0.65 g, 4.7 mmol) stirred in a DMF (15 ml) at 80 °C for 1 h. Then 3.4 mmol of 4-(3-bromopropoxy)benzoic acid methyl ester **4a** or 4-(4-bromobutoxy)benzoic acid methyl ester **4b** was added, and then stirred at the same temperature for 24 h. The reaction mass was evaporated in a vacuum, the residue was treated with water (100 ml) and extracted with chloroform (5 × 25 ml). The combined extract was evaporated under reduced pressure, the residue (oily liquid) was dissolved in ethanol (20 ml) containing NaOH (0.8 g, 20.00 mmol), and water (20 ml) were added. The resulting mixture was stirred at room temperature for 36 h, then evaporated under reduced pressure, the residue was dissolved in water (100 ml) and acidified

with conc. HCl up to pH 2. The precipitate that formed was filtered off, dried in air on a Petri dish, and purified by flash chromatography eluting with ethyl acetate. The proper fractions of the eluate were evaporated under reduced pressure, the solid residue was recrystallized from a mixture of ethyl acetate-hexane (1:1).

4-{3-[2,6-Dioxo-3-(4-phenoxybenzyl)-3,6-dihydropyrimidin-1(2*H*)-yl]propoxy}benzoic acid (5a).

Yield 66%, mp 161-162.5 °C, R_f 0.48 (Pr^iOH-ethyl acetate-NH_4OH, 9:6:5). ^1H NMR (300 MHz, DMSO-D_6) δ 2.02 (2H, quin, *J* = 7.0 Hz, CH_2), 4.00 (2H, t, *J* = 7.3 Hz, CH_2), 4.06 (2H, t, *J* = 6.0 Hz, CH_2), 4.90 (2H, s, CH_2), 5.73 (1H, d, *J* = 7.9 Hz, Ura-H-5), 6.89-7.01 (6H, m, aromatic H), 7.14 (1H, t, *J* = 7.5 Hz, H-4"), 7.33-7.41 (4H, m, aromatic H), 7.81-7.89 (3H, m, aromatic H, Ura-H-6). ^{13}C NMR (75 MHz, DMSO-D_6) δ 27.4, 38.5, 51.3, 66.5, 101.1, 114.6, 119.1, 119.2, 119.4, 121.1, 123.4, 124.1, 130.0, 130.1, 130.5, 131.8, 132.1, 133.3, 144.4, 151.7, 156.8, 162.5, 162.9, 167.4. HRMS, m/z: calculated for $C_{27}H_{24}N_2O_6$ [M + H]+ 473.1707; found 473.1703.

4-(3-{2,6-Dioxo-3-[4-(4-*tert*-butylphenoxy)benzyl]-3,6-dihydropyrimidin-1(2*H*)-yl}-propoxy)benzoic acid (5b).

Yield 63%, mp 169-171 °C, R_f 0.58 (Pr^iOH-ethyl acetate-NH_4OH, 9:6:5). ^1H NMR (300 MHz, DMSO-d_6) δ 1.27 (9H, s, CH_3 × 3), 2.01 (2H, quin, *J* = 6.5 Hz, CH_2), 4.00 (2H, t, *J* = 6.7 Hz, CH_2), 4.05 (2H, t, *J* = 6.2 Hz, CH_2), 4.89 (2H, s, CH_2), 5.73 (1H, d, *J* = 7.7 Hz, Ura-H-5), 6.88-6.97 (6H, m, aromatic H), 7.32-7.40 (4H, m, aromatic H), 7.81-7.88 (3H, m, aromatic H, Ura-H-6). ^{13}C NMR (75 MHz, DMSO-d_6) δ 27.4, 31.7, 38.5, 51.3, 66.6, 101.1, 114.6, 118.8, 123.4, 127.2, 130.0, 131.75, 131.80, 144.4, 146.4, 151.7, 154.5, 157.2, 162.5, 162.9, 167.4. HRMS, m/z: calculated for $C_{31}H_{32}N_2O_6$ [M + H]+ 529.2333; found 529.2328.

4-(3-{2,6-Dioxo-3-[4-(4-fluorophenoxy)benzyl]-3,6-dihydropyrimidin-1(2*H*)-yl}propoxy]-benzoic acid (5c).

Yield 60%, mp 191.5-193 °C, R_f 0.51 (Pr^iOH-ethyl acetate-NH_4OH, 9:6:5). ^1H NMR (300 MHz, DMSO-D_6) δ 1.99 (2H, quin, *J* = 6.4 Hz, CH_2), 3.99 (2H, t, *J* = 6.8 Hz, CH_2), 4.05 (2H, t, *J* = 6.1 Hz, CH_2), 4.91 (2H, s, CH_2), 5.73 (1H, d, *J* = 7.9 Hz, Ura-H-5), 6.89-7.02 (6H, m, aromatic H), 7.33-7.41 (4H, m, aromatic H), 7.79-7.88 (3H, m, aromatic H, Ura-H-6). ^{13}C NMR (75 MHz, DMSO-D_6) δ 27.4, 38.5, 51.6, 66.6, 101.2, 114.6, 118.0, 118.1, 119.2, 119.5, 122.8, 123.5, 124.1, 130.5, 130.8, 131.7, 139.4, 144.5, 151.7, 156.7, 157.4, 162.5, 162.9, 167.5. HRMS, m/z: calculated for $C_{27}H_{23}FN_2O_6$ [M + H]+ 491.1613; found 491.1610.

4-(3-{2,6-Dioxo-3-[4-(4-chlorophenoxy)benzyl]-3,6-dihydropyrimidin-1(2*H*)-yl}propoxy)-benzoic acid (5d).

Yield 66%, mp 202-203.5 °C, R_f 0.55 (Pr^iOH-ethyl acetate-NH_4OH, 9:6:5). ^1H NMR (300 MHz, DMSO-D_6) δ 2.02 (2H, quin, *J* = 7.2 Hz, CH_2), 4.00 (2H, t, *J* = 6.8 Hz, CH_2), 4.06 (2H, t, *J* = 6.1 Hz, CH_2), 4.90 (2H, s, CH_2), 5.73 (1H, d, *J* = 7.8 Hz, Ura-H-5), 6.89-7.03 (6H, m, aromatic H), 7.35-7.43 (4H, m, aromatic H), 7.82-7.88 (3H, m, aromatic H, Ura-H-6). ^{13}C NMR (75 MHz, DMSO-D_6) δ 27.4, 38.5, 51.3, 66.6, 101.2, 114.6, 119.3, 120.8, 123.4, 127.8, 129.3, 130.1, 130.3, 131.8, 132.6, 144.4, 151.7, 156.0, 156.3, 162.5, 162.9, 167.4. HRMS, m/z: calculated for $C_{27}H_{23}ClN_2O_6$ [M + H]+ 507.1317; found 507.1311.

4-(4-{2,6-Dioxo-3-[4-(4-chlorophenoxy)benzyl]-3,6-dihydropyrimidin-1(2*H*)-yl}butoxy)-benzoic acid (5e).

Yield 70%, mp 90-92 °C, R_f 0.56 (Pr^iOH-ethyl acetate-NH_4OH, 9:6:5). ^1H NMR (300 MHz, DMSO-D_6) δ 1.69-1.71 (4H, m, CH_2 × 2), 3.87 (2H, t, *J* = 6.6 Hz, CH_2), 4.05 (2H, t, *J* = 5.7 Hz, CH_2), 4.92 (2H, s, CH_2), 5.74 (1H, d, *J* = 7.8 Hz, Ura-H-5), 6.95-7.04 (6H, m, aromatic H), 7.34-7.43 (4H, m, aromatic H), 7.81-7.89 (3H, m, aromatic H, Ura-H-6). ^{13}C NMR (75 MHz, DMSO-D_6) δ 24.3, 26.5, 51.4, 67.9, 101.1, 114.7, 119.4, 120.8, 121.1, 123.3, 127.8, 130.1, 130.4, 131.8, 132.6, 144.5, 151.6, 156.0, 156.3, 162.7, 162.8, 167.4. HRMS, m/z: calculated for $C_{28}H_{25}ClN_2O_6$ [M + H]+ 521.1474; found 521.1469.

Antiviral activity

Viruses

Influenza virus A/California/7/2009(H1N1)pdm09 and SARS-CoV-2 culture hCoV-19/Russia/Moscow-PMVL-12/2020 (EPI_ISL_572398) GISAD: PMVL-12 was used. The culture was obtained from the State Collection of Viruses of the Russian Federation at the FSBI ‘National Research Center for Epidemiology and Microbiology N.F. Gamaleya’ of the Ministry of Health of Russia. Influenza virus was cultivated in the allantoic cavity of 9-10-day-old chicken embryos for 48 hs at 36°C. The infectious and hemagglutinating activity of the virus was determined according to the methods recommended by WHO [S1].

Study of the effect of drugs on the infectious titer of the influenza virus in MDCK cell culture

In the work, we used the MDCK cell culture provided by the Russian Collection of Cell Cultures at the FSBI ‘N.N. N.F. Gamaleya’ of the Ministry of Health of Russia. They were cultured in Eagle's MEM medium (IPVE RAMN) with a double set of amino acids and 5% fetal calf serum (HyClone),

10 mM glutamine and 4% gentamicin. Antiviral activity was studied against influenza virus A/California/7/2009 (H1N1)pdm09. Each concentration of the drug was tested in 2 parallel wells. The antiviral activity of the tested substances was taken into account by reducing the infectious titer of the influenza virus in the MDCK cell culture by cytopathic action (CPE) and in the hemagglutination reaction.

Study of the effect of compounds on the infectious titer of the SARS-CoV-2 virus in Vero E6 cell culture

Vero E6 cells were cultured in DMEM (Thermo Fischer Scientific), containing 2.5% fetal bovine serum in an atmosphere of 5% CO_2 in a humid atmosphere at 37 °C. Cells were seeded on 6-well plates at a density of 105; upon reaching 90% of the monolayer, the culture medium was replaced with fresh (0.5 ml perwell) containing SARS-CoV-2 (MOI 0.1) and left to shake every 20 min. After 2 hours, the medium was replaced with fresh medium containing 10 μM of the test substances. 4 days after infection, 0.5 ml of the medium was taken, RNA was isolated using the High Pure RNA Isolation Kit (Roche, Switzerland), and the levels of viral genomic RNA were determined by real-time RT-PCR as described [S2,S3].

References

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S2. M. V. Golikov, I. L. Karpenko, A. V. Lipatova, O. N. Ivanova, I. T. Fedyakina, V. F. Larichev, N. F. Zakirova, O. G. Leonova, V. I. Popenko, B. Bartosch, S. N. Kochetkov, O. A. Smirnova and A. V. Ivanov, *Antioxidants*, 2021, **11**, 97.

S3. S. Onikienko, M. Vinokurov, M. Yurinskaya, A. Zemlyanoi, S. Abkin, E. Shaykhutdinova, V. Palikov, A. Ivanov, O. Smirnova, I. Fedyakina, N. Bychkova, O. Zatsepina, D. Garbuz and M. Evgen'ev, *Biomedicines*, 2022, **10**, 2155.

Contents

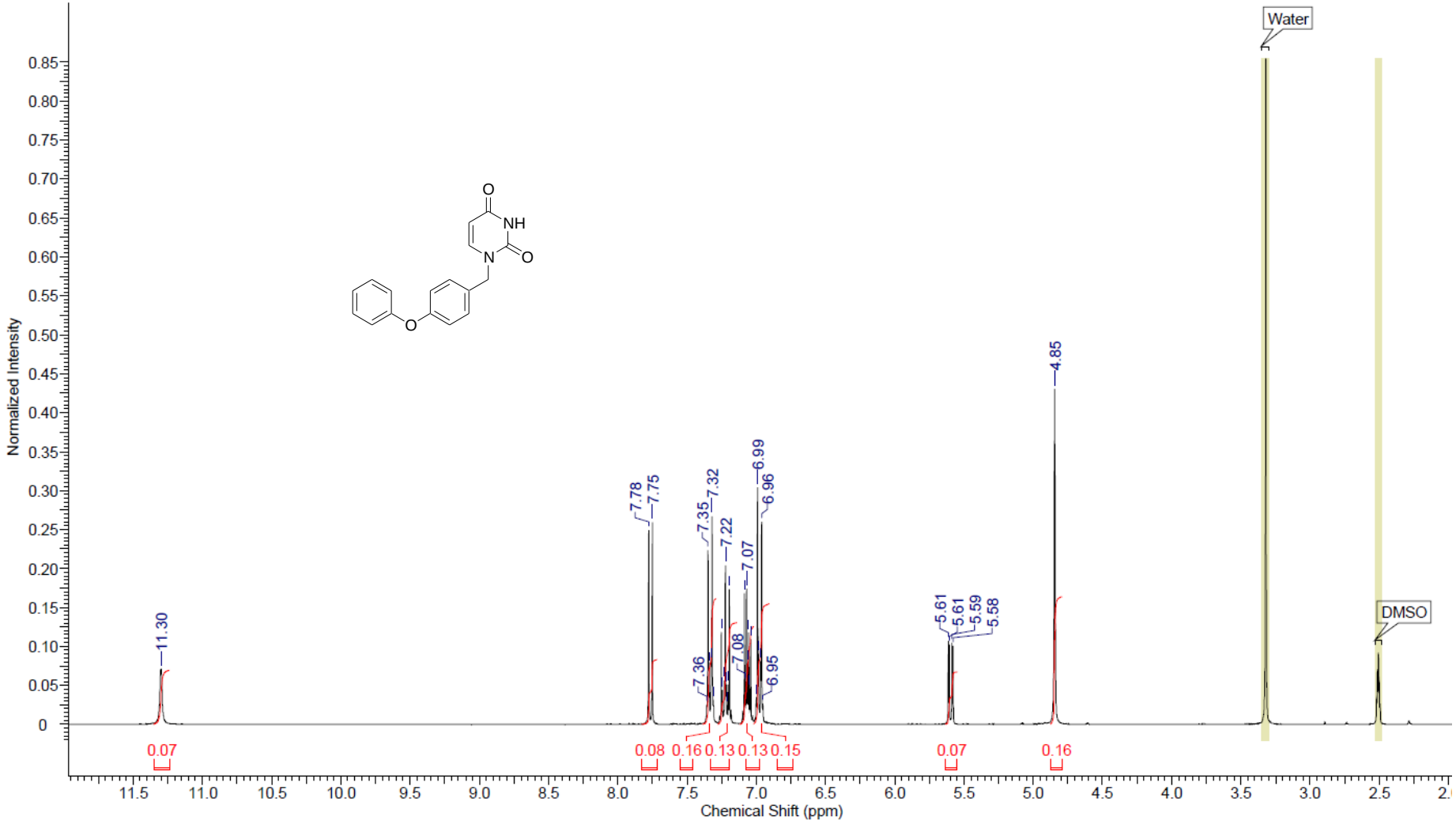

Figure S1 ^{1}H NMR spectrum of compound **3a** in DMSO-d_6 at 300 MHz.

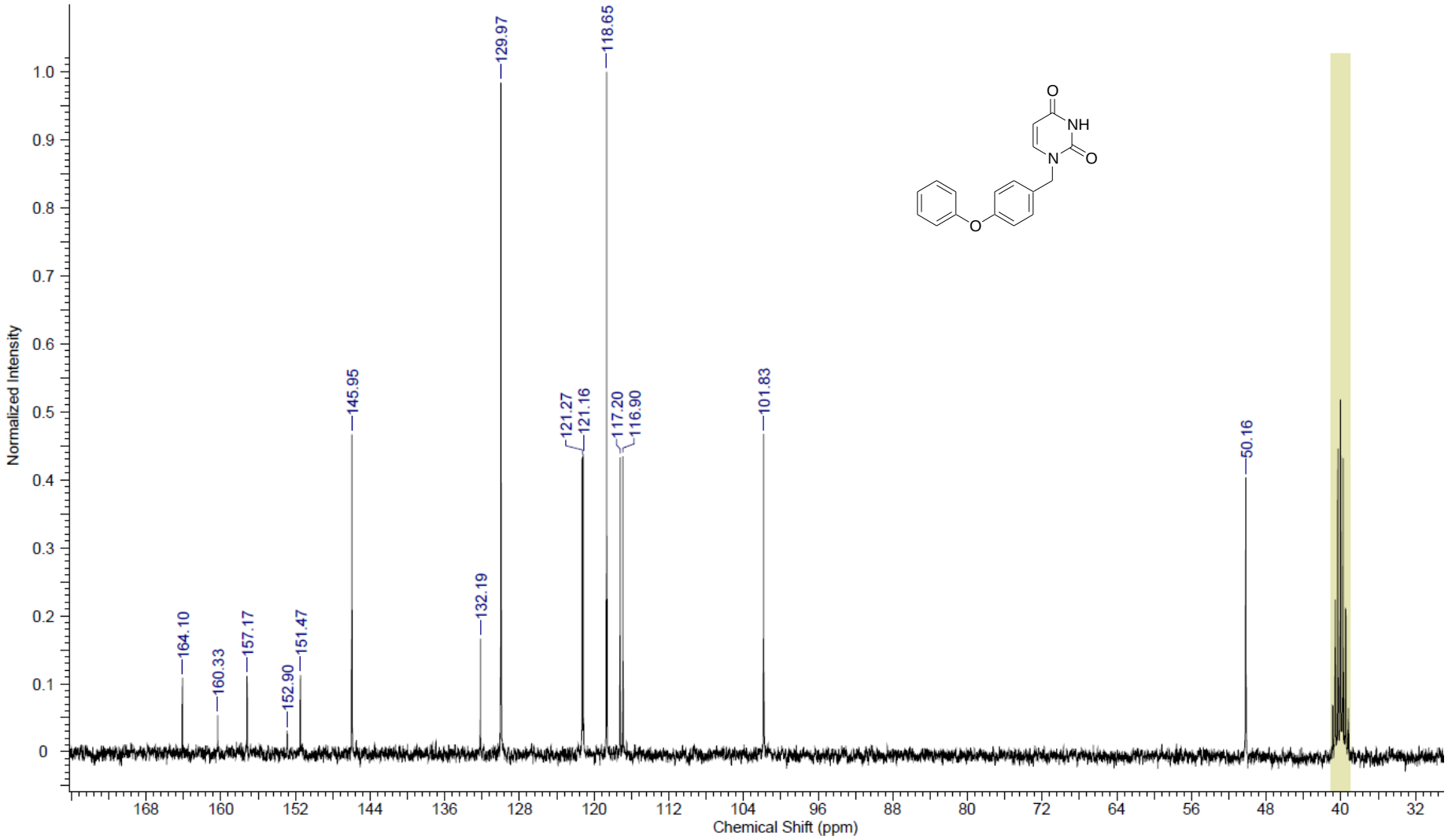

Figure S2 ^{13}C NMR spectrum of compound **3a** in DMSO-d_6 at 75 MHz.

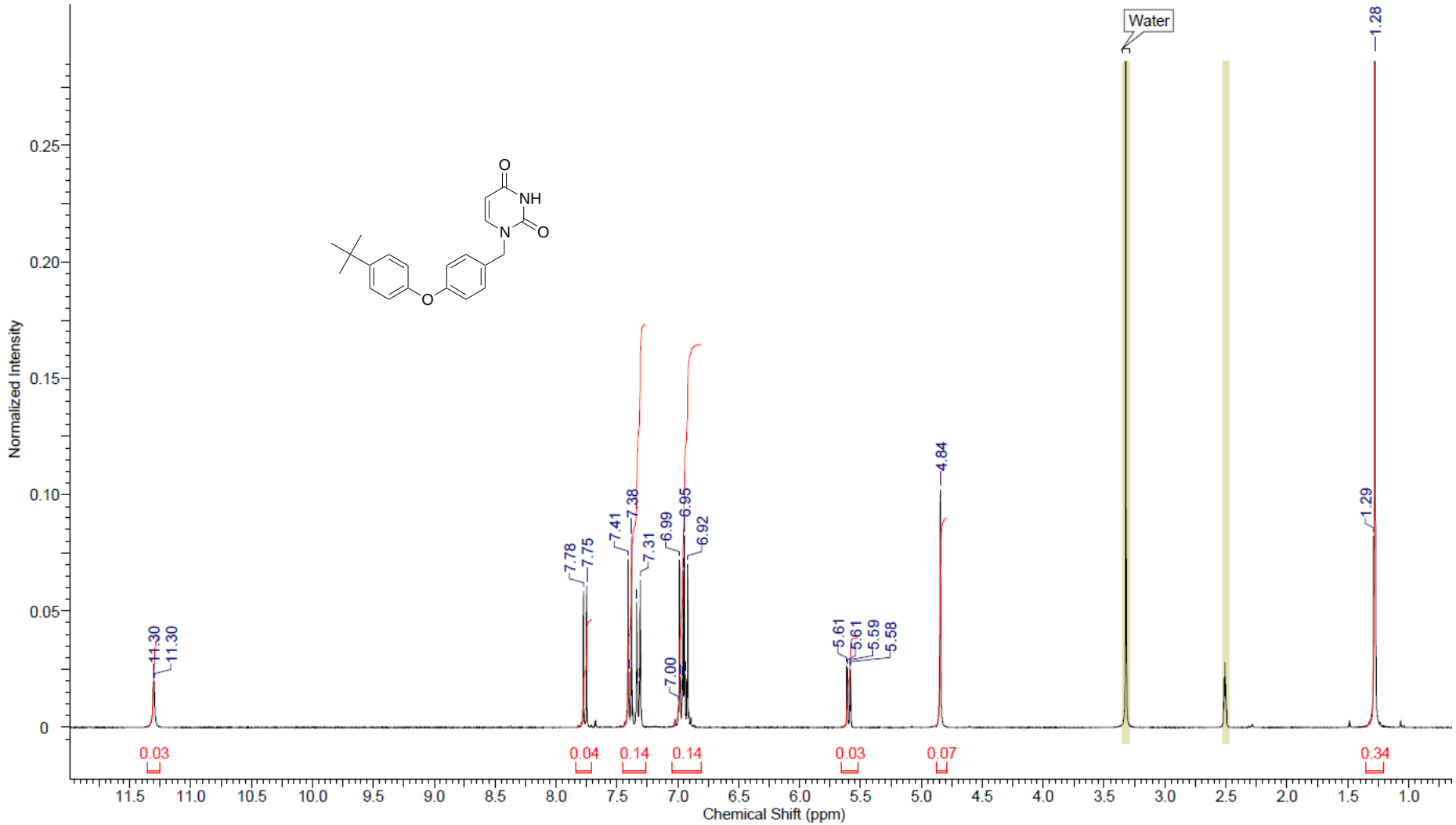

Figure S3 ^{1}H NMR spectrum of compound **3b** in DMSO-d_6 at 400 MHz.

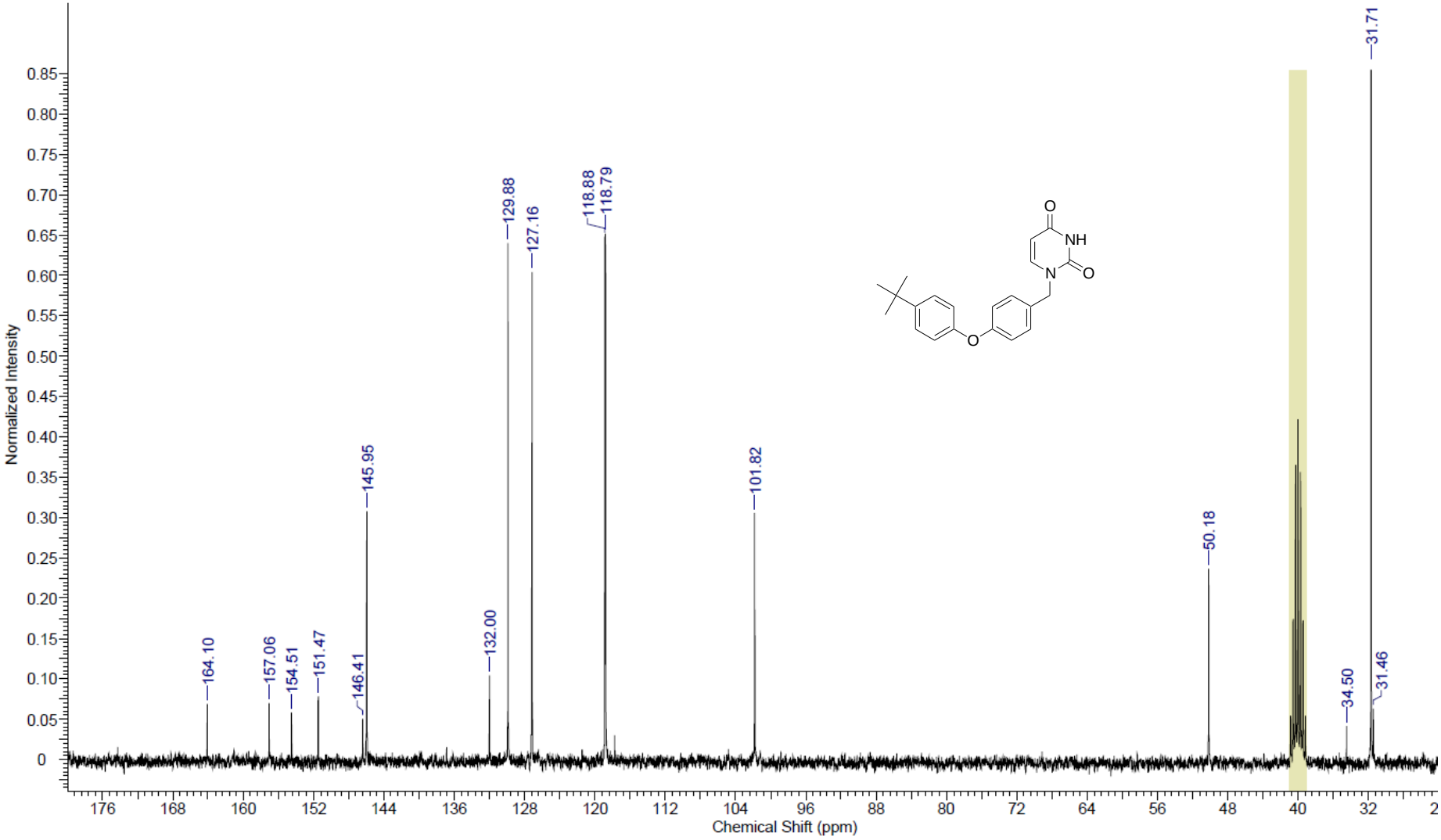

Figure S4 ^{13}C NMR spectrum of compound **3b** in DMSO-d_6 at 100 MHz.

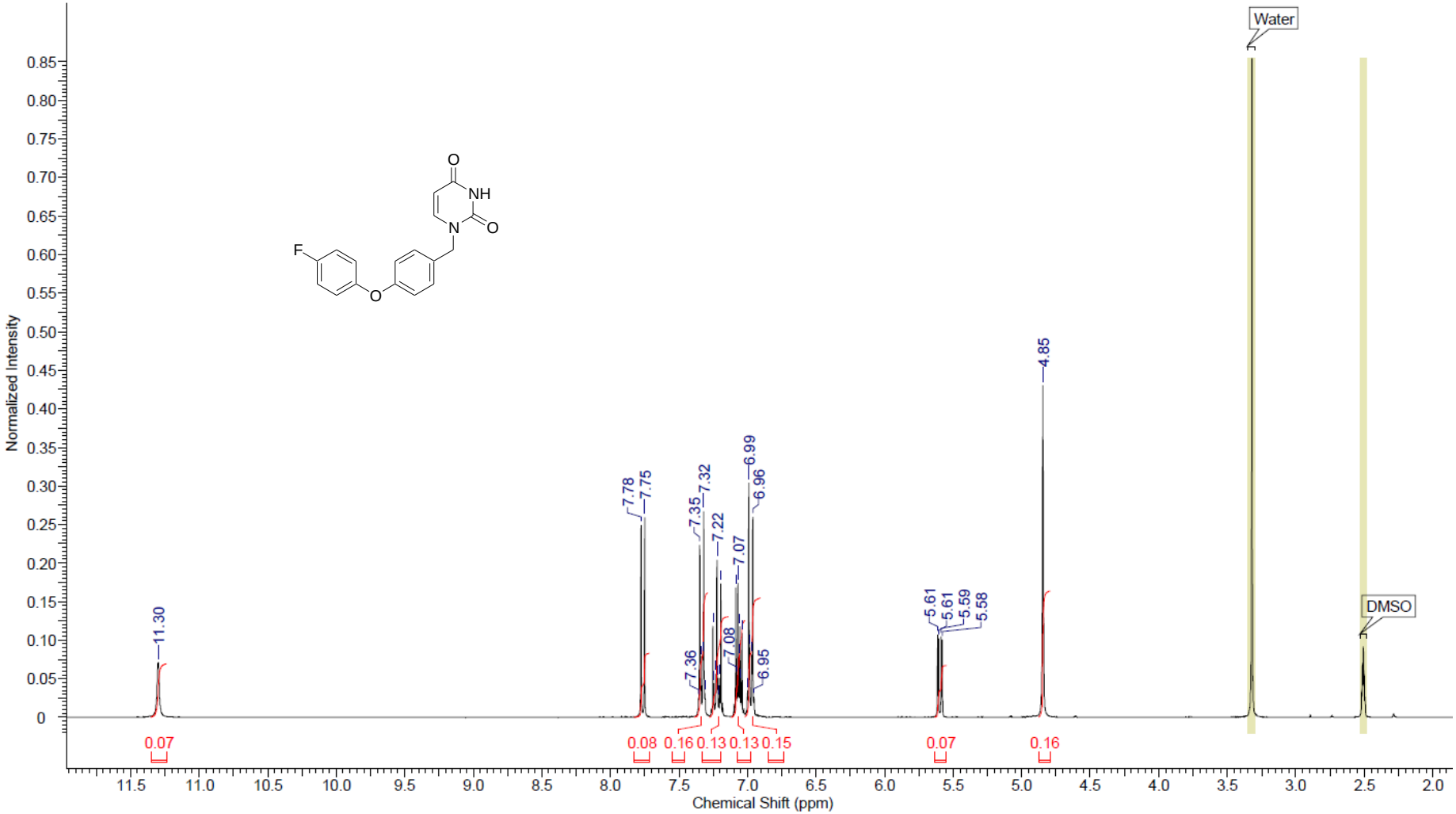

Figure S5 ^{1}H NMR spectrum of compound **3c** in DMSO-d_6 at 400 MHz.

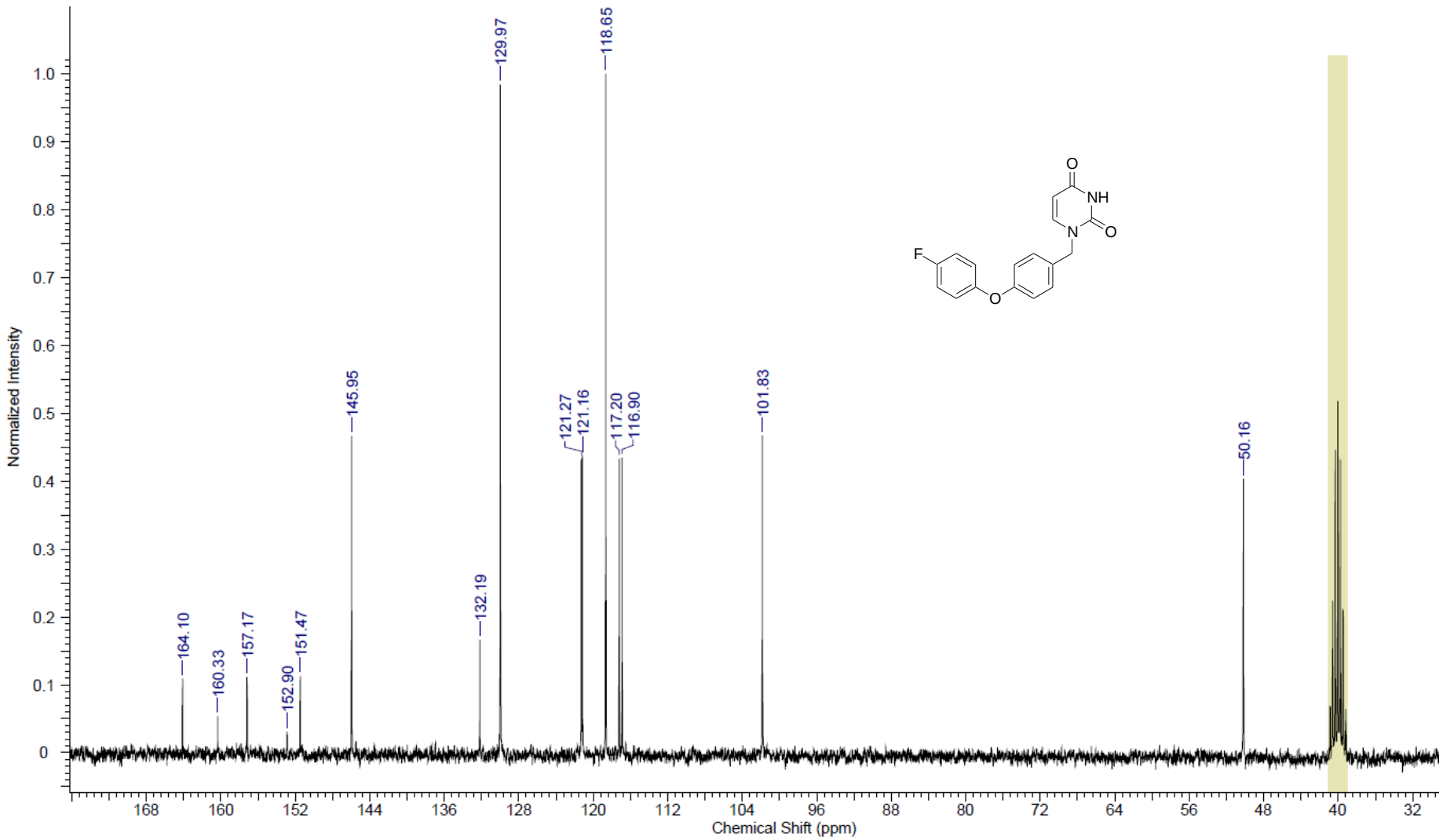

Figure S6 ^{13}C NMR spectrum of compound **3c** in DMSO-d_6 at 100 MHz.

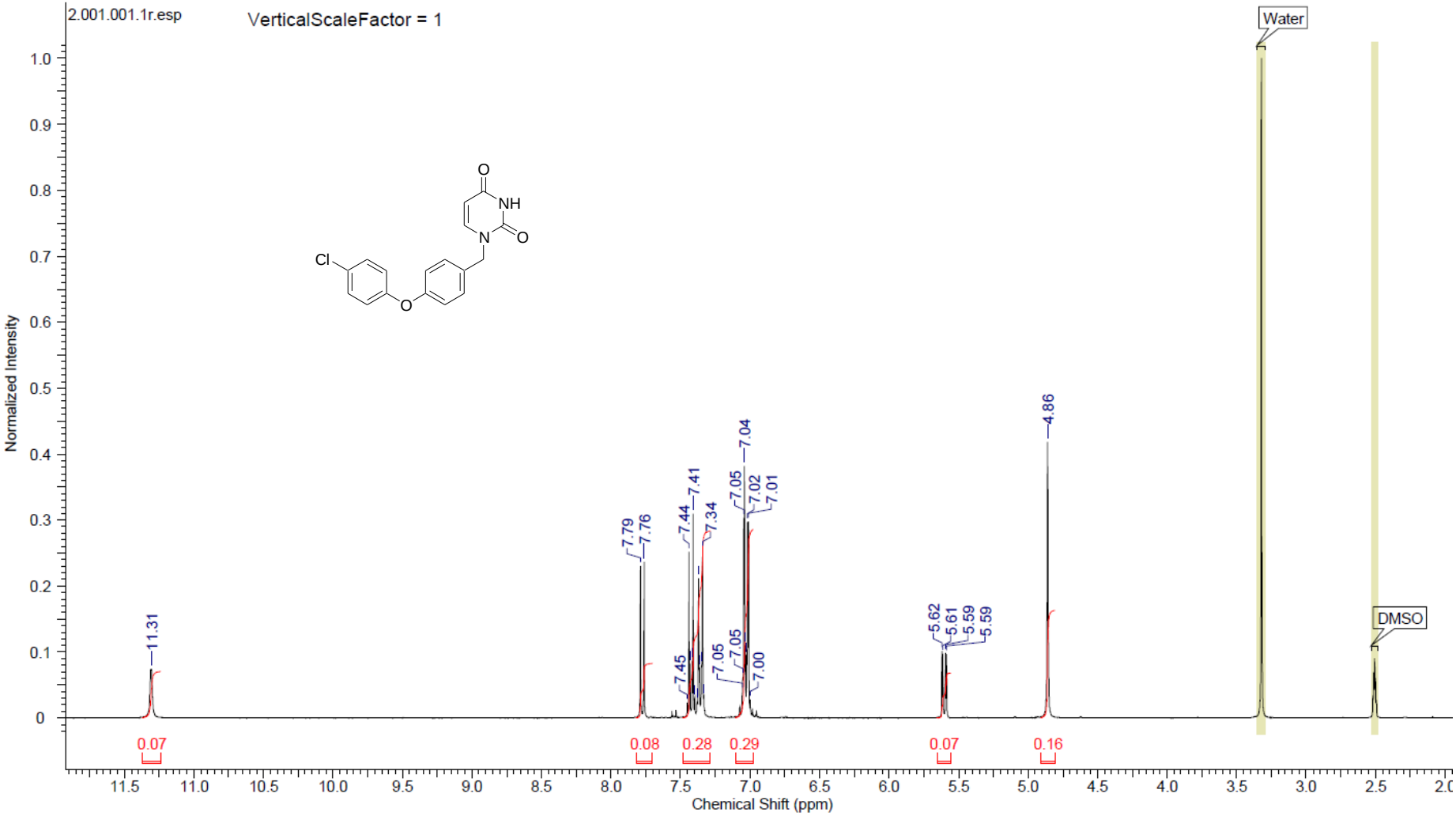

Figure S7 ^{1}H NMR spectrum of compound **3d** in DMSO-d_6 at 400 MHz.

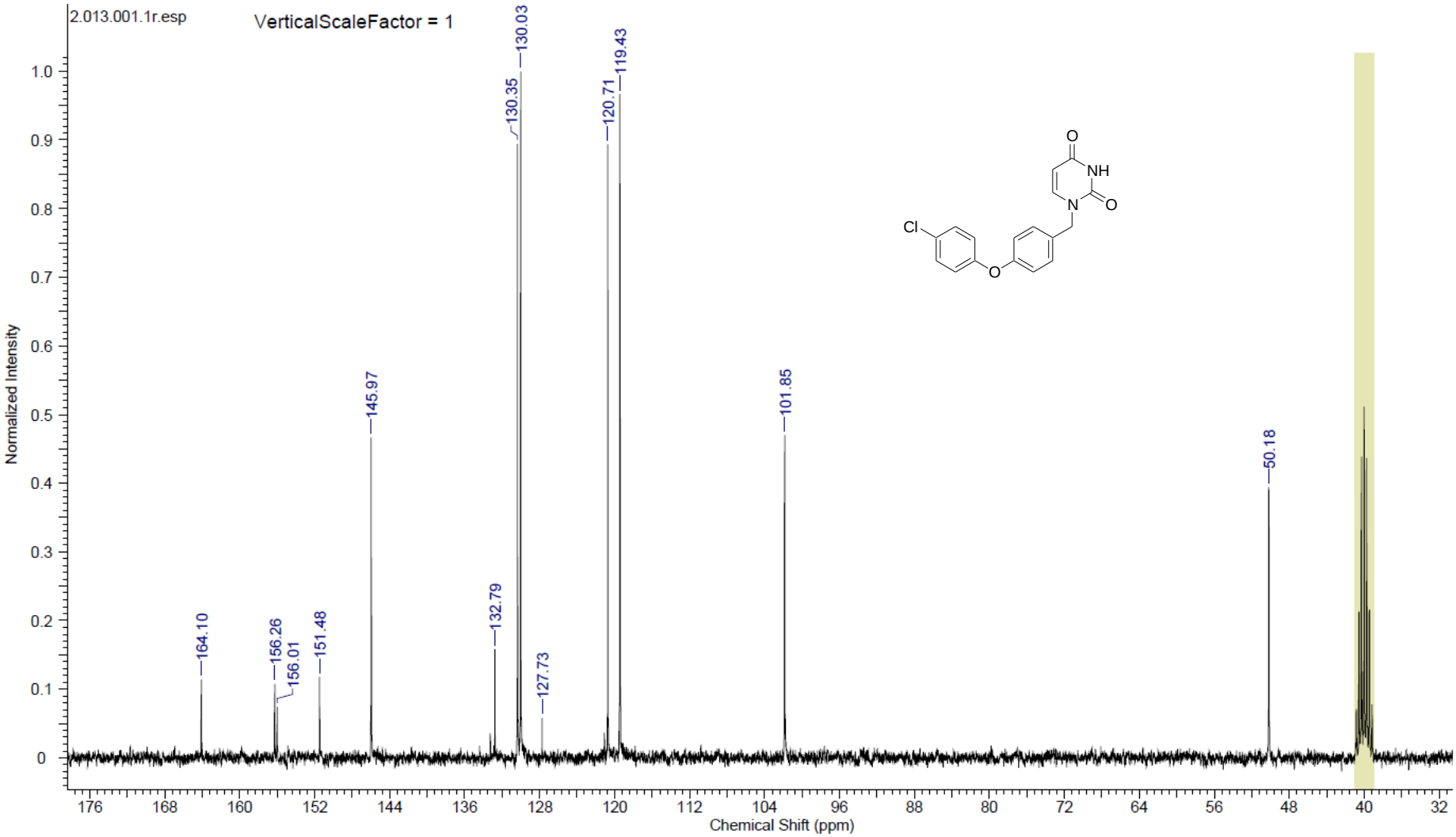

Figure S8 ^{13}C NMR spectrum of compound **3d** in DMSO-d_6 at 100 MHz.

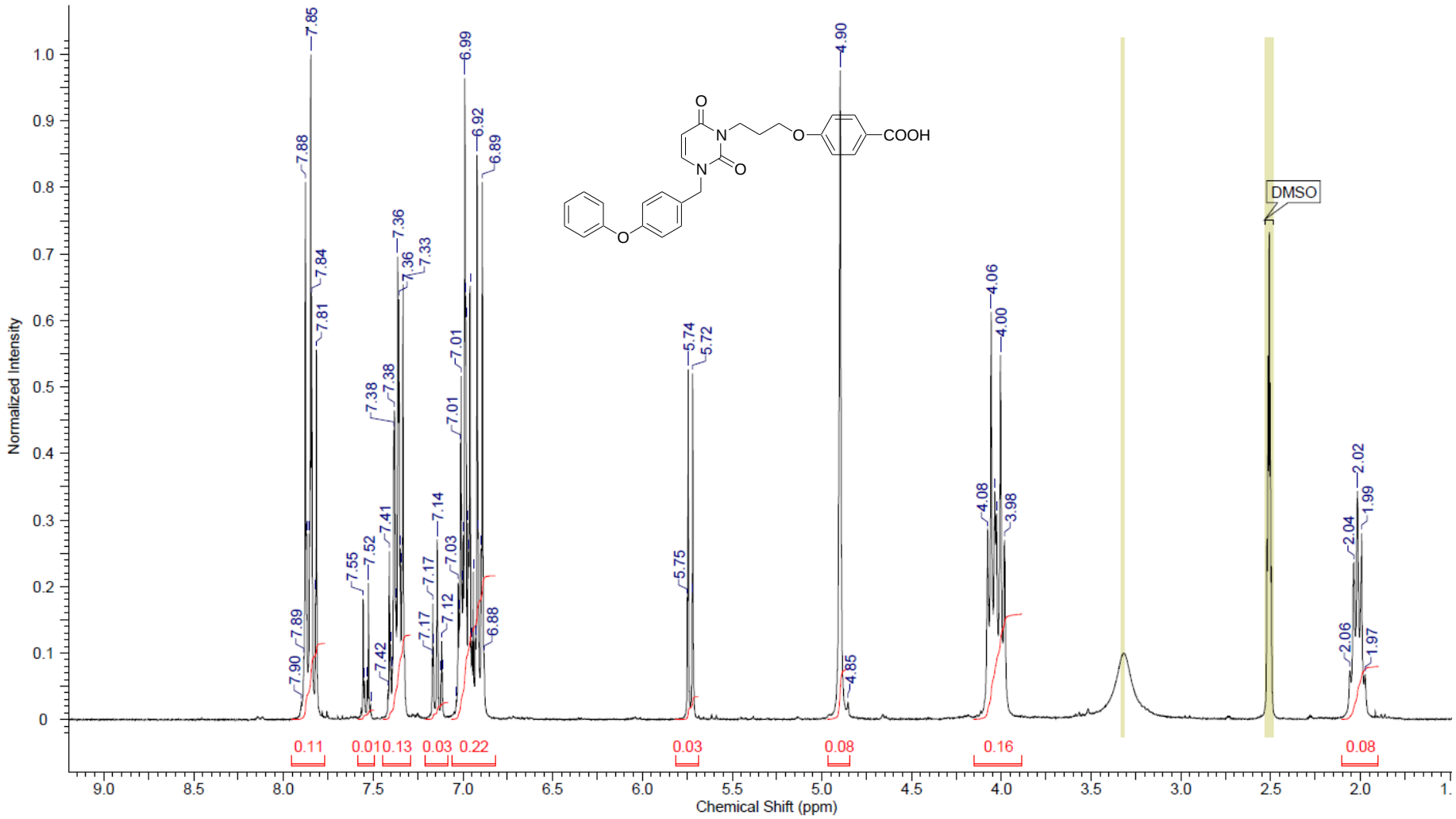

Figure S9 ^{1}H NMR spectrum of compound **5a** in DMSO-d_6 at 300 MHz.

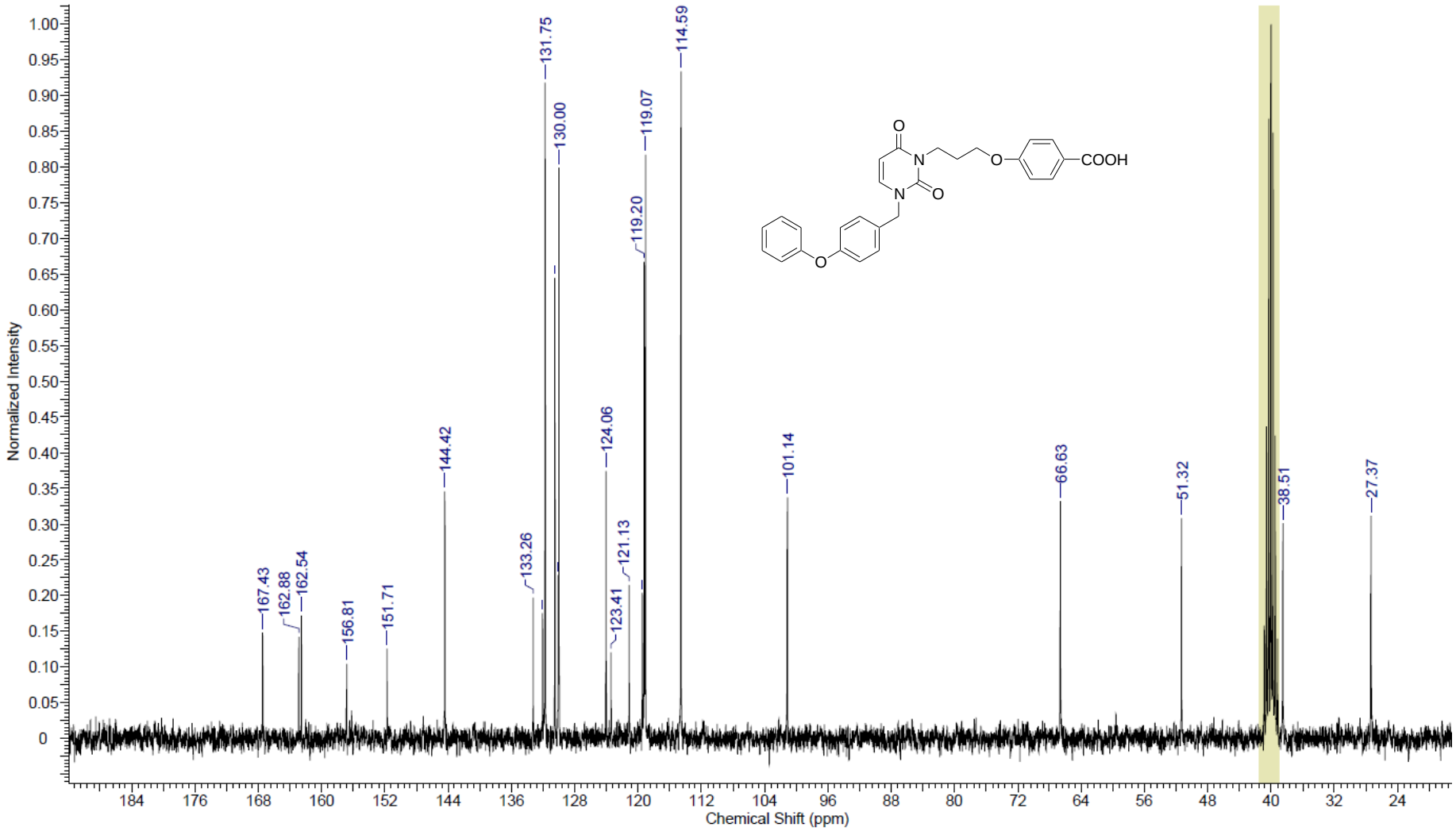

Figure S10 ^{13}C NMR spectrum of compound **5a** in DMSO-d_6 at 75 MHz.

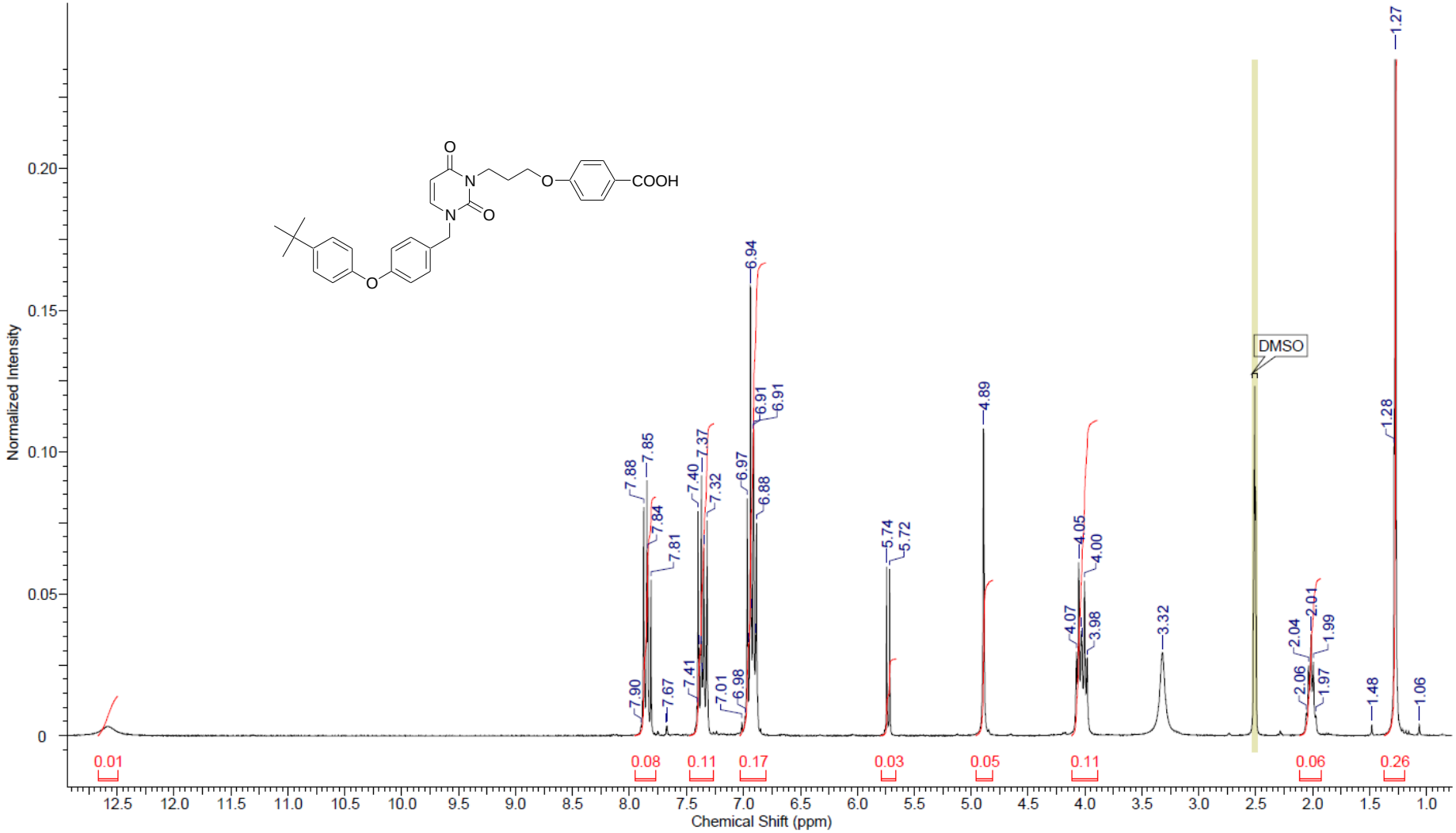

Figure S11 ^{1}H NMR spectrum of compound **5b** in DMSO-d_6 at 400 MHz.

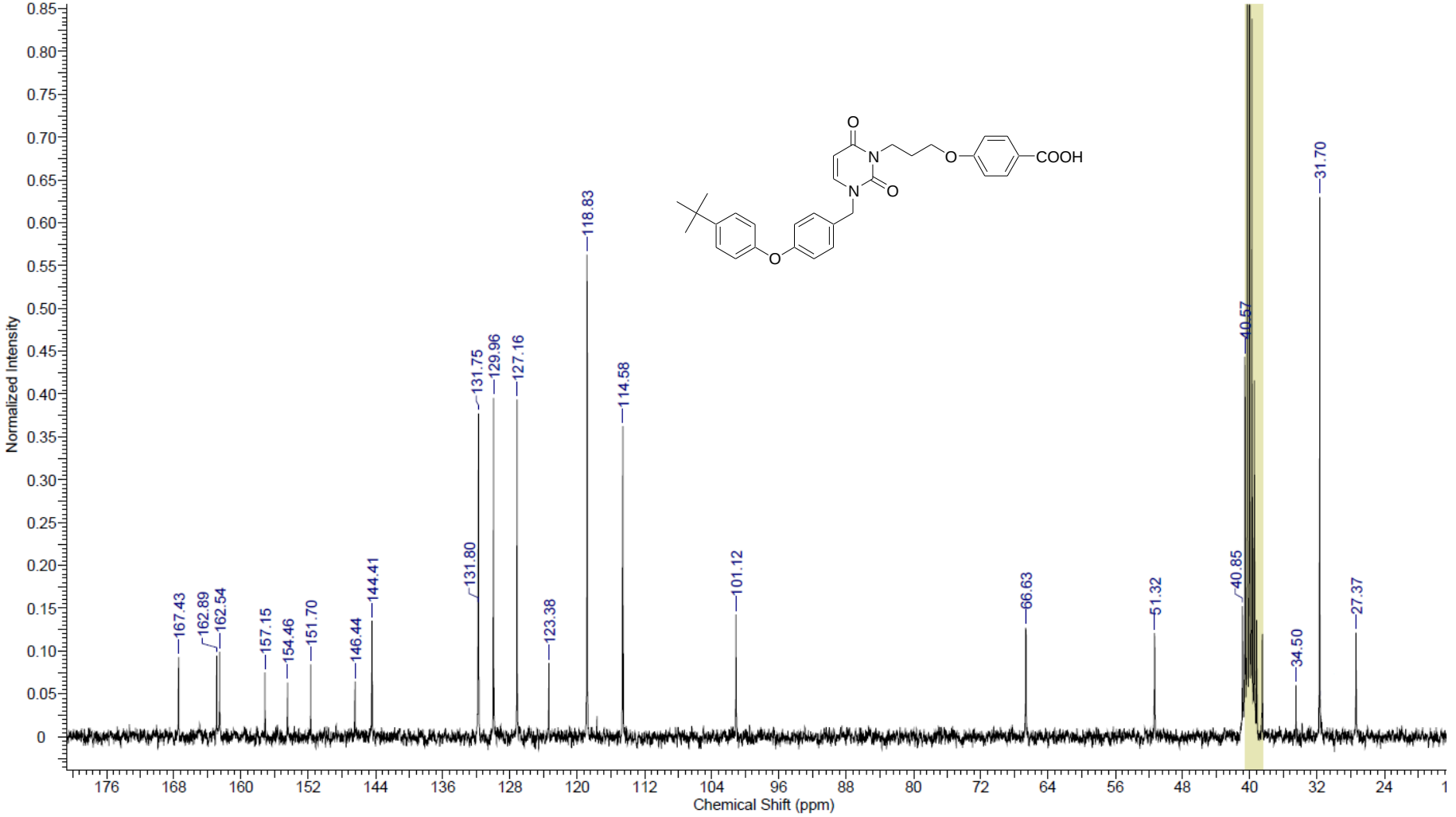

Figure S12 ^{13}C NMR spectrum of compound **5b** in DMSO-d_6 at 100 MHz.

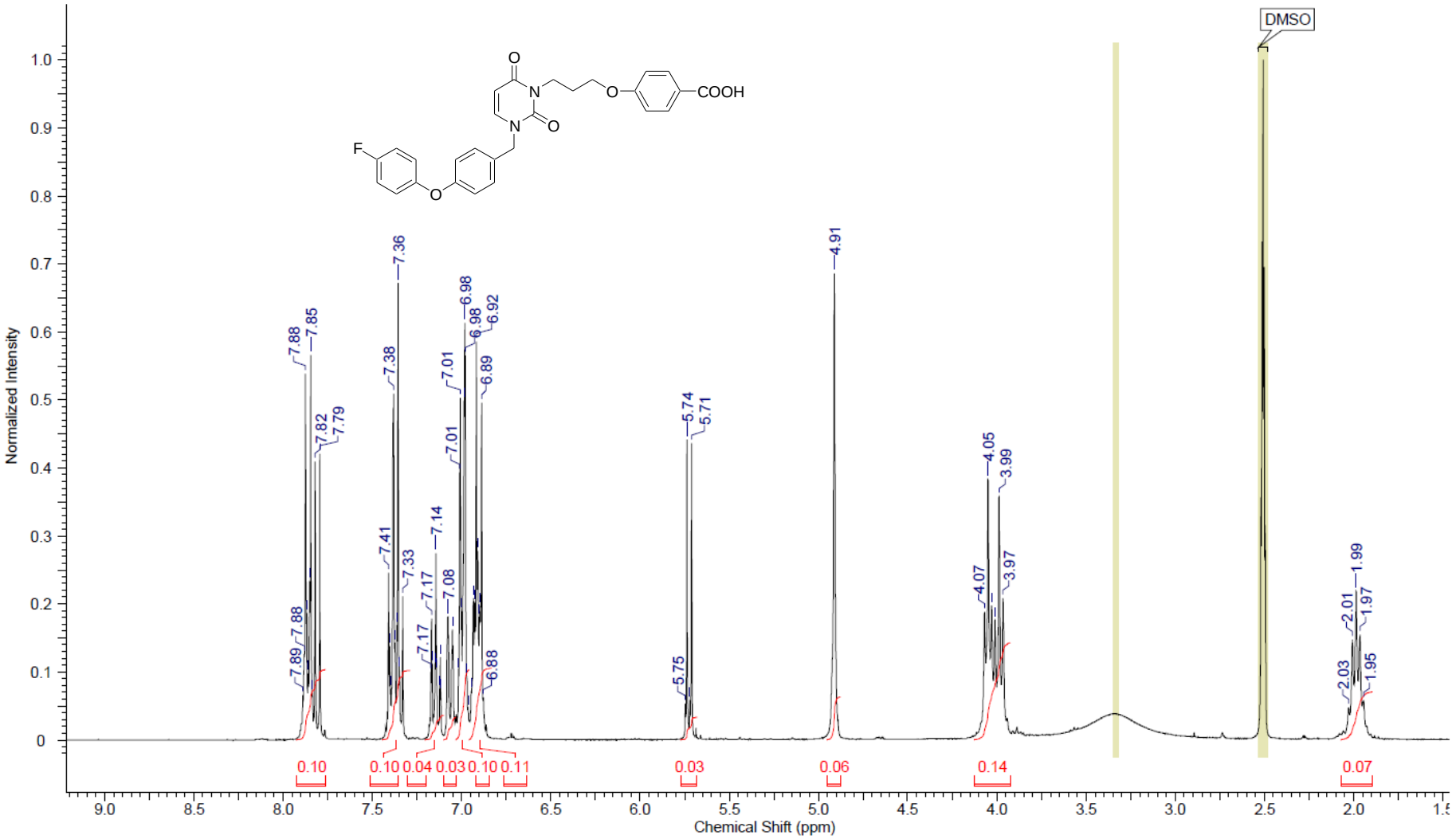

Figure S13 ^{1}H NMR spectrum of compound **5c** in DMSO-d_6 at 300 MHz.

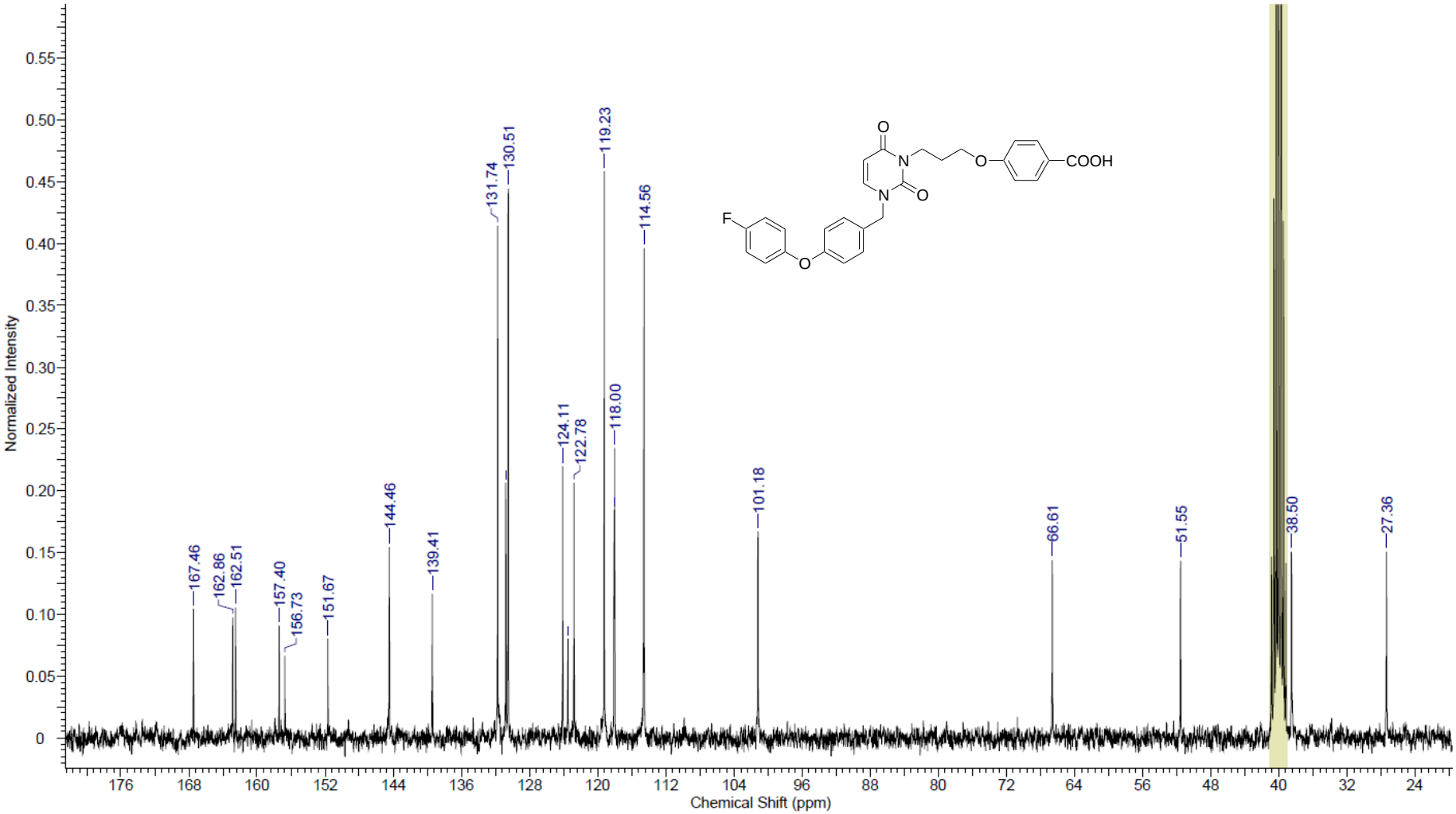

Figure S14 ^{13}C NMR spectrum of compound **5c** in DMSO-d_6 at 75 MHz.

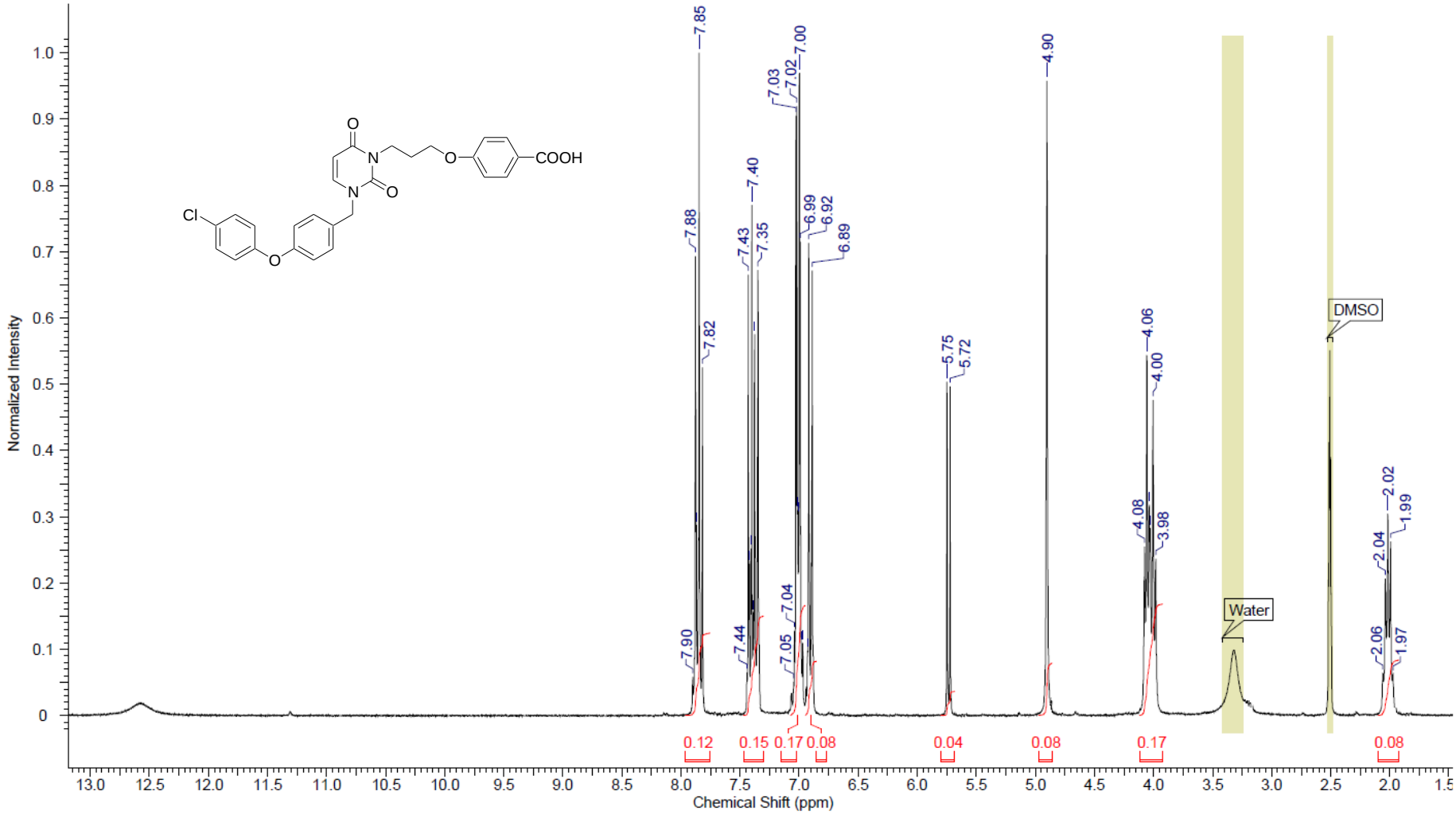

Figure S15 ^{1}H NMR spectrum of compound **5d** in DMSO-d_6 at 300 MHz.

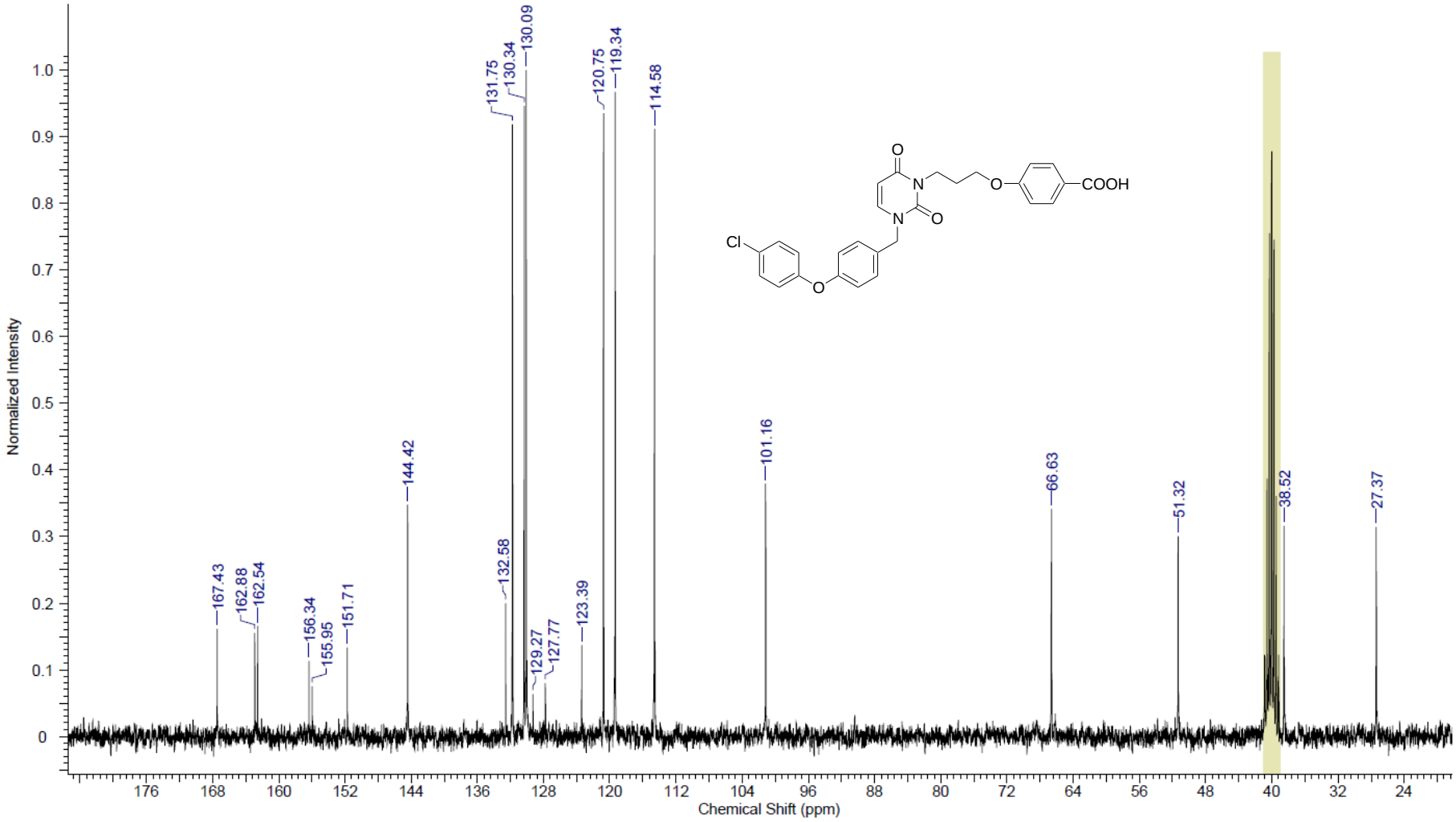

Figure S16 ^{13}C NMR spectrum of compound **5d** in DMSO-d_6 at 100 MHz.

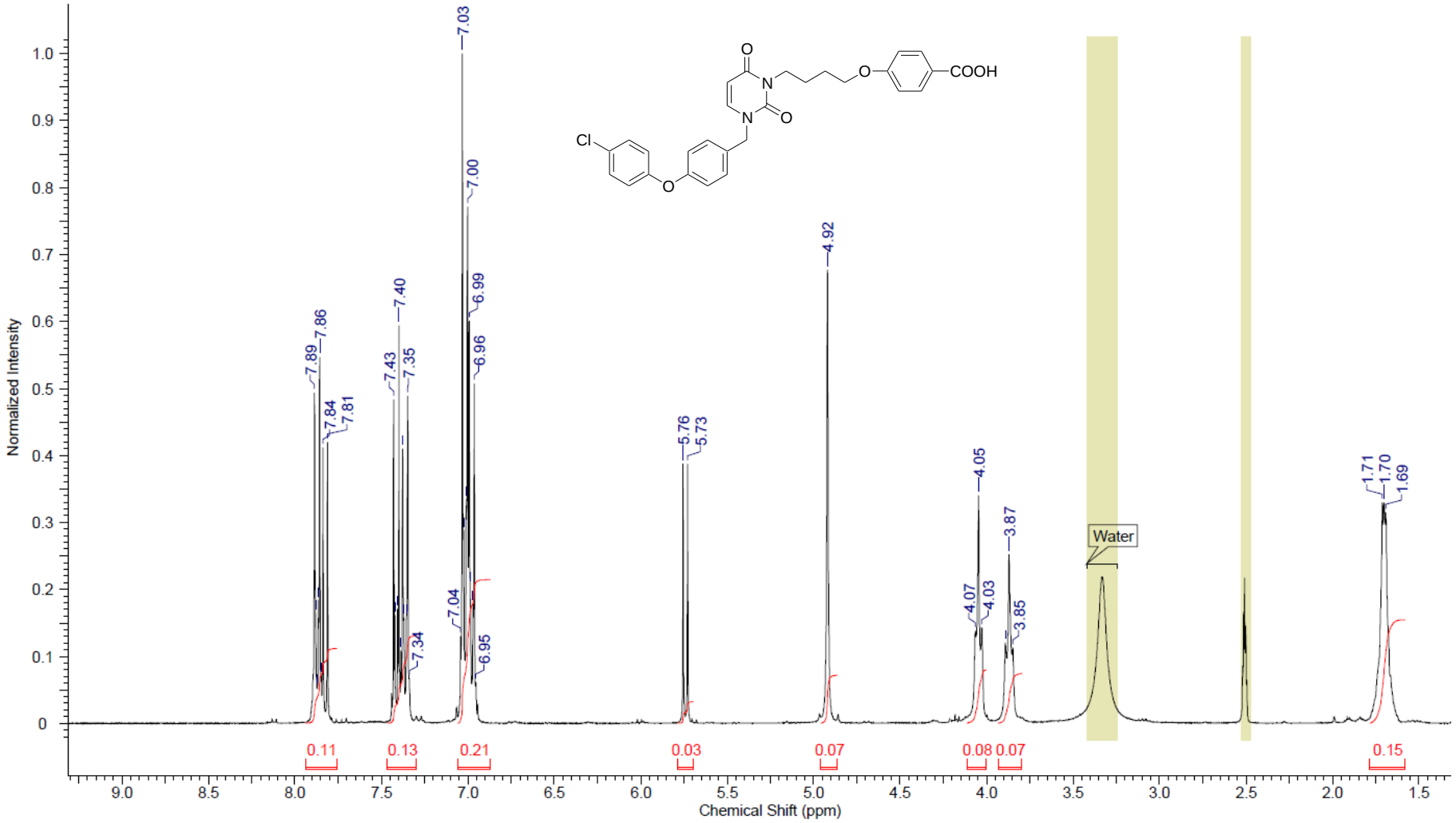

Figure S17 ^{1}H NMR spectrum of compound **5e** in DMSO-d_6 at 400 MHz.

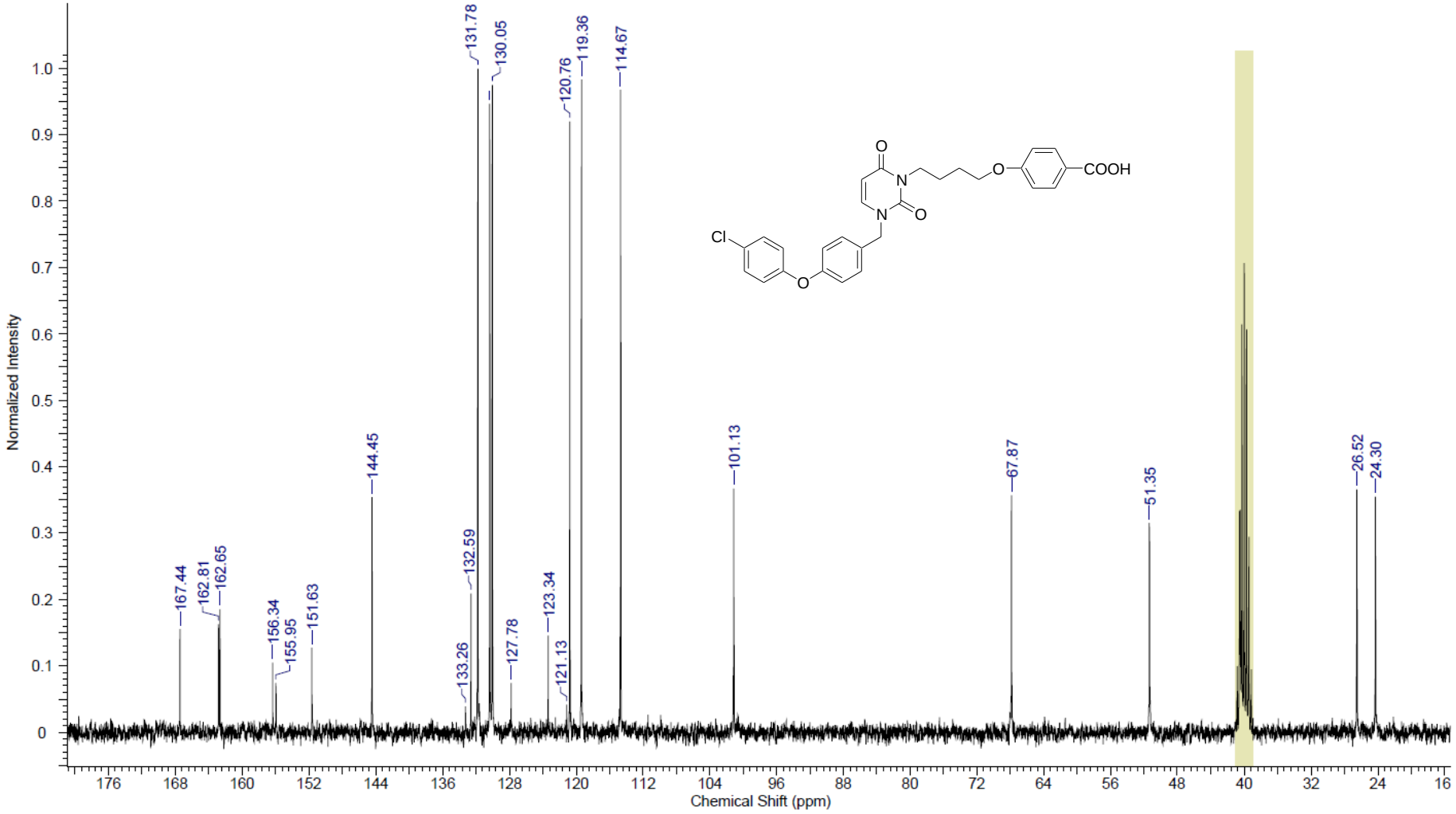

Figure S18 ^{13}C NMR spectrum of compound **5e** in DMSO-d_6 at 100 MHz.