Supporting Information

Design, synthesis and evaluation of the anti-breast cancer activity of 1,3-oxazolo[4,5-*d*]pyrimidine and 1,3-oxazolo[5,4-*d*]pyrimidine derivatives

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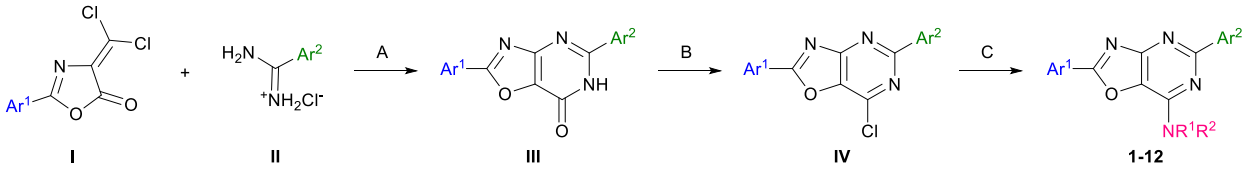
Experimental part 1. Chemistry

Materials and methods

^{1}H, ^{13}C and ^{19}F NMR spectra were obtained on a Bruker AVANCE DRX-500 or Varian Mercury spectrometer (TMS as internal reference) in $CDCl_3$, DMSO-d_6 or $CF_3C(O)OD$. Mass spectra were recorded on an Agilent 1100 Series LC-MS system equipped with a diode array detector Agilent LC\MSD SL (atmospheric pressure chemical ionization). M. p. was determined on a Fisher–Johns apparatus and are uncorrected. All reagents and solvents were purchased from commercial sources were used.

Synthesis of 1,3-oxazolo[4,5-*d*]pyrimidines (1–12)



General method for the synthesis of 1,3-oxazolo[4,5-*d*]pyrimidin-7(6H)-ones (III)

To a solution of 1,3-oxazol-5(4H)-one **I** (40 mmol)[1] in dry THF (100 ml), amidine hydrochloride **II** (40 mmol) was added followed by Et_3N (5.74 ml, 41 mmol). The mixture was stirred at r.t. for 72 h. The precipitate formed was filtered off, washed with H_2O, dried, dissolved in pyridine (60 ml) and refluxed for 10 h. The solvent was removed *in vacuo*. The residue was treated with H_2O, filtered off, dried, and recrystallized from DMF.

2,5-Diphenyl-1,3-oxazolo[4,5-d]pyrimidin-7(6H)-one (**IIIa**), 5-(4-methylphenyl)-2-phenyl-1,3-oxazolo[4,5-d]pyrimidin-7(6H)-one (**IIIb**), 2-(4-methylphenyl)-5-phenyl-1,3-oxazolo[4,5-d]pyrimidin-7(6H)-one (**IIIc**), 2,5-bis(4-methylphenyl)-1,3-oxazolo[4,5-d]pyrimidin-7(6H)-one (**IIId**), 5-(4-fluorophenyl)-2-(4-methylphenyl)-1,3-oxazolo[4,5-d]pyrimidin-7(6H)-one (**IIIe**), 5-(4-chlorophenyl)-2-(4-methylphenyl)-1,3-oxazolo[4,5-d]pyrimidin-7(6H)-one (**IIIf**) and 5-(4-bromophenyl)-2-(4-methylphenyl)-1,3-oxazolo[4,5-d]pyrimidin-7(6H)-one (**IIIg**) were synthesized according to the method described in the articles.[2-4]

General procedure for the synthesis of 2,5-diaryl-7-chloro-1,3-oxazolo[4,5-*d*]pyrimidines (IV)

A mixture of compound **III** (10 mmol), $POCl_3$ (30 mL), and Me_2NPh (2.42 g, 20 mmol) was refluxed for 3 h. After evaporation of $POCl_3$ excess the residue was recrystallized from 1,4-dioxane.

7-Chloro-2,5-diphenyl-1,3-oxazolo[4,5-d]pyrimidine (**IVa**), 7-chloro-5-(4-methylphenyl)-2-phenyl-1,3-oxazolo[4,5-d]pyrimidine (**IVb**), 7-chloro-2-(4-methylphenyl)-5-phenyl-1,3-oxazolo[4,5-d]pyrimidine (**IVc**), 7-chloro-2,5-bis(4-methylphenyl)-1,3-oxazolo[4,5-d]pyrimidine (**IVd**), 7-chloro-5-(4-fluorophenyl)-2-(4-methylphenyl)-1,3-oxazolo[4,5-d]pyrimidine (**IVe**), 7-chloro-5-(4-chlorophenyl)-2-(4-methylphenyl)-1,3-oxazolo[4,5-d]pyrimidine (**IVf**) and 7-chloro-5-(4-bromophenyl)-2-(4-methylphenyl)-1,3-oxazolo[4,5-d]pyrimidine (**IVg**) were synthesized according to the method described in the articles.[2-4]

General procedure for the synthesis of 7-amino-substituted 1,3-oxazolo[4,5-*d*]pyrimidines (1–12)

A mixture of compound **IV** (2 mmol), appropriate amine (2 mmol) and Et_3N (0.28 ml, 2 mmol) in dioxane (15 ml) was refluxed for 6 h. After removal of the solvent, the residue was triturated with water, filtered off, dried, and recrystallized from DMF/MeCN (1:3).

2,5-Diphenyl-7-(piperazin-1-yl)-1,3-oxazolo[4,5-d]pyrimidine **(1)** Color: White solid; Yield 87%, 0.62 g; m.p. 249-251°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53–8.46 (m, 2H, ArH), 8.29–8.19 (m, 2H, ArH), 7.63–7.39 (m, 6H, ArH), 4.10 (t, *J* = 5.0 Hz, 4H, CH_2, (piperazinyl)), 3.09 (t, *J* = 5.1 Hz, 4H, CH_2, (piperazinyl)). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.9, 161.6, 159.8, 147.2, 137.3, 131.6, 129.1, 128.2, 127.5, 127.3, 127.1, 125.4, 112.8, 45.2. MS *m/z* : 358.0 $[M+H]^+$.

7-(1,4-Diazepan-1-yl)-2,5-diphenyl-1,3-oxazolo[4,5-d]pyrimidine **(2)** was synthesized according to the method described in the article.[4] Color: White solid; Yield 78%; m.p. 213-215°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 8.40-8.38 (m, 2H, Ar-H), 8.23 (d, *J*=6.8 Hz, 2H, Ar-H), 7.70-7.66 (m, 3H, Ar-H), 7.51-7.49 (m, 3H, Ar-H), 4.09-4.05 (m, 4H, CH_2 (1,4-diazepanyl)), 3.05 (s, 2H, CH_2 (1,4-diazepanyl)), 2.78 (t, *J*=4.4 Hz, 2H, CH_2 (1,4-diazepanyl)), 1.91 (t, *J*=4.4 Hz, 2H, CH_2 (1,4-diazepan)) (NH proton has not been observed); ^{13}C NMR ($CF_3C(O)OD$, 125 MHz) δ (ppm) 170.8, 155.7, 151.9, 150.0, 136.4, 135.3, 130.1, 130.0, 128.9, 128.6, 128.1, 128.0, 122.3, 49.4, 46.9, 46.4, 44.2 25.0; MS, *m/z*: 372 $[M+1]^+$.

2-Phenyl-7-(piperazin-1-yl)-5-(4-methylphenyl)-1,3-oxazolo[4,5-d]pyrimidine **(3)** was synthesized according to the method described in the article.[3] Color: White solid; Yield 77%; m.p. 256-258°C; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ (ppm) 8.26-8.22 (m, 4H, Ar-H), 7.67-7.63 (m, 3H, Ar-H), 7.27 (d, 2H, Ar-H), 3.98 (s, 4H, CH_2 (piperazine)), 2.93 (s, 4H, CH_2

(piperazine)), 2.37 (s, 4H, CH_3, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 166.1, 158.6, 156.8, 153.2, 137.2, 131.8, 130.5, 129.3, 128.5, 127.8, 126.8, 125.9, 114.8, 45.7, 20.5.

7-(1,4-Diazepan-1-yl)-5-(4-methylphenyl)-2-phenyl-1,3-oxazolo[4,5-d]pyrimidine **(4)** was synthesized according to the method described in the article.[4] Color: White solid; Yield 72%; m.p. 240-243°C. ^{1}H NMR ($CF_3C(O)OD$, 400 MHz) δ (ppm) 8.45-8.32 (m, 4H, Ar-H), 8.04 (t, *J* = 6.4 Hz, 1H, Ar-H), 7.92-7.88 (m, *J* = 8.8 Hz, 2H, Ar-H), 7.71 (d, *J* = 7.6 Hz, 2H, Ar-H), 5.01-4.96 (m, 2 H, CH_2 (1,4-diazepanyl)), 4.81-4.79 (m, 2 H, CH_2 (1,4-diazepanyl)),4.28 (s, 1H, CH_2 (1,4-diazepanyl)), 4.15 (s, 1H, CH_2 (1,4-diazepanyl)), 4.00-3.96 (s, 2H, CH_2 (1,4-diazepanyl)), 2.90-2.78 (m, 2H, CH_2 (1,4-diazepanyl)), 2.71 (s, 3H, CH_3), (NH proton has not been observed); ^{13}C NMR ($CF_3C(O)OD$, 125 MHz) δ (ppm) 170.6, 155.7, 151.7, 149.9, 147.9, 136.3, 130.7, 130.0, 128.8, 128.1, 128.0, 125.7, 122.3, 49.3, 46.9, 46.4, 44.1, 25.0, 20.2; MS, *m/z*: 386 $[M+1]^+$.

5-Phenyl-7-(piperazin-1-yl)-2-(4-methylphenyl)-1,3-oxazolo[4,5-d]pyrimidine **(5)** was synthesized according to the method described in the article.[3] Color: White solid; Yield 74%; m.p. 273-275°C; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ (ppm) 8.38-8.36 (m, 2H, Ar-H), 8.12 (d, 2H, Ar-H), 7.49-7.43 (m, 5H, Ar-H), 3.98 (s, 4H, CH_2 (piperazine)), 2.92 (s, 4H, CH_2 (piperazine)), 2.43 (s, 3H, CH_3), (NH proton has not been observed). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 166.1, 158.6, 156.8, 153.2, 137.3, 131.8, 130.5, 129.3, 128.5, 127.8, 126.8, 125.9, 114.8, 45.7, 22.3.

2.3. *7-(1,4-Diazepan-1-yl)-2-(4-methylphenyl)-5-phenyl-1,3-oxazolo[4,5-d]pyrimidine* **(6)** was synthesized according to the method described in the article.[4] Color: Light yellow solid; Yield 73%; m.p. 251-254°C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 8.38-8.37 (m, 2H, Ar-H), 8.11 (d, *J* = 7.6 Hz, 2H, Ar-H), 7.50-7.45 (m, 5H, Ar-H), 4.10-4.05 (m, 4H, CH_2 (1,4-diazepanyl)), 3.08 (t, *J* = 5.3 Hz, 2H, CH_2 (1,4-diazepanyl)), 2.82 (t, *J* = 5.7 Hz, 2H, CH_2 (1,4-diazepanyl)), 2.44 (s, 3H, CH_3), 1.98-1.95 (s, 2H, CH_2 (1,4-diazepanyl)). ^{13}C NMR ($CF_3C(O)OD$, 125 MHz) δ (ppm) 171.1, 155.6, 151.9, 149.8, 149.6, 135.2, 130.9, 130.0, 129.0, 128.7, 127.9, 119.3, 119.2, 49.4, 47.4, 47.0, 46.9, 46.5, 46.4, 46.2, 44.2, 25.0, 24.2, 20.6; MS, *m/z*: 386 $[M+1]^+$.

7-(Piperazin-1-yl)-2,5-di(4-methylphenyl)-1,3-oxazolo[4,5-d]pyrimidine **(7)** was synthesized according to the method described in the article.[3] Color: Light yellow solid; Yield 73%; m.p. 279-281°C**;** ^{1}H-NMR (DMSO-d_6, 400 MHz): δ (ppm) 8.25 (d, 2H, Ar-H), 8.09 (d, 2H, Ar-H), 7.42 (d, 2H, Ar-H), 7.27 (d, 2H, Ar-H), 3.98 (s, 4H, CH_2 (piperazine)), 2.95 (s, 4H, CH_2 (piperazine)), 2.43 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 2.30 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 165.9, 158.4, 156.6, 153.0, 137.0, 131.5, 130.2, 129.1, 128.2, 127.6, 126.6, 125.7, 114.5, 45.5, 23.2, 21.8.

7-(1,4-Diazepan-1-yl)-2,5-bis(4-methylphenyl)-1,3-oxazolo[4,5-d]pyrimidine **(8)** was synthesized according to the method described in the article.[4] Color: White solid; Yield 75%; m.p. 244-247°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 8.25 (d, *J* = 6.8 Hz, 2H, Ar-H), 8.07 (d, *J* = 7.6 Hz, 2H, Ar-H), 7.43 (d, *J* = 7.6 Hz, 2H, Ar-H), 7.23 (d, *J* = 7.2 Hz, 2H, Ar-H), 4.03 (d, *J* = 16.0 Hz, 4H, CH_2 (1,4-diazepanyl)), 3.03 (bs, 2H, CH_2 (1,4-diazepanyl)), 2.78 (br s, 2H, CH_2 (1,4-diazepanyl)), 2.42 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 1.92 (bs, 2H, CH_2 (1,4-diazepanyl)) (NH proton has not been observed); ^{13}C NMR ($CF_3C(O)OD$, 125 MHz) δ (ppm) 170.9, 155.6, 151.8, 149.8, 149.5, 147.9, 130.9, 130.8, 129.0, 128.0, 127.7, 125.7, 119.3, 49.3, 47.4, 47.0, 46.9, 46.5, 46.4, 46.1, 44.1, 25.0, 24.2, 20.5, 20.2; MS, *m/z*: 400 $[M+1]^+$.

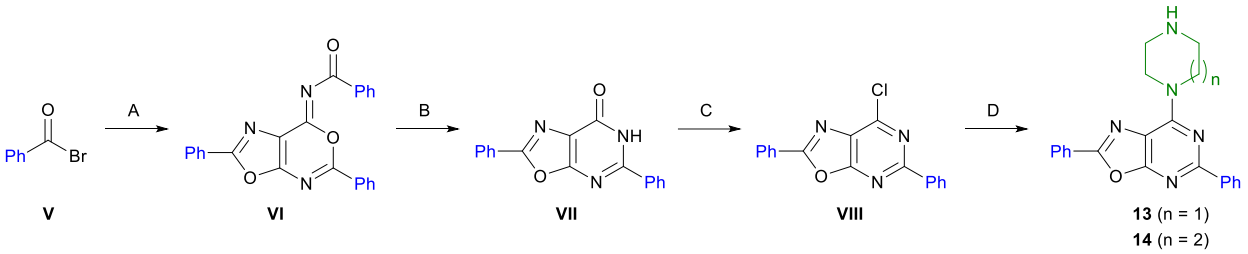
5-(4-Fluorophenyl)-7-(piperazin-1-yl)-2-(4-methylphenyl)-1,3-oxazolo[4,5-d]pyrimidine **(9)** Color: White solid; Yield 94%, 0.73 g; m.p. 279-281. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.50 – 8.46 (m, 2H, ArH), 8.12 (d, *J* = 7.9 Hz, 2H, ArH), 7.32 (d, *J* = 7.9 Hz, 2H, ArH), 7.13–7.09 (m, 2H, ArH), 4.07 (t, *J* = 5.0 Hz, 4H, CH_2, (piperazinyl)), 3.08 (t, *J* = 5.0 Hz, 4H, CH_2, (piperazinyl)), 2.44 (s, 3H, Me (4-MeC_6H_4)) (NH proton has not been observed). ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 165.3, 162.6, 159.7, 148.2, 143.4, 130.5, 130.4, 129.9, 128.1, 127.9, 123.6, 115.2, 115.1, 46.4, 46.3, 21.9. ^{19}F NMR (376 MHz, DMSO-d_6) δ -114.26. MS *m/z* : 390.2 $[M+H]^+$.

7-(1,4-Diazepan-1-yl)-5-(4-fluorophenyl)-2-(4-methylphenyl)-1,3-oxazolo[4,5-d]pyrimidine **(10)** Color: White solid; Yield 85%; m.p. 256-258 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.49 (dd, *J* = 8.6, 5.7 Hz, 2H, ArH), 8.11 (d, *J* = 8.0 Hz, 2H, ArH), 7.33 (d, *J* = 8.0 Hz, 2H, ArH), 7.11 (t, *J* = 8.6 Hz, 2H, ArH), 4.14 – 4.09 (m, 4H, CH_2 (1,4-diazepanyl)), 3.18 (t, *J* = 5.4 Hz, CH_2 (1,4-diazepanyl)), 2.93 (t, *J* = 5.9 Hz, 2H, CH_2 (1,4-diazepanyl)), 2.45 (s, 3H, Me (4-MeC_6H_4)), 2.06 (p, *J* = 6.1 Hz, 2H, CH_2 (1,4-diazepanyl)). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.3, 161.2, 158.5, 147.9, 143.0, 129.9, 127.5, 123.0, 115.2, 115.1, 47.5, 21.3. ^{19}F NMR (376 MHz, DMSO-d_6) δ -114.38. MS *m/z* : 404.0 $[M+H]^+$.

5-(4-Chlorophenyl)-7-(1,4-diazepan-1-yl)-2-(4-methylphenyl)-1,3-oxazolo[4,5-d]pyrimidine **(11)** Color: White solid; Yield 83%, 0.70 g; mp 264-266 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.42 (d, *J* = 8.5 Hz, 2H, ArH), 8.09 (d, *J* = 8.1 Hz, 2H, ArH), 7.42 – 7.35 (m, 2H, ArH), 7.32 (d, *J* = 8.0 Hz, 2H, ArH), 4.17 – 3.99 (m, 4H, CH_2 (1,4-diazepanyl)), 3.16 (t, *J* = 5.3 Hz, 2H, CH_2 (1,4-diazepanyl)), 2.92 (t, *J* = 5.7 Hz, 2H, CH_2 (1,4-diazepanyl)), 2.44 (s, 3H, Me (4-MeC_6H_4)), 2.04 (p, *J* = 6.1 Hz, 2H, CH_2 (1,4-diazepanyl)). ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 165.3, 162.2, 159.7, 148.2, 143.3, 137.0, 136.1, 129.9, 129.8, 128.5, 128.0, 123.7, 48.3, 21.9. MS *m/z* : 420.0 $[M+H]^+$.

5-(4-Bromophenyl)-7-(1,4-diazepan-1-yl)-2-(4-methylphenyl)-1,3-oxazolo[4,5-d]pyrimidine **(12)** Color: White solid; Yield 88%, 0.81 g; mp 265-267 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.35 (d, *J* = 8.5 Hz, 2H, ArH), 8.09 (d, *J* = 8.1 Hz, 2H, ArH), 7.55 (d, *J* = 8.5 Hz, 2H, ArH), 7.32 (d, *J* = 8.0 Hz, 2H, ArH), 4.12 – 4.07 (m, 4H, CH_2 (1,4-diazepanyl)), 3.17 (t, *J* = 5.3 Hz, 2H, CH_2 (1,4-diazepanyl)), 2.92 (t, *J* = 6.0 Hz, 2H, CH_2 (1,4-diazepanyl)), 2.44 (s, 3H, Me (4-MeC_6H_4)), 2.05 (p, *J* = 6.1 Hz, 2H, CH_2 (1,4-diazepanyl)). ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 165.3, 162.2, 159.8, 148.3, 143.4, 137.4, 131.4, 130.1, 129.9, 128.0, 124.6, 123.7, 21.9, 5.2. MS *m/z* : 464.0 $[M+H]^+$.

Synthesis of 1,3-oxazolo[5,4-*d*]pyrimidines (13, 14)



The preparation of *N*-[(7*Z*)-2,5-diphenyl-7*H*-1,3-oxazolo[5,4-*d*][1,3]oxazin-7-ylidene]benzamide (VI)

Compound **VI** was prepared by a literature procedure.[5] A solution of benzoyl bromide **V** (7 mL, 59 mmol, 3.00 equiv.) in anhydrous ether (35 mL), in which was suspended silver cyanide (7.96 g, 59 mmol, 3.00 equiv.), was stirred and refluxed for 21 h. The solids were collected and washed with cold ether. Yellow product was dissolved in hot chloroform and separated from the silver salts. The solvent was removed in vacuo.

The preparation of 2,5-diphenyl-1,3-oxazolo[5,4-*d*]pyrimidin-7(6*H*)-one (VII)

Compound **VII** was obtained by a published procedure.[5] Trimer **VI** (7.00 g, 18 mmol, 1.00 equiv.) was stirred at r.t. for 16 h in anhydrous methanol (70 mL) containing sodium methoxide (2.16 g, 36 mmol, 2.00 equiv.). The solid was filtered, washed with methanol and dried. The resulting sodium salt was converted into **VII** by stirring in 50% aqueous acetic acid (50 mL) at r.t. for 1 h.

The preparation of 7-chloro-2,5-diphenyl-1,3-oxazolo[5,4-*d*]pyrimidine (VIII)

Compound **VIII** was obtained by a published procedure.[5] A mixture of compound **VII** (4.12 g, 14 mmol, 1.00 equiv.), $POCl_3$ (50 ml), and Me_2NPh (4 mL, 28 mmol, 2.00 equiv.) was refluxed for 3 h. After evaporation of $POCl_3$ excess the solid residue was recrystallized from 1,4-dioxane.

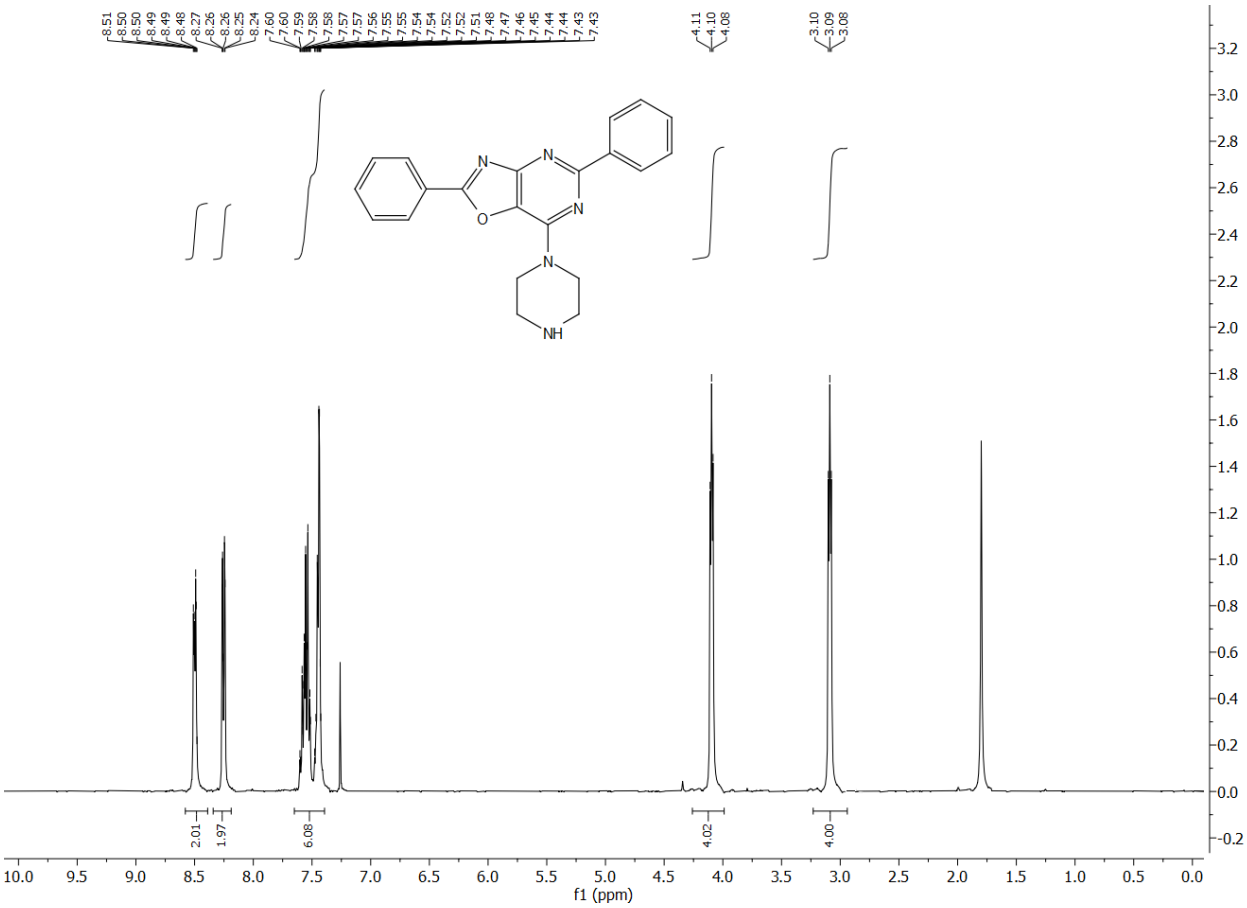
General procedure for the synthesis of 7-amino-substituted 1,3-oxazolo[5,4-*d*]pyrimidines 13, 14

A mixture of compound **VIII** (2 mmol), appropriate amine (2 mmol), and Et_3N (0.28 ml, 2 mmol) in dioxane (15 ml) was refluxed for 6 h. After removal of the solvent, the residue was triturated with water, filtered off, dried, and recrystallized from DMF/MeCN (1:3).

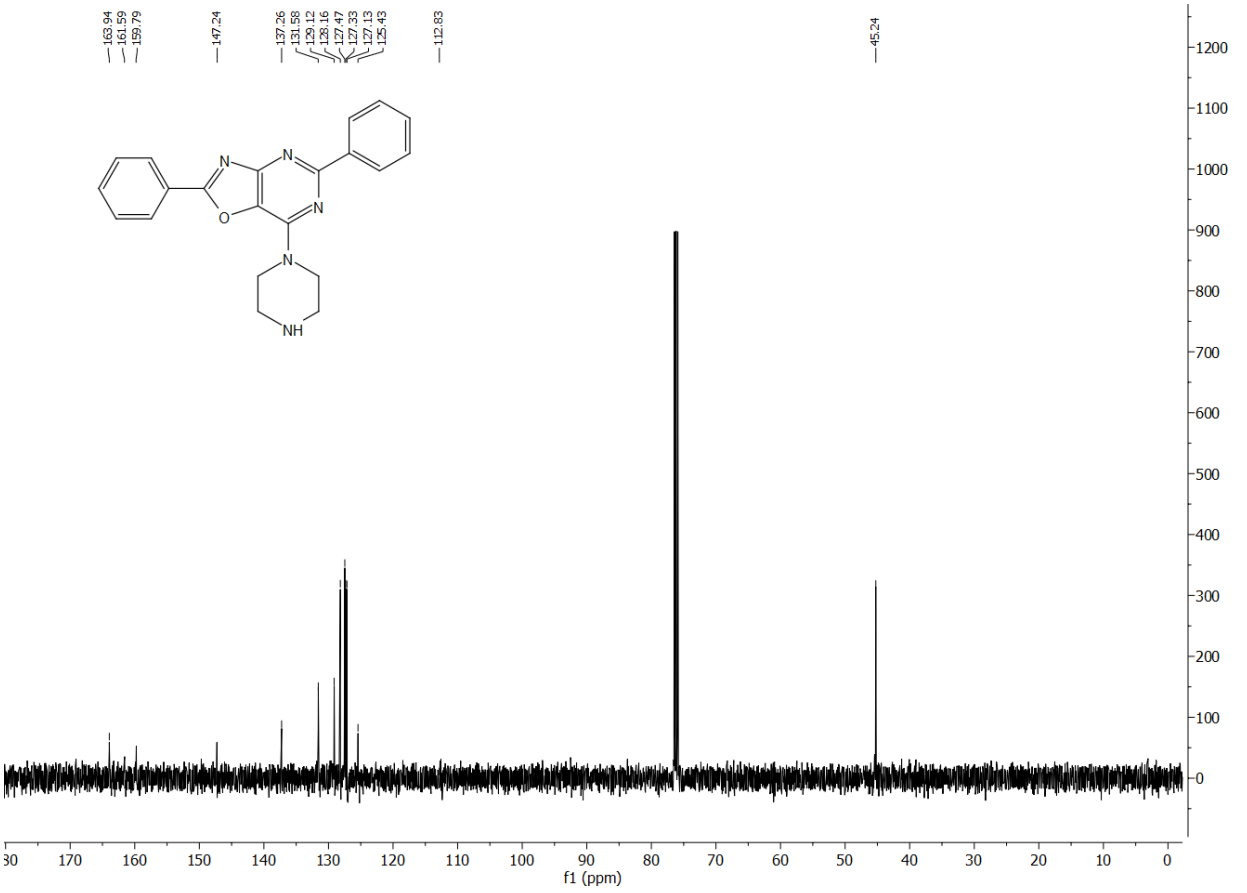
2,5-Diphenyl-7-(piperazin-1-yl)-1,3-oxazolo[5,4-d]pyrimidine **(13)** Color: White solid; Yield 98%, 0.70 g; mp 189–191 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.35–8.38 (m, 2H, ArH), 8.13–8.18 (m, 2H, ArH), 7.58–7.65 (m, 3H, ArH), 7.49–7.51 (m, 3H, ArH), 4.21 (bs, 4H, CH_2 (piperazinyl)), 2.88-2.90 (m, 4H, CH_2 (piperazinyl)) (NH proton has not been observed). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 166.1, 158.6, 156.8, 153.2, 137.3, 131.8, 130.5, 129.3, 128.5, 127.8, 126.8, 125.9, 114.8, 45.7. MS *m/z* : 358.0 $[M+H]^+$.

7-(1,4-Diazepan-1-yl)-2,5-diphenyl-1,3-oxazolo[5,4-d]pyrimidine **(14)** Color: White solid; Yield 95%, 0.71 g; mp 150–152 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.35 (d, *J* = 4.3 Hz, 2H, ArH), 8.11 (dd, *J* = 6.5, 3.1 Hz, 2H, ArH), 7.60 (dd, *J* = 5.2, 1.8 Hz, 3H, ArH), 7.50 (dd, *J* = 5.0, 1.6 Hz, 3H, ArH), 4.31 (d, *J* = 5.4 Hz, 2H, CH_2 (1,4-diazepanyl)), 4.04 – 4.01 (m, 2H, CH_2 (1,4-diazepanyl)), 3.01 – 2.98 (m, 2H, CH_2 (1,4-diazepanyl)), 2.76 – 2.71 (m, 2H, CH_2 (1,4-diazepanyl)), 1.90 (s, 2H, CH_2 (1,4-diazepanyl)). ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 165.9, 158.7, 156.9, 153.6, 137.4, 131.6, 130.4, 129.3, 128.5, 127.8, 126.7, 126.1, 114.7, 47.5, 47.8, 47.1, 30.2, 28.5. MS *m/z* : 372.0 $[M+H]^+$.

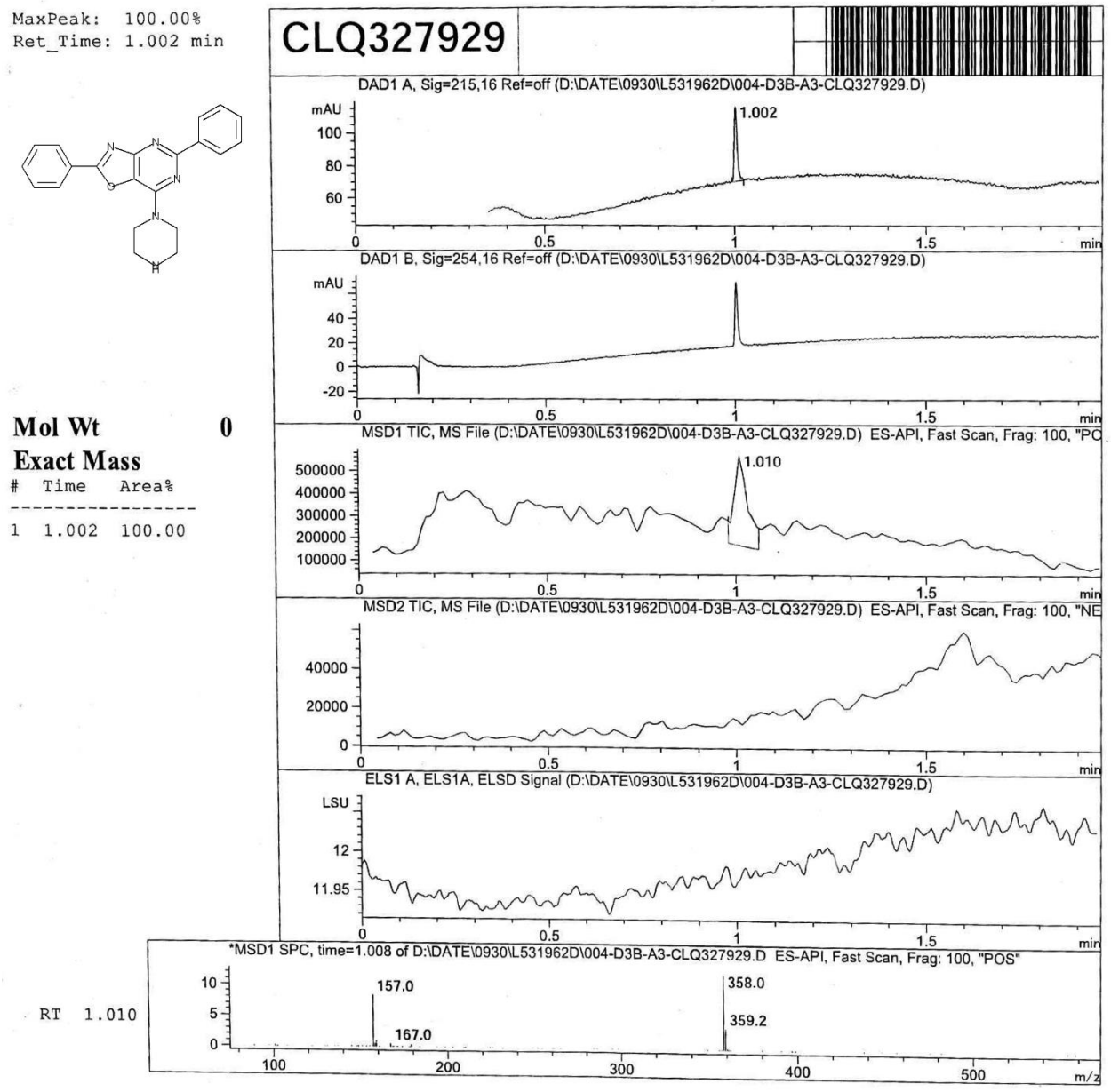
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound (**1**):



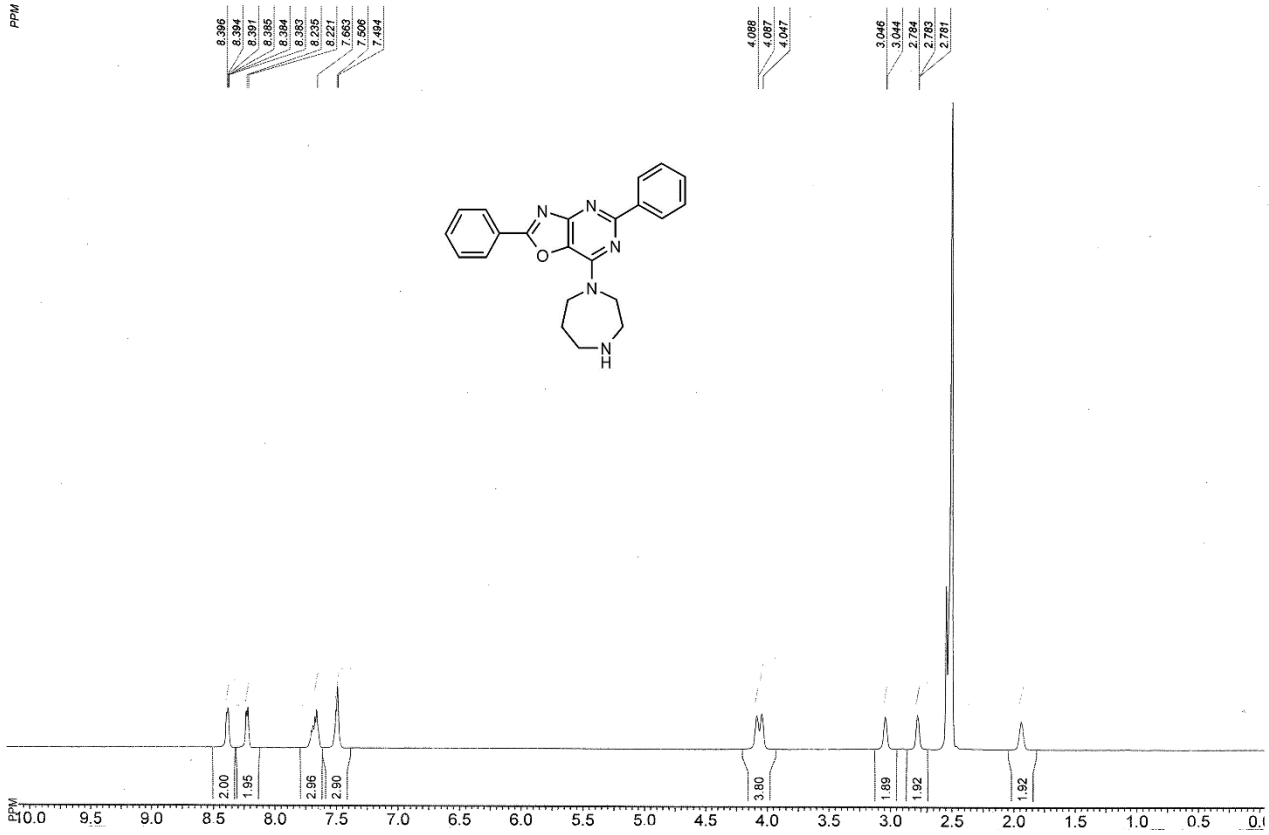
^{13}C NMR (126 MHz, $CDCl_3$) spectrum of compound (**1**):



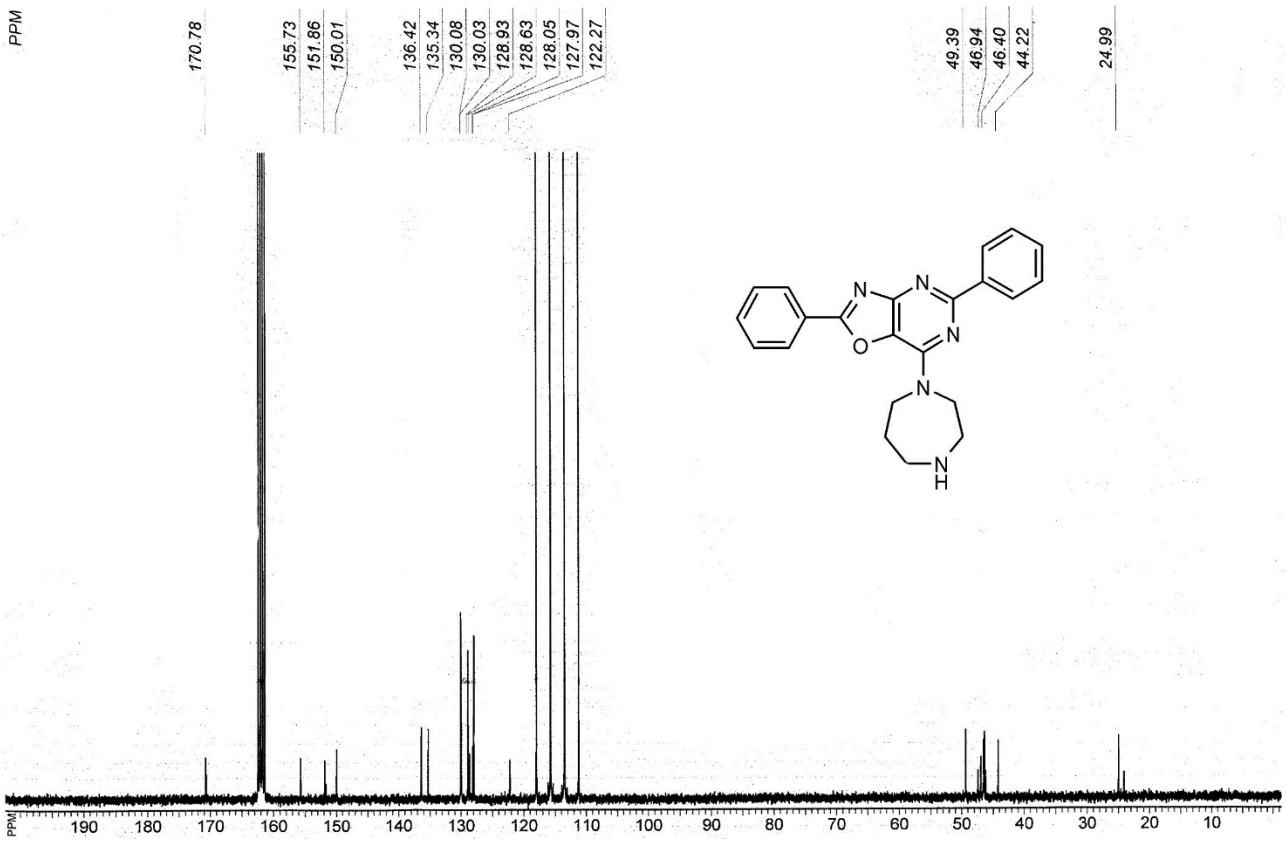
LCMS spectrum of compound (**1**):



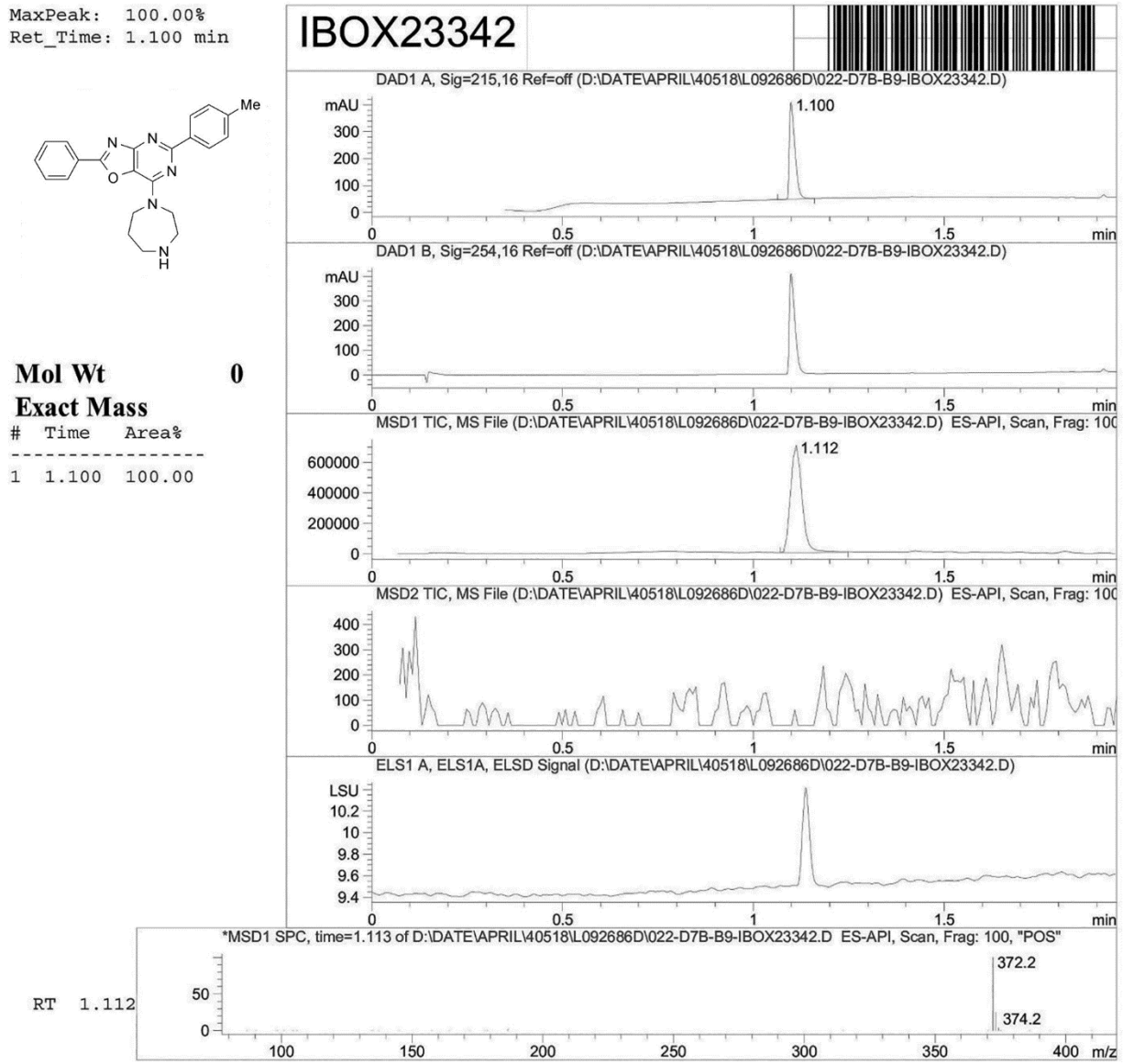
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound (**2**):



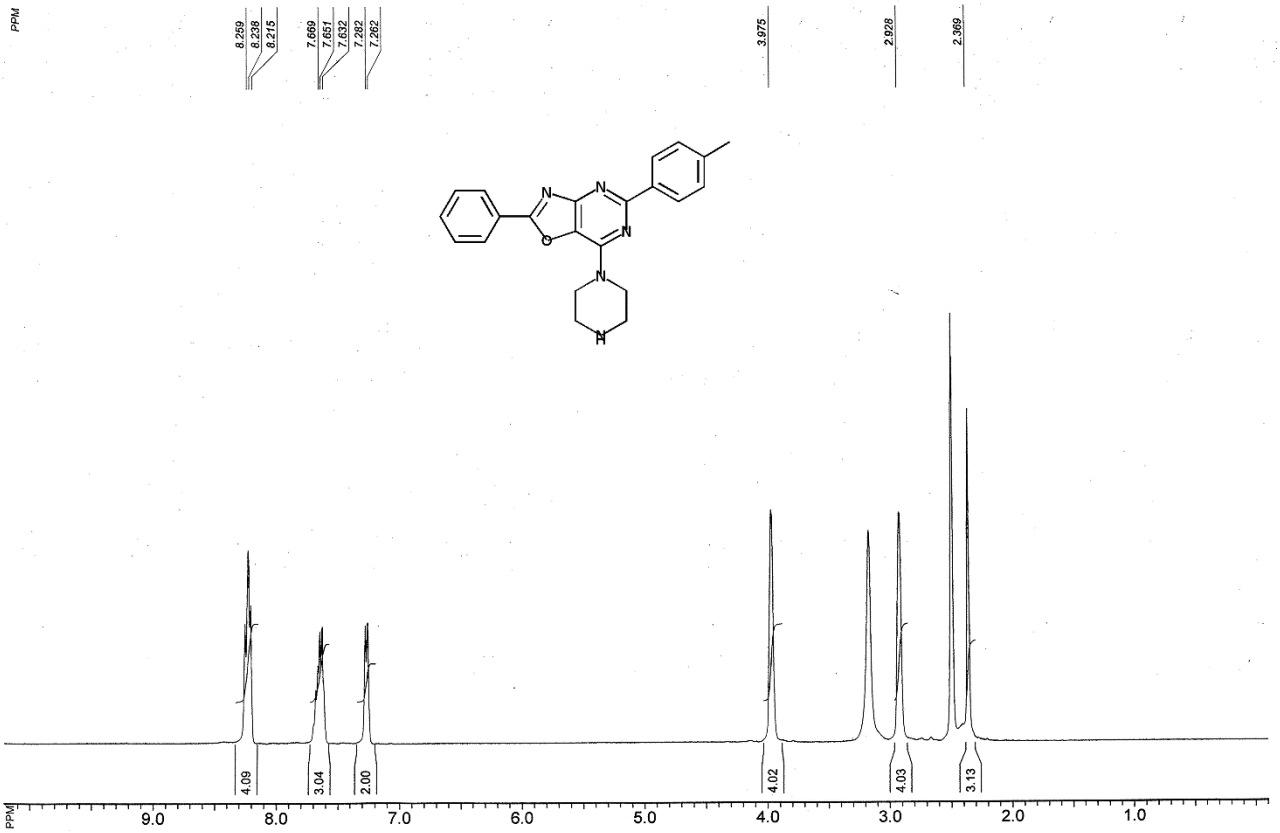
^{13}C NMR (126 MHz, CF_3COOD) spectrum of compound (**2**):



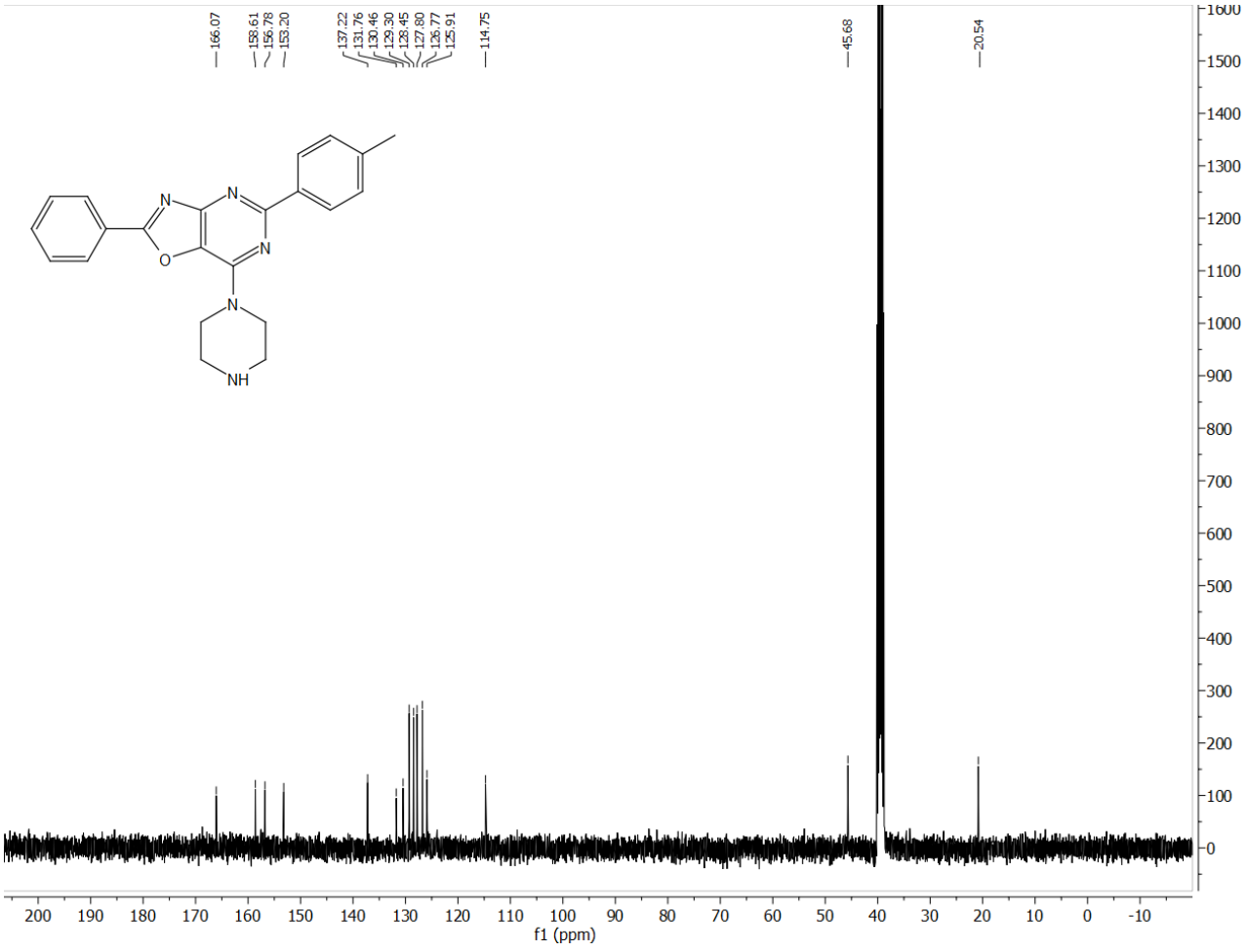
LCMS spectrum of compound (**2**):



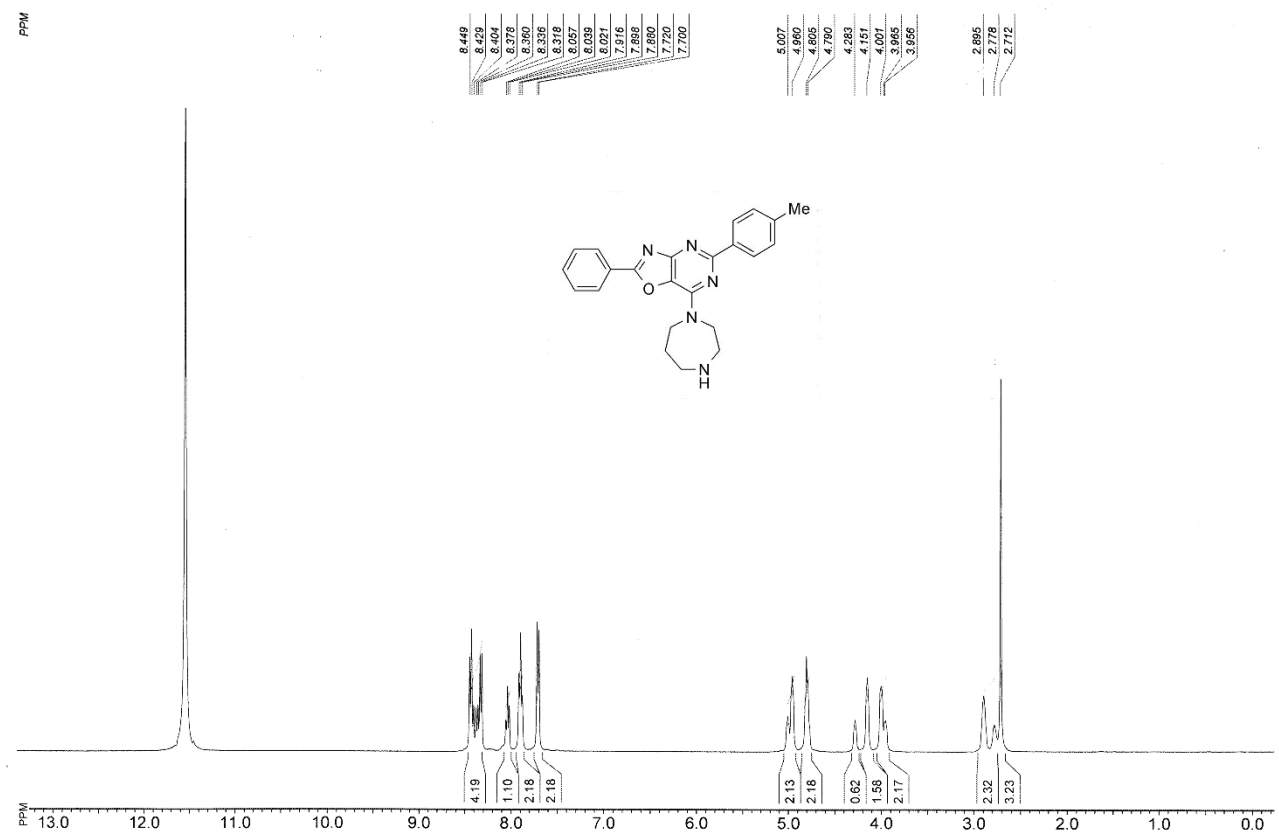
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound (**3**):



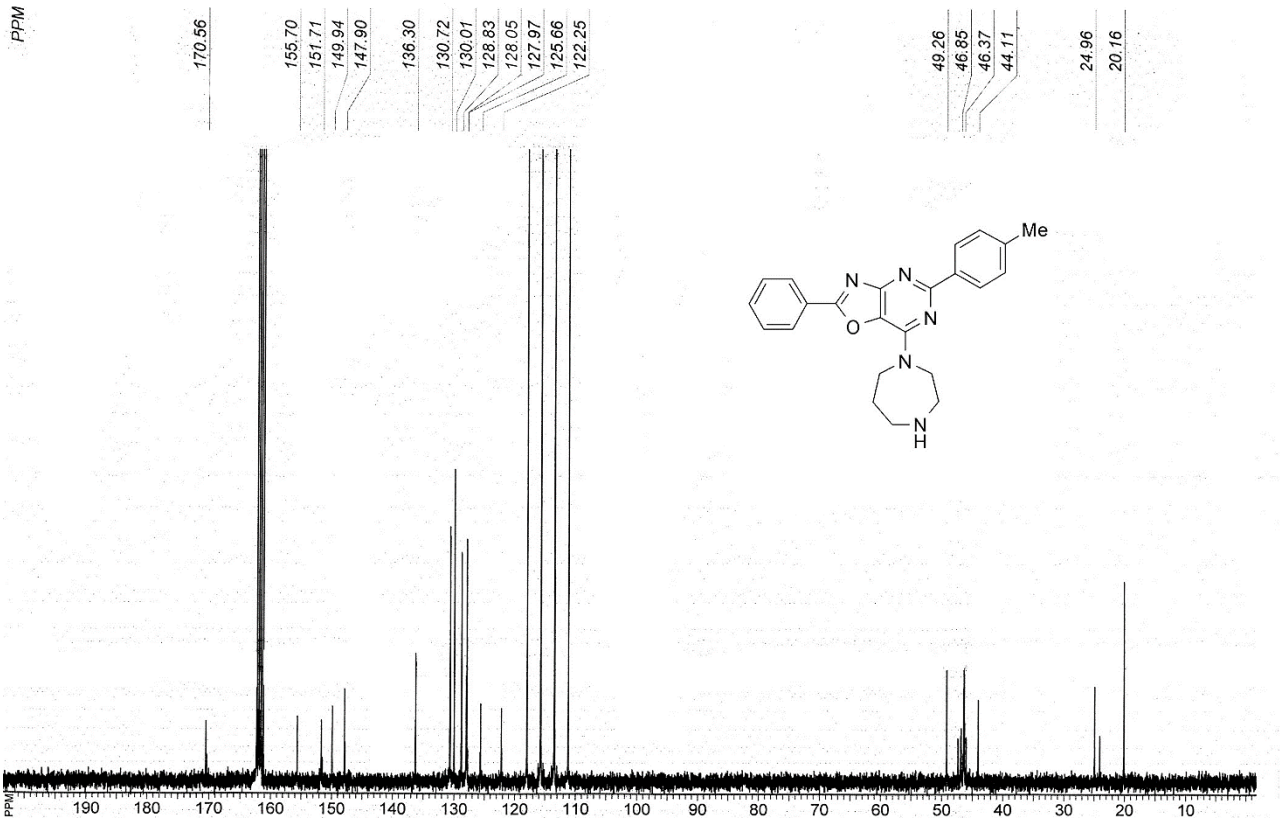
^{13}C NMR (126 MHz, DMSO-d_6) spectrum of compound (**3**):



^{1}H NMR (400 MHz, CF_3COOD) spectrum of compound (**4**):



^{13}C NMR (126 MHz, CF_3COOD) spectrum of compound (**4**):



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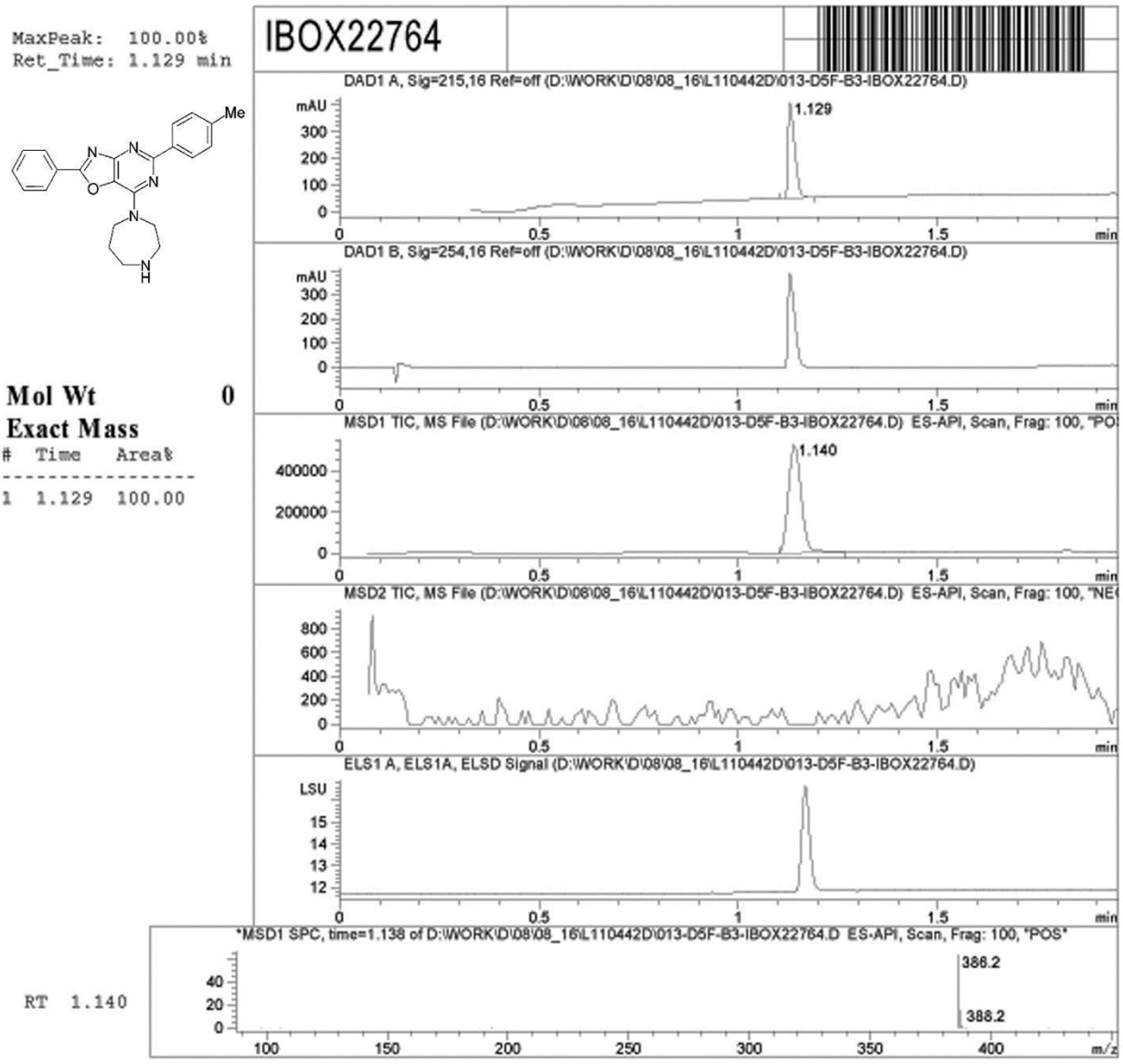
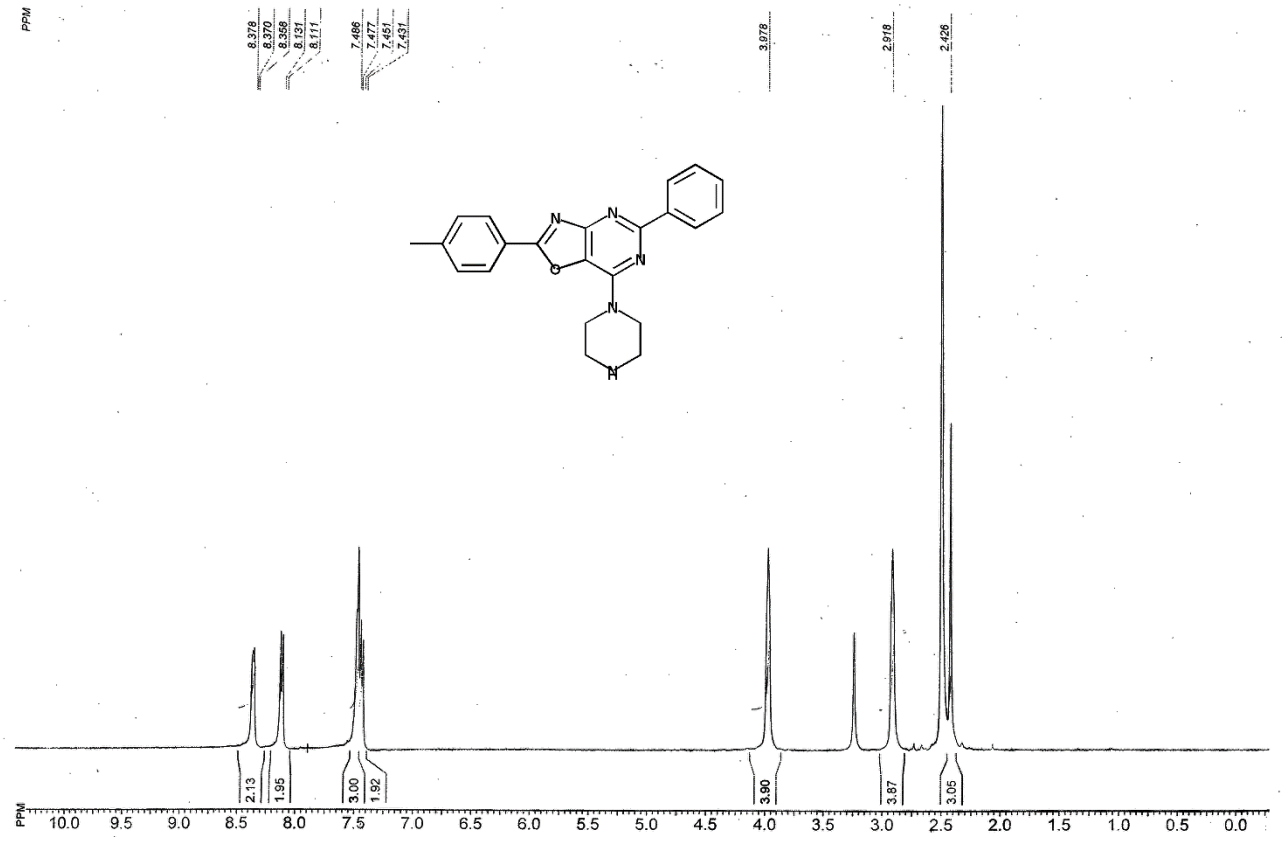
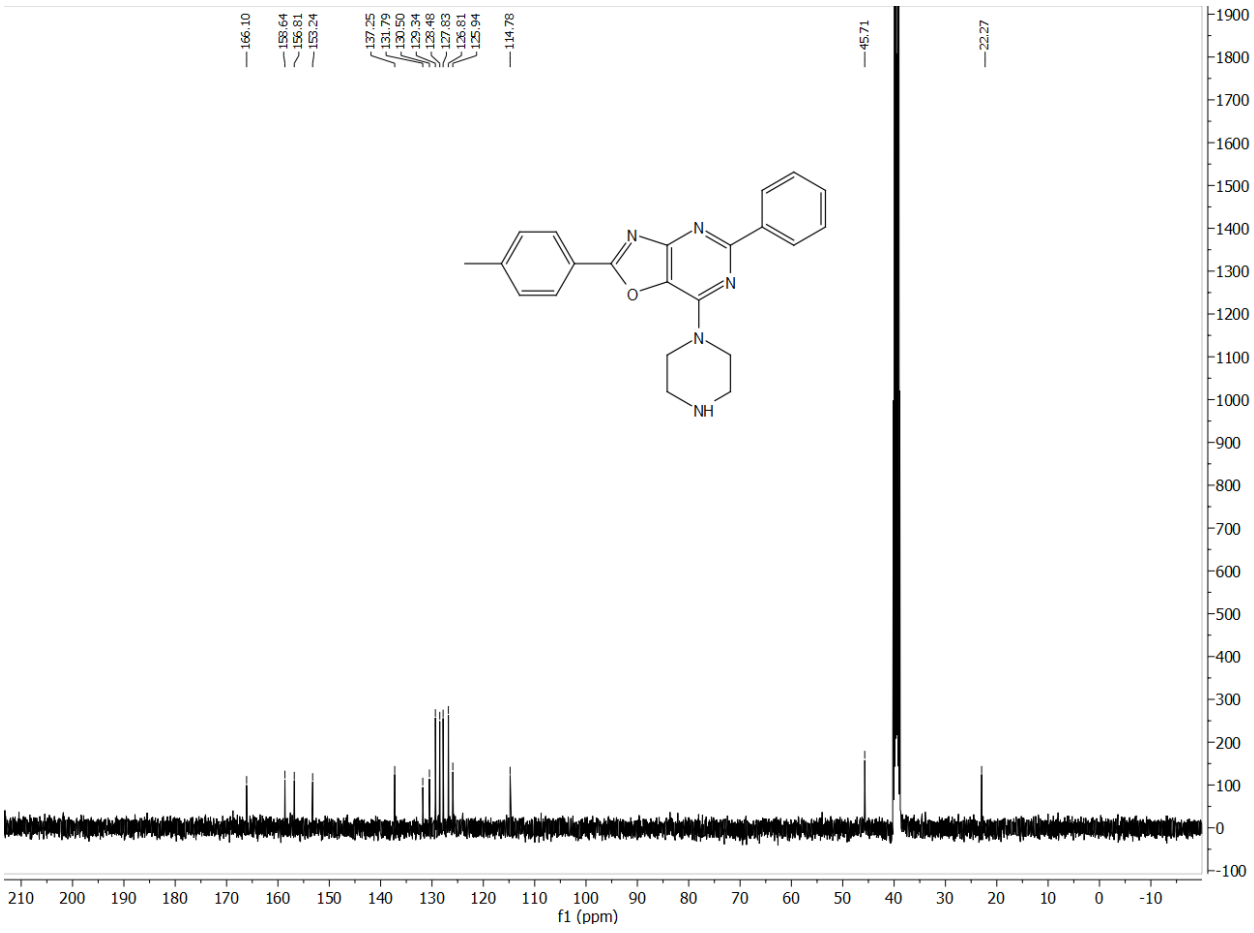


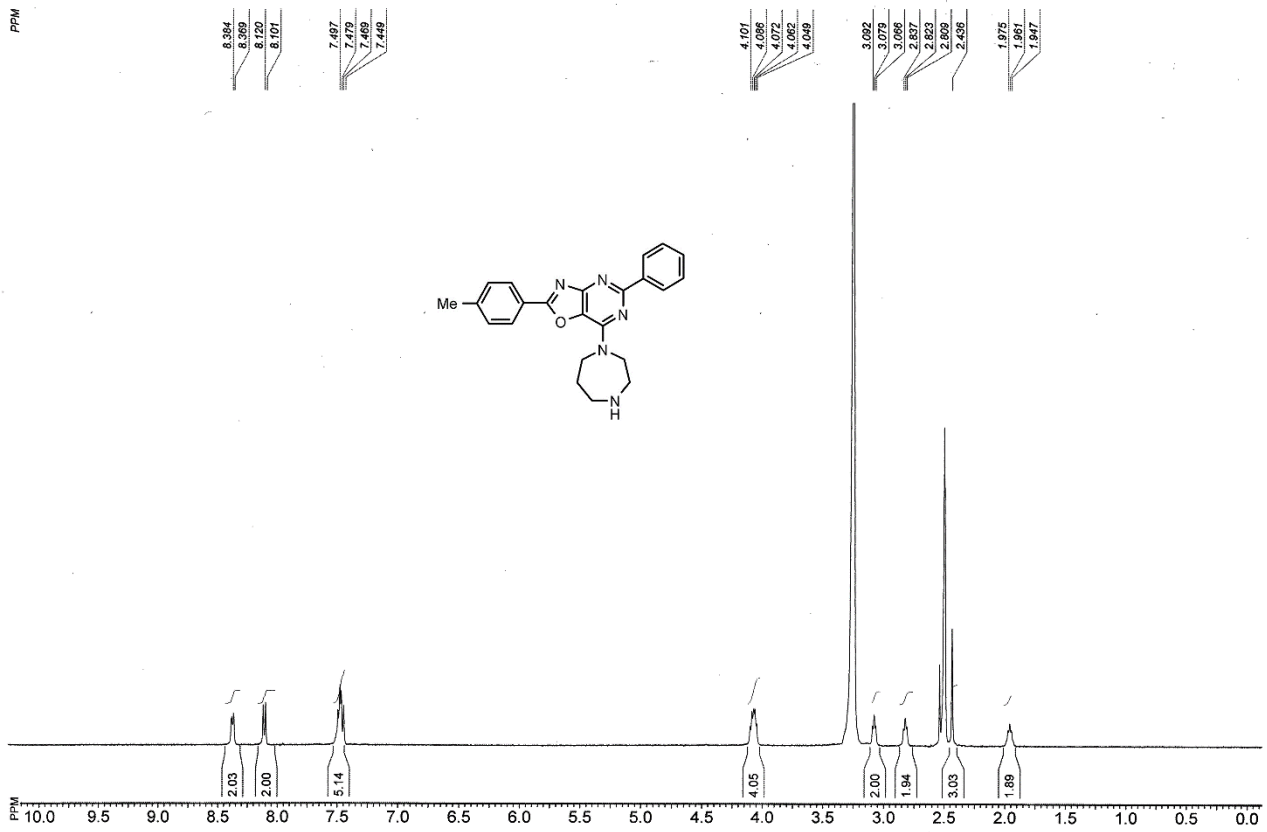
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound (**5**):



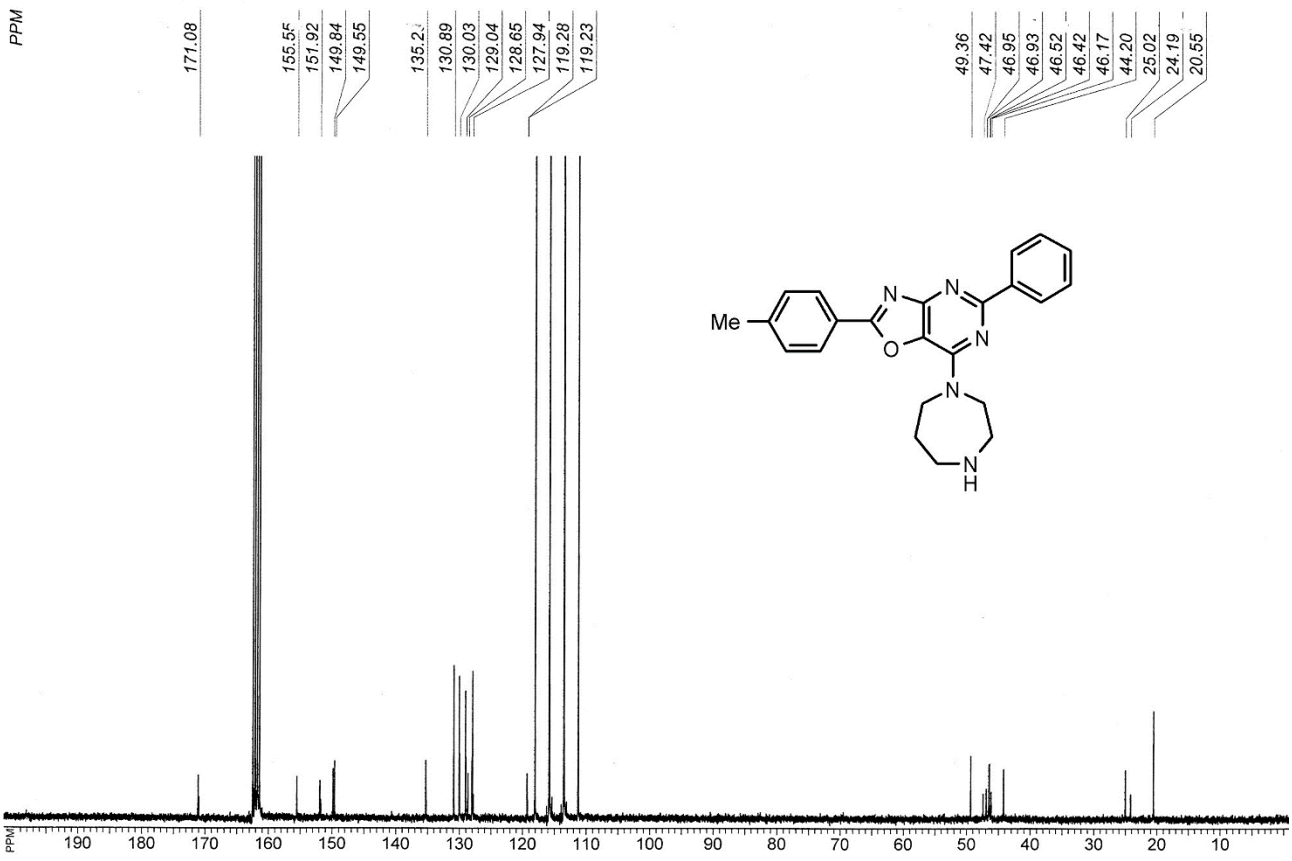
^{13}C NMR (126 MHz, DMSO-d_6) spectrum of compound (**5**):



^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound (**6**):



^{13}C NMR (126 MHz, CF_3COOD) spectrum of compound (**6**):



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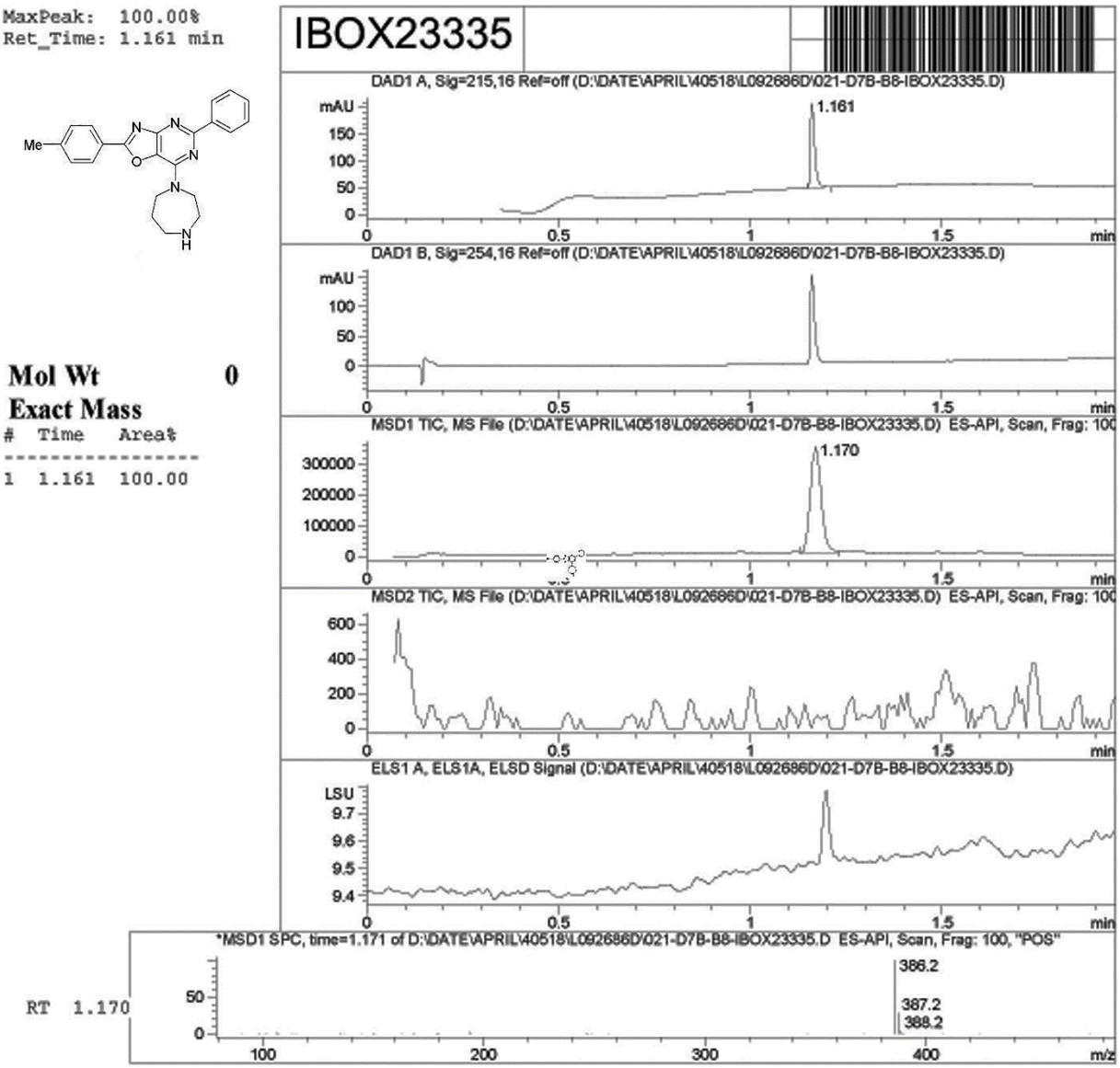
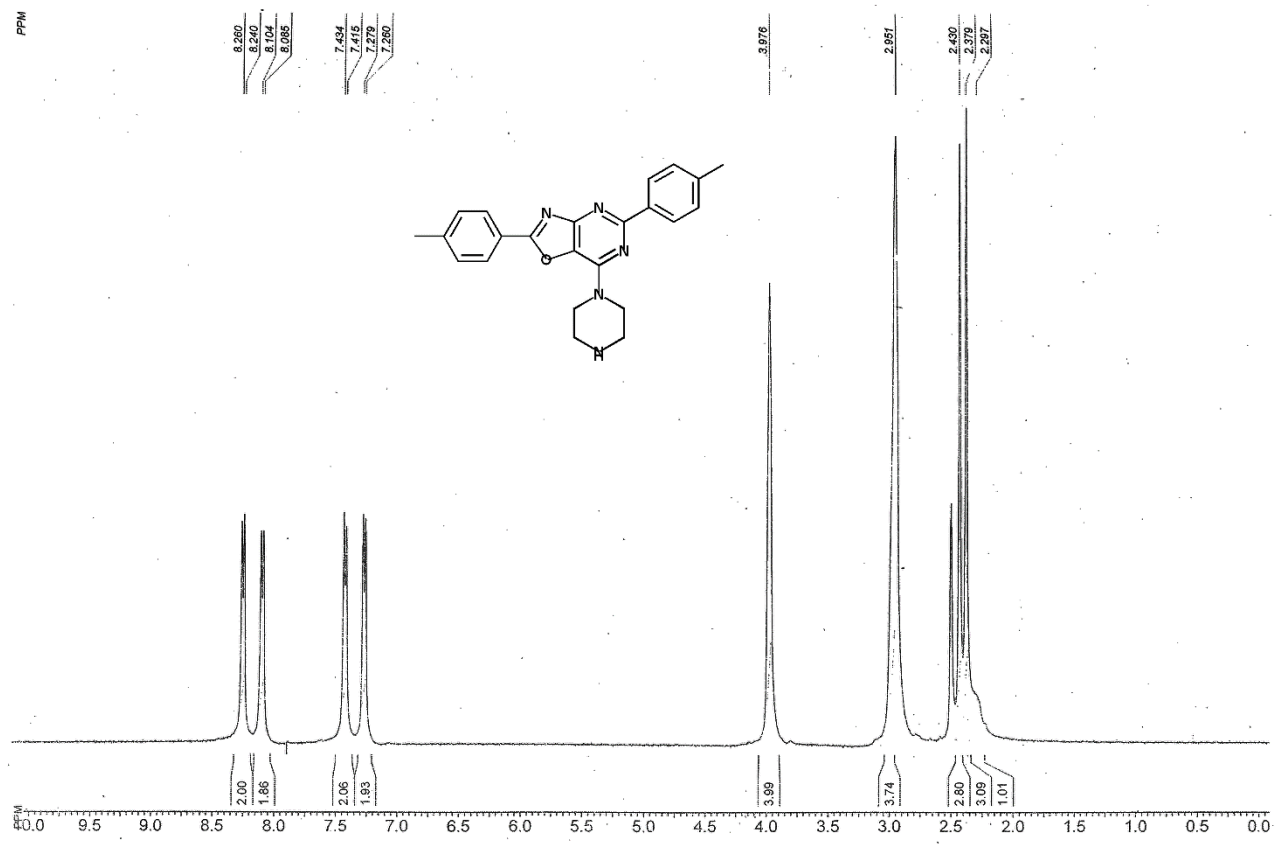
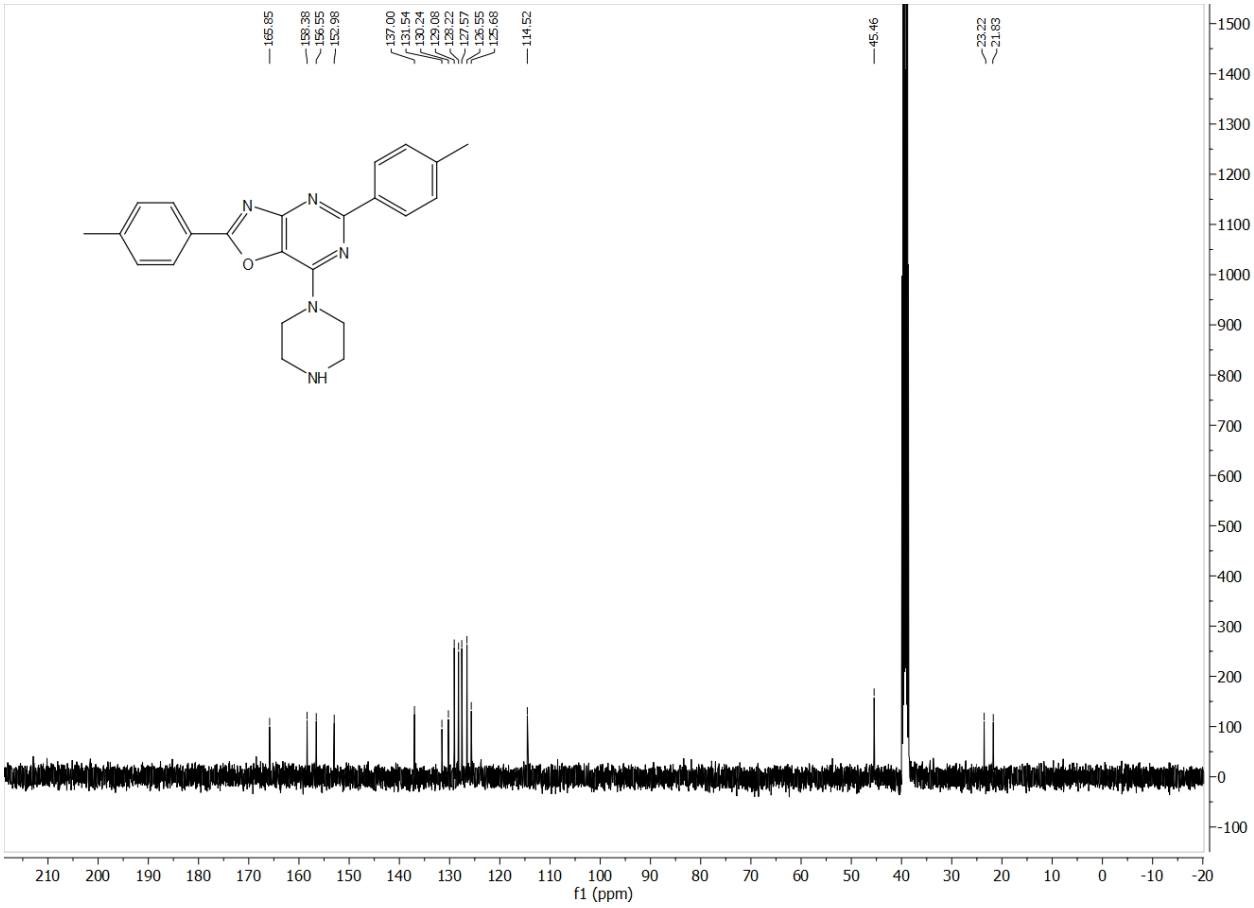


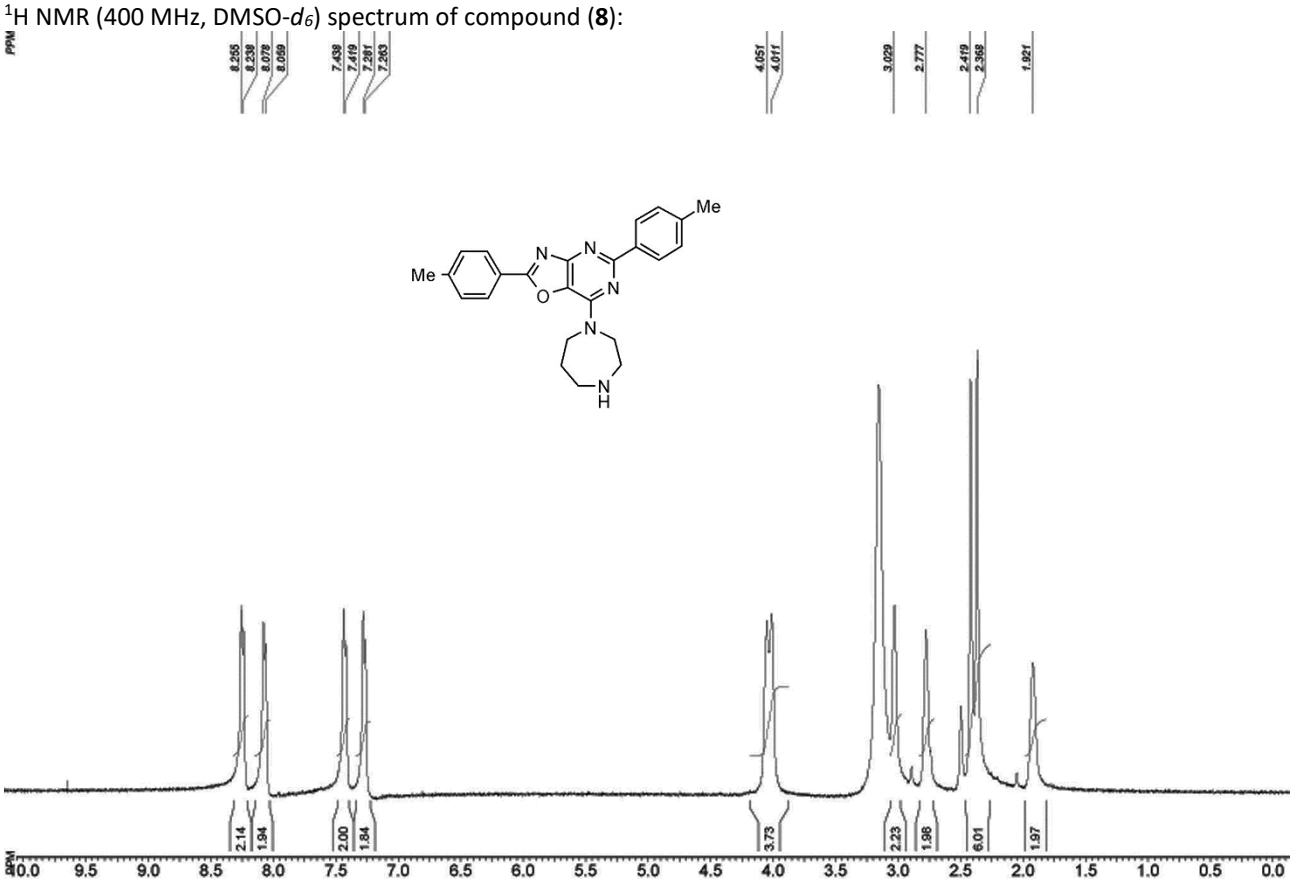
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound (**7**):



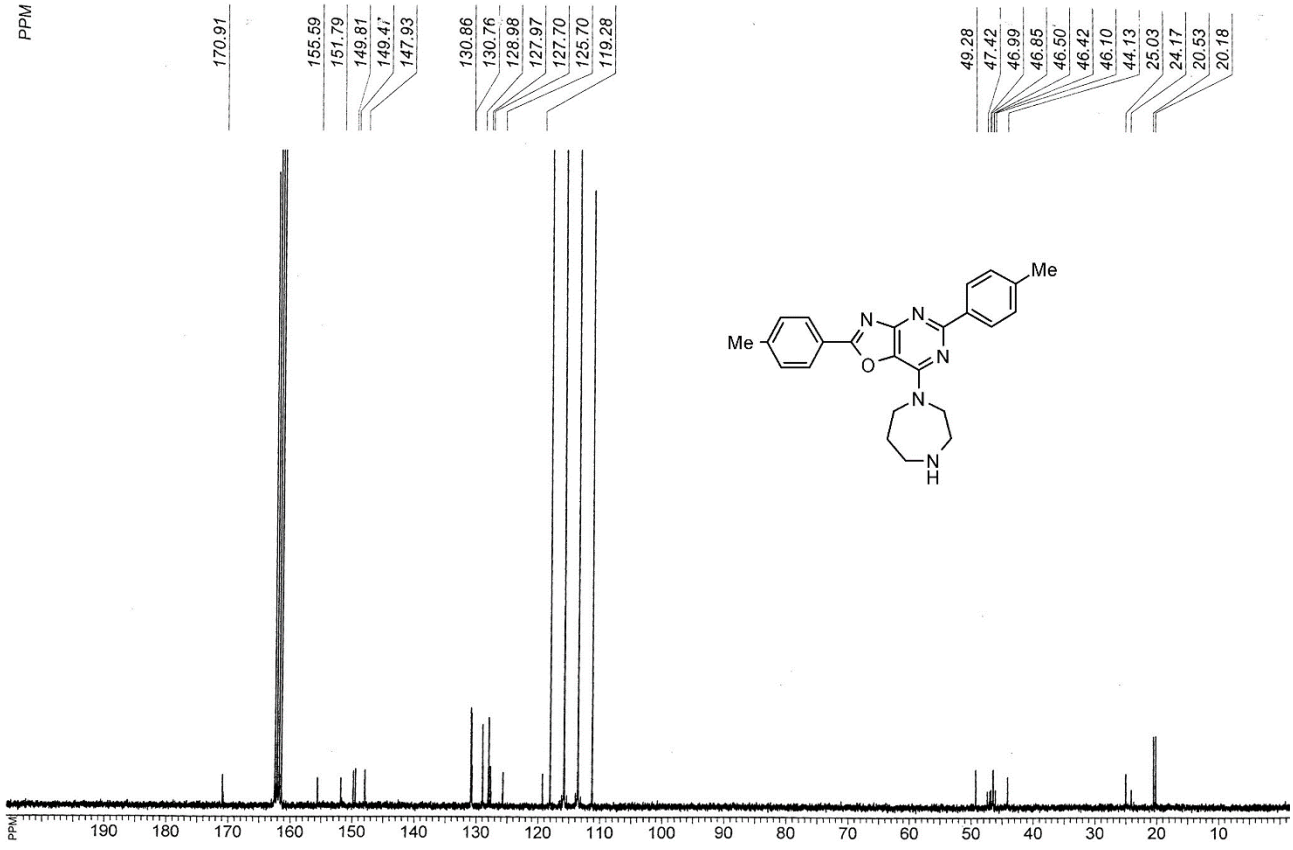
^{13}C NMR (126 MHz, DMSO-d_6) spectrum of compound (**7**):



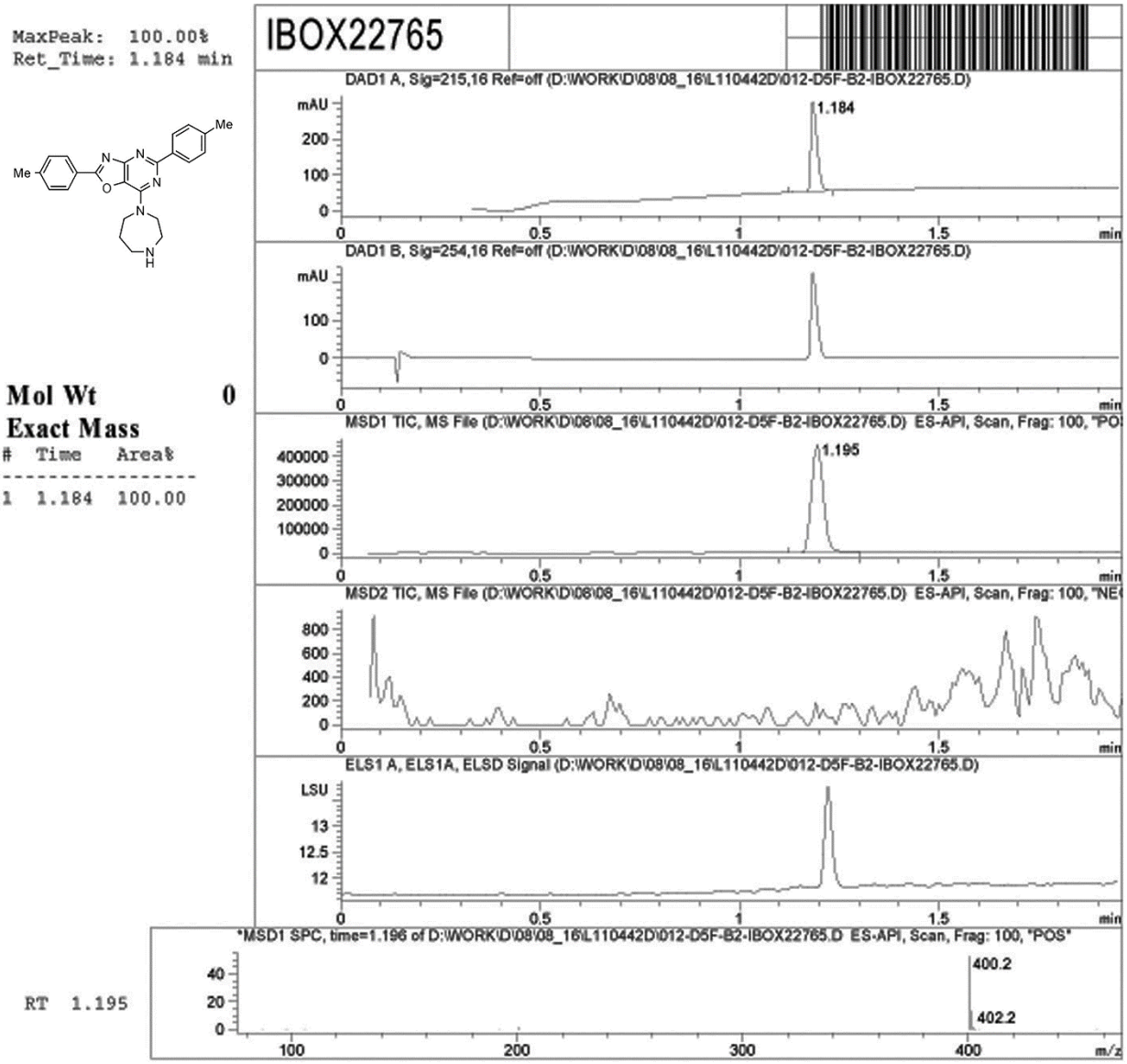
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound (**8**):



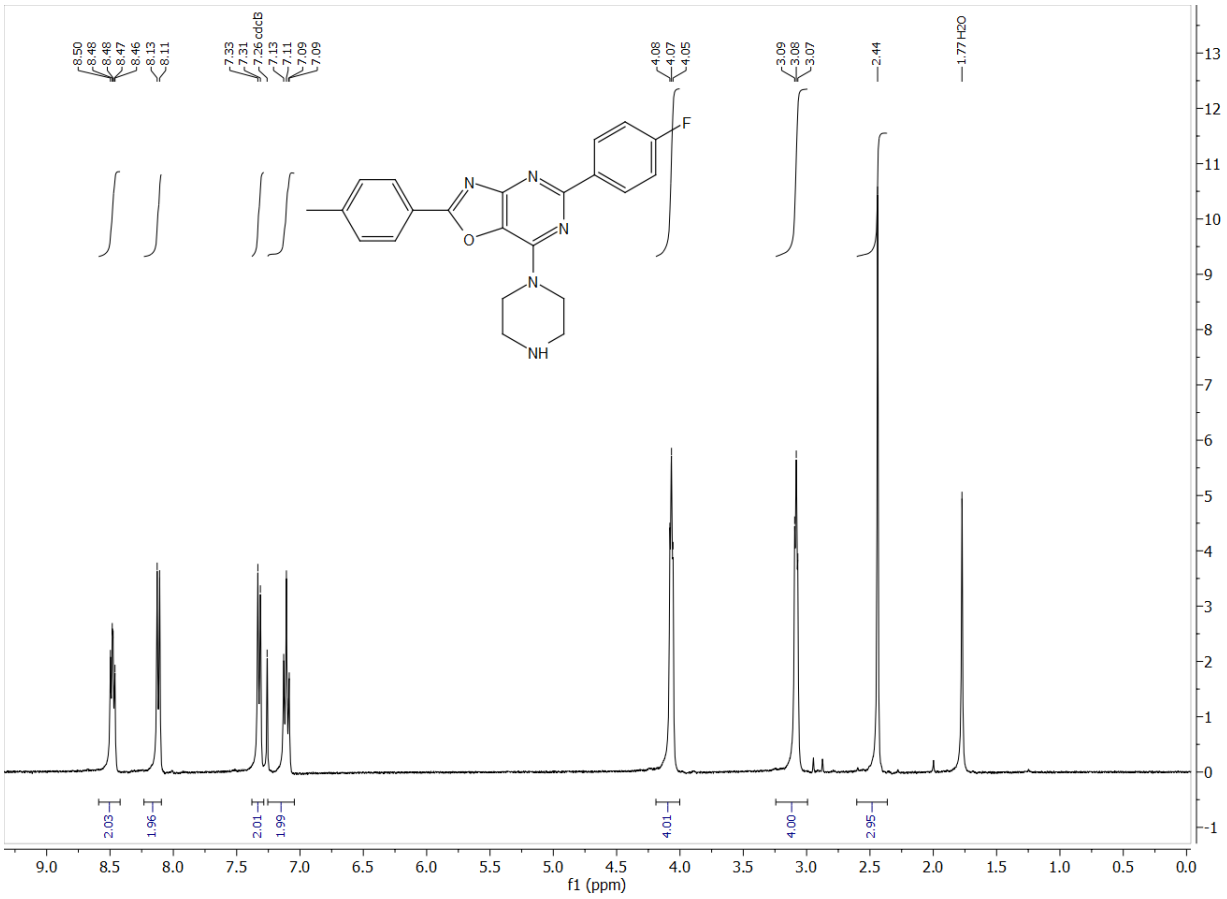
^{13}C NMR (126 MHz, CF_3COOD) spectrum of compound (**8**):



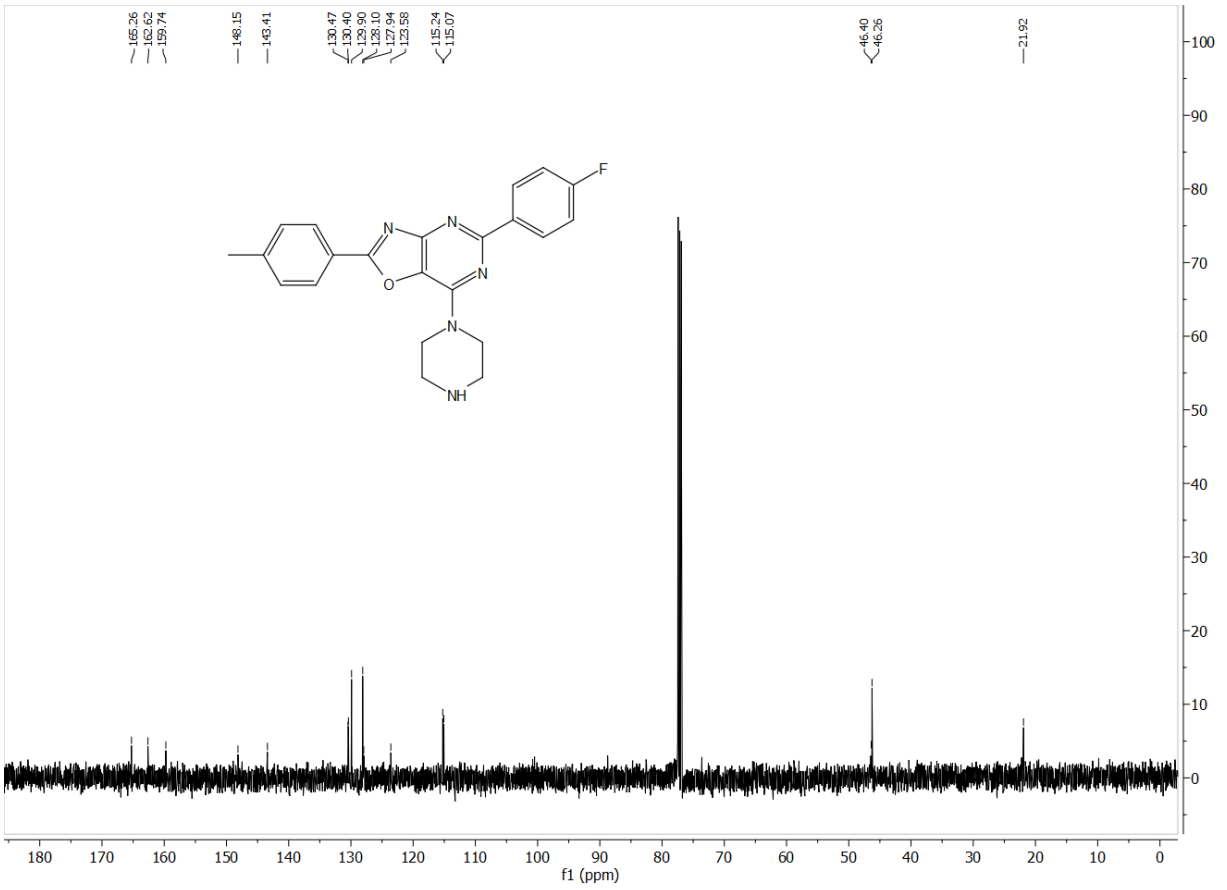
LCMS spectrum of compound (**8**):



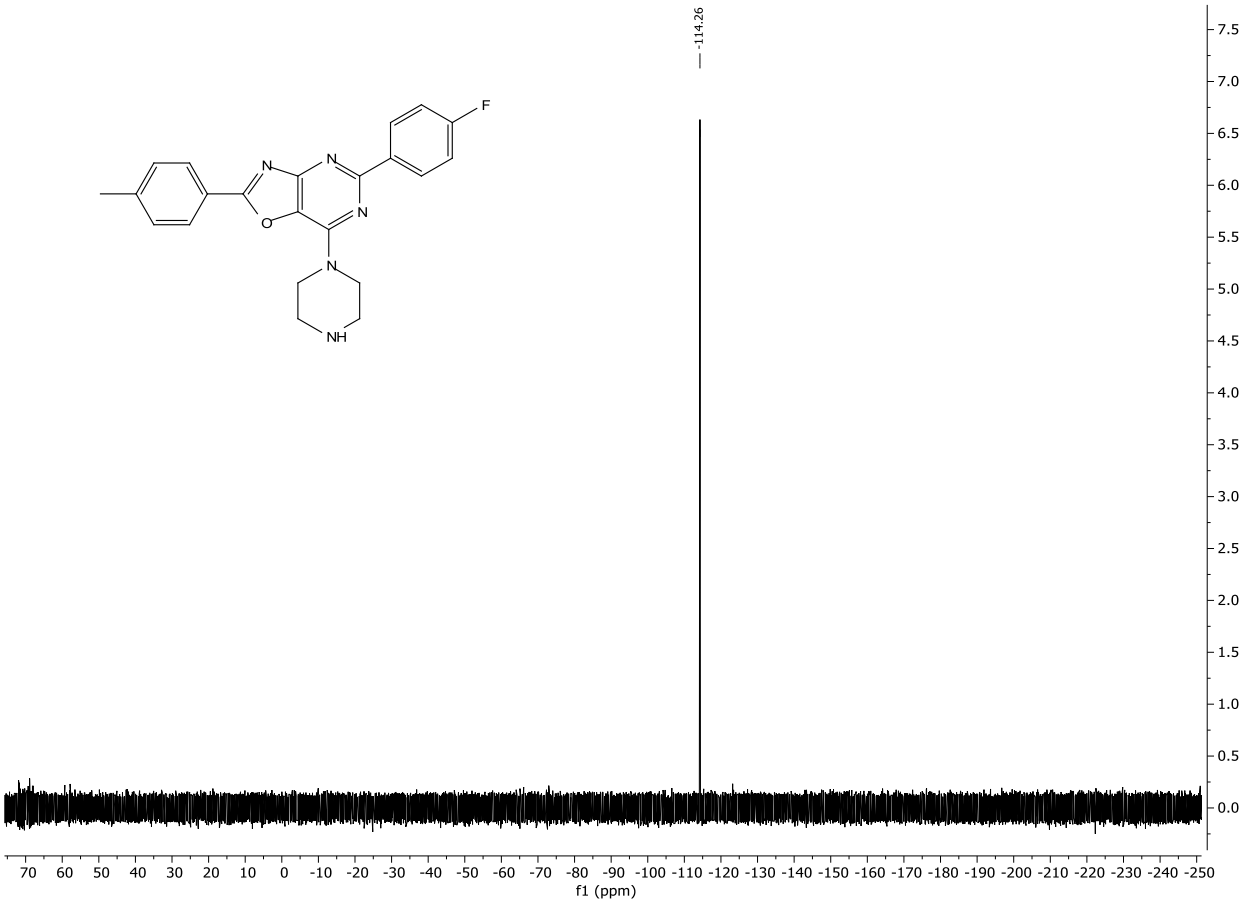
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound (**9**):



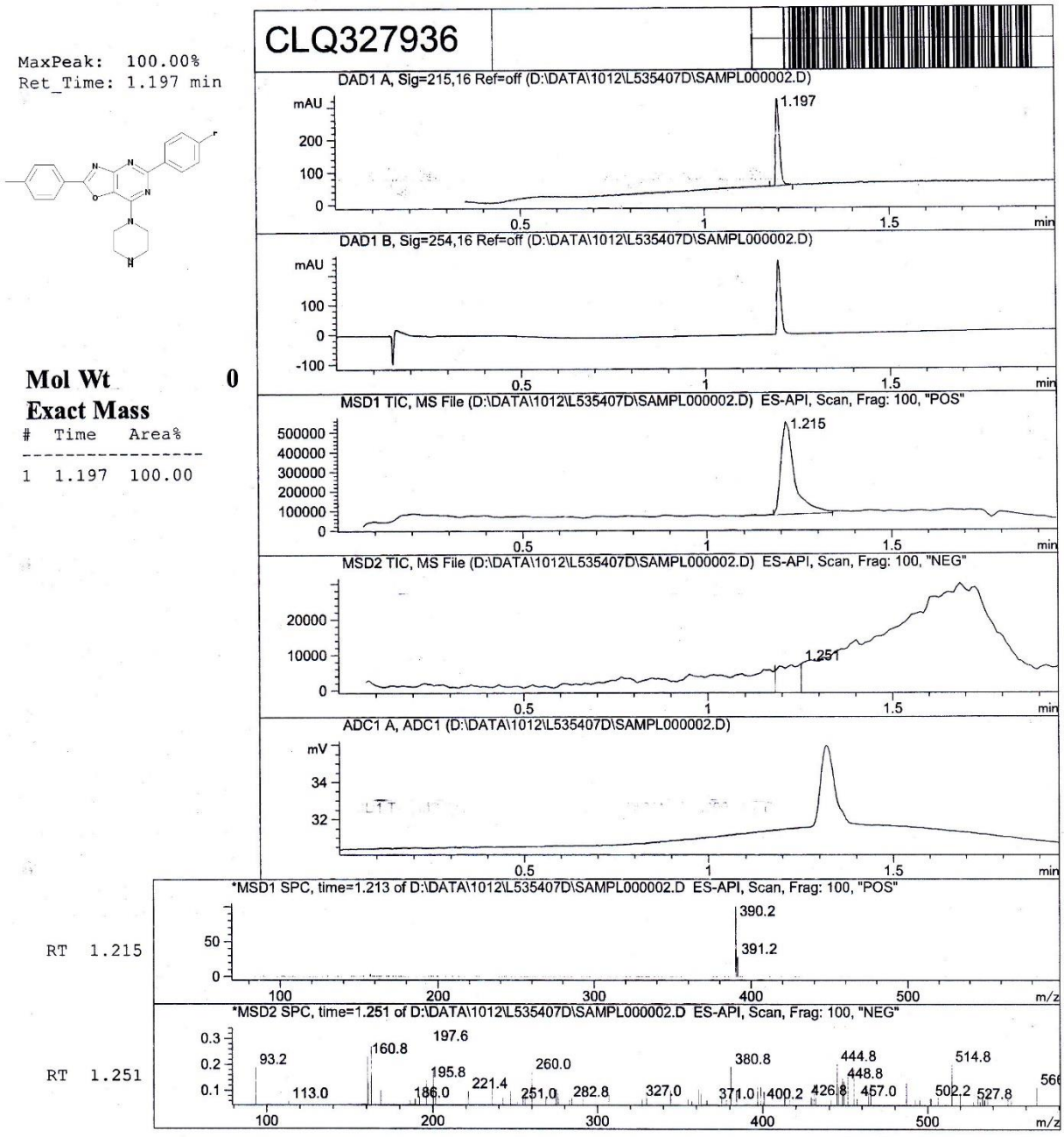
^{13}C NMR (126 MHz, $CDCl_3$) spectrum of compound (**9**):



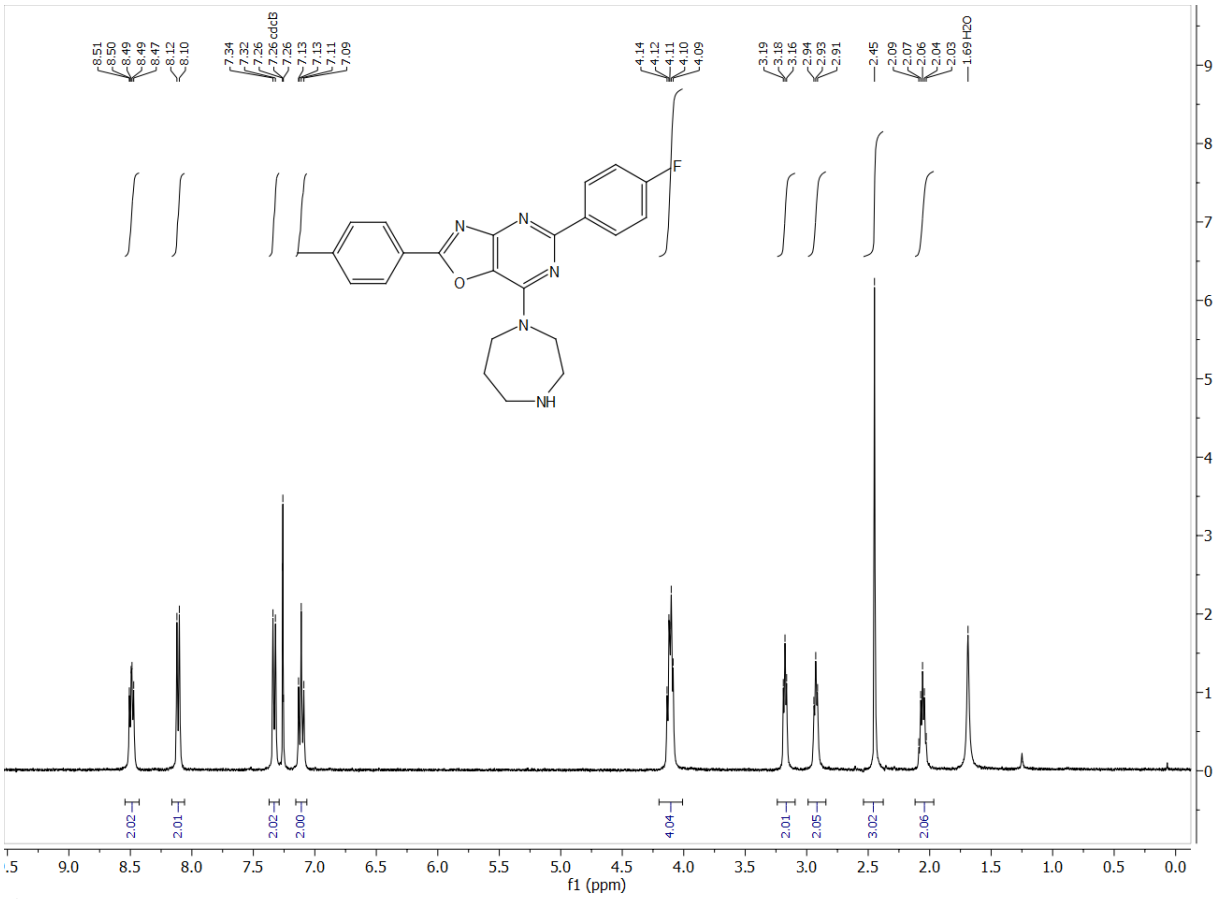
NMR (376 MHz, DMSO-d_6) spectrum of compound (**9**):



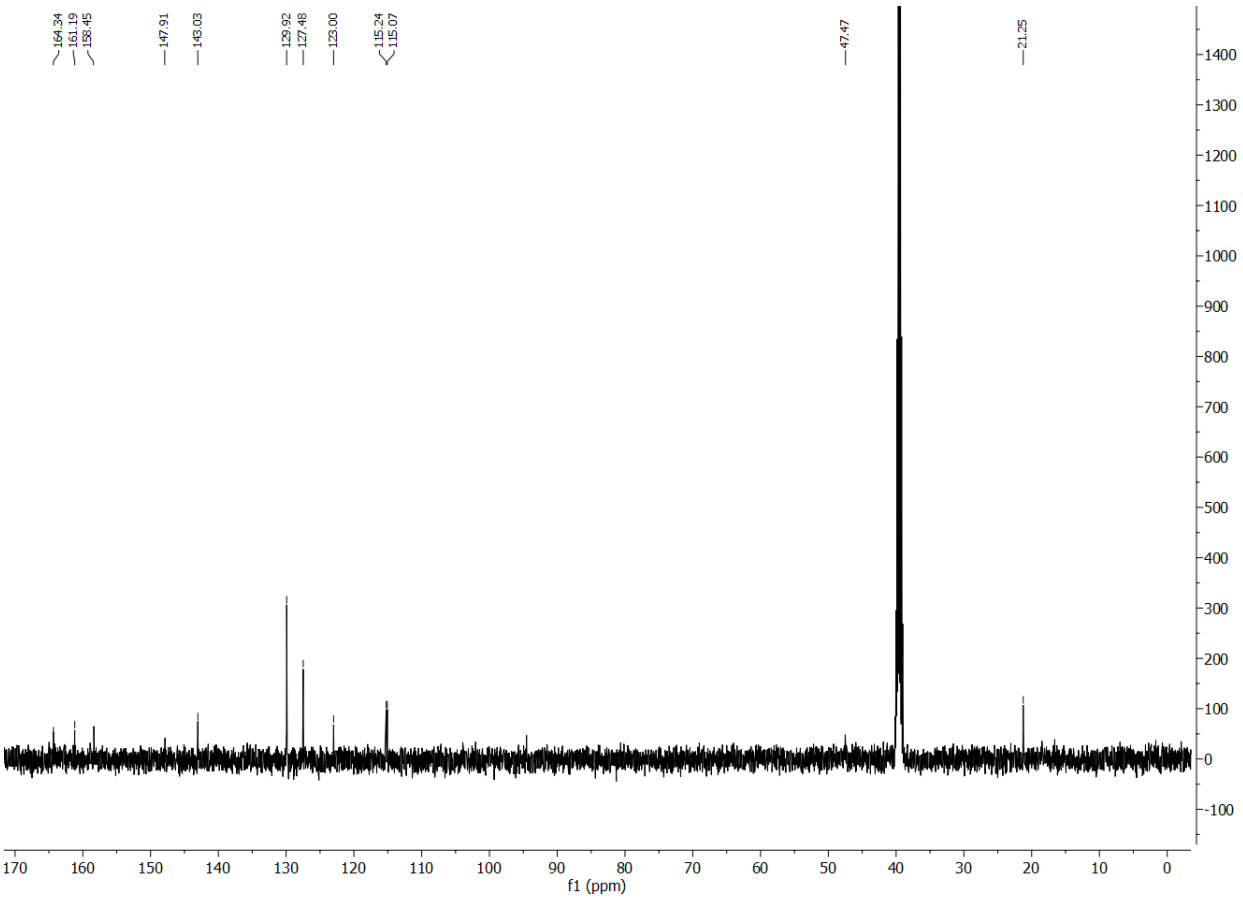
LCMS spectrum of compound (**9**):



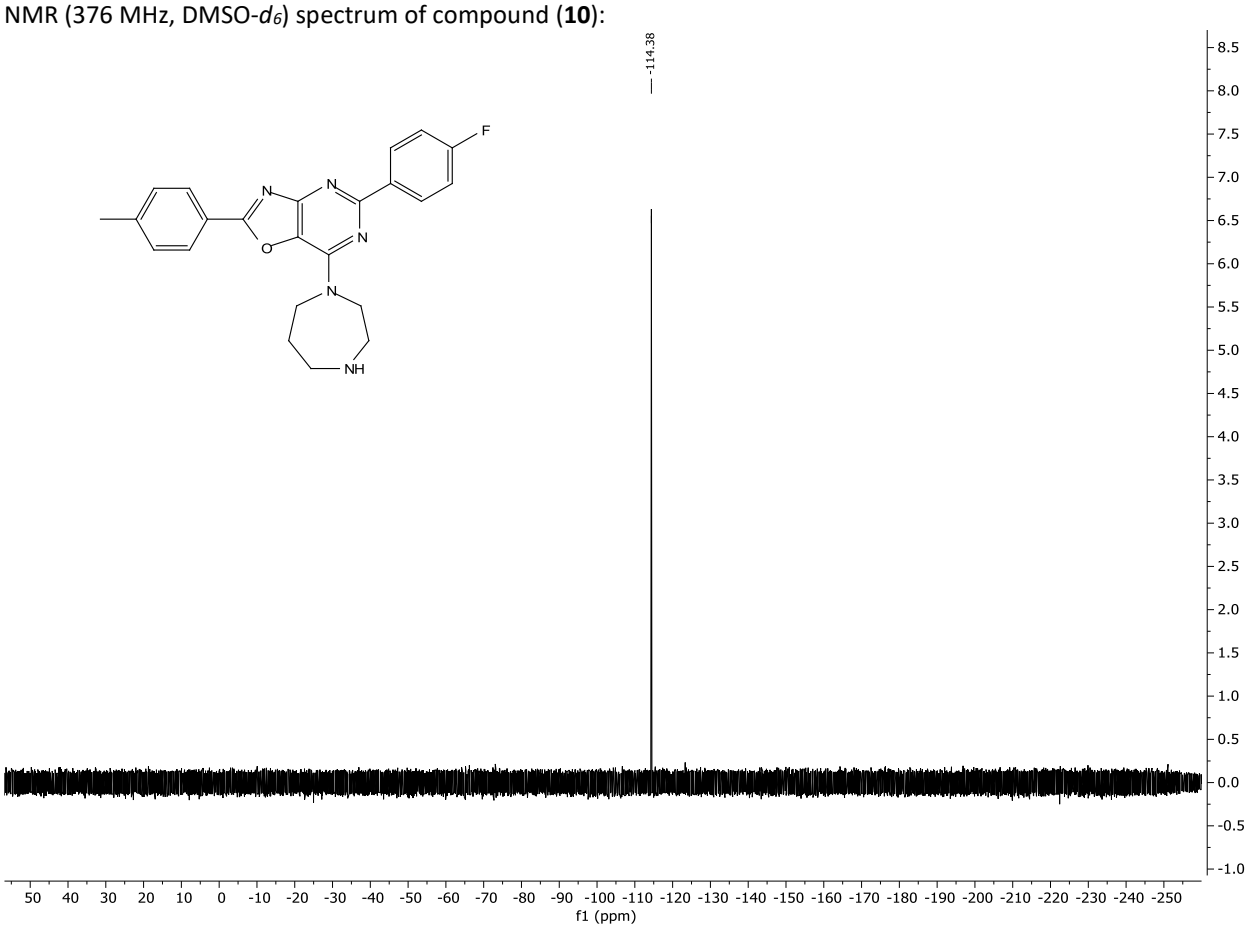
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound (**10**):



^{13}C NMR (126 MHz, DMSO-d_6) spectrum of compound (**10**):

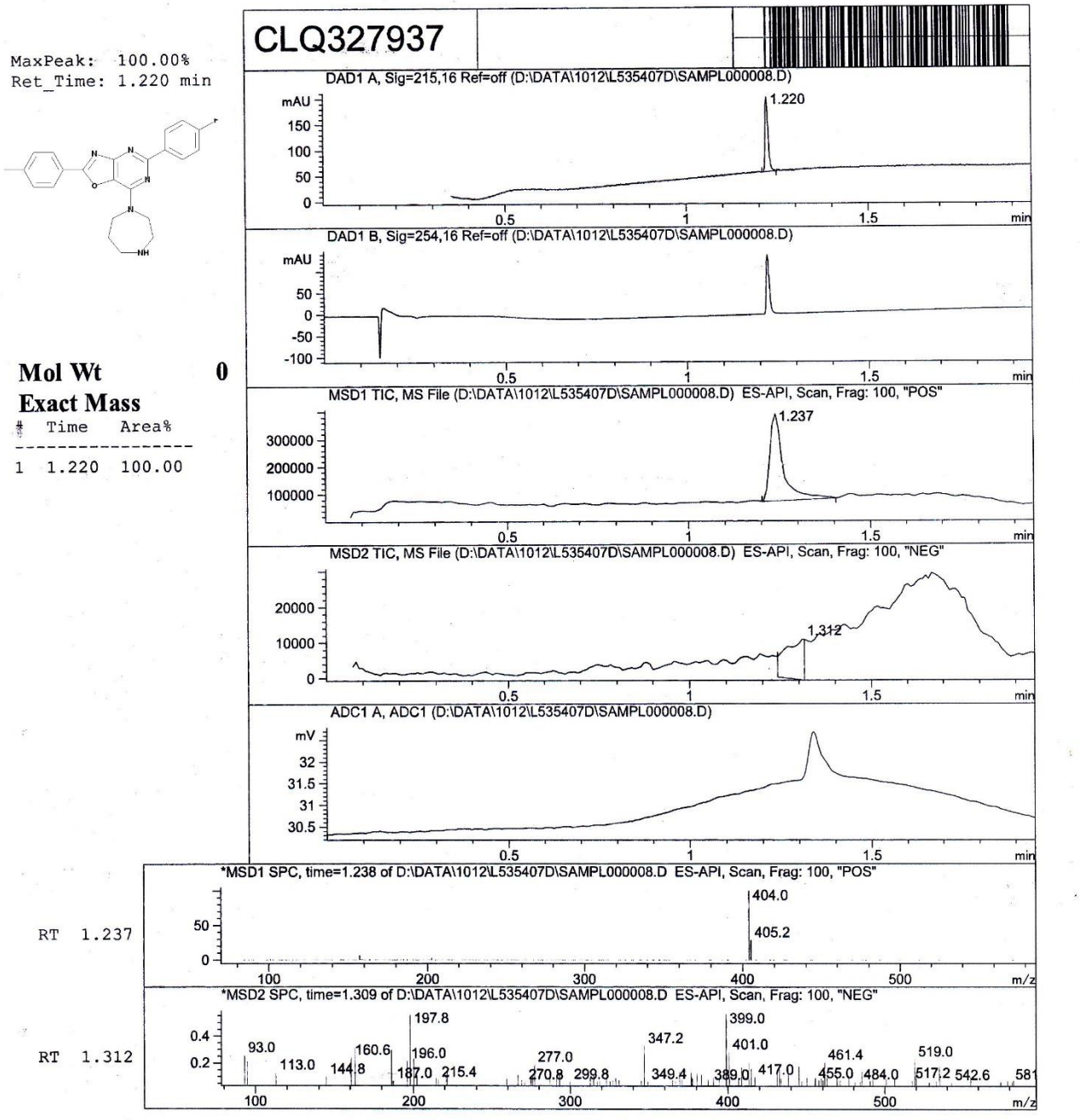


NMR (376 MHz, DMSO-d_6) spectrum of compound (**10**):

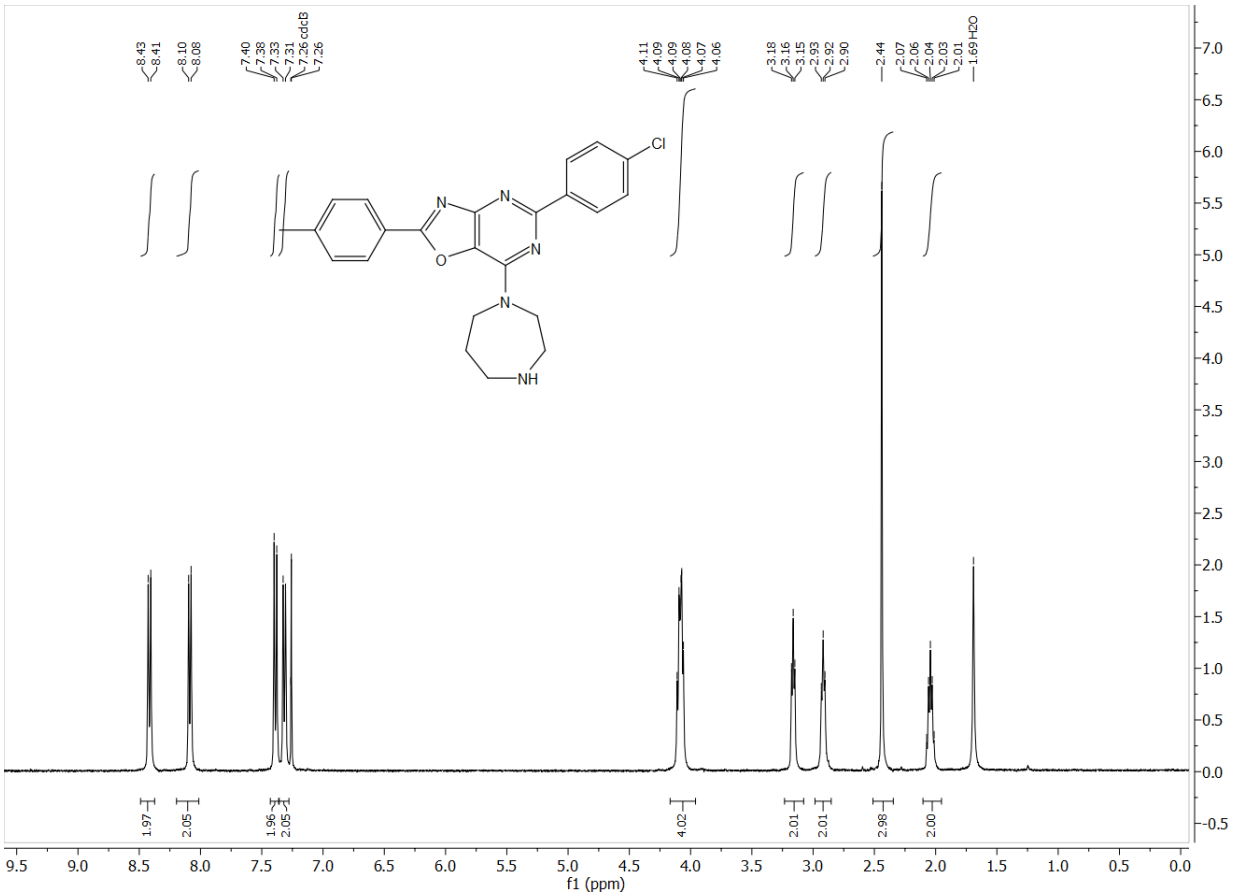


^{19}F

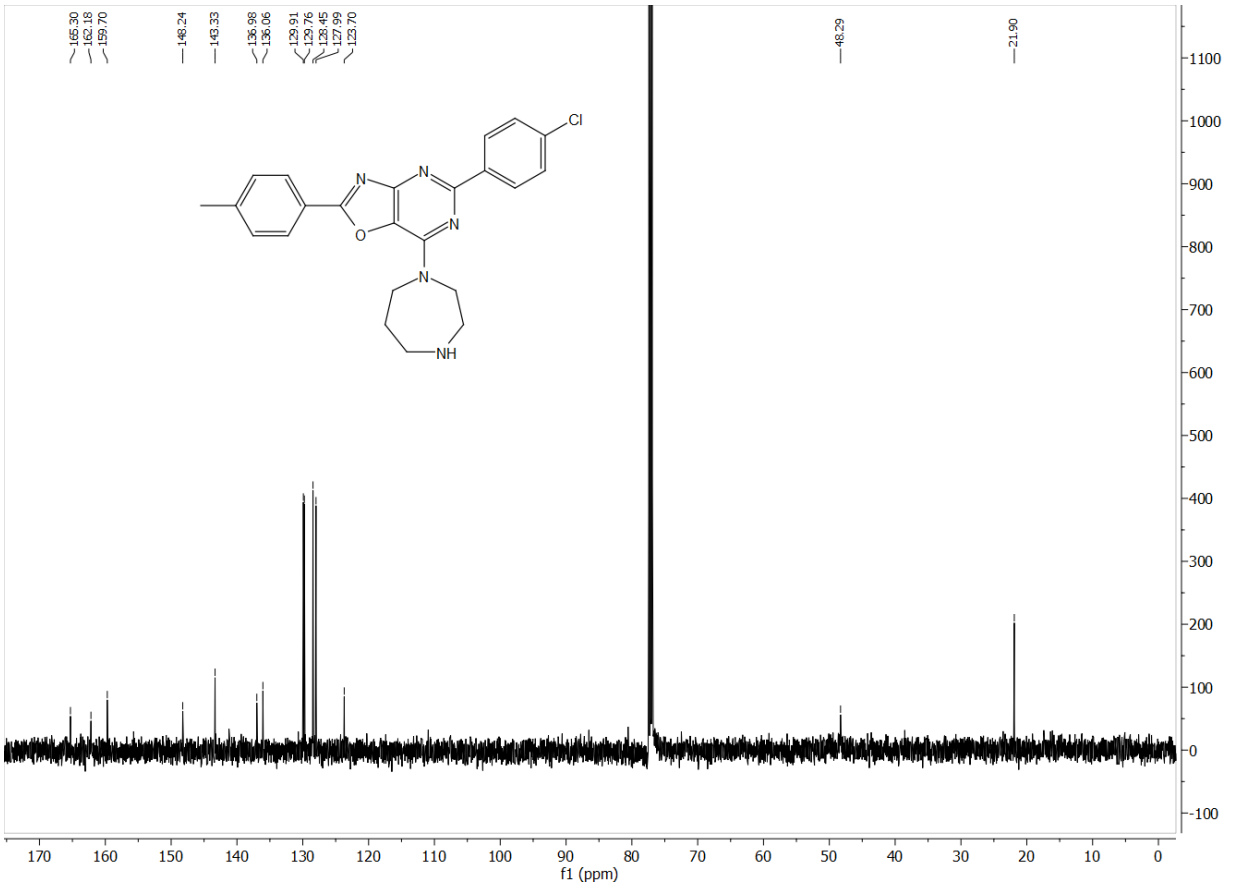
LSMS spectrum of compound (**10**):



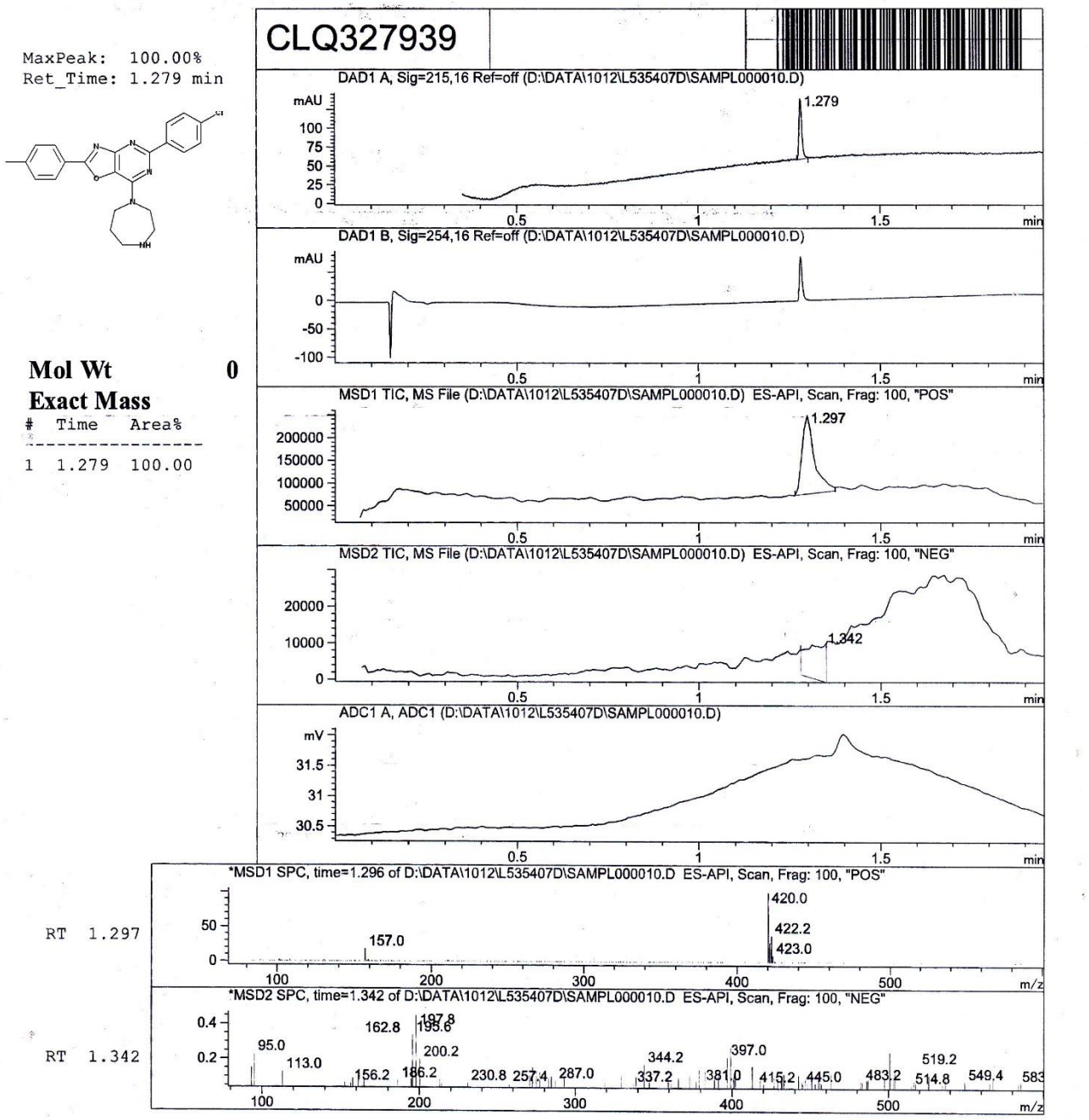
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound (**11**):



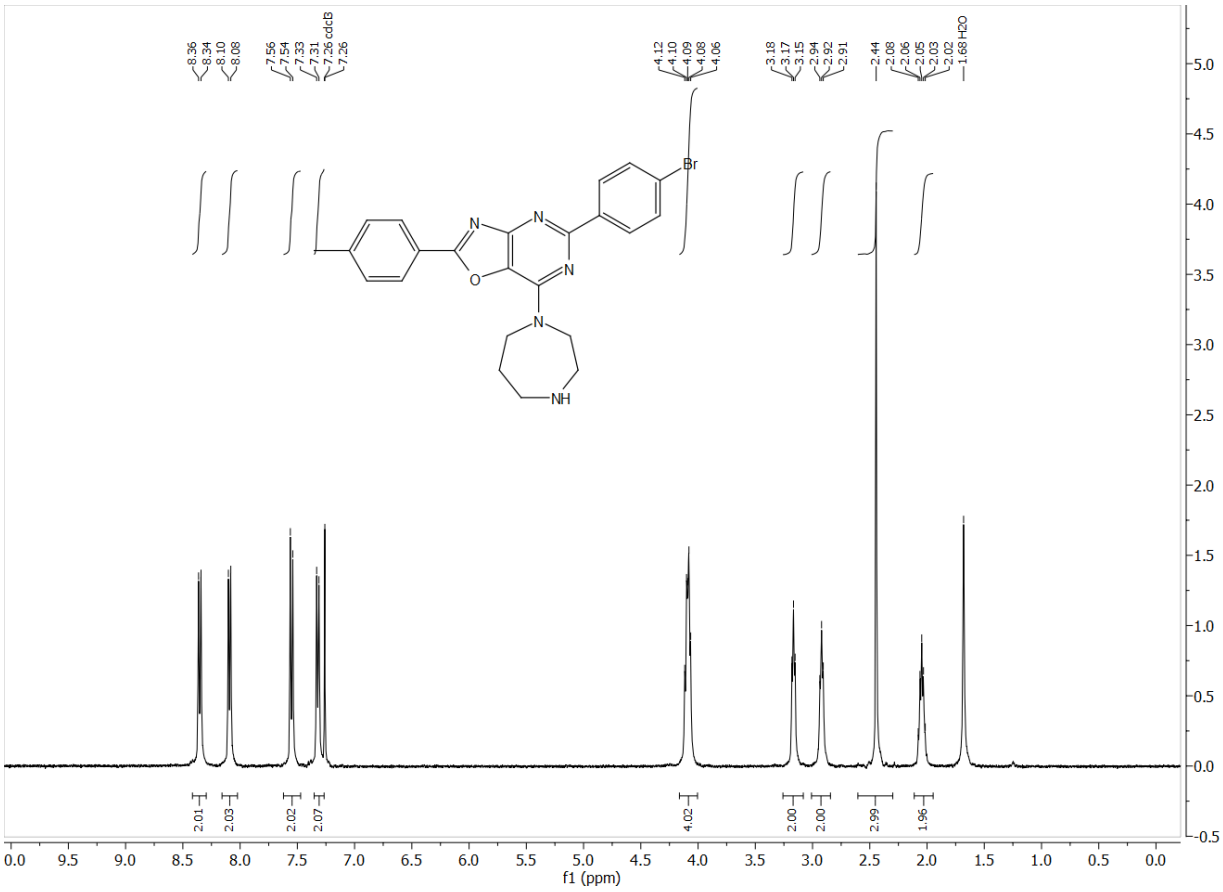
^{13}C NMR (126 MHz, $CDCl_3$) spectrum of compound (**11**):



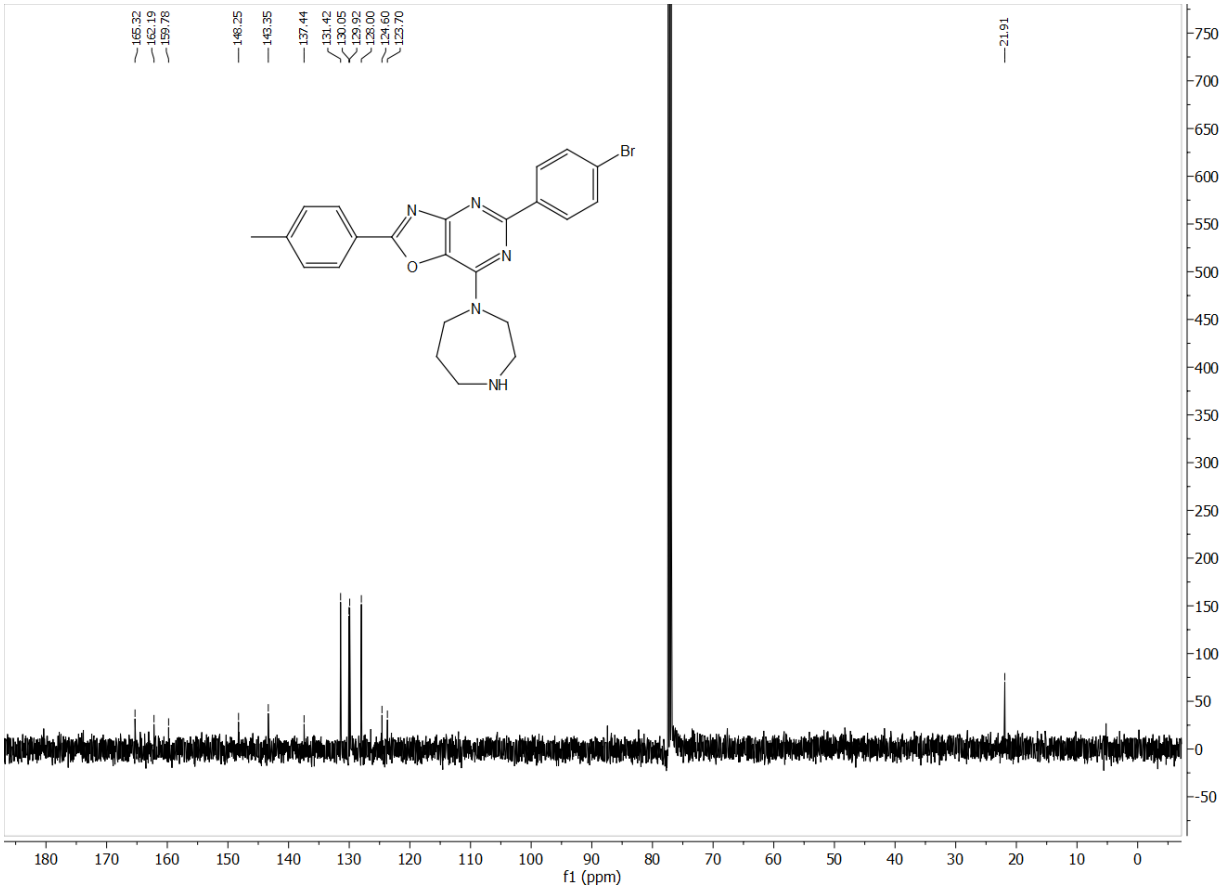
LSMS spectrum of compound (**11**):



^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound (**12**):



^{13}C NMR (126 MHz, $CDCl_3$) spectrum of compound (**12**):



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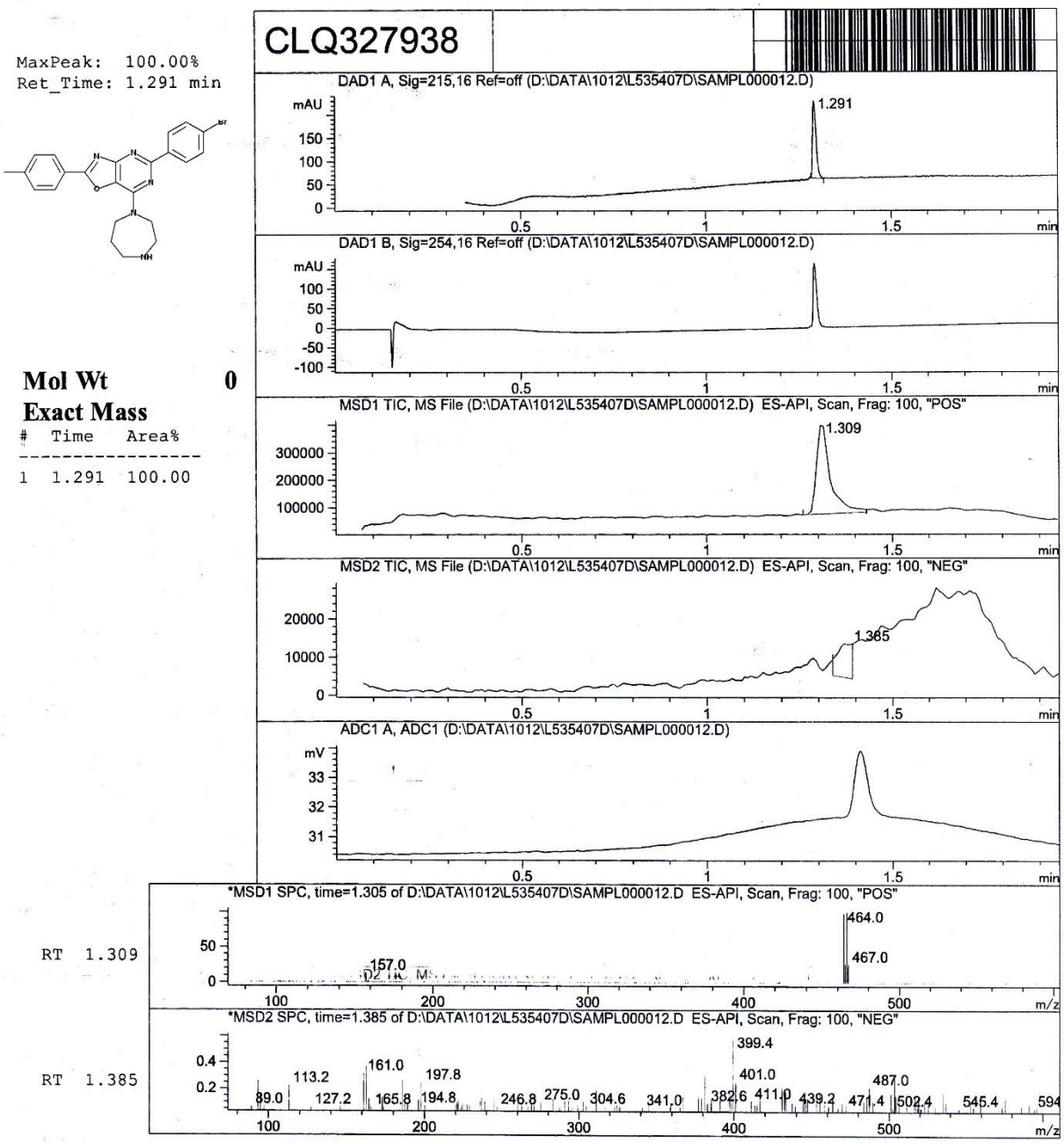
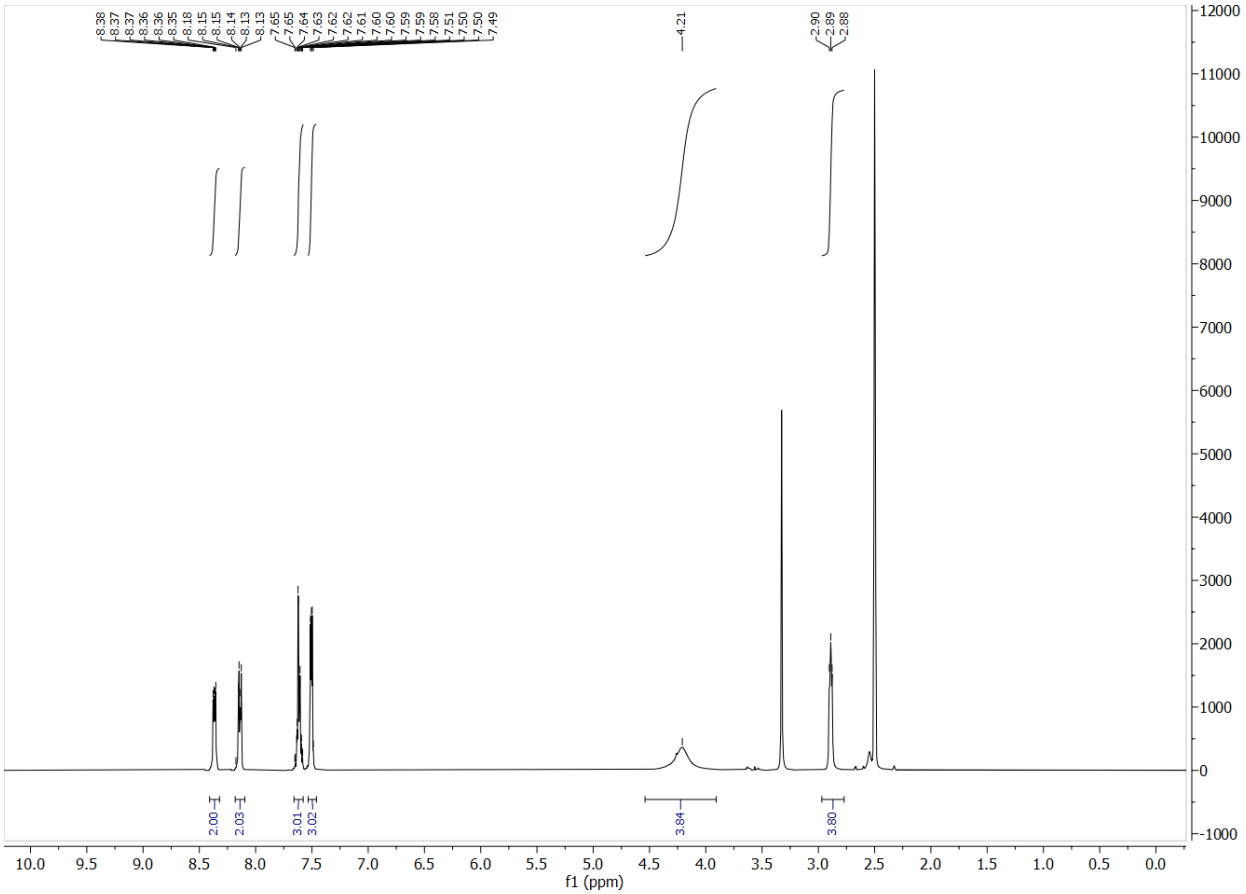
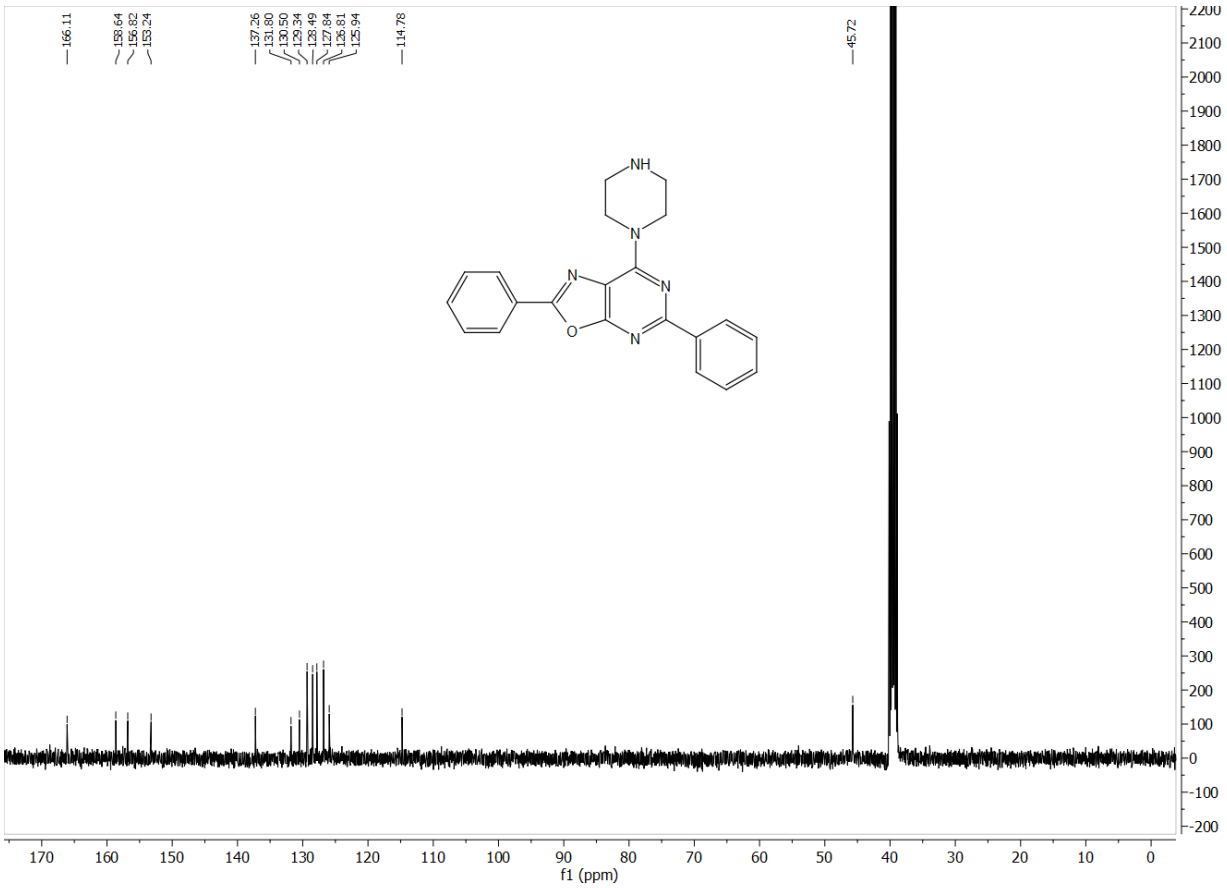


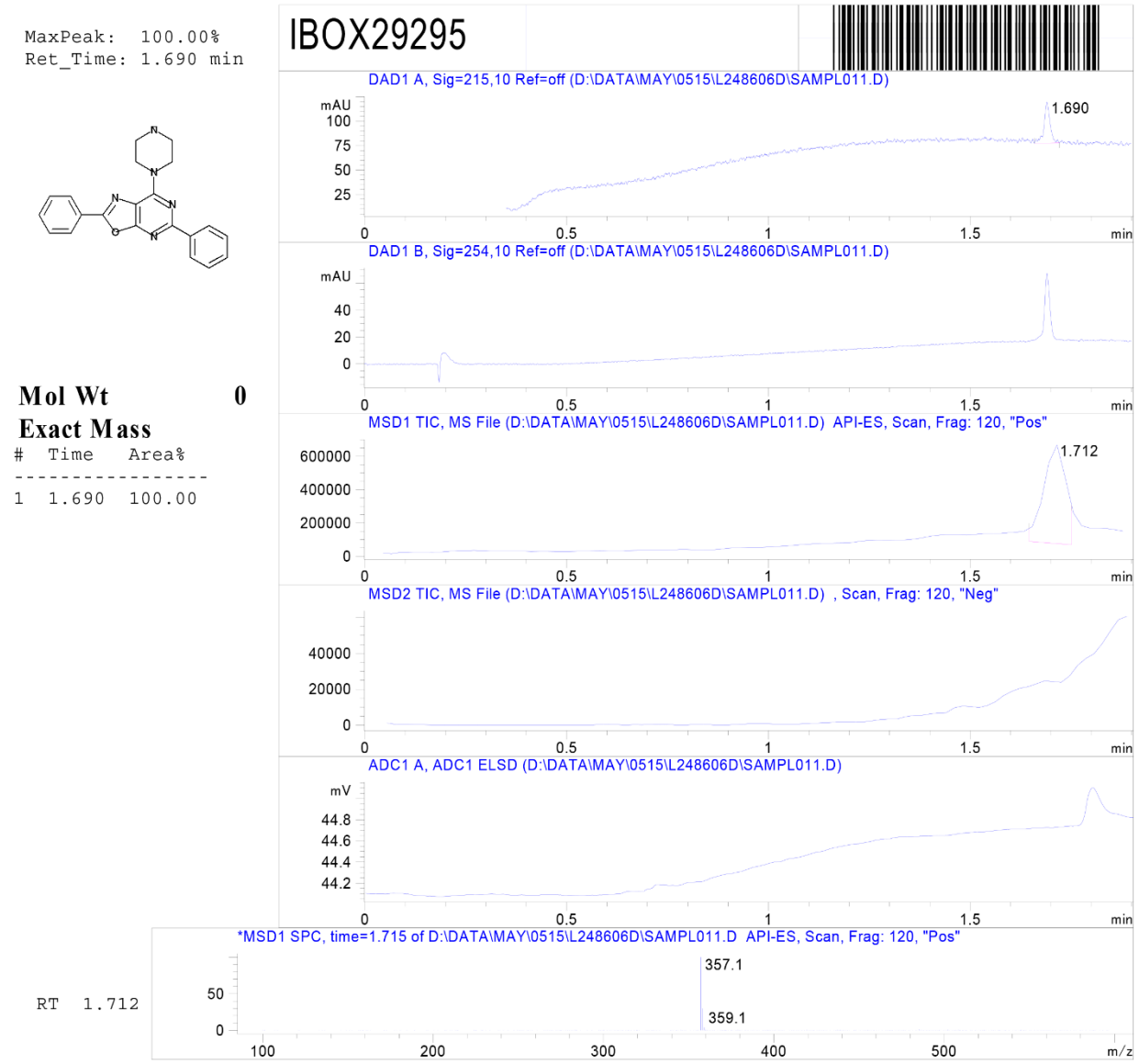
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound (**13**):



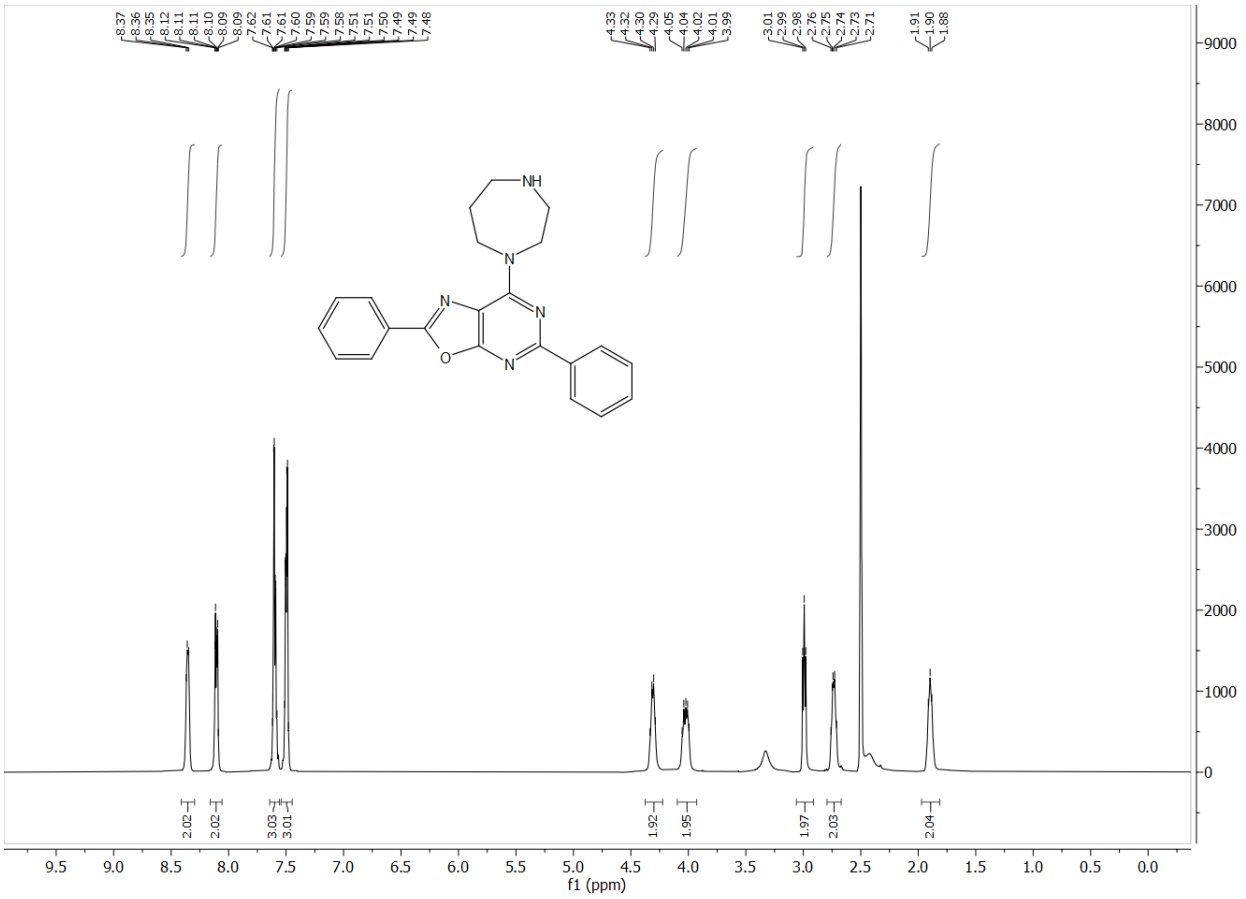
^{13}C NMR (126 MHz, DMSO-d_6) spectrum of compound (**13**):



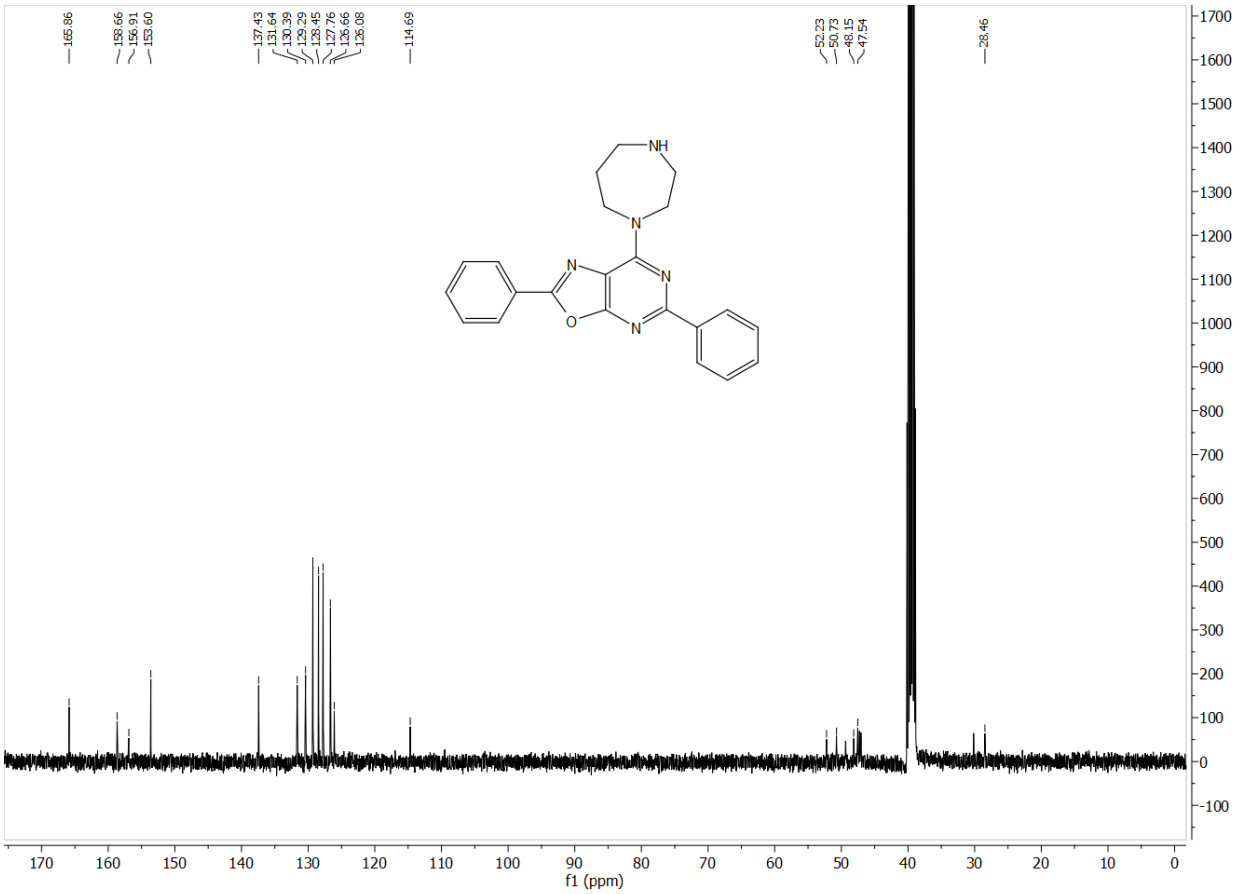
LSMS spectrum of compound (**13**):



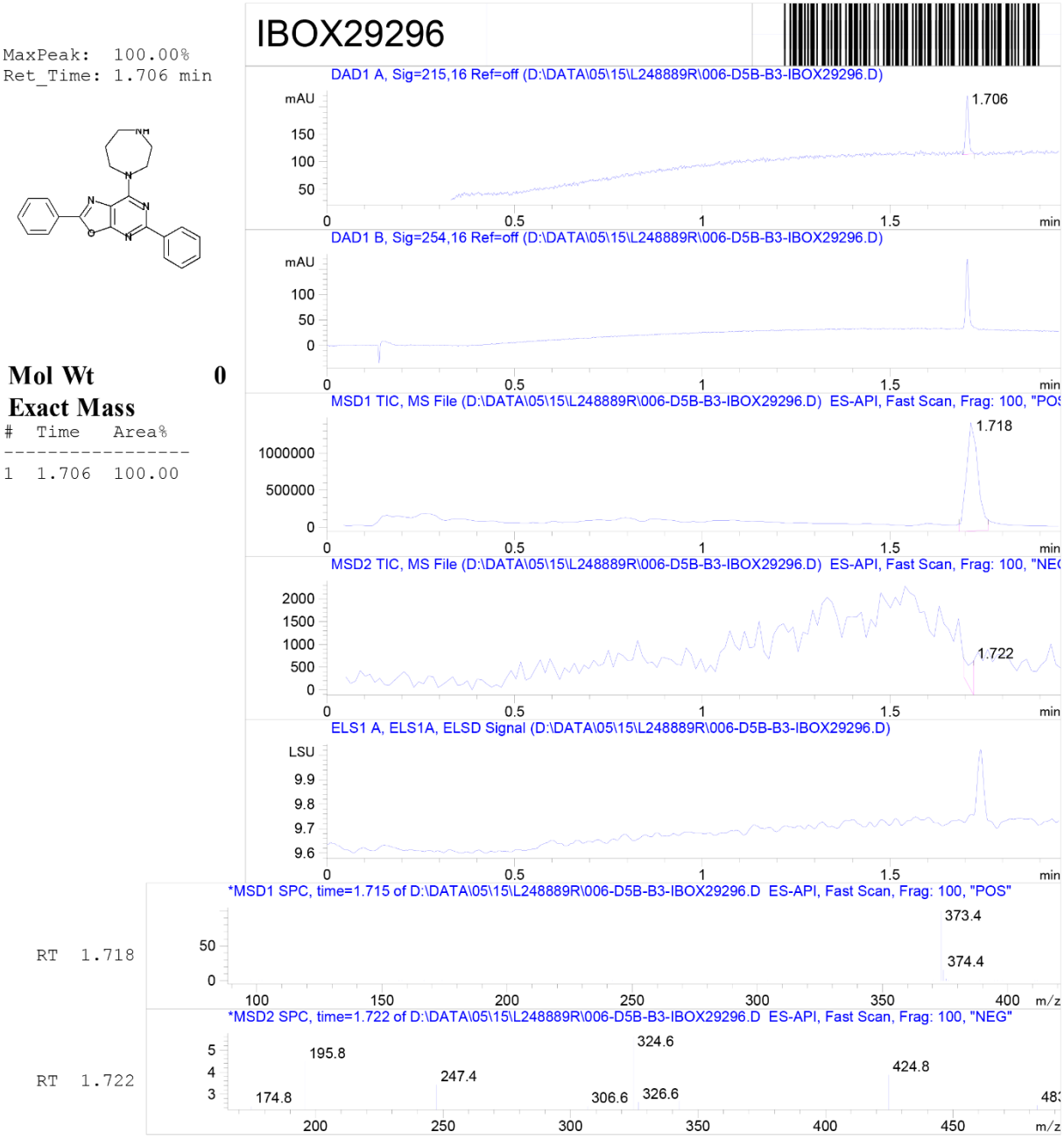
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound (**14**):



^{13}C NMR (126 MHz, DMSO-d_6) spectrum of compound (**14**):



LSMS spectrum of compound (**14**):



Experimental part 2. Biological assay

One-dose assay

Synthesized compounds (**1-14**) were submitted to the National Cancer Institute (NCI), Bethesda, Maryland, U.S.A. under the Developmental Therapeutic Program DTP. Breast cancer cell lines of the NCI subpanel engaged a total of 6 different human tumor cell lines.

Primary *in vitro* one-dose anticancer screening was initiated by cell inoculating into a series of standard 96-well microtiter plates at 5000-40000 cells/well in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine (day 0), and then preincubated in absence of drug at 37 °C and 5% CO_2 for 24 h. Test compounds were then added to the plates at one concentration of 10^{-5} M (day 1) followed by incubation for a further 48 h under the same conditions. Then the media were removed, and the cells were fixed *in situ*, washed, and dried (day 3). The sulforhodamine B assay was used for cell density determination, based on the measurement of cellular protein content. After an incubation period, cell monolayers were fixed with 10% (wt/vol) trichloroacetic acid and stained for 30 min, after which the excess dye was removed by washing repeatedly with 1% (vol/vol) acetic acid. The bound stain was resolubilized in 10 mM Tris base solution and measured spectrophotometrically on automated microplate readers for OD determination at 510 nm.

Five-dose assay

The screening compounds were tested against Breast cancer cell lines of the NCI subpanel. Cells of all Breast cancer lines were incubated at five different concentrations (0.01, 0.1, 1, 10, and 100 μM) of the tested compounds. The outcomes were used to create three response parameters (GI_{50}, TGI, and LC_{50}) that were calculated for each cell line. The GI_{50} value (drug potency) is the measure of the sensitivity of a cell to the effect of the drug and corresponds to the concentration of the compound causing a 50% decrease in net cell growth. TGI (drug efficacy) refers to the maximum effect of a drug and is the concentration of the study drug that causes total inhibition of cell growth. The LC_{50} value (cytotoxic activity) is the concentration of the compound causing a net 50% loss of initial cells at the end of the incubation period of 48 h.

The three dose-response parameters GI_{50}, TGI and LC_{50} were calculated for each experimental compound. Data calculations were made according to the method described by the NCI/NIH Development Therapeutics Program.[6] ***(i)*** The GI_{50} is determined as the drug concentrations result in a 50 % and 0% growth at 48 h drug exposure. Growth inhibition is calculated from: $[(T-T_0)/(C-T_0)] \times 100 = 50$. ***(ii)*** The TGI is calculated from: $100 \times (T - T_0)/(C - T_0) = 0$. Thus, the TGI signifies a cytostatic effect. ***(iii)*** The LC_{50}, which signifies a cytotoxic effect, is calculated as: $[(T-T_0)/T_0] \times 100 = -50$, when $T < T_0$.

COMPARE correlations

The graph of mean values for each compound was subsequently used to run the COMPARE algorithm from the Developmental Therapeutics Program, NCI, and calculate the correlation coefficient with respect to compounds from the standard agent database with a known mechanism of action.[7] Pairwise correlation coefficients of greater than 0.3 were used as the cut-off for assessing whether two agents were likely to share a similar mechanism of action. Briefly, vectors of GI_{50}, TGI, and LC_{50} concentrations for the tested compound were correlated with the set of average GI_{50}, TGI, and LC_{50} vectors for all public NCI-60 vectors for the full public standard agents database.

Cell Lines, Cell Proliferation and Viability Assay

All the cell and virus studies were conducted with protocols approved by the Institutional Biosafety Committee of the Legacy Research Institute and in accordance with the principles outlined by the NIH. The MDA-MB-231 (HTB-26) cell line was purchased from the American Type Culture Collection (AATC, Manassas, VA) and used to investigate the phenotypical changes after introducing engineered CRISPR/Cas9 modification of ADK isoforms.[8] The previously established MDA-ADK-L-KD and MDA-ADK-SD cell lines with the corresponding knockdown of ADK-L and ADK-S isoforms, together with unmodified wild-type MDA-ADK-231 (MDA-ADK-WT) (Figure 1) were used for proliferation and viability evaluation.

The above three MDA cell lines were treated with 5-Iodotubercidin (5-ITU, 1745, Tocris, at 1 μM and 3 μM dose), and our synthesized compound 1 (COMP1, at 10 μM and 30 μM), which were dissolved in 0.225% DMSO (as vehicle control). Cell proliferation was quantified by Trypan Blue cell counting[9] after seeding, cell counting was performed every 24 h for several consecutive days. MTT assay was performed to evaluate the cell viability of WT cells versus mutant cells using MTT assay Kit (Cell Proliferation Kit I, Sigma Aldrich, St. Louis, MO) according to the manufacturer's protocol.

Western blot assay

To quantify expression changes of ADK isoforms in the patient specimens and cultured cells, Western blot assays were conducted as described.[8] Briefly, patient specimens and harvested cultured cells were homogenized and digested using RIPA buffer (10 mM Tris-Cl, pH 8.0, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS, 140 mM NaCl, 1 mM PMSF) to prepare extracts. Extracts were standardized to 40 μg protein per lane and electrophoresed in a 10% Tris-glycine gel. After transfer, membranes were incubated in primary antibody anti-ADK antibody (#A304-280A, 1:5,000; Bethyl

lab, Montgomery, TX) followed by incubation with peroxidase-conjugated anti-rabbit antibody (#7074, 1:8,000, Cell Signaling, Boston, MA). The anti-ADK antibody was used to detect both isoforms of ADK distinguished by their molecular weights. To normalize ADK immunoreactivity to protein loading, a mouse monoclonal anti-α-tubulin antibody (# sc-8035, 1:5,000; Santa Cruz, Santa Cruz, CA, USA) or GAPDH antibody (1:5,000; #14C10, Cell Signaling, Boston, MA) was used to reprobe the same blot and the OD ratio of ADK to α-tubulin or GAPDH was calculated. The intensity of immunoblots was quantified using Image Lab software (BioRad, Herculer, California, USA).

Statistical data analysis

Statistical analysis was performed with ANOVA and t-test using GraphPad-Prism8 software. Statistical analysis of the curve fitting was performed using the program Statistica (v6.0 for Windows). A $p< 0.05$ was accepted as statistical significance. The data were expressed as means ± SEM.

COMPARE correlations

The graph of mean values for each of compounds was subsequently used to run the COMPARE algorithm from the Developmental Therapeutics Program, NCI, and calculate the correlation coefficient with respect to compounds from the standard agent database with a known mechanism of action.[10] Pairwise correlation coefficients of greater than 0.3 were used as the cut-off for assessing whether two agents were likely to share a similar mechanism of action. Briefly, vectors of GI_{50}, TGI, and LC_{50} concentrations for tested compound were correlated with the set of average GI_{50}, TGI, and LC_{50} vectors for all public NCI-60 vectors for the full public standard agents database.

Molecular docking

The oxazolopyrimidine derivatives were evaluated *in silico* as possible inhibitors of adenosine kinase (ADK). For this purpose, the compounds were docked by AutoDock Vina software[11] to adenosine binding site of A subunit of ADK. The file of the crystal structure of the enzyme was downloaded from RCSB Protein Data Bank (RCSB PDB, rcsb.org).[15] Ligand and water molecules were removed from this file. The adding of hydrogen atoms and Gasteiger partial charges to the amino acid residues of the enzyme and preparing of pdbqt file were performed by AutoDockTools 1.5.6 software.[16] The flexible ligands were docked to the rigid enzyme. The grid box dimensions were 25 × 25 × 25, and grid center was centered on ligand in the PDB crystal (6.943, 2.078 and 33.383). Three-dimensional structures of 1,3-oxazolo[4,5-*d*]pyrimidine and 1,3-oxazolo[5,4-*d*]pyrimidine derivatives with protonated piperazine or diazepane substituents (pH 7.4) were optimized by Avogadro software using MMFF94s force field[17] and converted to pdbqt format by AutoDockTools 1.5.6 software. Discovery Studio 3.5 visualizer (Accelrys Software Inc., San Diego, CA, USA) was used for analysis of the compounds binding modes.

References

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