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Antibacterial activity of 2-amino-3-cyanopyridine derivatives

Ibrahim Mamedov, Farid Naghiyev, Abel Maharramov, Owens Uwangue, Anne Farewell, Per Sunnerhagen and Mate Erdelyi

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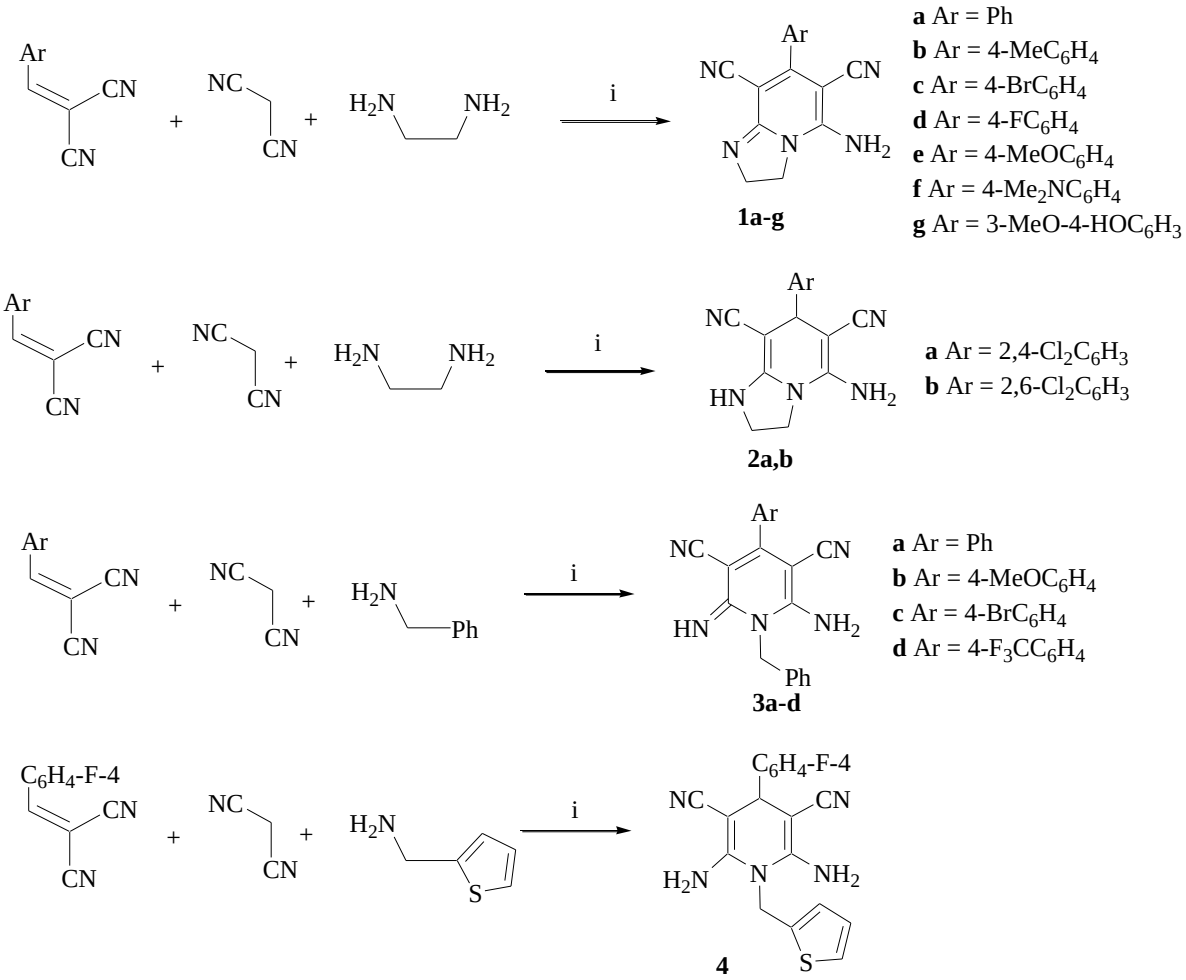
1. Synthesis

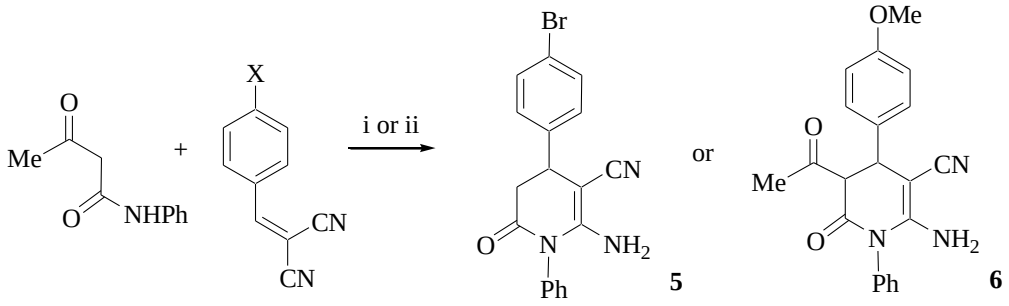
Synthesis of compounds 1-3 (Scheme S1). Arylidenemalononitrile (5.1 mmol) and malononitrile (5.2 mmol) were dissolved in MeOH (25 ml) and were stirred for 5-7 minutes, followed by addition of ethylenediamine (in case of **1a-g, 2a,b**) or benzylamine (in case of **3a-d**) (5.2 mmol) under vigorous stirring. The reaction was allowed to proceed for 48-72 h (TLC control, EtOAc/hexane, 2:1). In the course of the solvent evaporation, the product crystalized and precipitated. The crystals were filtered off and recrystallized from a mixture of EtOH/H_2O (3:2) to afford the product in high purity of 50-72% yield.

Synthesis of compound 4 (Scheme S1). A mixture of 2-(4-fluorobenzylidene)malononitrile (5.1 mmol) and malononitrile (5.2 mmol) were dissolved in MeOH (35 ml), and this was stirred for 5-7 min. (2-Thienyl)methylamine (5.2 mmol) was added under vigorous stirring (TLC control, EtOAc/hexane, 2:1). The reaction was allowed to proceed for 48-72 hours. Upon evaporation of the solvent, the product crystallized and the crystals precipitated. The crystals were filtered off using filter paper and recrystallized from a 3:2 EtOH/H_2O mixture to afford product **4** in 84% yield.



Scheme 1 *Reagents and conditions:* i, MeOH, room temperature, 48-72 h.

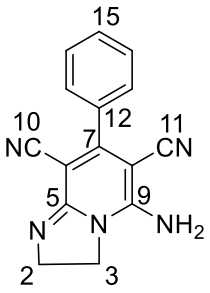
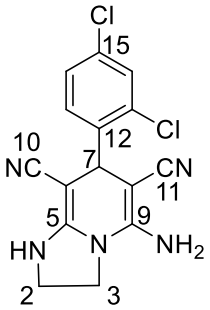
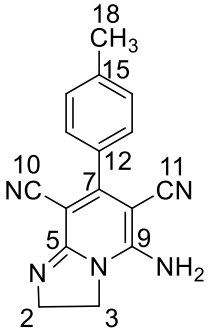
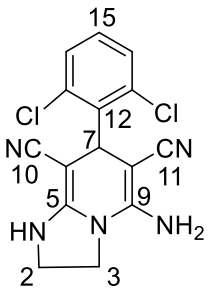
Synthesis of compounds 5 and 6 (Scheme S2)**.** 2-(4-Bromobenzylidene)malononitrile (3 mmol) or 2-(4-methoxybenzylidene)malononitrile) (3 mmol) and acetoacetanilide (3.1 mmol) were dissolved in MeOH (20 ml), and 7 mol% piperazine hydrate (or 3-4 drops piperidine) was added. The mixture was stirred for 5 min at room temperature and was heated for 5-7 min (TLC control, EtOAc/hexane, 2:1). In the course of evaporation of the solvent, the product crystalized and the crystals precipitated. The crystals were filtered and recrystallized from EtOH:H_2O (3:2) to provide **5** or **6** in 77 and 71% yields, respectively.

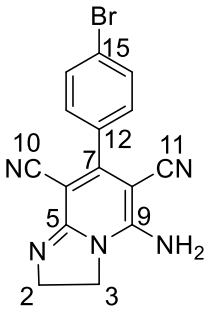
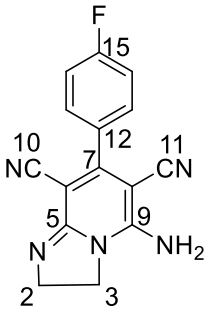
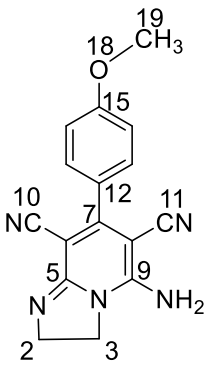
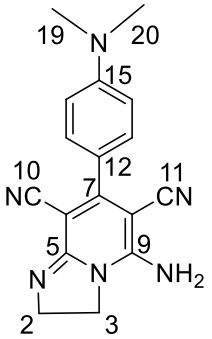


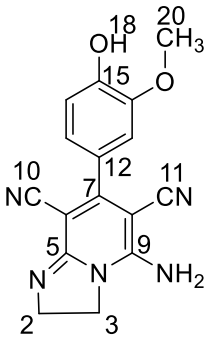
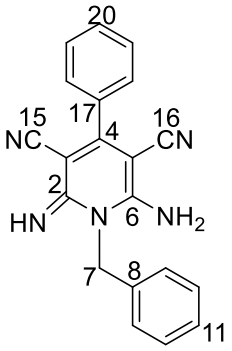
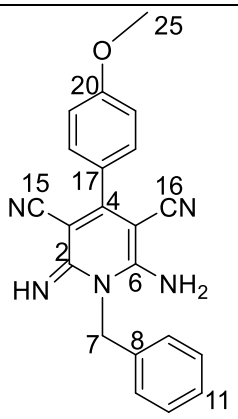
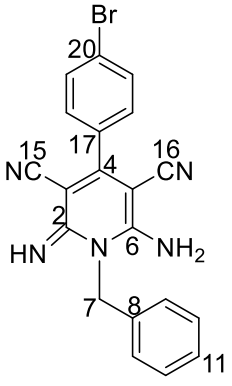
Scheme 2 *Reagents and conditions:* i, piperazine (7 mol.%), MeOH, room temperature, 5 min, then Δ, 5-7 min; ii, the same, with piperidine in place of piperazine.

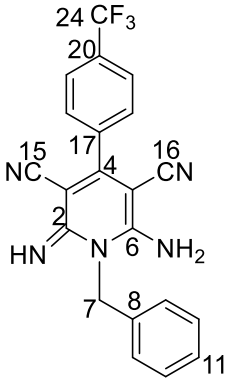
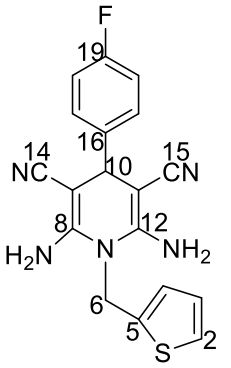
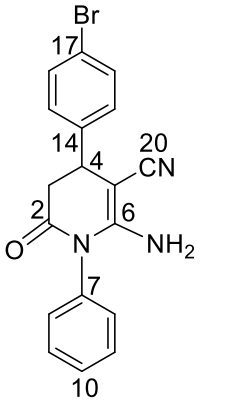
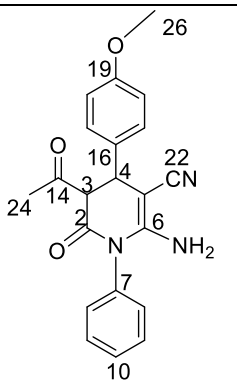
2. Materials and methods

Characterization data for compounds 1-6

	**5-Amino-7-phenyl-2,3-dihydroimidazo[1,2-*a*]pyridine-6,8-dicarbonitrile (1a).** ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.97 (s, 4H, CH_2-2/3), 7.62-7.39 (m, 5H, H13-17), 8.03 (br s, 2H, NH_2-18); ^{13}C NMR (75 MHz, DMSO-d_6) δ 46.8 (C-2), 52.8 (C-3), 72.0 (C-6), 80.1 (C-8), 117.0 (CN-10), 117.3 (CN-11), 128.3 (CH-14 and C16), 129.0 (CH-13 and C17), 130.4 (CH-15), 135.6 (C-12), 152.7 (C-9), 154.4 (C-7), 160.1 (C-5); m.p. = 325°C.
	**5-Amino-7-(2,4-dichlorophenyl)-1,2,3,7-tetrahydroimidazo[1,2-*a*]pyridine-6,8-dicarbonitrile (2a).** ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.51 (*t*, 2H, CH_2-2, $^{3}J_{H,H}$ = 7.2 Hz); 3.84 (*t*, 2H, CH_2-3, $^{3}J_{H,H}$ = 7.2 Hz), 4.74 (s, 1H, CH-7), 6.41 (s, 2H, NH_2-20), 7.35-7.50 (m, 3H, H13-14, H-16), 7.53 (s, 1H, NH-1); ^{13}C NMR (75 MHz, DMSO-d_6) δ 37.6 (CH-7), 42.4 (CH_2-2), 45.1 (CH_2-3), 52.1 (C-8), 55.1 (C-6), 121.1 (CN-10), 121.7 (CN-11), 128.4 (C-16), 129.1 (C-14), 132.4 (C-17), 132.9 (C-15), 133.0 (C-13), 142.8 (C-12), 151.6 (C-9), 154.3 (C-5); m.p. = 254°C. X-ray crystallographic data: CCDC 1585393.
	**5-Amino-7-(*p*-tolyl)-2,3-dihydroimidazo[1,2-*a*]pyridine-6,8-dicarbonitrile (1b).** ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.38 (s, 3H, CH_3-18), 3.94 (s, 4H, CH_2-2/3), 7.31-7.37 (s, 5H, H14-16 and H13-17), 8.09 (s, 2H, NH_2-19); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.8 (CH_3-18), 47.0 (CH_2-2), 53.9 (CH_2-3), 72.2 (C-6), 81.0 (C-8), 117.1 (CN-10), 117.3 (CN-11), 128.5 (C-14 and C-16), 129.9 (C-13 and C-17), 133.2 (C-12), 140.1 (C-15), 152.2 (C-9), 155.0 (C-7), 160.0 (C-5); m.p. = 347°C. X-ray crystallographic data: CCDC 1821874.
	**5-Amino-7-(2,6-dichlorophenyl)-1,2,3,7-tetrahydroimidazo[1,2-*a*]pyridine-6,8-dicarbonitrile (2b):** ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.51 (t, 2H, CH_2-2, $^{3}J_{H,H}$ = 7.2 Hz), 3.87 (t, 2H, CH_2-3, $^{3}J_{H,H}$ = 7.2 Hz), 5.36 (s, 1H, CH-7), 6.37 (s, 2H, NH_2-20), 7.25-7.45 (m, 3H, H14-16), 7.50 (s, 1H, NH-1); ^{13}C NMR (75 MHz, DMSO-d_6) δ 37.3 (CH-7), 42.3 (CH_2-2), 44.9 (CH_2-3), 49.5 (C-8), 52.6 (C-6), 121.0 (CN-10), 121.6 (CN-11), 128.8 (C-16), 129.6 (C-14), 131.3 (C-15), 134.8 (C-17), 136.3 (C-13), 138.1 (C-12), 152.3 (C-9), 155.0 (C-5); m.p. = 265°C. X-ray crystallographic data: CCDC 1820010.

	**5-Amino-7-(4-bromophenyl)-2,3-dihydroimidazo[1,2-*a*]pyridine-6,8-dicarbonitrile (1c):** ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.59 (s, 2H, NH_2-19), 3.92 (m, 4H, CH_2-2/3), 7.38 (2H, H13-17, AA' part fo AA'XX'), 7.71 (2H, H14-16, XX' part of AA'XX'); ^{13}C NMR (75 MHz, DMSO-d_6) δ 46.7 (CH_2-2), 52.8 (CH_2-3), 71.7 (C-6), 80.6 (C-8), 116.9 (CN-10), 117.1 (CN-11), 123.9 (C-15), 130.6 (C-13 and C-17), 132.1 (C-14 and C-16), 134.7 (C-12), 152.6 (C-9), 154.3 (C-5), 158.9 (C-7); m.p. = 278°C.
	**5-Amino-7-(4-fluorophenyl)-2,3-dihydroimidazo[1,2-*a*]pyridine-6,8-dicarbonitrile (1d):** ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.53 (s, 1H, NH_2-19), 3.92 (s, 4H, CH_2-2/3), 7.35 (2H, H14-16, AA' part of AA'XX'), 7.50 (2H, H13-17, XX' part of AA'XX'); ^{13}C NMR (75 MHz, DMSO-d_6) δ 46.7 (CH_2-2), 52.7 (CH_2-3), 72.0 (C-6), 81.0 (C-8), 116.0 (d, $^{2}J_{CF}$ = 12.9 Hz, C-14 and C16), 117.0 (CN-10), 117.2 (CN-11), 130.9 (d, $^{3}J_{CF}$ = 8.6 Hz, C-13 and C17), 131.9 (C-12), 152.6 (C-9), 154.3 (C-7), 159.1 (C-5), 161.7 (d, $^{1}J_{CF}$ = 340 Hz, C-15); m.p. = 310°C.
	**5-Amino-7-(4-methoxyphenyl)-2,3-dihydroimidazo[1,2-*a*]pyridine-6,8-dicarbonitrile (1e):** ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.81 (s, 3H, CH_3O-19), 3.91 (s, 4H, CH_2-2/3), 7.06 (d, 2H, H14-16 $^{3}J_{H,H}$ = 8.7 Hz), 7.40 (d, 2H, H13-17, $^{3}J_{H,H}$ = 8.4 Hz), 8.00 (s, 2H, NH_2-20); ^{13}C NMR (75 MHz, DMSO-d_6) δ 46.7 (CH_2-2), 52.7 (CH_2-3), 55.7 (OCH_3-19), 71.9 (C-6), 80.5 (C-8), 114.3 (C-14 and C-16), 117.3 (CN-10), 117.5 (CN-11), 127.5 (C-12), 130.1 (C-13 and C-17), 152.9 (C-9), 154.4 (C-5), 159.7 (C-7), 160.9 (C-15); m.p.=286°C. X-ray crystallographic data: CCDC 1819988.
	**5-Amino-7-[4-(dimethylamino)phenyl]-2,3-dihydroimidazo[1,2-*a*]pyridine-6,8-dicarbonitrile (1f):** ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.98 (s, 6H, CH_3-19/20), 3.89 (m, 4H, CH_2-2/3), 6.77 (2H, H14-16, AA' part of AA'XX'), 7.29 (2H, H17-13, XX' part of AA'XX'), 7.95 (s, 1H, NH_2-21); ^{13}C NMR (75 MHz, DMSO-d_6) δ 40.3 (C-19 and C-20), 46.2 (CH_2-2), 46.2 (CH_2-3), 71.6 (C-6), 80.2 (C-8), 111.1 (C-14 and C-16), 117.3 (CN-10), 117.5 (CN-11), 121.4 (C-12), 129.3 (C-13 and C-17), 151.3 (C-9), 152.7 (C-15), 154.1 (C-7), 159.6 (C-5); m.p. = 367°C. HR-MS: calcd. for $C_{17}H_{17}N_6$, m/z = 305.1515, found: 305.1514 ($[M+H]^+$).

	**5-Amino-7-(4-hydroxy-3-methoxyphenyl)-2,3-dihydroimidazo[1,2-*a*]pyridine-6,8-dicarbonitrile (1g):** ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.74 (s, 1H, OH-18), 3.79 (s, 3H, CH_3O-20), 3.91 (m, 4H, $2CH_2$-2/3), 6.89-7.02 (m, 3H, H-13, H-14 and H-17), 8.17 (s, 2H, NH_2-21); ^{13}C NMR (75 MHz, DMSO-d_6) δ 46.7 (CH_2-2), 52.5 (CH_2-3), 56.2 (OCH_3-20), 72.0 (C-8), 80.2 (C-6), 112.9 (C-17), 115.7 (C-14), 117.4 (CN-10), 117.6 (CN-11), 121.7 (C-13), 126.0 (C-12), 147.5 (C-15), 148.7 (C-16), 153.0 (C-7), 154.4 (C-5), 160.0 (C-9); m.p. = 279°C.
	**6-Amino-1-benzyl-2-imino-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (3a):** ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.62 (d, 2H, CH_2-7, $^{3}J_{H,H}$ = 5.7 Hz), 7.24-7.53 (m, 12H, H9-H13, H18-22, H-20, and NH_2-23), 8.10 (t, 1H, H-N-1, $^{3}J_{H,H}$ = 5.7 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 44.3 (CH_2-7), 79.7 (C-3), 80.8 (C-5), 116.9 (CN-15), 117.0 (CN-16), 127.2 (C18 and C-22), 128.3 (C-11), 128.6 (C-9 and C-13), 128.7 (C-19 and C-21), 129.0 (C-10 and C-12), 130.3 (C-20), 135.5 (C-17), 139.9 (C-8), 159.4 (C-2), 160.1 (C-6), 161.4 (C-4); m.p. = 177°C. HR-MS: calcd. for $C_{20}H_{16}N_5$, m/z = 326.1406, found: 326.1405 ($[M+H]^+$).
	**6-Amino-1-benzyl-2-imino-4-(4-methoxyphenyl)-1,2-dihydropyridine-3,5-dicarbonitrile (3b):** ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.83 (s, 3H, CH_3O-25), 4.60 (d, 2H, CH_2-7, $^{3}J_{H,H}$ = 6 Hz), 7.08 (AA′ part of AA′XX′, 2H, H18-22), 7.21-7.46 (m, 9H, H9-13, H19-21, NH_2-23), 8.03 (t, 1H, H-N-1, $^{3}J_{H,H}$ = 6 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 44.2 (CH_2-7), 55.7 (OCH_3-25), 79.6 (C-5), 80.7 (C-3), 114.4 (C-18 and C-22), 117.1 (CN-15), 117.2 (CN-16), 127.2 (C-11), 127.5 (C-17), 128.3 (C-9 and C-13), 128.6 (C-10 and C-12), 130.5 (C-19 and C-21), 140.0 (C-8), 159.5 (C-2), 159.7 (C-6), 160.9 (C-4), 161.5 (C-20); m.p. = 186°C. HR-MS: calcd. for $C_{21}H_{18}N_5O$, m/z = 356.1511, found: 356.1511 ($[M+H]^+$).
	**6-Amino-1-benzyl-4-(4-bromophenyl)-2-imino-1,2-dihydropyridine-3,5-dicarbonitrile (3c):** ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.59 (d, 2H, CH_2-7, $^{3}J_{H,H}$ = 5.7 Hz), 7.21-7.47 (m, 7H, H9-13, H18-22), 7.75 (AA′ part of AA′XX′, 2H, H19-21, NH_2-23), 8.14 (t, 1H, HN-14, $^{3}J_{H,H}$ = 5.7 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 44.2 (CH_2-7), 79.5 (C-5), 80.7 (C-3), 116.7 (CN-15), 116.8 (CN-16), 124.0 (C-20), 127.2 (C-11), 128.2 (C9 and C-13), 128.3 (C10 and C-12), 131.0 (C18 and C-22), 132.1 (C19 and C-21), 134.8 (C-17), 139.9 (C-8), 159.0 (C-2), 159.2 (C-6), 161.3 (C-4); m.p. = 226°C. HR-MS: calcd. for $C_{20}H_{15}BrN_5$, m/z = 404.0511, found: 404.0511($[M+H]^+$).

	**6-Amino-1-benzyl-2-imino-4-(4-trifluoromethylphenyl)-1,2-dihydropyridine-3,5-dicarbonitrile (3d):** ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.62 (d, 2H, CH_2-7, $^{3}J_{H,H}$ = 5.7 Hz), 7.24-7.80 (m, 7H, H9-13, H18-22), 7.93 (AA′ part of AA′XX′, 2H, H19-21, NH_2-23), 8.20 (t, 1H, H-N-14, $^{3}J_{H,H}$ = 5.7 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 44.3 (CH_2-7), 79.6 (C-5), 80.7 (C-3), 116.6 (CN-15), 116.7 (CN-16), 126.0 (C-11), 126.1 (q, $^{2}J_{C,F}$ = 45.8 Hz, C-20), 127.2 (C-19 and C-21), 128.3 (C-10 and C-12), 128.6 (C-9 and C-13), 128.8 (q, $^{1}J_{CF}$ = 548 Hz, C-24), 129.9 (C-18 and C-22), 139.7 (C-8), 139.8 (C-17), 158.7 (C-2), 159.2 (C-4), 161.2 (C-6); m.p. = 219°C. HR-MS: calcd. for $C_{21}H_{15}N_5F_3$, m/z = 394.1280, found: 394.1279([M+H]$^{+}$).
	**2,6-Diamino-4-(4-fluorophenyl)-1-(thiophen-2-ylmethyl)-1,4-dihydropyridine-3,5-dicarbonitrile (4):** ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.96 (s, 1H, CH-10), 5.17 (s, 2H, CH_2-6), 6.37 (s, 4H, 2NH_2-13/22), 6.65-7.52 (m, 7H, H17-21, H18-20, H-3, H-4, H-2); ^{13}C NMR (75 MHz, DMSO-d_6): δ 39.3 (CH-10), 42.9 (CH_2-6), 63.1 (C-9 and C-11), 115.1 (d, $^{2}J_{C,F}$ = 22.5 Hz, C-18 and C-20), 121.6 (CN-14 and CN-15), 126.9 (C-3), 127.4 (C-4), 128.2 (C-2), 128.85 (d, $^{3}J_{C,F}$ = 7.5 Hz, C-17 and C21), 137.6 (C-5), 141.7 (d, $^{4}J_{C,F}$= 3.0 Hz, C-16), 152.5 (C-8 and C-12), 161.3 (d, $^{1}J_{C,F}$= 240.8 Hz, C-19); m.p. = 205°C. HR-MS: calcd. for $C_{18}H_{14}FN_5S$, m/z = 352.0954, found: 352.1036 ([M+H]$^{+}$).
	**2-Amino-4-(4-bromophenyl)-6-oxo-1-phenyl-1,4,5,6-tetrahydropyridine-3-carbonitrile (5):** ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.79 (d, $^{3}J_{H,H}$=5.1 Hz, 1H, CH_2-3a), 3.17 (d, , $^{3}J_{H,H}$ = 6.6 Hz, 1H, CH_2-3b), 3.96 (dd, $^{3}J_{H,H}$ = 5.1, 6.6 Hz 1H, CH-4,), 5.86 (s, 2H, NH_2-21); 7.22-7.62 (m, 9H, H8-12, H15-19 and H16-18); ^{13}C NMR (DMSO-d_6) δ 35.9 (CH-4), 40.3 (CH_2-3), 59.1 (C-5), 120.7 (CN-20), 121.3 (C-17), 129.4 (C-10), 129.7 (C-8 and C-12), 129.8 (C-9 and C-11), 130.0 (C-16 and C-18), 132.2 (C-15 and C-19), 135.4 (C-7), 142.1 (C-14), 155.2 (C-6), 169.0 (C-2); m.p. = 209°C. HR-MS: calcd. for $C_{18}H_{14}BrN_3O$, m/z = 368.0320, found: 368.0379 ([M+H]$^{+}$).
	**5-Acetyl-2-amino-4-(4-methoxyphenyl)-6-oxo-1-phenyl-1,4,5,6-tetrahydropyridine-3-carbonitrile (6):** ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.33 (s, 3H, CH_3-24), 3.75 (s, 3H, CH_3O-26), 4.08 (d, , $^{3}J_{H,H}$ = 4.5 Hz, 1H, CH-3), 4.27-4.28 (d, $^{3}J_{H,H}$ = 4.5 Hz, 1H, CH-4), 5.92 (s, 2H, NH_2-23), 6.96-7.52 (m, 9H, H8-12, H17-H21 and H18-H20); ^{13}C NMR (DMSO-d_6) δ 29.0 (CH_3-24), 38.1 (CH-4), 55.5 (CH_3O-26), 58.31 (C-5), 63.4 (CH-3), 114.7 (C-18 and C-20), 121.1 (CN-22), 128.7 (C-8 and C-12), 129.5 (C-17 and C-21), 129.6 (C-10), 130.1 (C-9 and C-11), 132.4 (C-16), 135.2 (C-7), 154.5 (C-6), 159.0 (C-19), 167.2 (C-2), 203.0 (C-14); m.p. = 224°C. HR-MS: calcd. for $C_{21}H_{19}N_3O_3$, m/z = 362.1426, found: 362.1486 ([M+H]$^{+}$).

3. Antimicrobial activity

Purified substances were re-dissolved at 10 mg ml^{-1} in 100 % DMSO and stored at -20°C. *Escherichia coli* (ATCC 25922) and *Bacillus subtilis* (NBRC 1M470) were grown at 37ºC in Mueller-Hinton medium supplemented with Ca^{2+} and Mg^{2+}. For *in vivo* screening of antibacterial activity, a culture of bacterial cells was grown to OD 600_{nm} = 0.5. The culture was diluted 10× with pre-warmed medium, and the substances to be tested added to the culture medium for a final concentration of 200 μg ml^{-1}, each at 100 μl in a 96-well microtiter plate. Cultures with substances to be tested were then incubated at 37ºC without agitation for 18 h. To measure cell respiratory activity, 12 μl of AlamarBlue® solution (commercial name of resazurin solution, ThermoFisher) was added to each well, and incubation at 37ºC was continued for 1 h. Then, fluorescence was measured using a POLARstar Omega microplate-reader from BMG Labtech with excitation filter set to 544 nm and emission filter to 590 nm. Cells exposed to equivalent concentration of DMSO only were used as positive control. All tests of compound activity were performed in three independent replicates. All compounds where a reduction of fluorescence by at least 50 % relative to the solvent control was observed in any of the species were followed up by additional tests for more accurate determination of the degree of antibacterial activity.

Compound **3c** showed 577 μg ml^{-1} and 288 μg ml^{-1} MIC activities, against *E. coli* and *B. subtilis*, respectively. All other compounds were observed to possess no activities up to 200 μg ml^{-1} concentration.

4. Cytotoxicity assay

Human MCF-7 cells were grown in Dulbecco´s Modified Eagle Medium supplemented with 10 % fetal calf serum and kept in exponential growth. Before the assay, cells were reseeded into 96 well microtiter plates at a density allowing continued exponential growth, and allowed to settle for 24 h. The compounds to be investigated were added from a stock solution in DMSO, for a final concentration of 0.3 % v/v of the solvent in the culture medium. After 24 h of incubation in presence of the compound, cell viability was assayed using PrestoBlue™ Cell Viability Reagent (ThermoFisher) according to the manufacturer's instructions. A Polar Star Omega plate reader (BMG Lab Tech) was used to measure resorufin fluorescence at 544 nm excitation / 590 nm emission. Survival was expressed as percentage of the solvent-only control. EC_{50} values for each compound were calculated, from three independent replicate experiments, using 3-fold dilution intervals.

5. NMR spectra

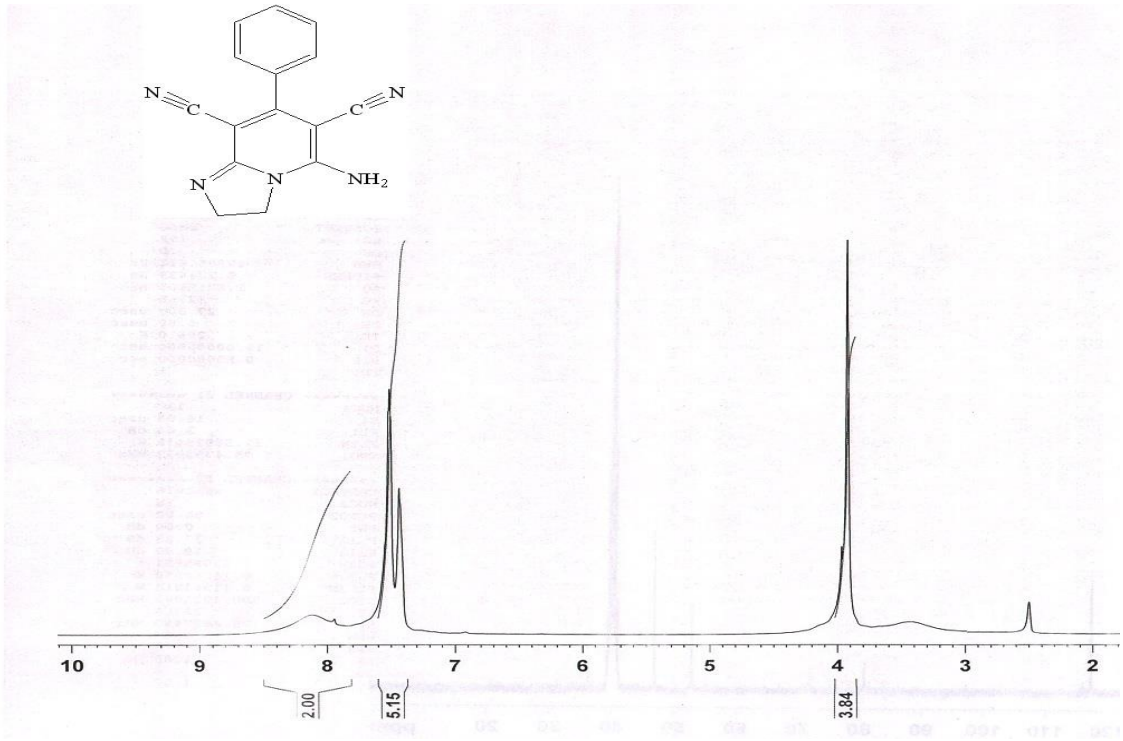


Figure S1. ^{1}H NMR spectrum of compound **1a** observed for DMSO-d_6 solution at 300 MHz and 25°C.

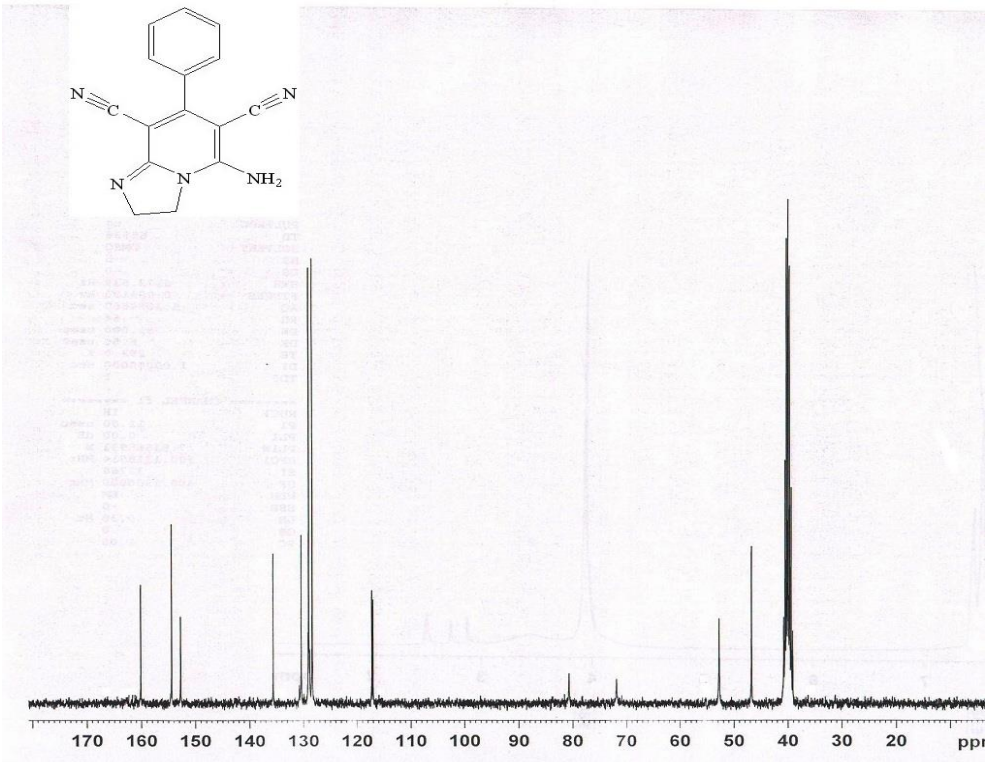


Figure S2. ^{13}C NMR spectrum of compound **1a** observed for DMSO-d_6 solution at 75 MHz and 25°C.

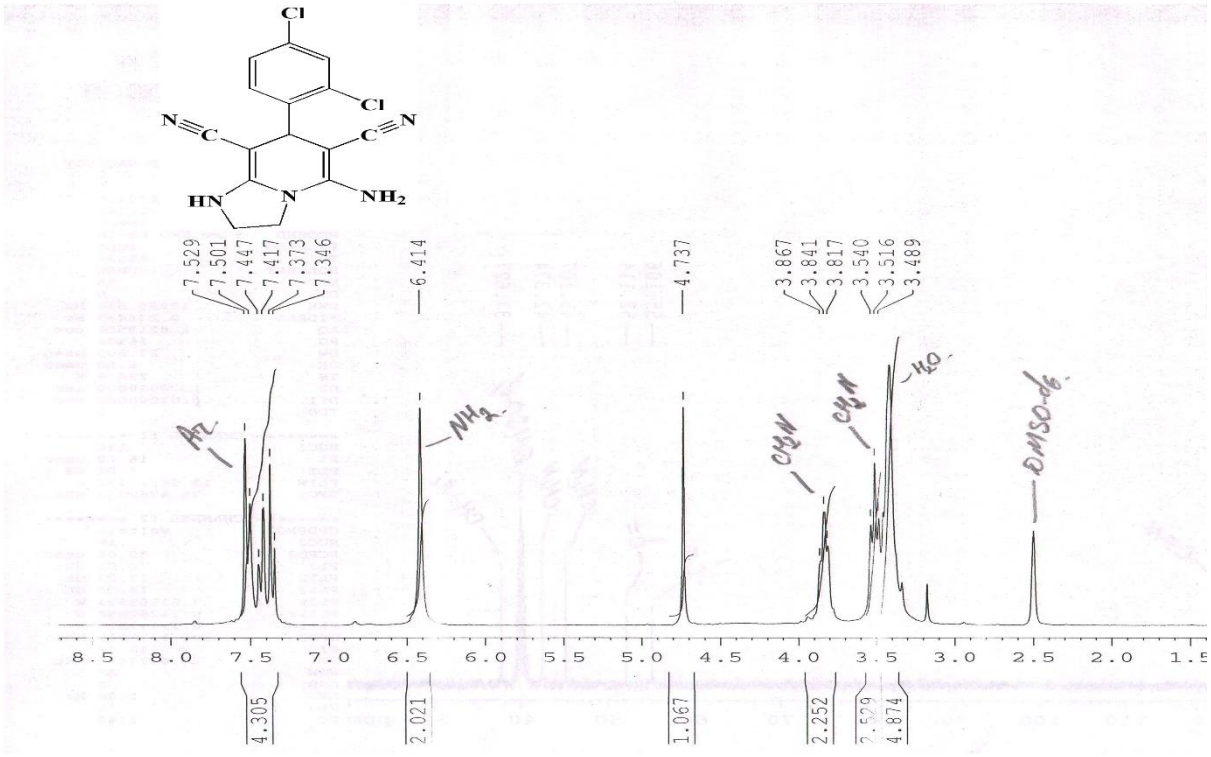


Figure S3. [1]H NMR spectrum of compound **2a** observed for DMSO-d_6 solution at 300 MHz and 25°C.

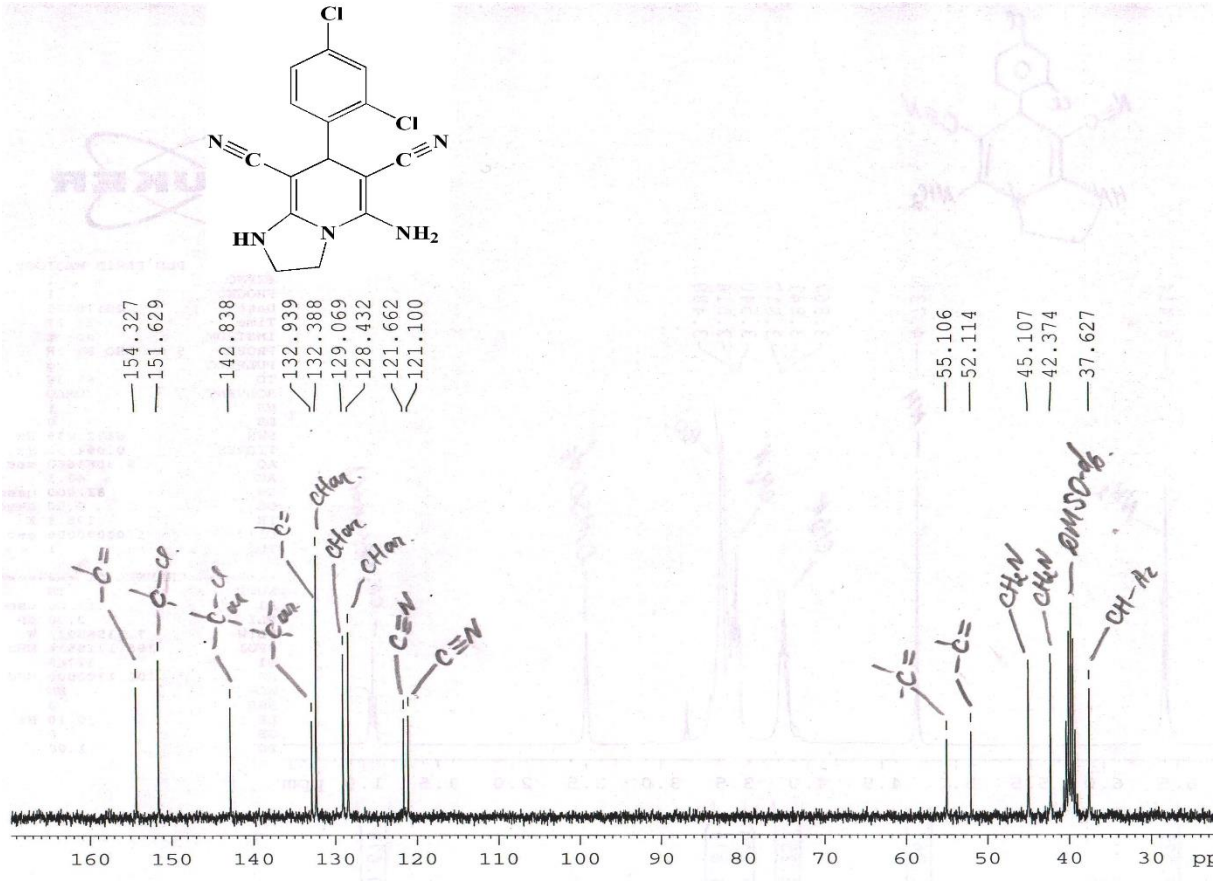


Figure S4. [13]C NMR spectrum of compound **2a** observed for DMSO-d_6 solution at 75 MHz and 25°C.

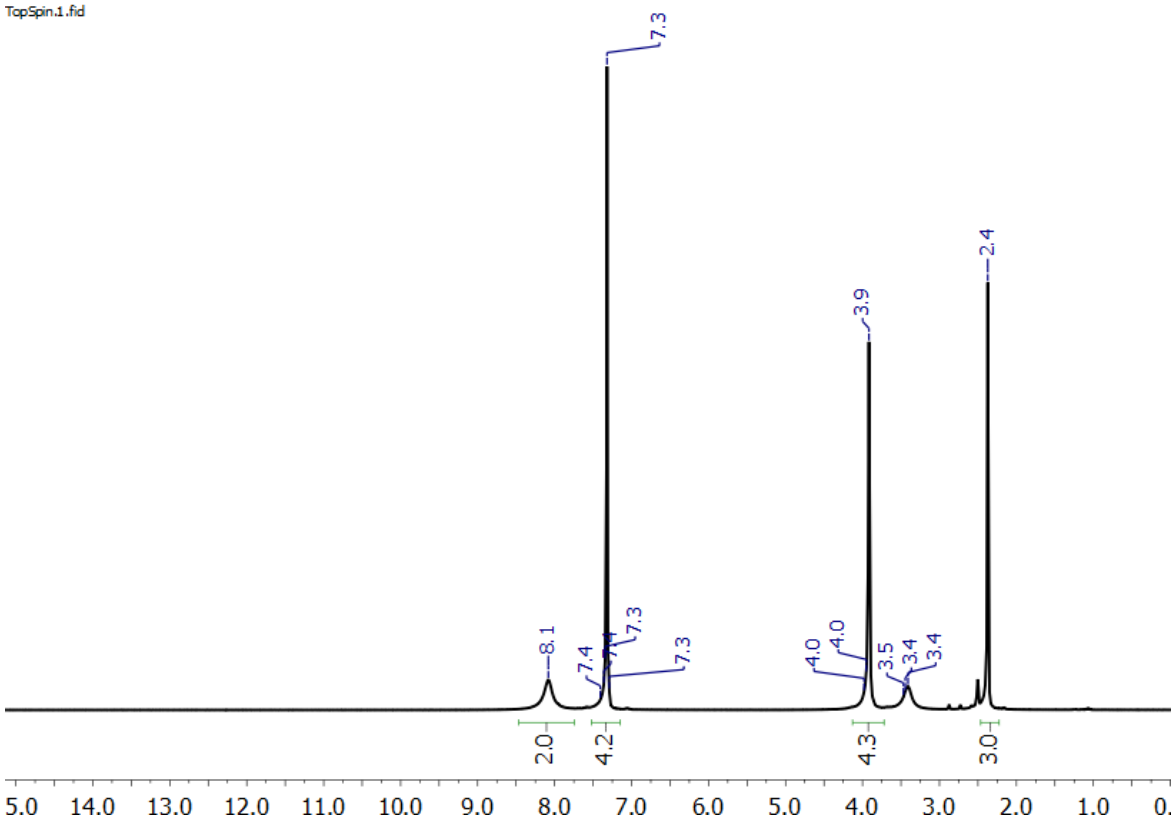


Figure S5. [1]H NMR spectrum of compound **1b** observed for DMSO-d_6 solution at 300 MHz and 25°C.

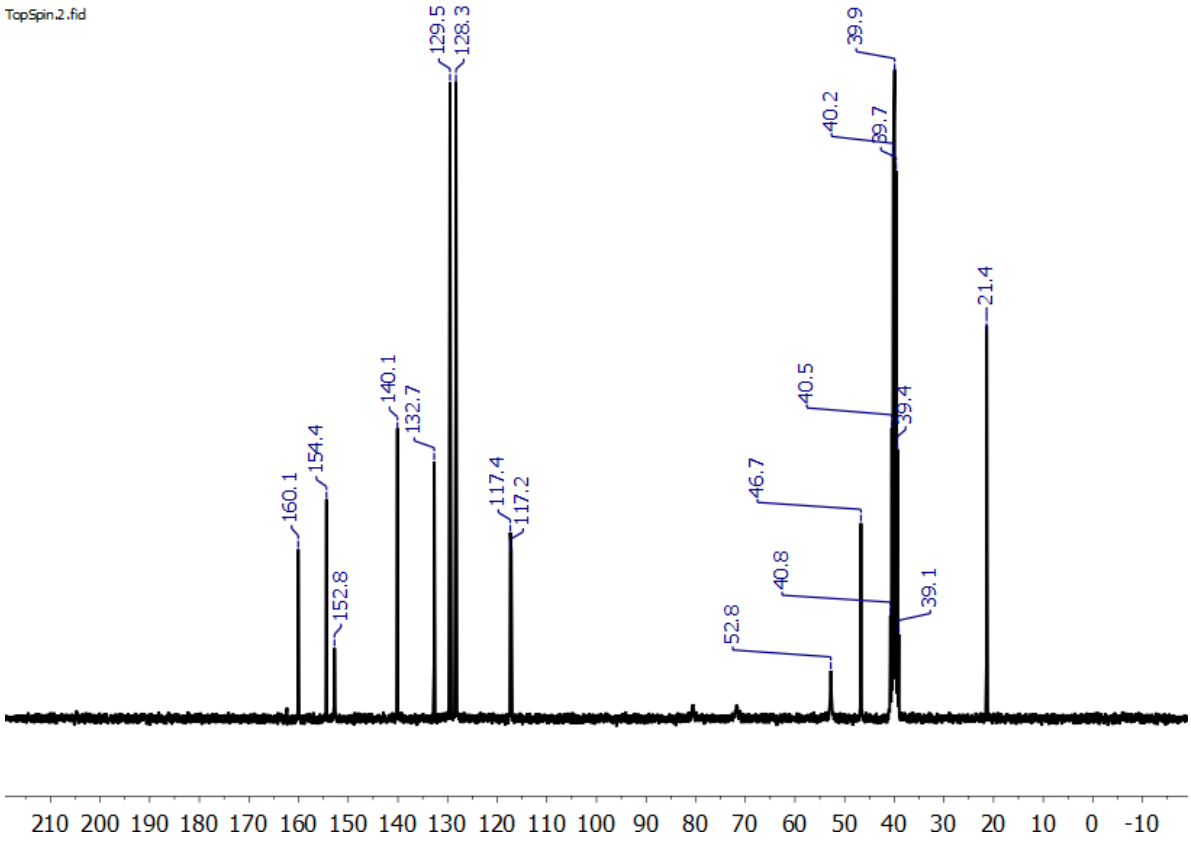


Figure S6. [13]C NMR spectrum of compound **1b** observed for DMSO-d_6 solution at 75 MHz and 25°C.

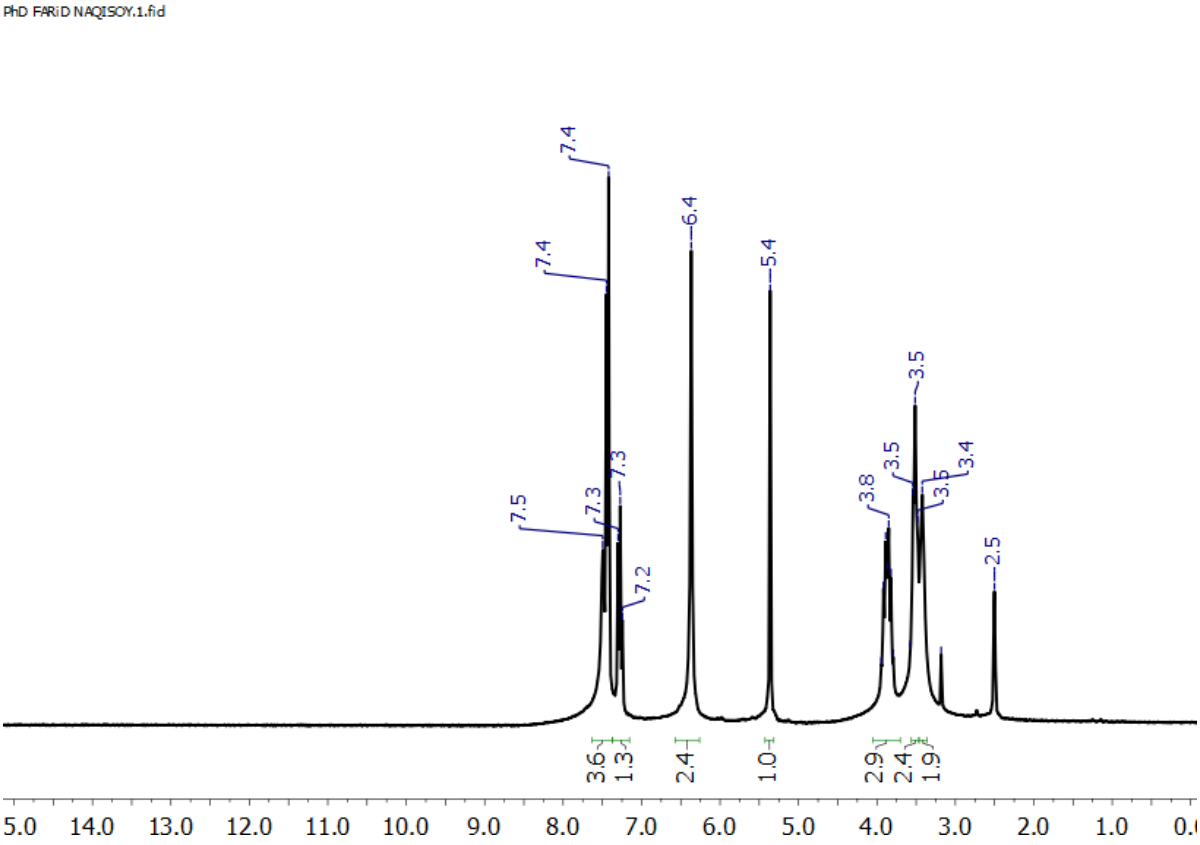


Figure S7. [1]H NMR spectrum of compound **2b** observed for DMSO-d_6 solution at 300 MHz and 25°C.

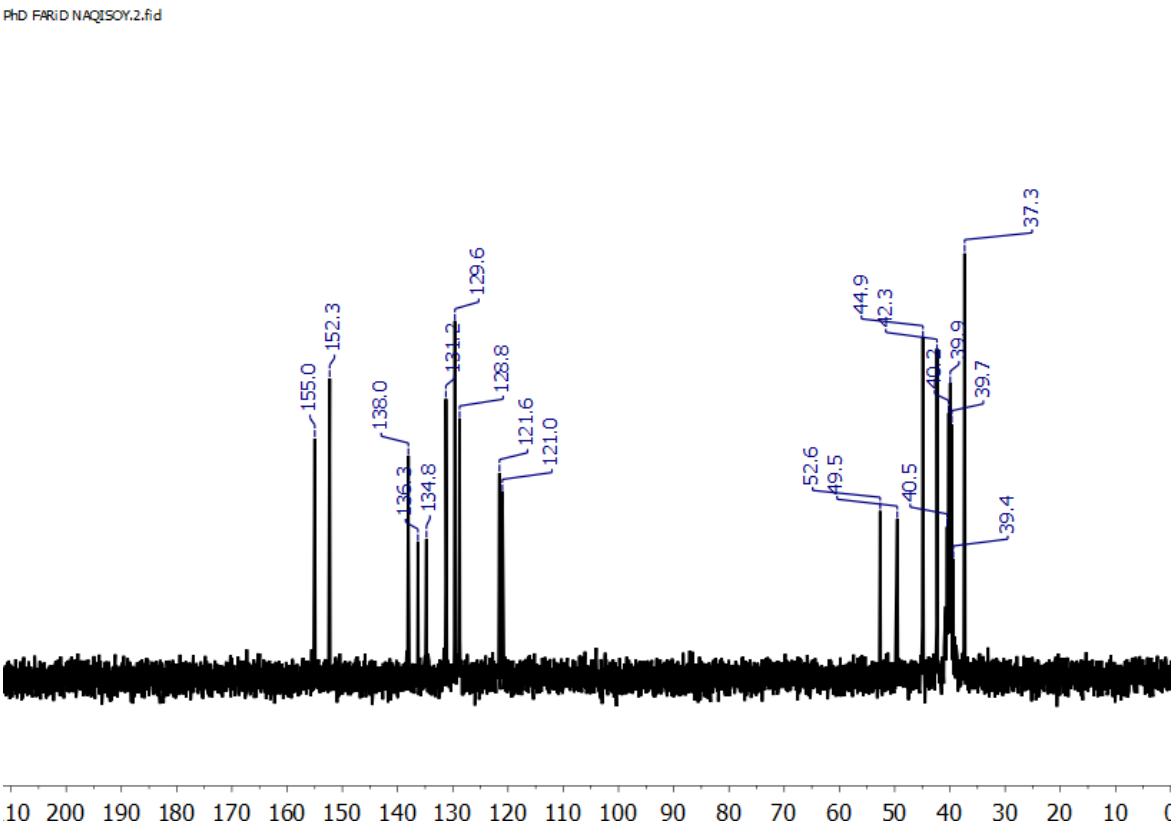


Figure S8. [13]C NMR spectrum of compound **2b** observed for DMSO-d_6 solution at 75 MHz and 25°C.

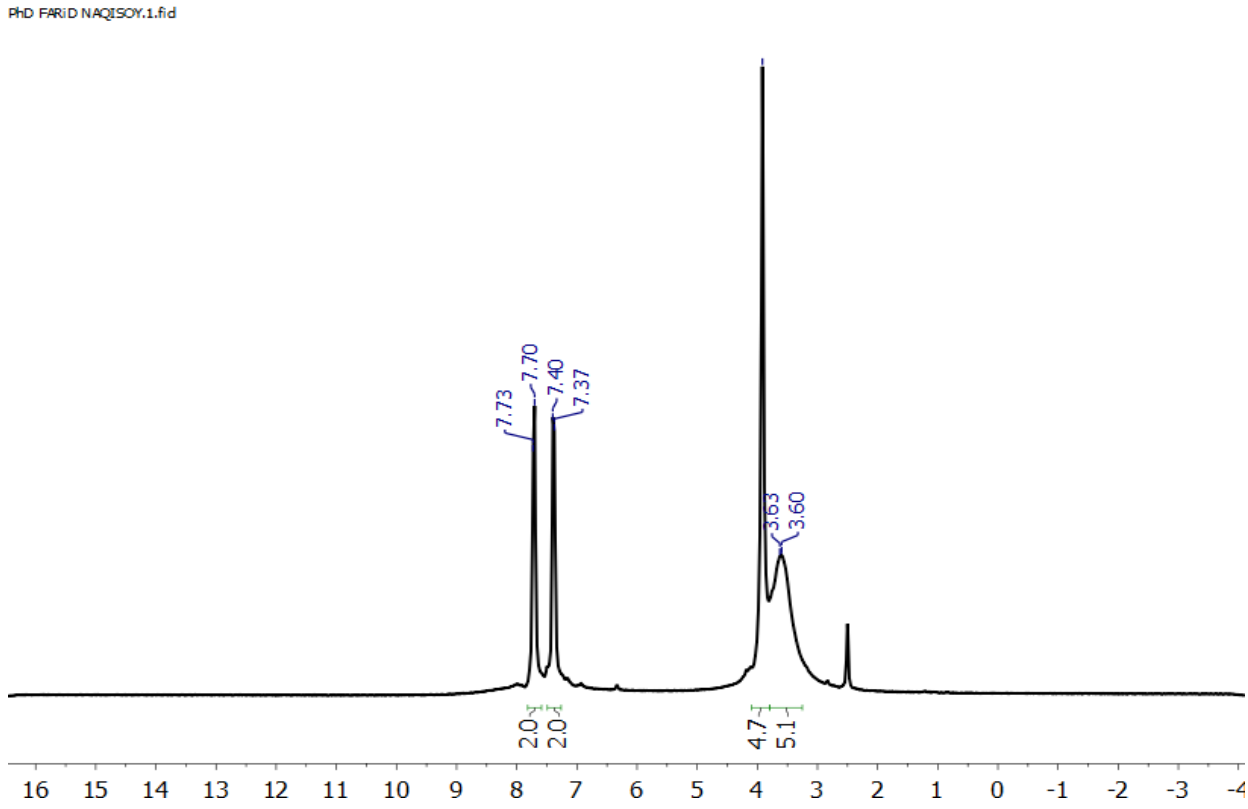


Figure S9. [1]H NMR spectrum of compound **1c** observed for DMSO-d_6 solution at 300 MHz and 25°C.

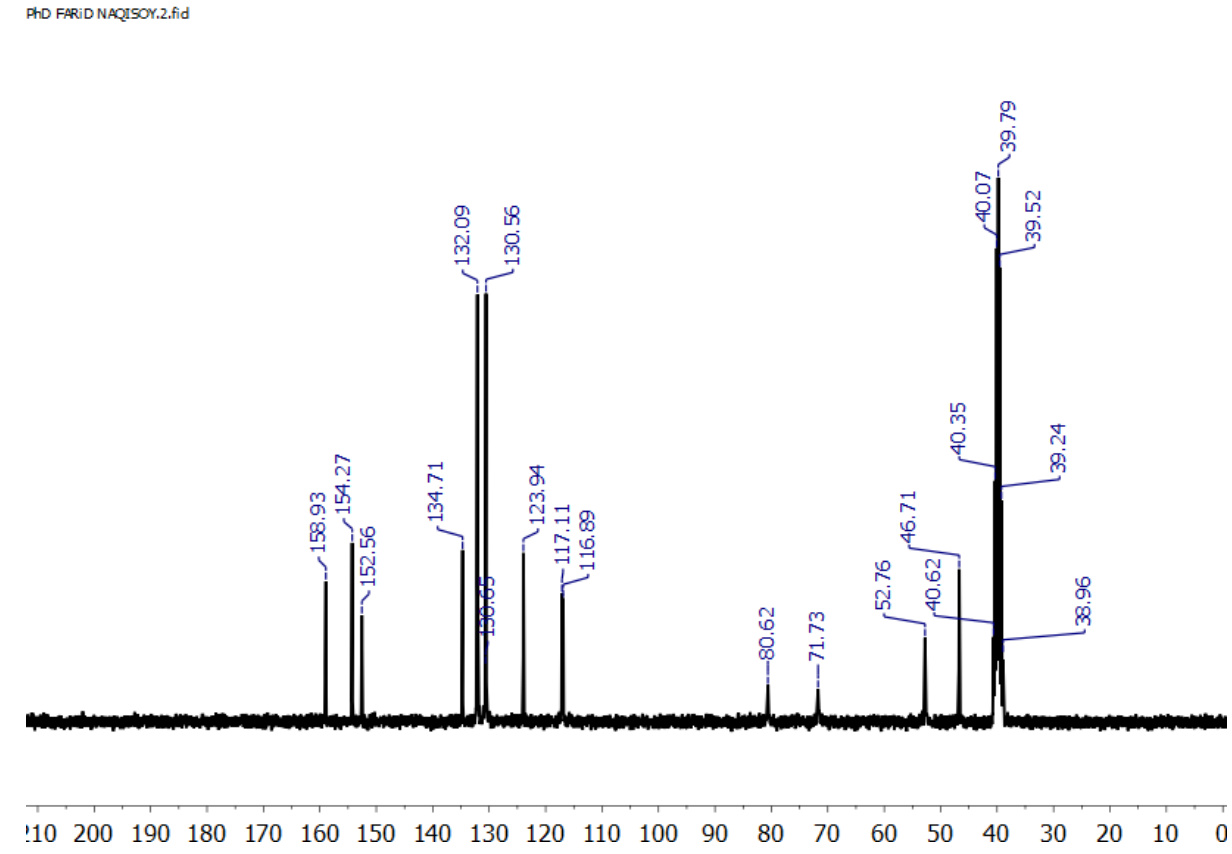


Figure S10. [13]C NMR spectrum of compound **1c** observed for DMSO-d_6 solution at 75 MHz and 25°C.

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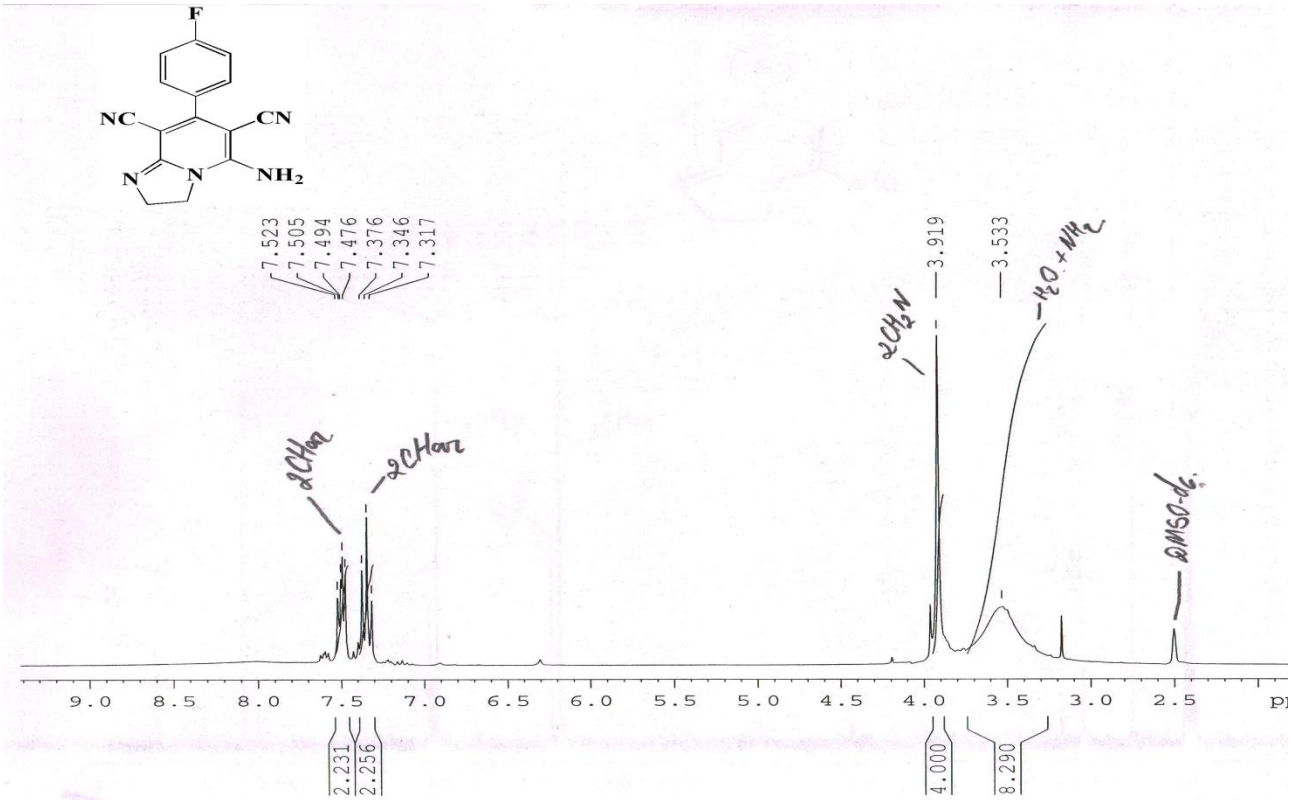


Figure S11. [1]H NMR spectrum of compound **1d** observed for DMSO-d_6 solution at 300 MHz and 25°C.

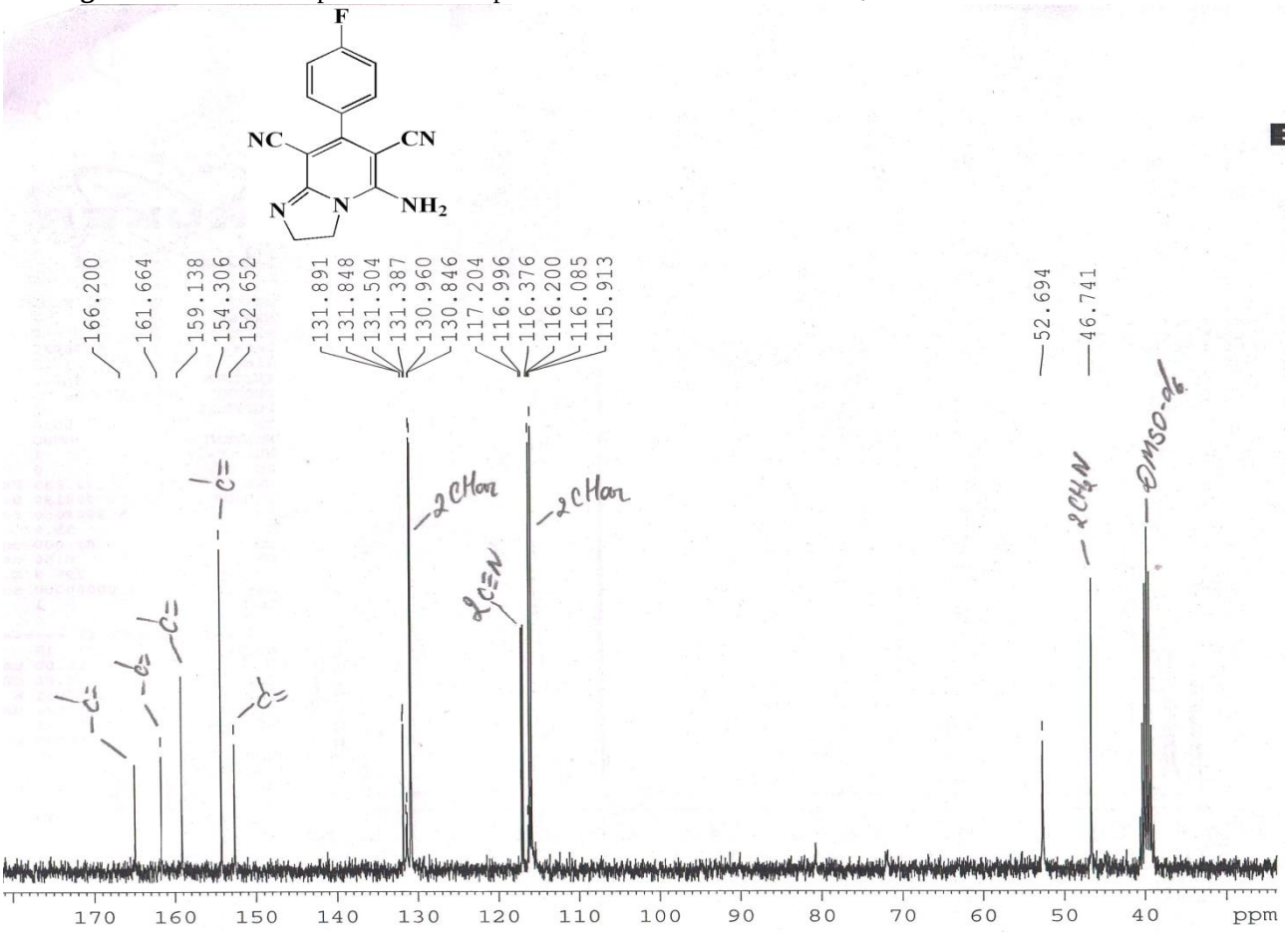


Figure S12. [13]C NMR spectrum of compound **1d** observed for DMSO-d_6 solution at 75 MHz and 25°C.

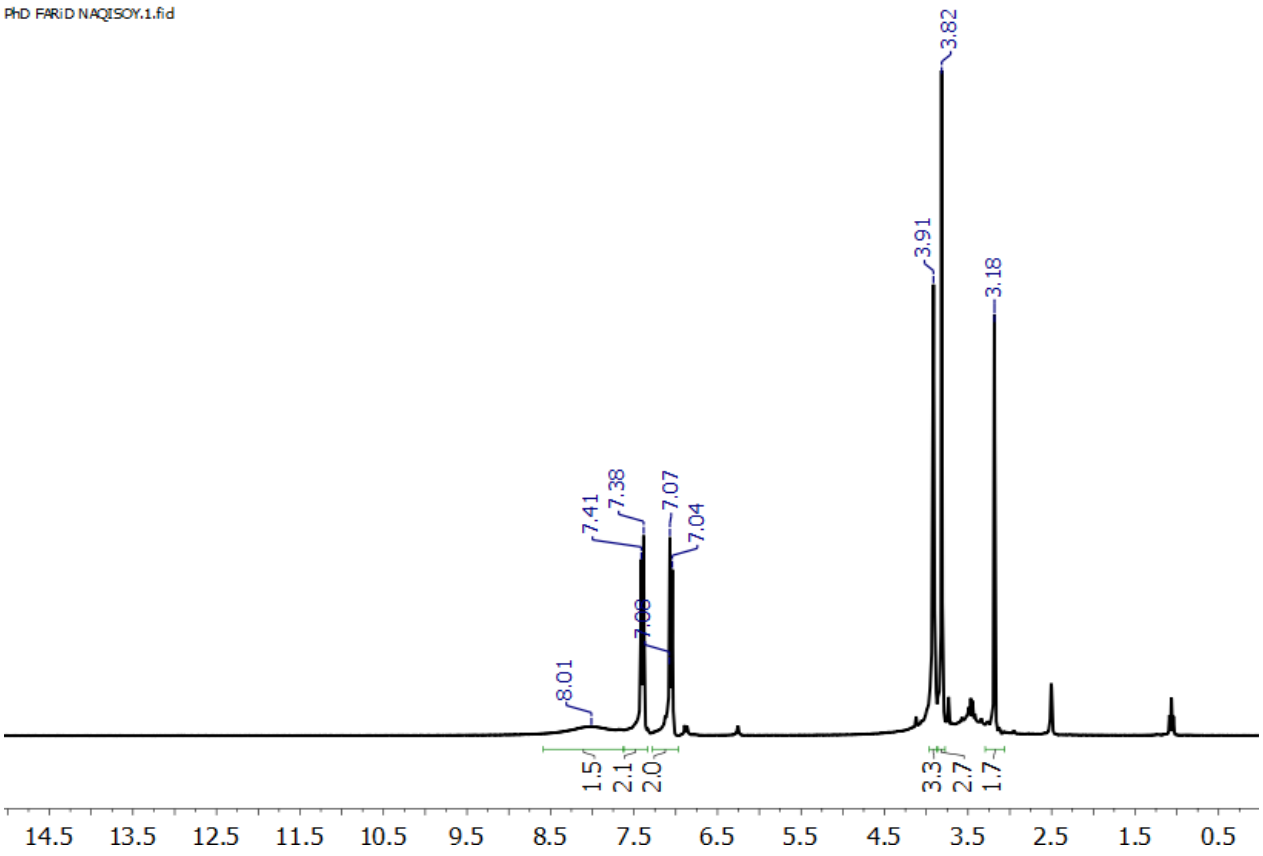


Figure S13. [1]H NMR spectrum of compound **1e** observed for DMSO-d_6 solution at 300 MHz and 25°C.

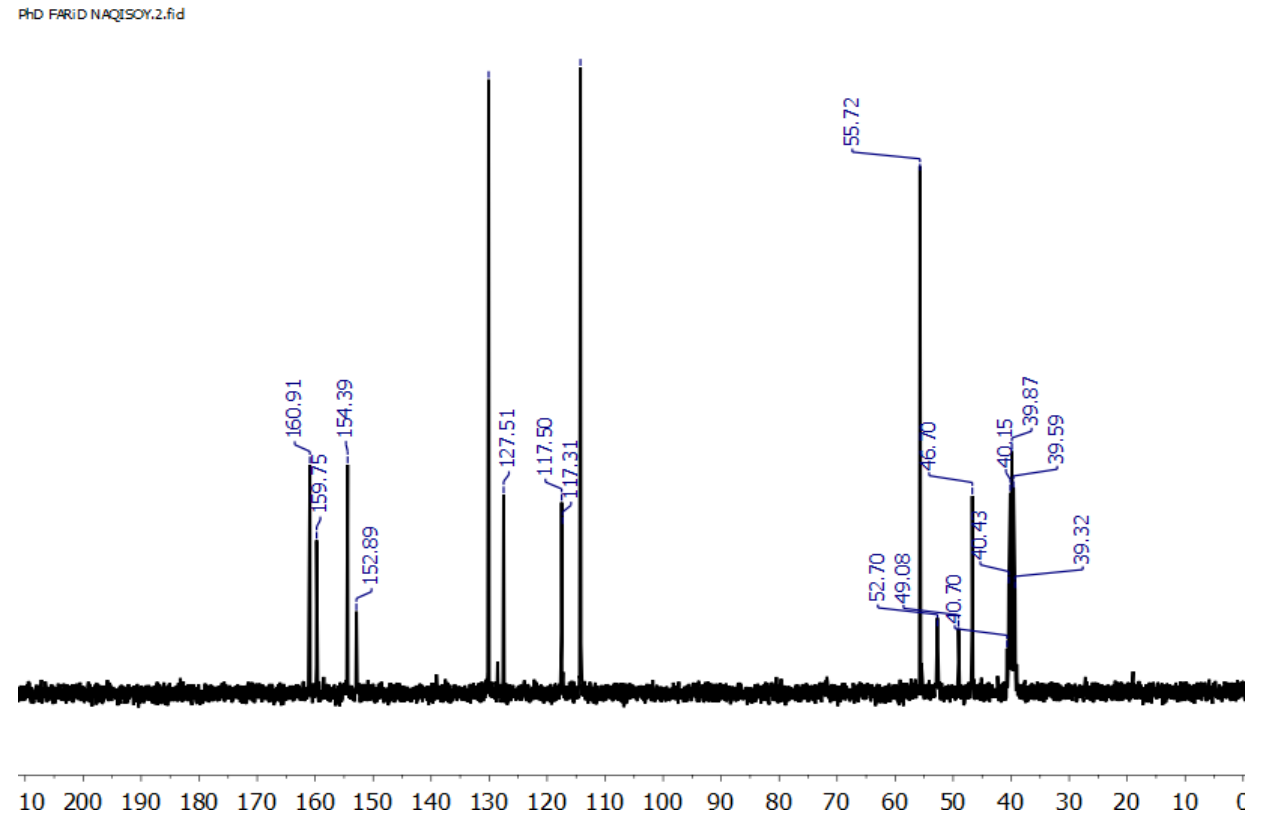


Figure S14. [13]C NMR spectrum of compound **1e** observed for DMSO-d_6 solution at 75 MHz and 25°C.

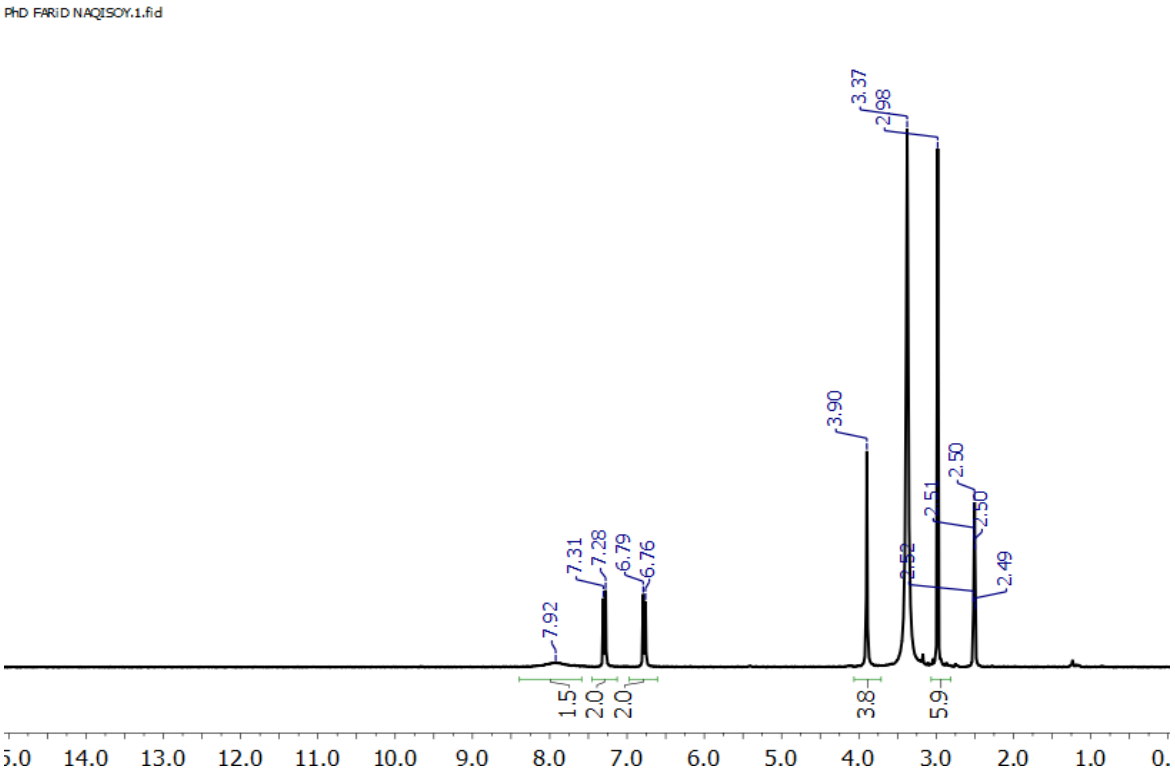


Figure S15. [1]H NMR spectrum of compound **1f** observed for DMSO-d_6 solution at 300 MHz and 25°C.

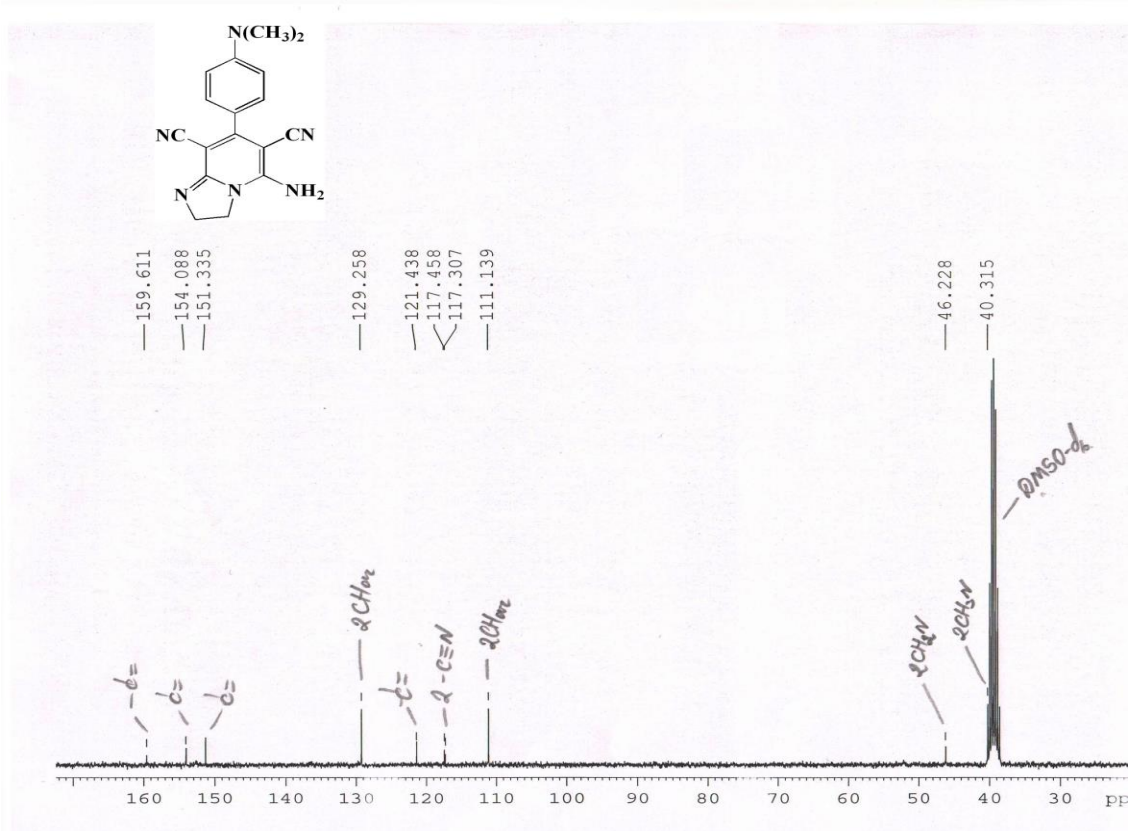


Figure S16. [13]C NMR spectrum of compound **1f** observed for DMSO-d_6 solution at 75 MHz and 25°C.

.

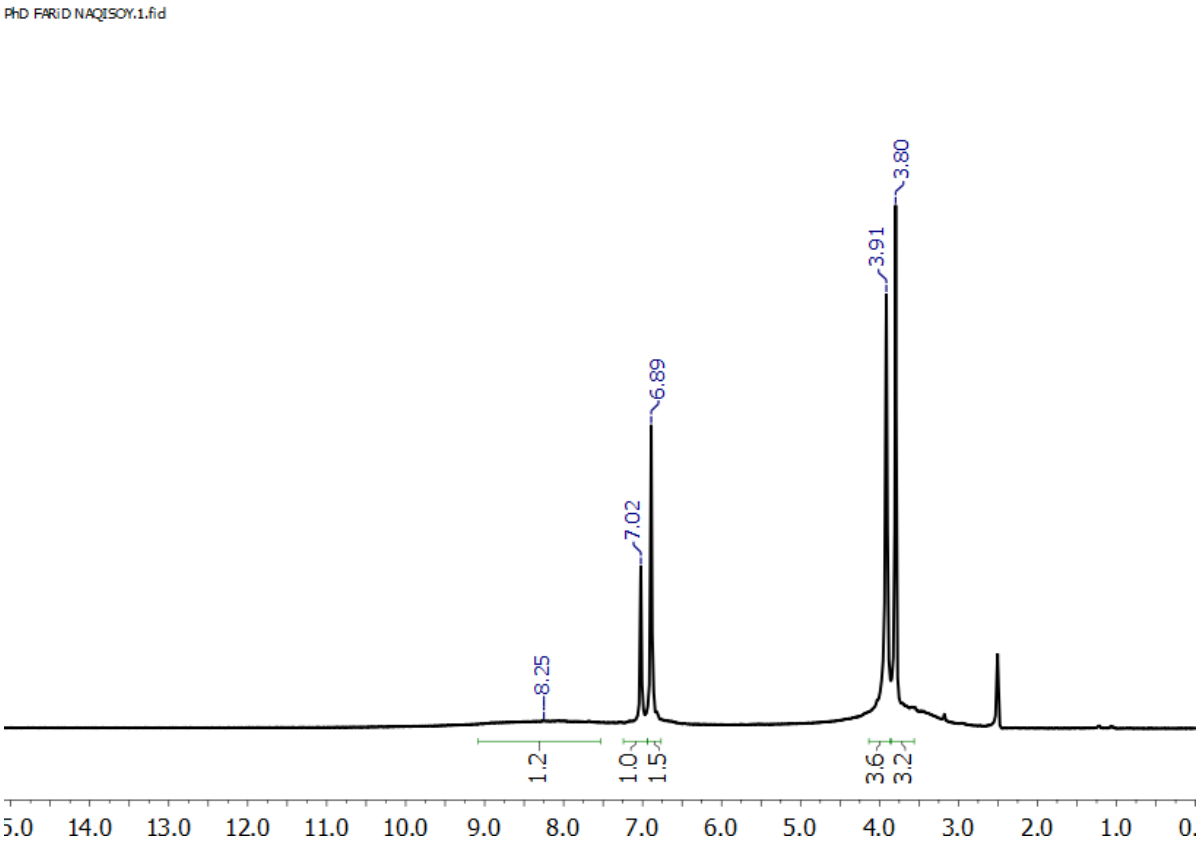


Figure S17. [1]H NMR spectrum of compound **1g** observed for DMSO-d_6 solution at 300 MHz and 25°C.

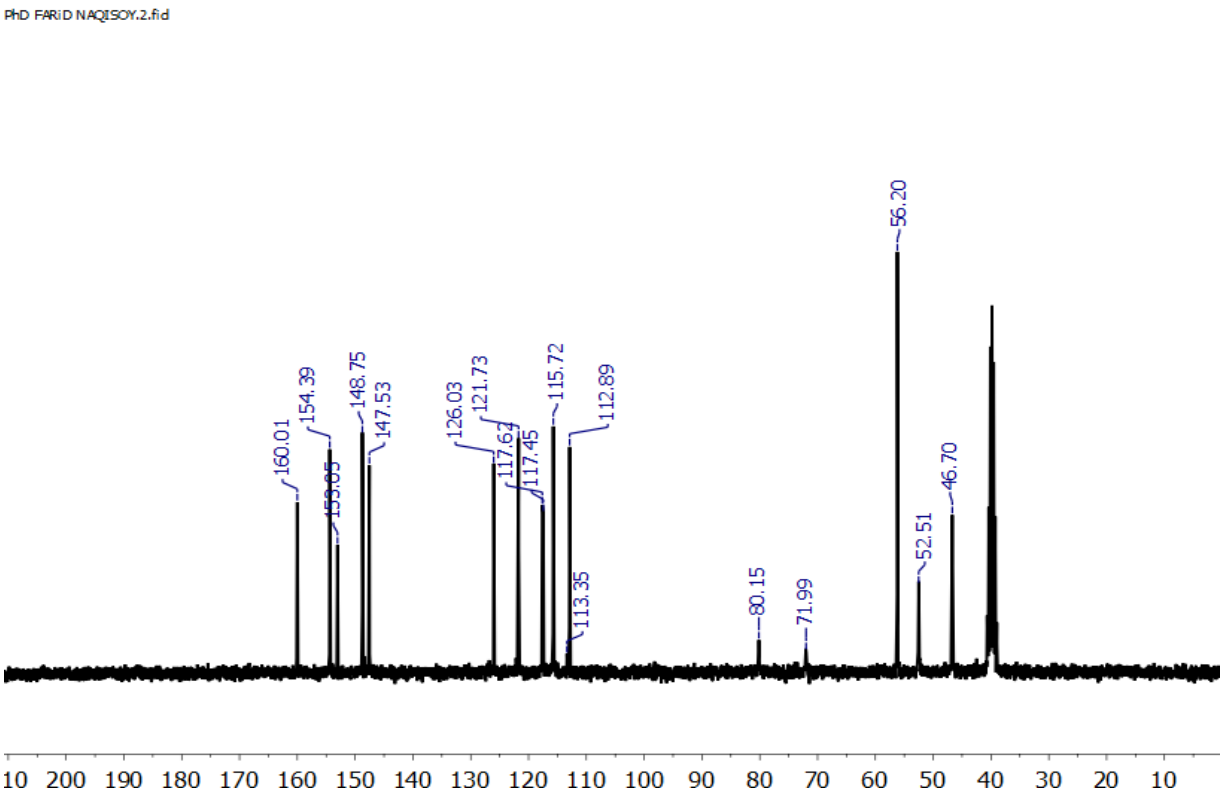


Figure S18. [13]C NMR spectrum of compound **1g** observed for DMSO-d_6 solution at 75 MHz and 25°C.

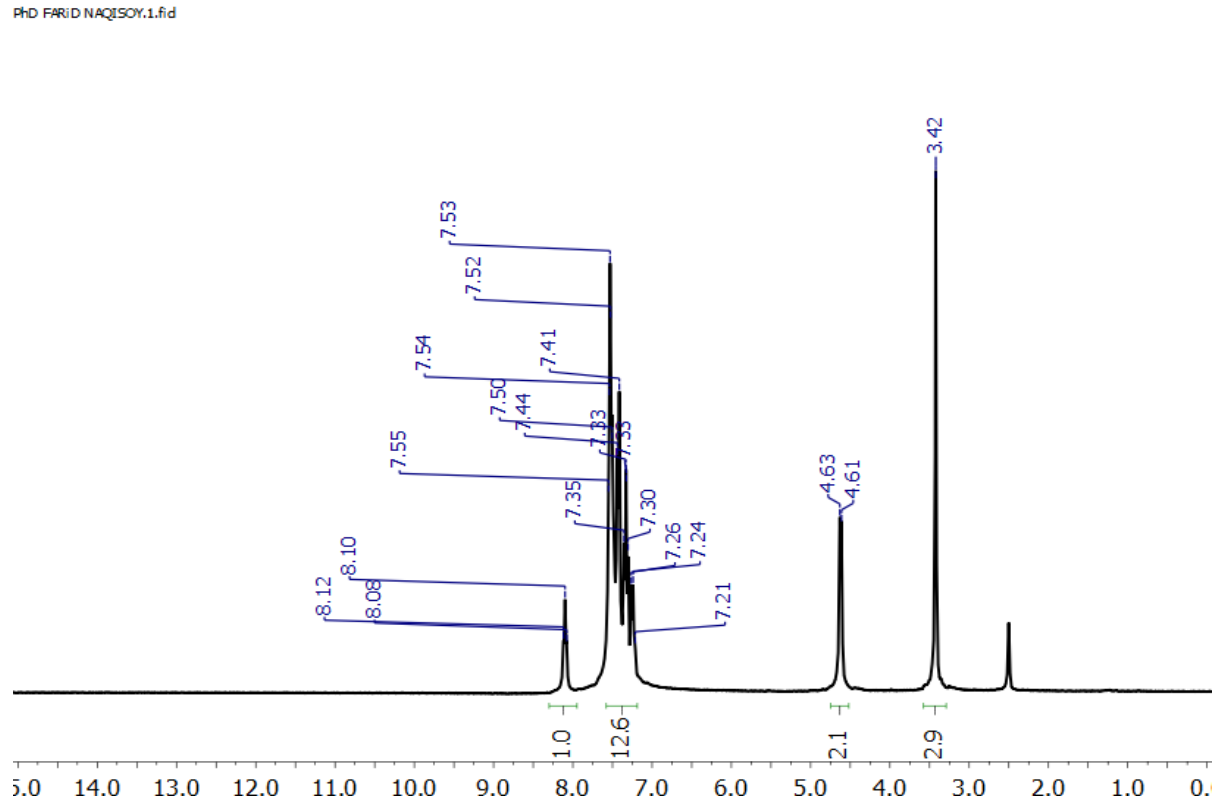


Figure S19. ^{1}H NMR spectrum of compound **3a** observed for DMSO-d_6 solution at 300 MHz and 25°C.

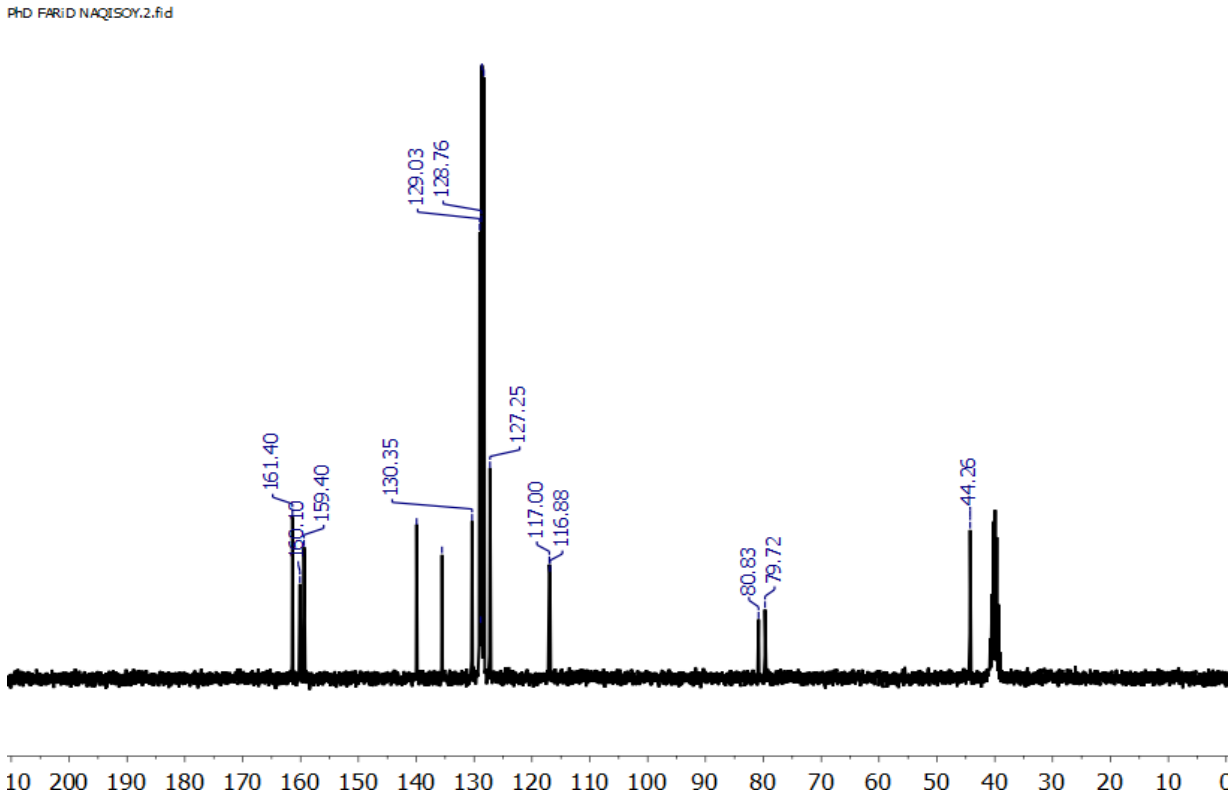


Figure S20. ^{13}C NMR spectrum of compound **3a** observed for DMSO-d_6 solution at 75 MHz and 25°C.

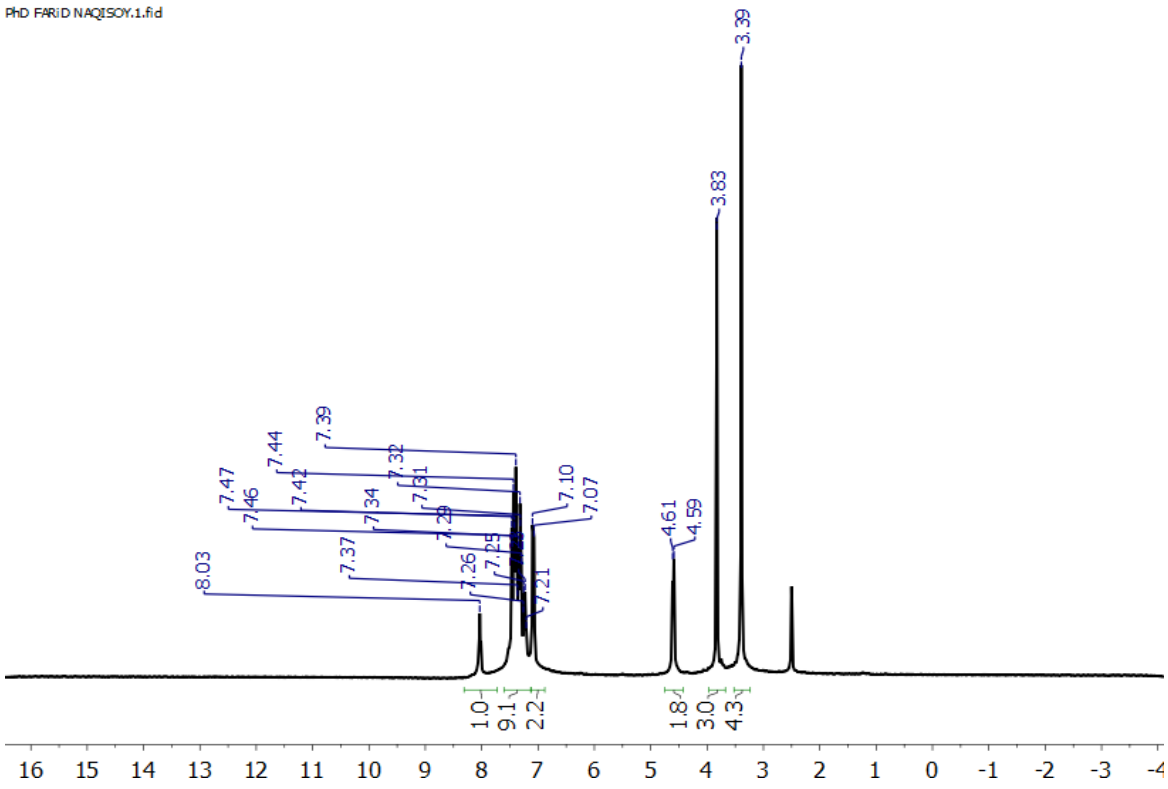


Figure S21. [1]H NMR spectrum of compound **3b** observed for DMSO-d_6 solution at 300 MHz and 25°C.

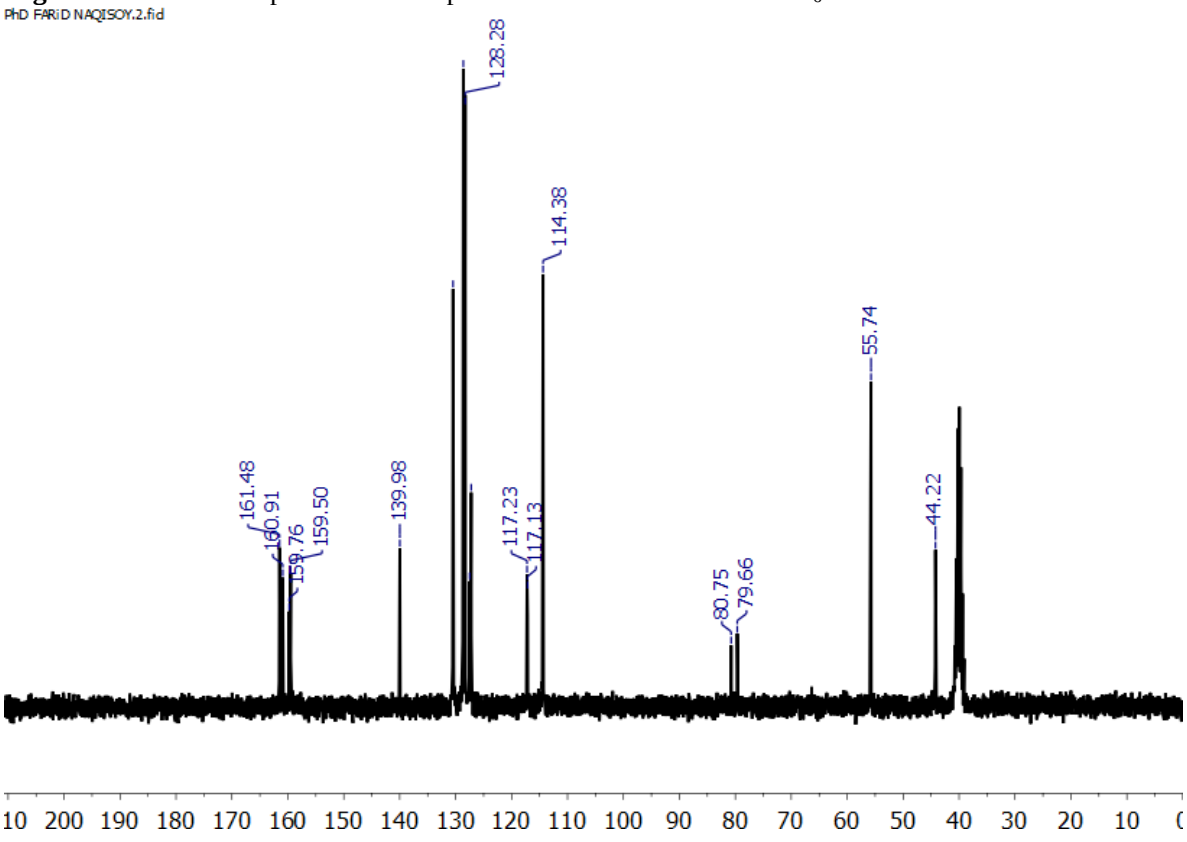


Figure S22. [13]C NMR spectrum of compound **3b** observed for DMSO-d_6 solution at 75 MHz and 25°C.

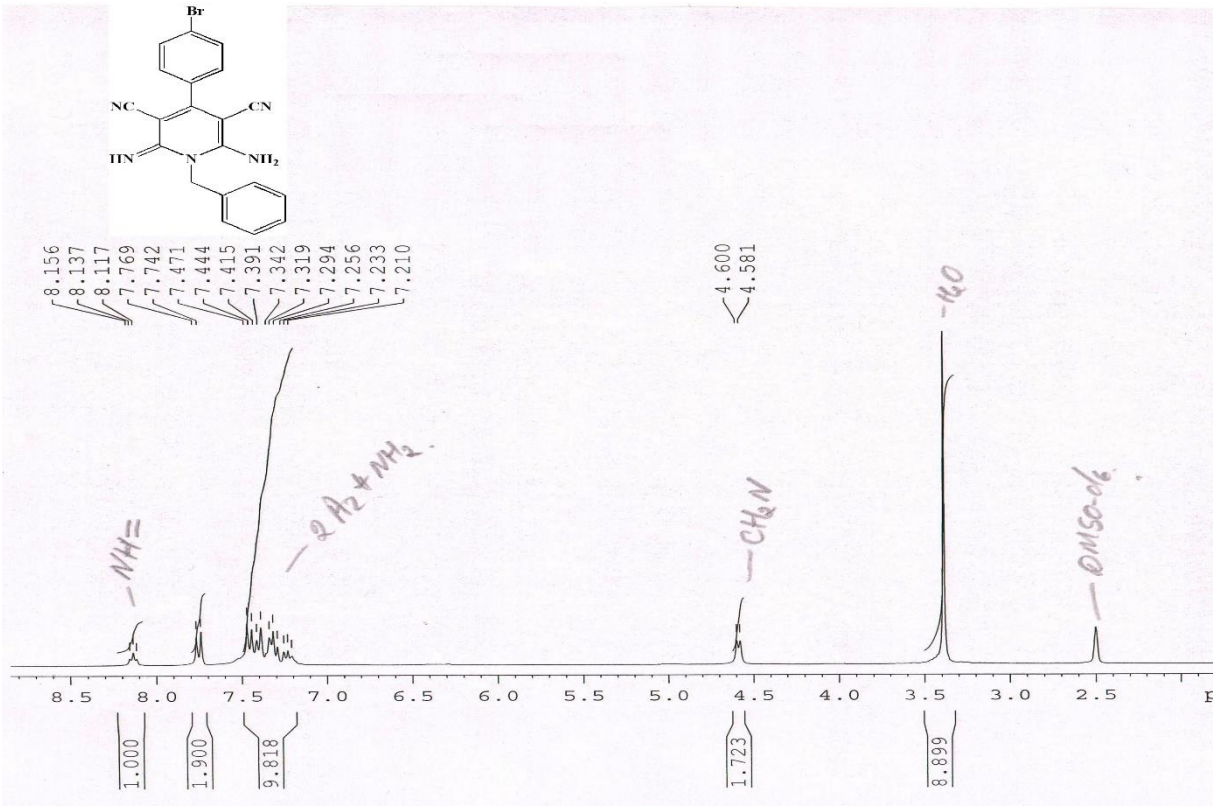


Figure S23. [1]H NMR spectrum of compound **3c** observed for DMSO-d_6 solution at 300 MHz and 25°C.

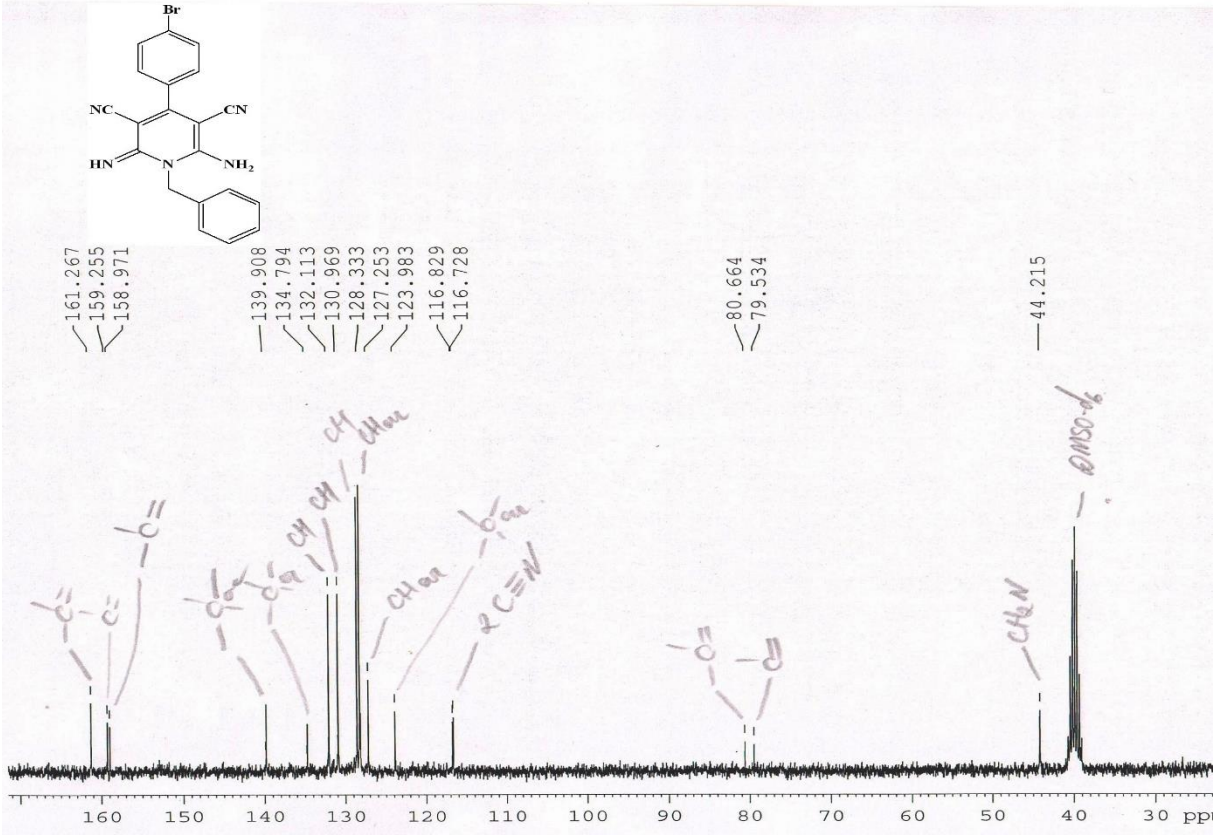


Figure S24. [13]C NMR spectrum of compound **3c** observed for DMSO-d_6 solution at 75 MHz and 25°C.

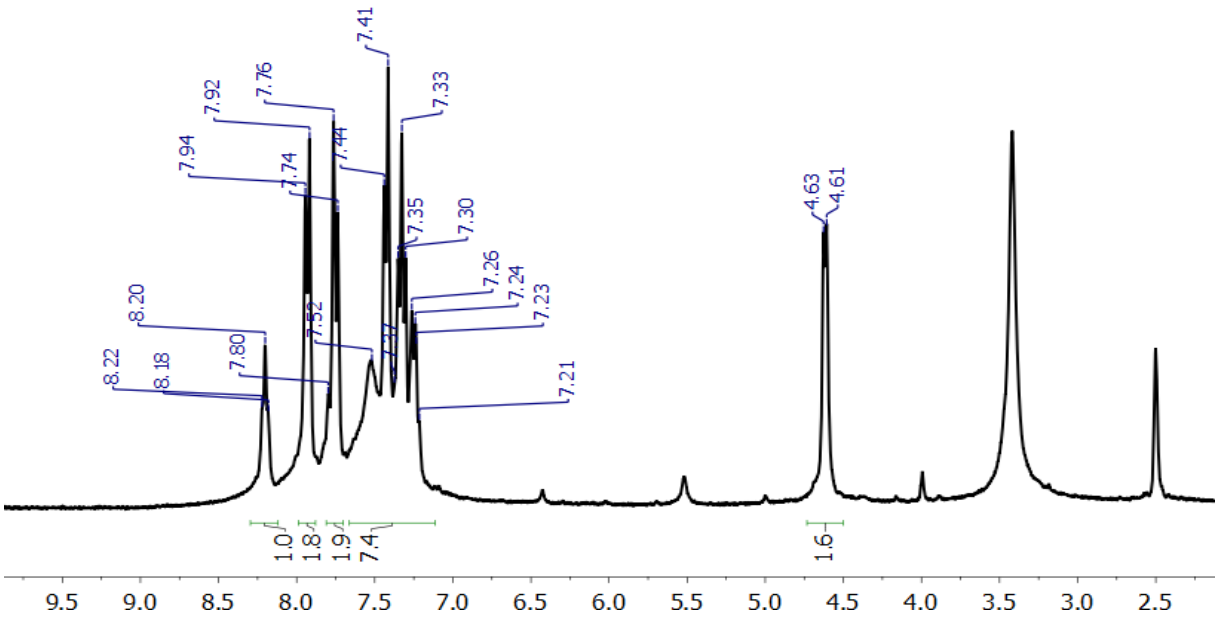


Figure S25. ^{1}H NMR spectrum of compound **3d** observed for DMSO-d_6 solution at 300 MHz and 25°C.

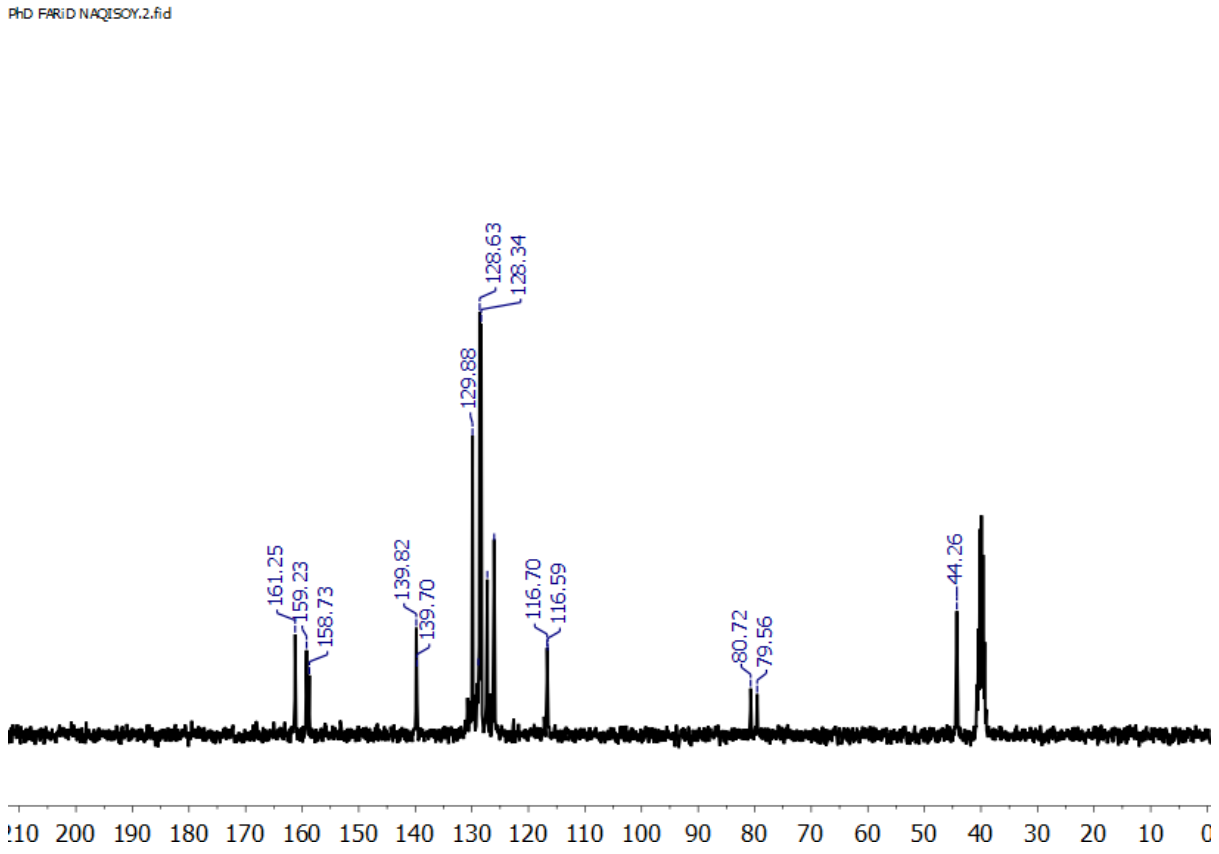


Figure S26. ^{13}C NMR spectrum of compound **3d** observed for DMSO-d_6 solution at 75 MHz and 25°C.

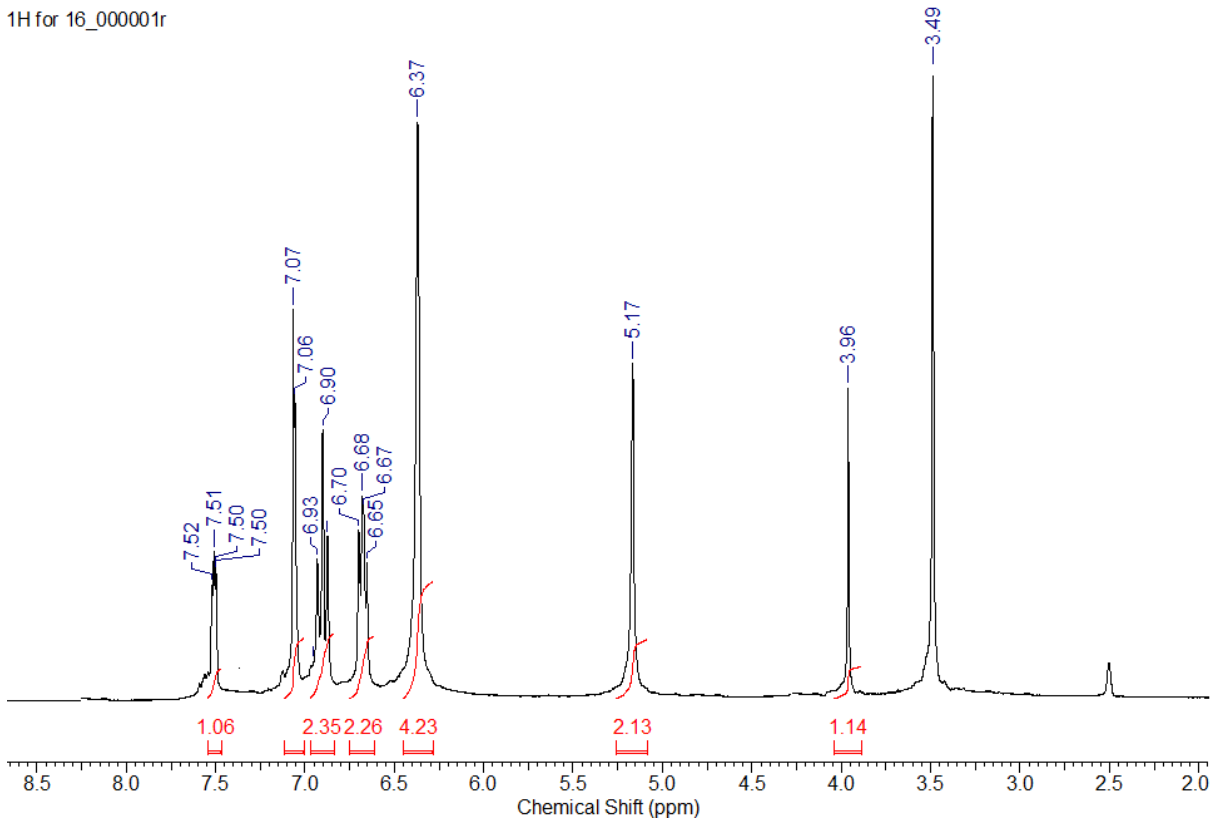


Figure S27. [1]H NMR spectrum of compound **4** observed for DMSO-d_6 solution at 300 MHz and 25°C.

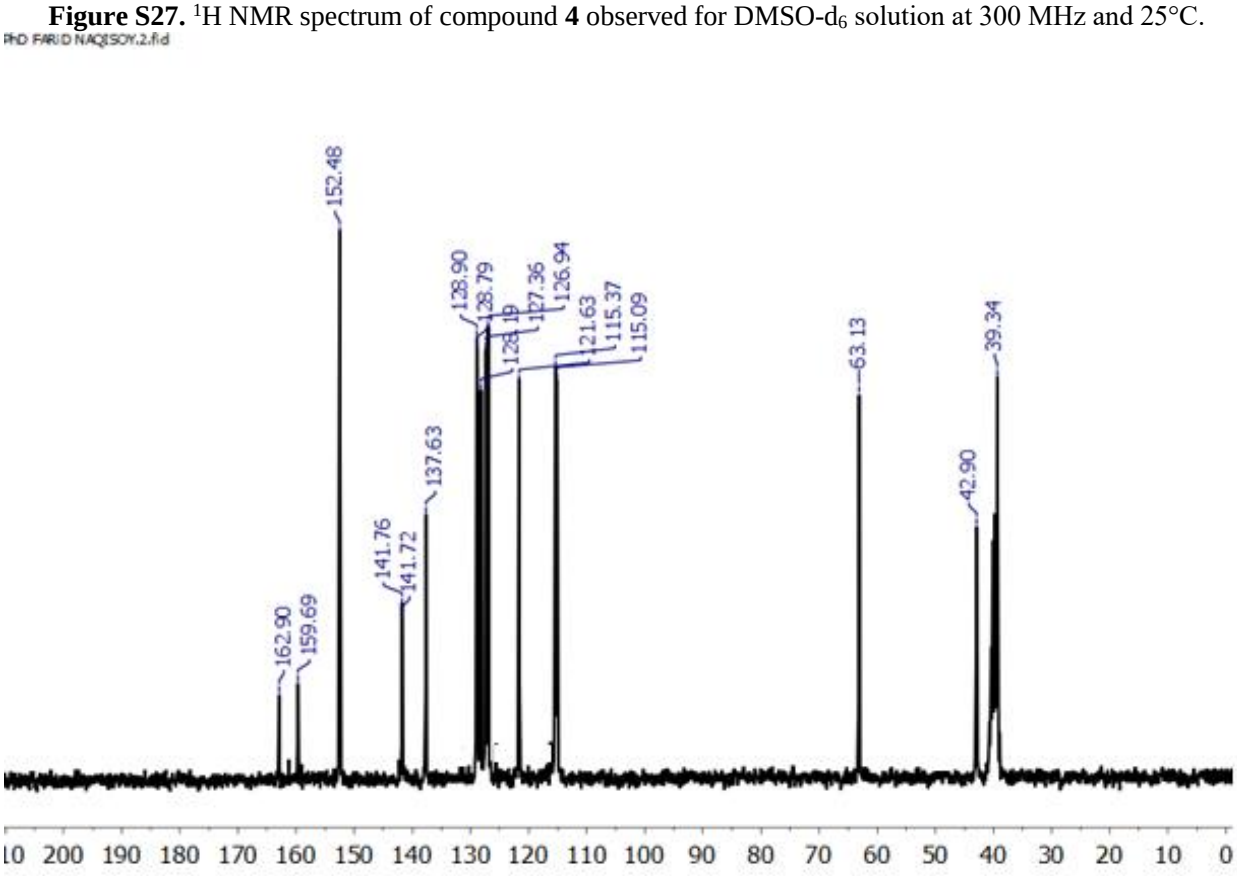


Figure S28. [13]C NMR spectrum of compound **4** observed for DMSO-d_6 solution at 75 MHz and 25°C.

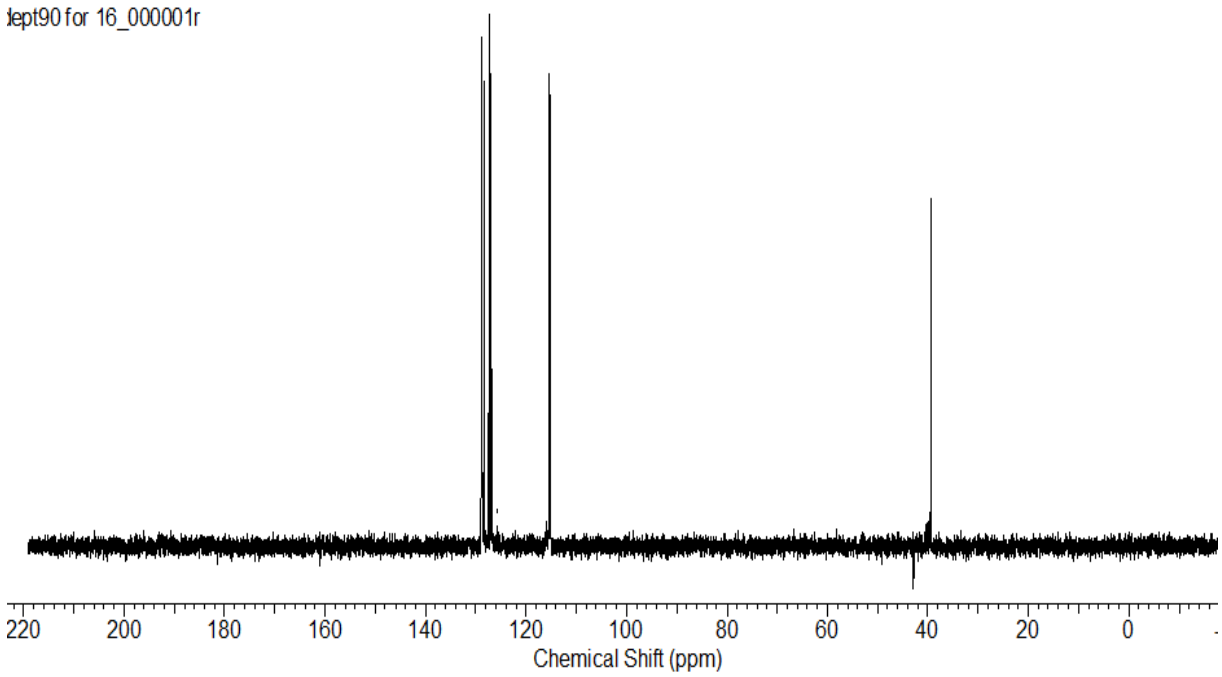


Figure S29. DEPT 90 ^{13}C NMR spectrum of compound **4** observed for DMSO-d_6 solution at 75 MHz and 25°C.

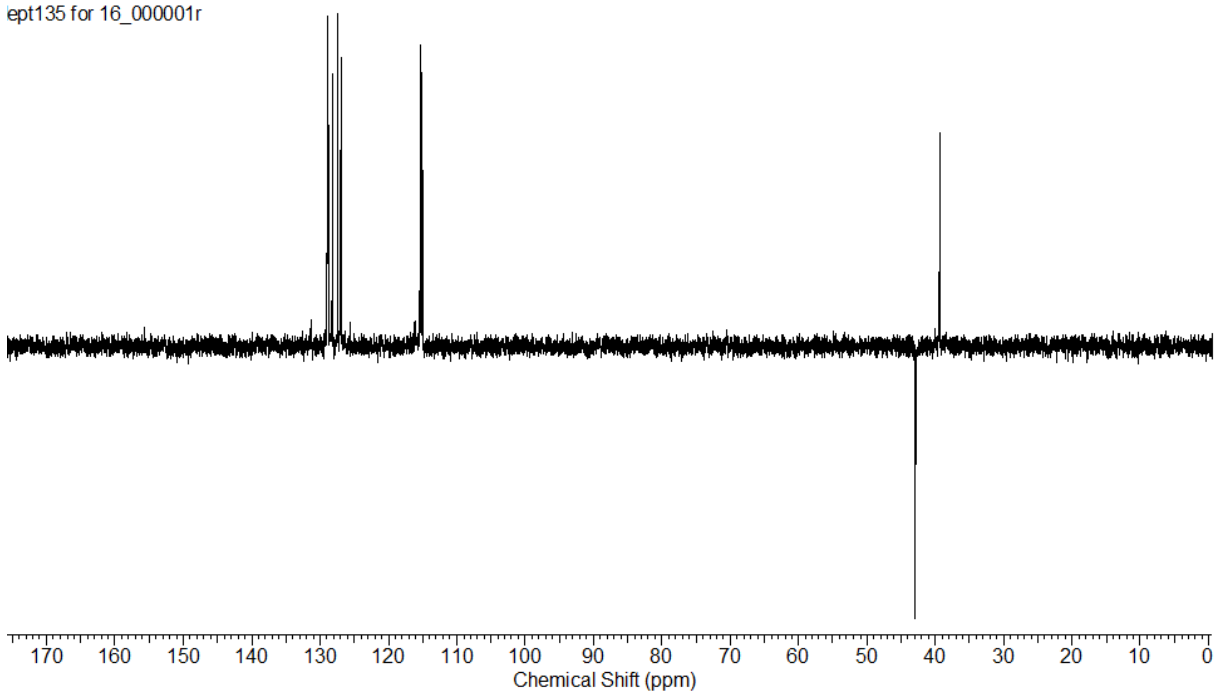


Figure S30. DEPT 135 ^{13}C NMR spectrum of compound **4** observed for DMSO-d_6 solution at 75 MHz and 25°C.

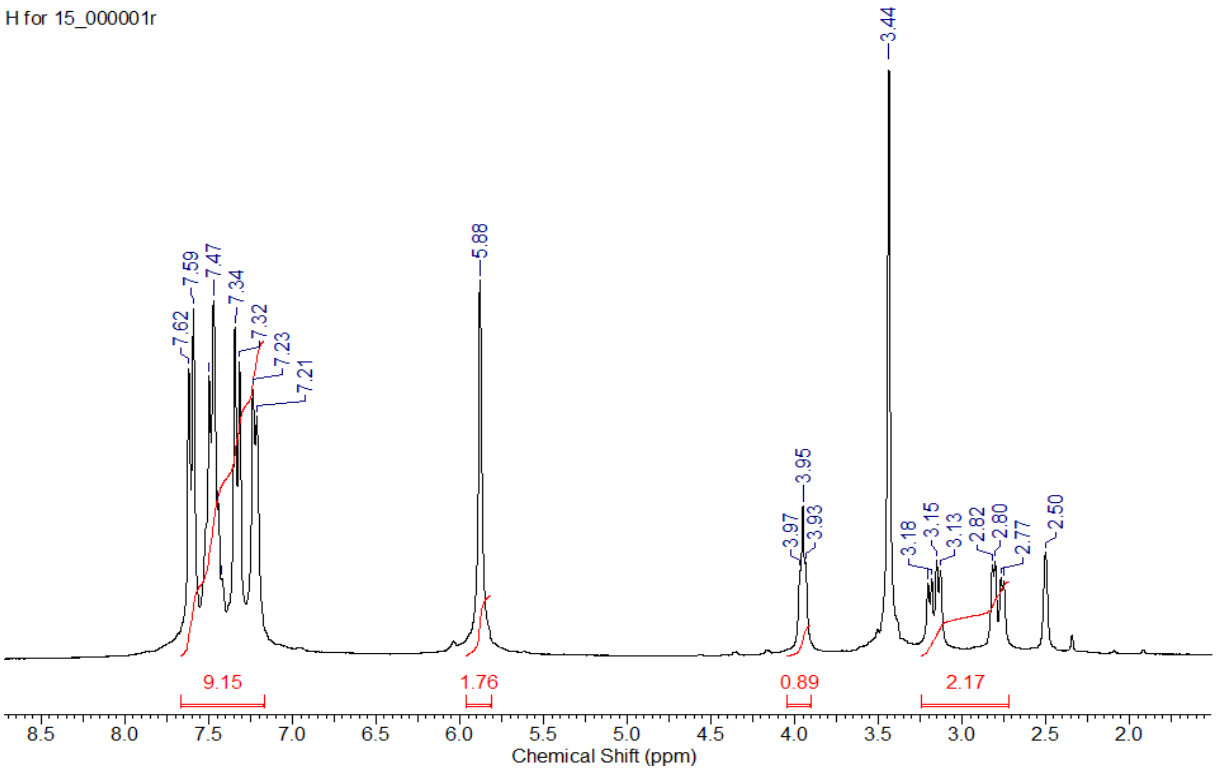


Figure S31. ^{1}H NMR spectrum of compound **5** observed for DMSO-d_6 solution at 300 MHz and 25°C.

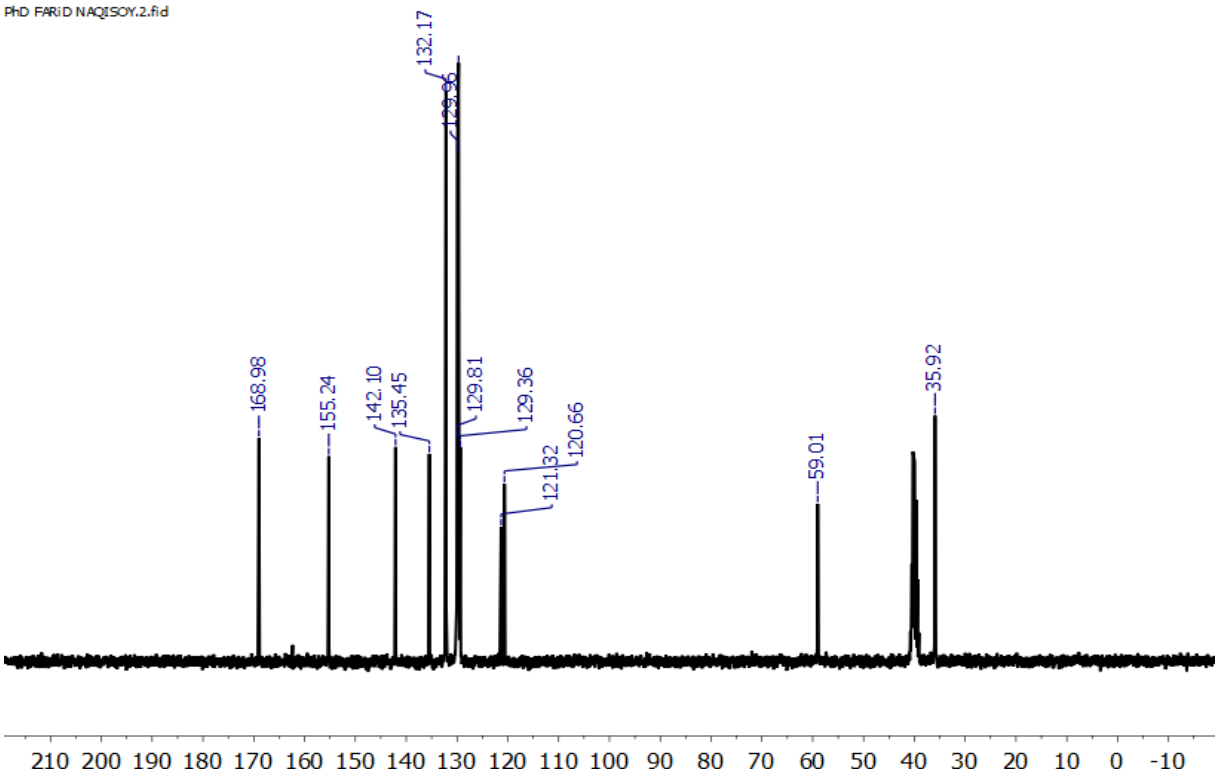


Figure S32. ^{13}C NMR spectrum of compound **5** observed for DMSO-d_6 solution at 75 MHz and 25°C.

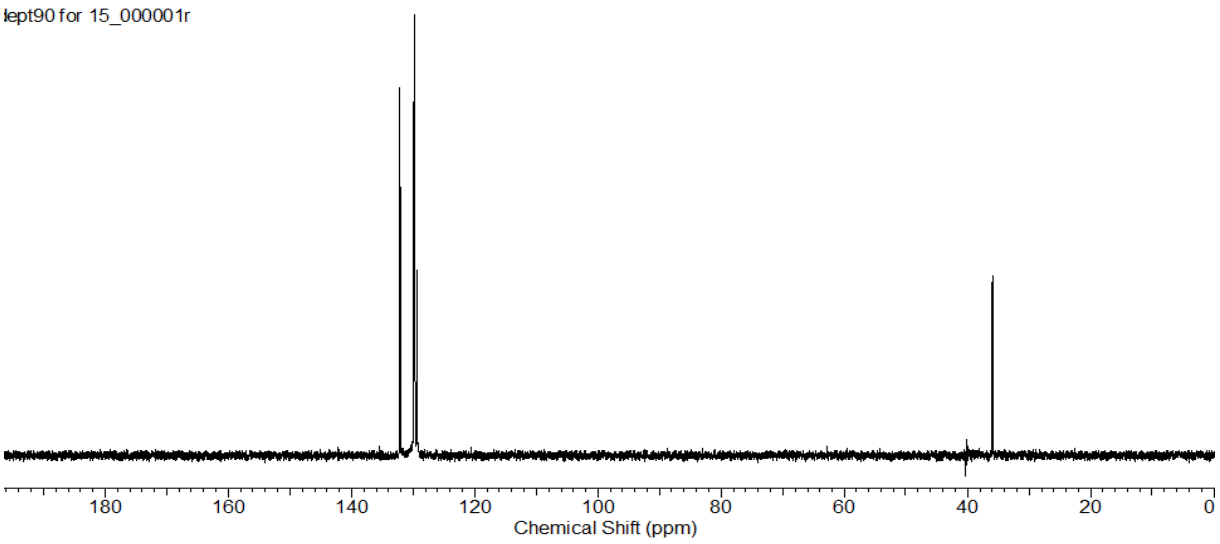


Figure S33. DEPT 90 ^{13}C NMR spectrum of compound **5** observed for DMSO-d_6 solution at 75 MHz and 25°C.

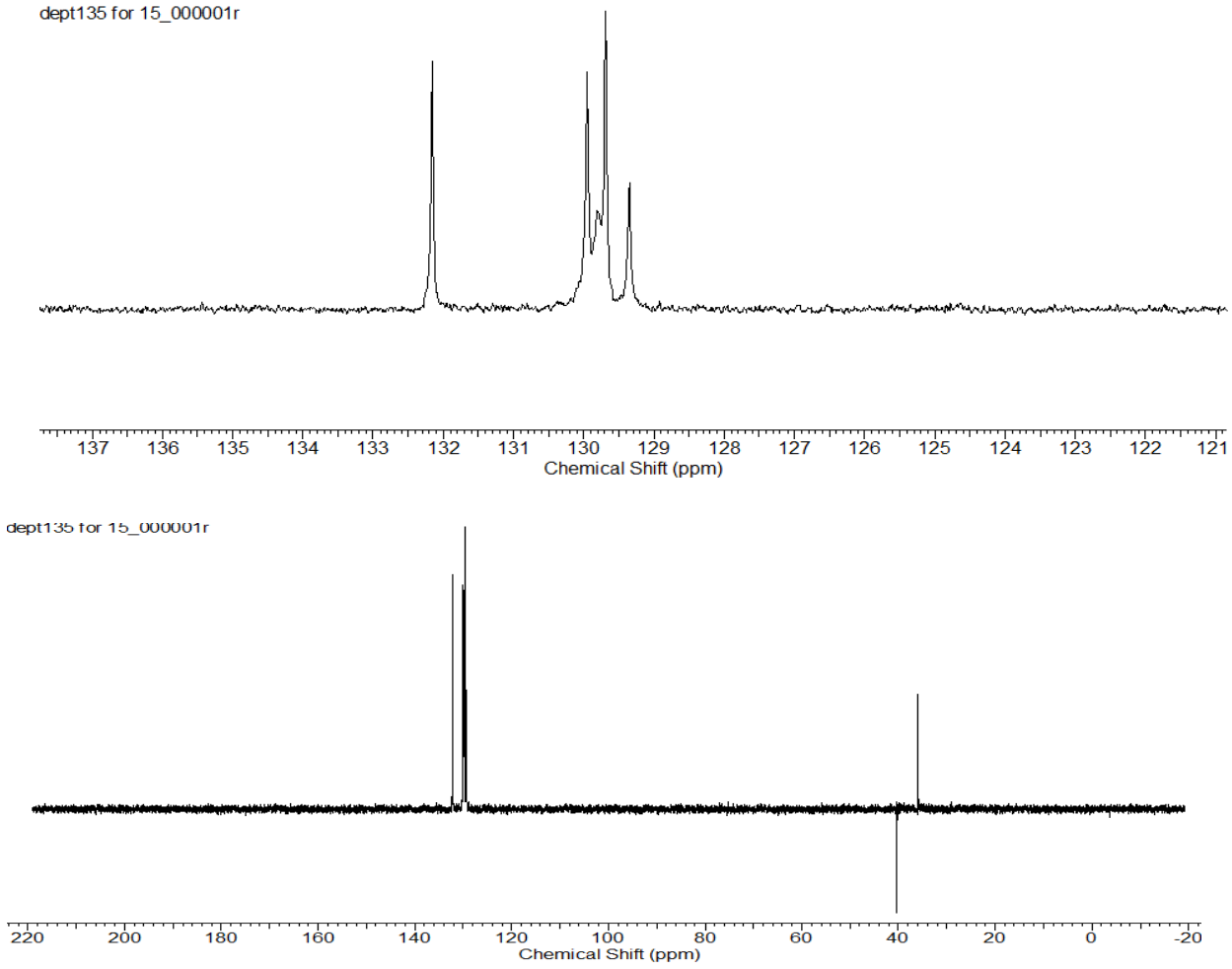


Figure S34. DEPT 135 ^{13}C NMR spectrum of compound **5** observed for DMSO-d_6 solution at 75 MHz and 25°C.

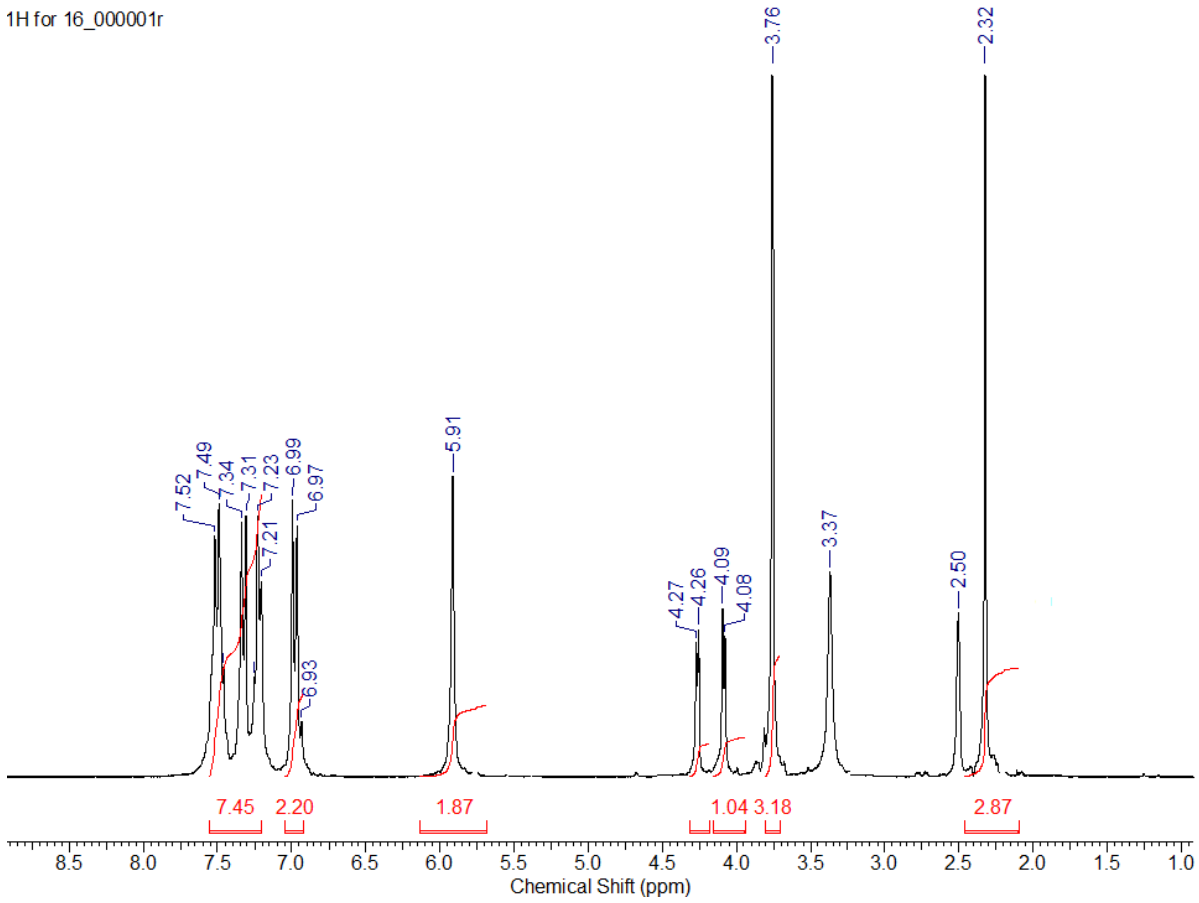


Figure S35. [1]H NMR spectrum of compound **6** observed for DMSO-d_6 solution at 300 MHz and 25°C.

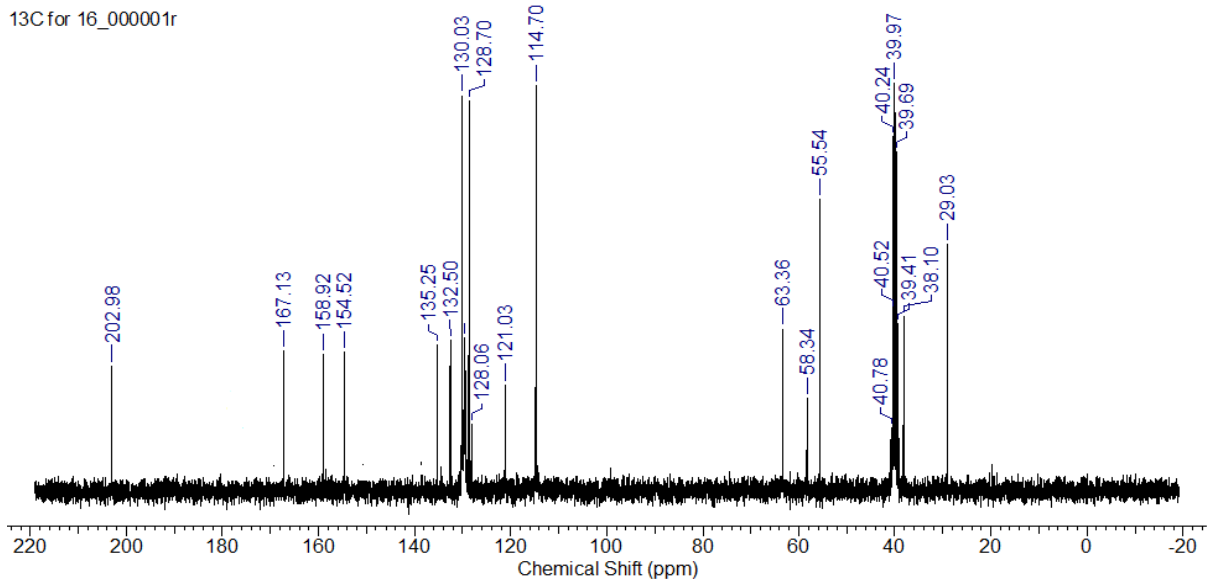


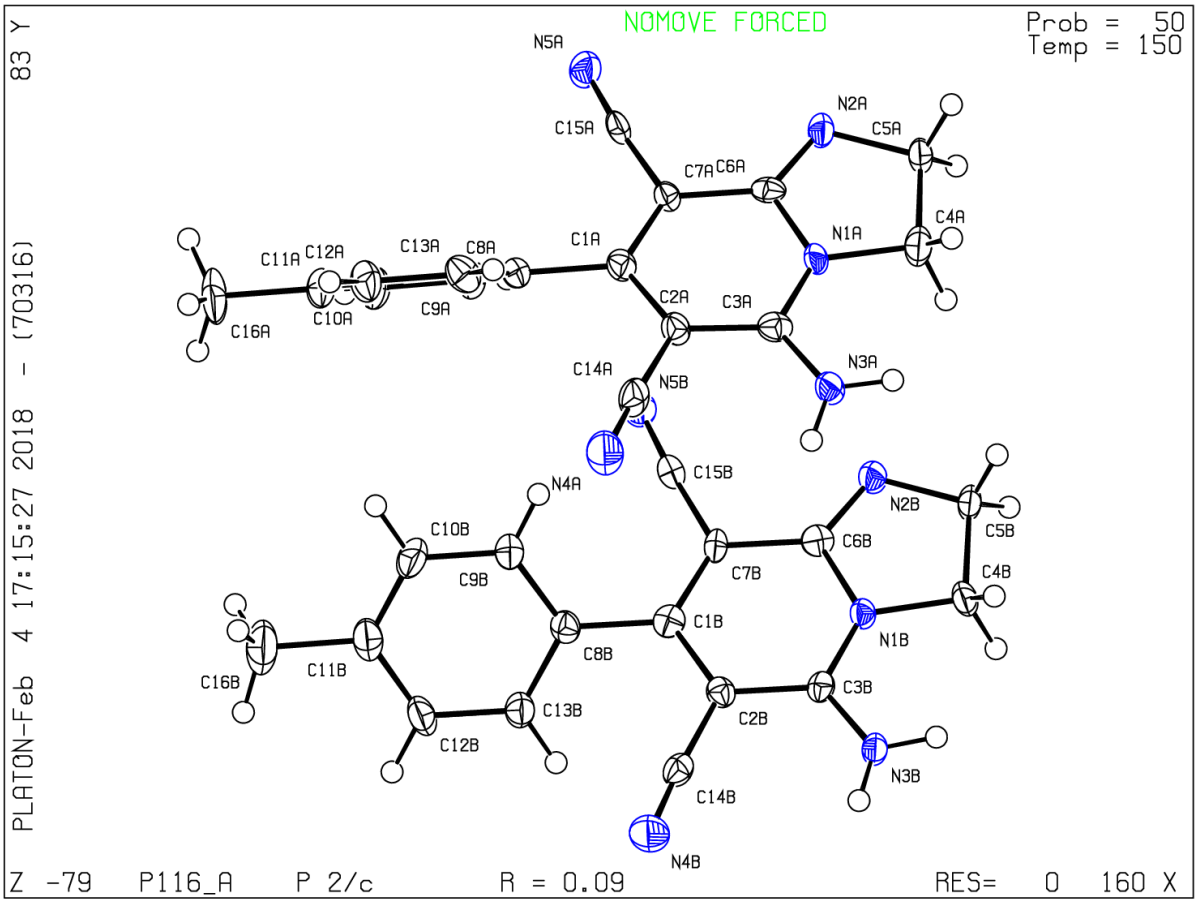
Figure S36. [13]C NMR spectrum of compound **6** observed for DMSO-d_6 solution at 75 MHz and 25°C.

6. X-ray crystallographic structure analyses

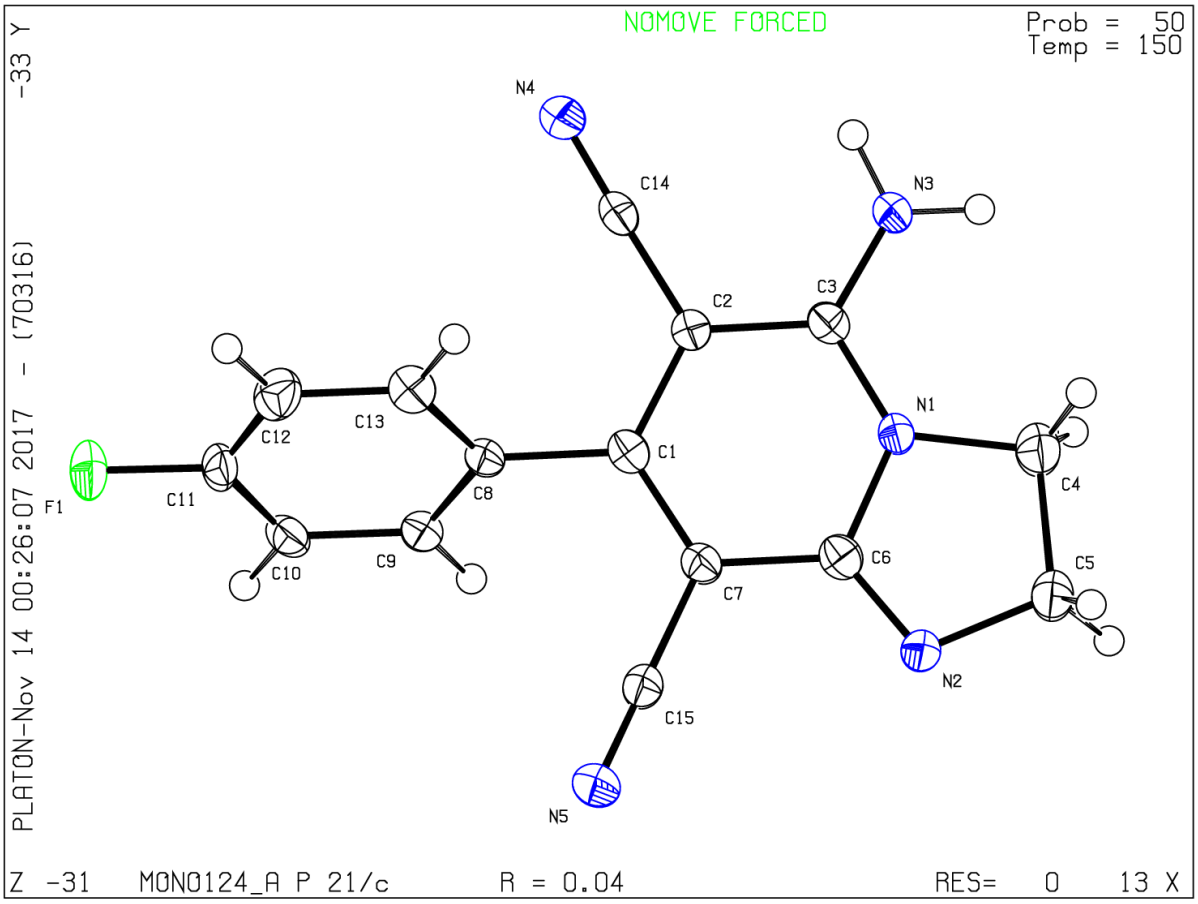
Single crystals of compounds **1b**, **1d**, **1e** and **2b** were obtained by crystallization form EtOH:H_2O (3:2), which provided colorless plates.

A crystals of **1b**, **1d**, **1e** and **2b** were immersed in cryo-oil, mounted in a Nylon loop, and the intensity data were collected at 150(2) K on a Smart Apex II diffractometer using Mo Kα radiation (λ = 0.71073 E). The diffraction experiments were carried out on a Bruker APEX II CCD diffractometer. The program SHELXTL was used for collecting frames of data, indexing reflections, and for the determination of the lattice parameters, SAINTP-for integration of the intensity of reflections and scaling, SADABS-for absorption correction, and SHELXTL-for the space group and structure determination, least-squares refinements on *F*2.

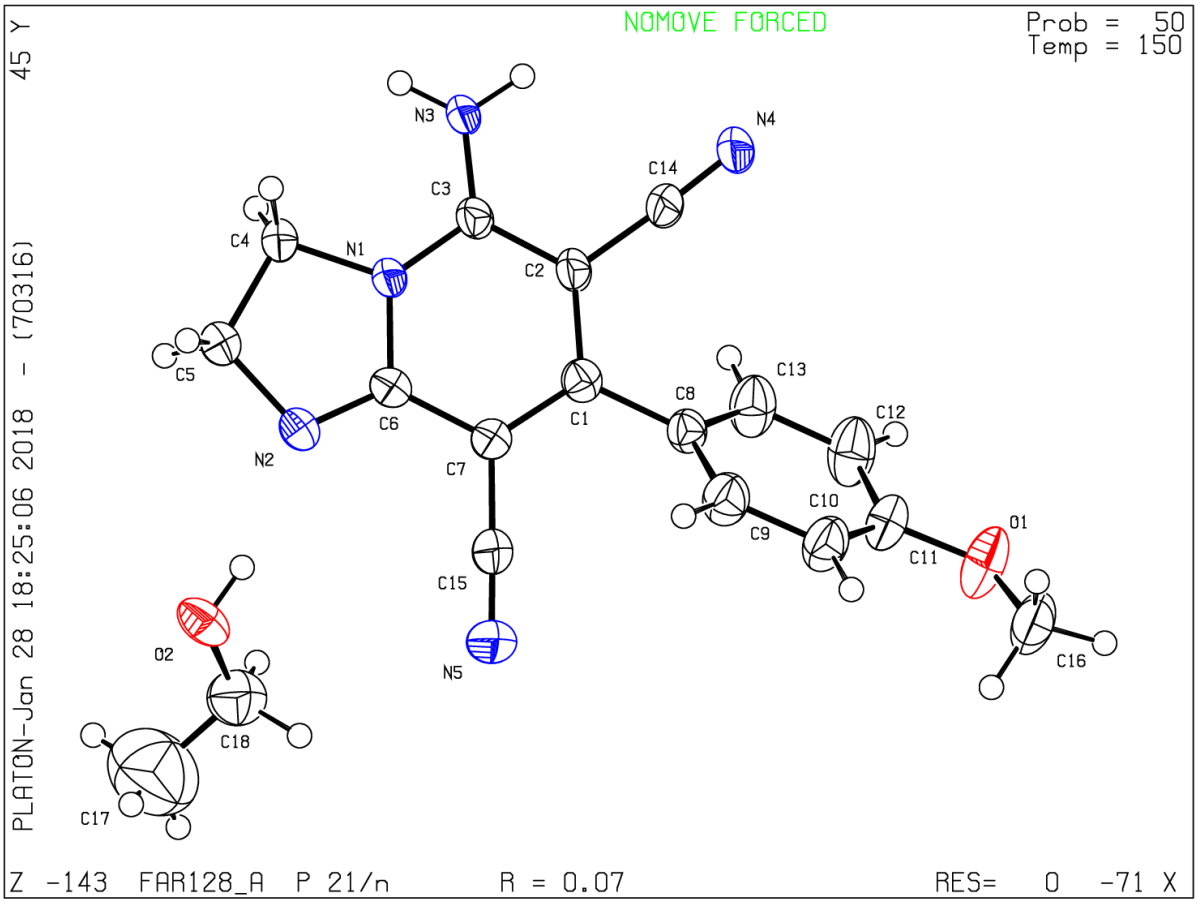
Crystal data for compound 1b: $C_{16}H_{13}N_5$, F_W = 275.31, monoclinic, space group *C*2/*c*, *a* = 15.488(2), *b* = 13.5651(16), *c* = 14.7827(17) Å, β = 117.812(4)°, *V* = 2747.0(6) $Å^3$, *Z* = 8, ρ_{calc} = 1.331 Mg/m^3, μ = 0.084 mm^{-1}, F_{000} = 1152, crystal size 0.180 x 0.160 x 0.160 mm^3, θ range = 2.113-24.996°, 16760 collected reflections, 4767 independent reflections, 98.3 % data completeness, no restraints, 4767 parameters, Goodness-of-fit (F^2) = 1.057, Final *R* indices [$I>2\sigma(I)$]: *R*1 = 0.0881, w*R*2 = 0.2015. *R* indices (all data): *R*1 = 0.1183, w*R*2 = 0.2174. Largest residual electron densities: 0.518 and -0.372 e.$Å^{-3}$. CCDC 1821874.



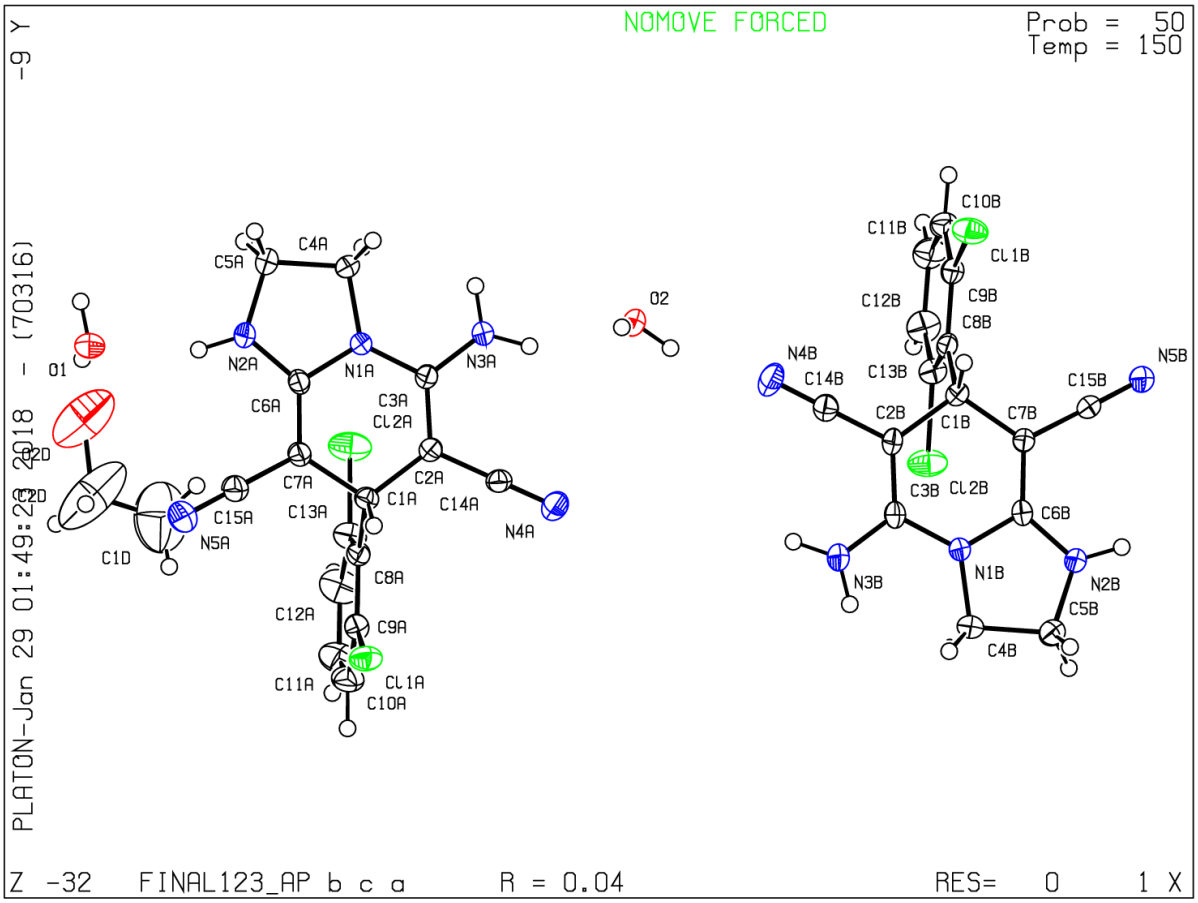
Crystal data for compound 1d: $C_{15}H_{10}FN_5$, F_W = 279.28, monoclinic, space group $P2_1/c$, a = 8.0209(5), b = 19.5305(13), c = 8.0469(4) Å, β = 92.536(3)°, V = 1259.33(13) Å^3, Z = 4, ρ_{calc} = 1.473 Mg/m^3, μ = 0.104 mm^{-1}, F_{000} = 576, crystal size 0.210 x 0.190 x 0.130 mm^3, θ range = 2.740-26.381°, 7761 collected reflections, 2550 independent reflections, 99.3 % data completeness, no restraints, 2550 parameters, Goodness-of-fit (F^2) = 1.043, Final R indices [$I>2\sigma(I)$]: $R1$ = 0.0390, w$R2$ = 0.0996. R indices (all data): $R1$ = 0.0564, w$R2$ = 0.1092. Largest residual electron densities: 0.213 and -0.278 e.Å^{-3}. CCDC 1585393.



Crystal data for compound 1e: $C_{16}H_{13}N_5O,C_2H_6O$, F_W = 337.38, monoclinic, space group $P2_1/n$, a = 6.3986(3), b = 11.1344(6), c = 24.0603(12) Å, β = 93.946(2)°, V = 1710.10(15) Å^3, Z = 4, ρ_{calc} = 1.310 Mg/m^3, μ = 0.089 mm^{-1}, F_{000} = 712, crystal size 0.230 x 0.160 x 0.130 mm^3, θ range = 1.697-25.496°, 23920 collected reflections, 3174 independent reflections, 99.6 % data completeness, no restraints, 3174 parameters, Goodness-of-fit (F^2) = 1.017, Final R indices [$I>2\sigma(I)$]: $R1$ = 0.0664, w$R2$ = 0.1573. R indices (all data): $R1$ = 0.0960, w$R2$ = 0.1811. Largest residual electron densities: 0.596 and -0.480 e.Å^{-3}. CCDC 1819988.

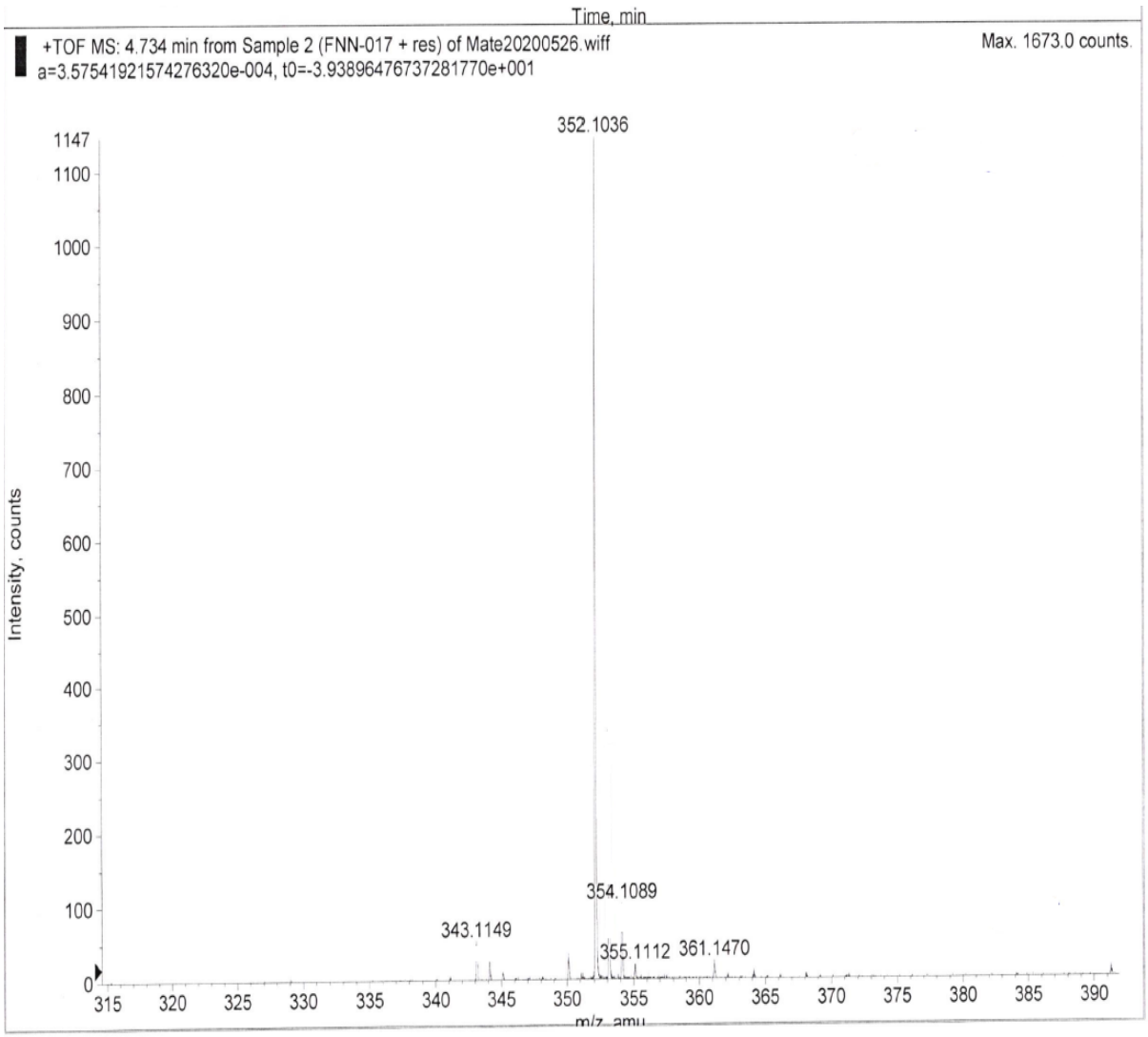


Crystal data for compound 2b: $2(C_{15}H_{11}Cl_2N_5)$, C_2H_6O, $2(H_2O)$, F_W = 746.47, orthorhombic, space group Pbca, a = 17.2195(9), b = 19.0819(11), c = 20.7497(12) Å, β = 90°, V = 6818.0 (7) Å^3, Z = 8, ρ_{calc} = 1.454 Mg/m^3, μ = 0.398 mm^{-1}, F_{000} = 3088, crystal size 0.180 x 0.120 x 0.090 mm^3, θ range = 2.350-25.998°, 95516 collected reflections, 6694 independent reflections, 99.8 % data completeness, no restraints, 6694 parameters, Goodness-of-fit (F^2) = 1.058, Final R indices [$I>2\sigma(I)$]: $R1$ = 0.0436, w$R2$ = 0.1203. R indices (all data): $R1$ = 0.0598, w$R2$ = 0.1338. Largest residual electron densities: 0.733 and -0.592 e.Å^{-3}. CCDC 1820010.

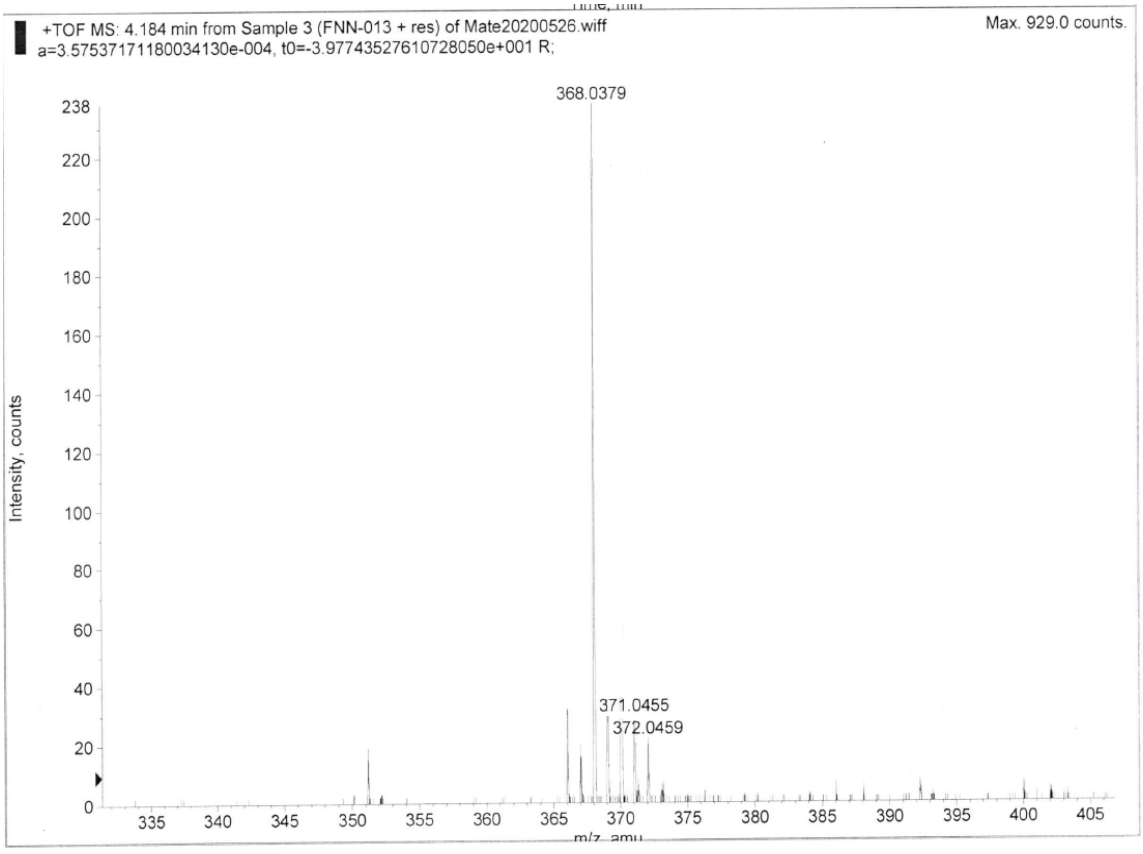


7. HRMS analyses

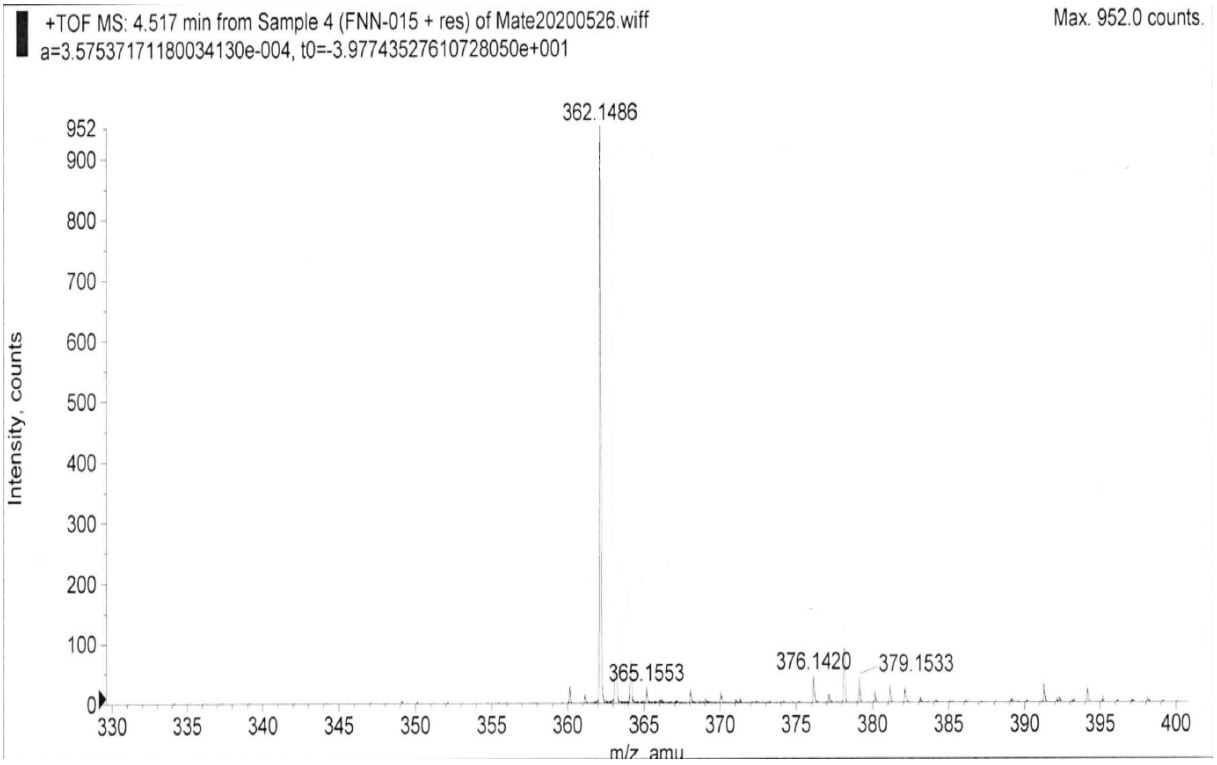
HRMS data have been obtained by Stenhagens Analys AB, Gothenburg, on a Sciex Qstar XL for solutions in acetonitrile/water.



HRMS analysis for **4**



HRMS analysis for **5**



HRMS analysis for **6**