

**Novel biologically active *N*-substituted benzimidazole derived Schiff bases: design,
synthesis and biological evaluation**

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N-hexyl-2-nitroaniline **7**

^{13}C NMR (151 MHz, DMSO- d_6): δ/ppm = 145.7, 137.1, 131.3, 126.7, 115.5, 115.0, 42.7, 31.4, 28.7, 26.5, 22.5, 14.4; Anal. Calcd. for $\text{C}_{12}\text{H}_{18}\text{N}_2\text{O}_2$: C, 64.84; H, 8.16; N, 12.60; O, 14.39. Found: C, 64.89; H, 8.23; N, 12.51; O, 14.29%.

3-N-(hexylamino)-4-nitrobenzonitrile **8**

^{13}C NMR (75 MHz, DMSO- d_6): δ/ppm = 146.8, 130.5, 118.2, 96.0, 42.3, 30.8, 27.9, 25.8, 21.9; Anal. Calcd. for $\text{C}_{13}\text{H}_{17}\text{N}_3\text{O}_2$: C, 63.14; H, 6.93; N, 16.99; O, 12.94. Found: C, 63.20; H, 6.88; N, 16.91; O, 12.89%.

4.1.3. General method for preparation of compounds **15–16**

*N*¹-hexylbenzene-1,2-diamine **15**

^{13}C NMR (151 MHz, DMSO- d_6): δ/ppm = 136.1, 135.0, 117.5, 116.5, 114.0, 109.6, 43.4, 31.2, 28.8, 26.5, 22.1, 13.9; Anal. Calcd. for $\text{C}_{12}\text{H}_{20}\text{N}_2$: C, 74.95; H, 10.48; N, 14.57. Found: C, 74.88; H, 10.54; N, 14.66%.

3-amino-4-N-(hexylamino)benzonitrile **16**

^{13}C NMR (75 MHz, DMSO- d_6): δ/ppm = 140.5, 135.5, 123.4, 121.6, 115.2, 108.8, 96.7, 43.2, 31.6, 28.8, 26.8, 22.6, 14.4; Anal. Calcd. for $\text{C}_{13}\text{H}_{19}\text{N}_3$: C, 71.85; H, 8.81; N, 19.34. Found: C, 71.94; H, 8.87; N, 19.41%.

4.1.4. General method for preparation of compounds **23 and 24**

2-amino-1-hexylbenzimidazole **23**

^{13}C NMR (151 MHz, DMSO- d_6): δ/ppm = 151.6, 131.9, 122.9, 121.9, 112.9, 109.9, 42.4, 31.3, 28.2, 26.0, 22.5, 14.3; Anal. Calcd. for $\text{C}_{13}\text{H}_{19}\text{N}_3$: C, 71.85; H, 8.81; N, 19.34. Found: C, 71.87; H, 8.79; N, 19.29%.

2-amino-6-cyano-1-hexylbenzimidazole **24**

^{13}C NMR (151 MHz, DMSO- d_6): δ/ppm = 157.1, 143.2, 138.3, 121.2, 102.5, 42.1, 31.4, 28.7, 26.1, 22.5; Anal. Calcd. for $\text{C}_{14}\text{H}_{18}\text{N}_4$: C, 69.39; H, 7.49; N, 23.12. Found: C, 69.45; H, 7.40; N, 23.07%.

4.1.5. General method for preparation of Schiff bases 28–45

(E)-4-(((1-isobutyl-1*H*-benzo[d]imidazol-2-yl)imino)methyl)-*N,N*-dimethylaniline **28**

¹³C NMR (151 MHz, DMSO-*d*₆): δ/ppm = 150.4, 131.2 (2C), 129.1 (2C), 124.0, 123.4, 112.0 (2C), 111.2 (3C), 49.1, 27.7, 19.7 (2C); Anal. Calcd. for C₂₀H₂₄N₄: C, 74.97; H, 7.55; N, 17.48. Found: C, 74.95; H, 7.59; N, 17.41%.

(E)-2-((4-(dimethylamino)benzylidene)amino)-1-isobutyl-1*H*-benzo[d]imidazole-6-carbonitrile **29**

¹³C NMR (100 MHz, DMSO-*d*₆) (δ/ppm): 166.2, 159.0, 154.3, 141.6, 138.7, 132.6, 125.2, 122.7, 120.6, 112.1, 104.1, 49.8, 29.3, 20.3; Anal. Calcd. for C₂₁H₂₃N₅: C, 73.02; H, 6.71; N, 20.27. Found: C, 73.10; H, 6.68; N, 20.32%.

(E)-5-(diethylamino)-2-(((1-isobutyl-1*H*-benzo[d]imidazol-2-yl)imino)methyl)phenol **30**

¹³C NMR (151 MHz, DMSO-*d*₆): δ/ppm = 163.5, 153.8, 152.9, 135.0, 121.8, 121.3, 117.9, 111.2, 110.0, 108.5, 105.0, 104.4, 96.6, 95.9, 49.4, 44.1 (2C), 28.9, 19.9 (2C), 12.5 (2C); Anal. Calcd. for C₂₂H₂₈N₄O: C, 72.50; H, 7.74; N, 15.37; O, 4.39. Found: C, 72.48; H, 7.70; N, 15.32; O, 4.44%.

(E)-2-((4-(diethylamino)-2-hydroxybenzylidene)amino)-1-isobutyl-1*H*-benzo[d]imidazole-6-carbonitrile **31**

¹³C NMR (151 MHz, DMSO-*d*₆): δ/ppm = 164.3, 153.9 (2C), 141.6, 138.7, 125.3, 122.6, 120.6, 111.8 (2C), 109.1, 105.9 (2C), 104.1, 97.0 (2C), 50.0, 44.7, 29.4, 20.3, 13.0; Anal. Calcd. for C₂₃H₂₇N₅O: C, 70.92; H, 6.99; N, 17.98; O, 4.11. Found: C, 70.97; H, 6.91; N, 17.93; O, 4.08%.

(E)-1-isobutyl-2-((4-nitrobenzylidene)amino)-1*H*-benzo[d]imidazole-6-carbonitrile **32**

¹³C NMR (151 MHz, DMSO-*d*₆) (δ/ppm): 165.2, 156.2, 149.8, 140.6, 140.2, 138.2, 130.9 (2C), 125.7, 124.2 (2C), 123.9, 119.8, 112.4, 104.6, 49.6, 29.0, 19.8 (2C); Anal. Calcd. for C₁₉H₁₇N₅O₂: C, 65.69; H, 4.93; N, 20.16; O, 9.21. Found: C, 65.72; H, 4.87; N, 20.09; O, 9.34%.

(E)-*N,N*-dimethyl-4-(((1-methyl-1*H*-benzo[d]imidazol-2-yl)imino)methyl)aniline **33**

¹³C NMR (151 MHz, DMSO-*d*₆): δ/ppm = 190.4, 154.7, 132.0, 125.0, 123.8, 123.1, 112.2, 112.1, 111.6, 111.0, 110.4, 19.8, 9.1 (2C); Anal. Calcd. for C₁₇H₁₈N₄: C, 71.27; H, 5.65; N, 23.09. Found: C, 71.30; H, 5.61; N, 23.16%.

(E)-2-((4-(dimethylamino)benzylidene)amino)-1-methyl-1*H*-benzo[d]imidazole-6-carbonitrile **34**

¹³C NMR (100 MHz, DMSO-*d*₆) (δ/ppm): 166.4, 158.9, 154.3, 141.7, 139.0, 132.8, 125.2, 122.9, 122.8, 122.6, 120.7, 118.2, 112.0, 111.6, 108.8, 104.1, 29.5 (2C), 29.1; Anal. Calcd. for C₁₈H₁₇N₅: C, 71.27; H, 5.65; N, 23.09. Found: C, 71.30; H, 5.61; N, 23.16%.

(E)-5-(diethylamino)-2-(((1-methyl-1*H*-benzo[d]imidazol-2-yl)imino)methyl)phenol **35**

¹³C NMR (151 MHz, DMSO-*d*₆): δ/ppm = 164.5, 163.9, 153.4, 141.9 (2C), 135.8, 122.3, 121.7, 118.3, 110.2, 109.1, 105.5, 97.0, 44.6 (2C), 29.1, 13.0 (2C); Anal. Calcd. for C₁₉H₂₂N₄O: C, 70.78; H, 6.88; N, 17.38; O, 4.96. Found: C, 70.71; H, 6.79; N, 17.44; O, 5.03%.

(E)-2-((4-(diethylamino)-2-hydroxybenzylidene)amino)-1-methyl-1*H*-benzo[d]imidazole-6-carbonitrile **36**

¹³C NMR (100 MHz, DMSO-*d*₆) (δ/ppm): 165.3, 164.2, 157.6, 153.9, 141.7, 138.9, 125.2, 122.4, 120.7, 111.5, 109.2, 105.9, 104.1, 96.9, 44.7 (2C), 29.5, 13.0 (2C); Anal. Calcd. for C₂₀H₂₁N₅O: C, 69.14; H, 6.09; N, 20.16; O, 4.61. Found: C, 69.19; H, 5.98; N, 20.13; O, 4.71%.

(E)-1-methyl-2-((4-nitrobenzylidene)amino)-1*H*-benzo[d]imidazole-6-carbonitrile **37**

¹³C NMR (151 MHz, DMSO-*d*₆): δ/ppm = 165.9, 156.7, 150.4, 141.2, 140.7, 139.1, 131.5 (2C), 126.2, 124.7 (2C), 124.3, 120.3, 112.6, 105.1, 29.9; Anal. Calcd. for C₁₆H₁₁N₅O₂: C, 62.95; H, 3.63; N, 22.94; O, 10.48. Found: C, 62.91; H, 3.69; N, 22.88; O, 10.41%.

(E)-*N,N*-dimethyl-4-(((1-phenyl-1*H*-benzo[d]imidazol-2-yl)imino)methyl)aniline **38**

¹³C NMR (151 MHz, DMSO-*d*₆): δ/ppm = 165.5, 156.2, 153.9, 142.1, 135.9, 135.6, 132.3, 129.7 (2C), 128.2, 127.4 (2C), 123.1, 122.9, 122.6, 118.9, 112.0, 110.4; Anal. Calcd. for C₂₂H₂₀N₄: C, 77.62; H, 5.92; N, 16.46. Found: C, 77.68; H, 5.98; N, 16.39%.

(E)-2-((4-(dimethylamino)benzylidene)amino)-1-phenyl-1*H*-benzo[d]imidazole-6-carbonitrile **39**

¹³C NMR (151 MHz, DMSO-*d*₆): δ/ppm = 167.1, 158.6, 154.4, 141.9, 138.6, 135.0, 129.8 (2C), 128.8, 127.5 (2C), 126.1, 123.2, 122.6, 120.4, 112.0, 111.7, 105.0; Anal. Calcd. for C₂₃H₁₉N₅: C, 75.59; H, 5.24; N, 19.16. Found: C, 75.65; H, 5.30; N, 19.09%.

(E)-5-(diethylamino)-2-(((1-phenyl-1*H*-benzo[d]imidazol-2-yl)imino)methyl)phenol **40**

¹³C NMR (75 MHz, DMSO-*d*₆) (δ/ppm): 164.4, 163.5, 153.0, 141.6, 135.6, 135.1, 135.0, 129.7 (2C), 128.2, 126.9 (2C), 122.6, 122.1, 118.3, 109.7, 108.5, 105.0, 96.5, 44.1, 12.5; Anal. Calcd. for C₂₄H₂₄N₄O: C, 74.97; H, 6.29; N, 14.57; O, 4.16. Found: C, 74.89; H, 6.19; N, 14.51; O, 4.20%.

(E)-2-((4-(diethylamino)-2-hydroxybenzylidene)amino)-1-phenyl-1H-benzo[d]imidazole-6-carbonitrile **41**

¹³C NMR (100 MHz, DMSO-*d*₆) (δ/ppm): 165.8, 164.4, 156.8, 154.0, 142.0, 138.6, 136.4, 134.7, 130.3, 129.3, 127.8, 126.2, 122.9, 120.4, 111.5, 109.1, 105.9, 105.0, 96.9, 44.7 (2C), 13.0 (2C); Anal. Calcd. for C₂₅H₂₃N₅O: C, 73.33; H, 5.66; N, 17.10; O, 3.91. Found: C, 73.36; H, 5.69; N, 17.15; O, 4.02%.

(E)-4-(((1-hexyl-1H-benzo[d]imidazol-2-yl)imino)methyl)-N,N-dimethylaniline **42**

42a: ¹³C NMR (151 MHz, DMSO-*d*₆) (δ/ppm): 165.8, 153.8, 137.8, 124.7, 122.4 (2C), 122.4, 122.2, 120.1, 117.7, 111.5 (2C), 111.1 (2C), 108.4, 103.6, 41.9, 30.5, 28.9, 25.5, 21.9, 13.8; **42b**: ¹³C NMR (151 MHz, DMSO-*d*₆) (δ/ppm): 156.6, 142.8, 141.2 (2C), 132.1, 123.0, 128.1, 122.4 (2C), 120.7, 117.7 (2C), 111.1, 111.0, 102.0, 41.6, 30.9, 28.2, 25.6, 22.0, 13.8; Anal. Calcd. for C₂₂H₂₈N₄: C, 75.82; H, 8.10; N, 16.08. Found: C, 75.78; H, 8.16; N, 16.14%.

(E)-2-((4-(dimethylamino)benzylidene)amino)-1-hexyl-1H-benzo[d]imidazole-6-carbonitrile **43**

¹³C NMR (151 MHz, DMSO-*d*₆) (δ/ppm): 165.77, 158.29, 156.63, 153.81, 142.75, 141.22, 137.80, 137.78, 132.15, 129.99, 128.12, 124.68, 122.38, 122.35, 122.20, 120.65, 120.12, 117.68, 111.64, 111.54, 111.14, 111.03, 108.41, 103.57, 101.96, 41.96, 41.60, 30.85, 30.50, 28.90, 28.23, 25.57, 25.50, 21.96, 21.90, 13.80, 13.75; Anal. Calcd. for C₂₃H₂₇N₅: C, 73.96; H, 7.29; N, 18.75. Found: C, 74.01; H, 7.24; N, 18.70%.

(E)-5-(diethylamino)-2-(((1-hexyl-1H-benzo[d]imidazol-2-yl)imino)methyl)phenol **44**

¹³C NMR (100 MHz, DMSO-*d*₆) (δ/ppm): 164.0, 163.9, 154.6, 154.3, 153.4, 141.9, 135.1, 122.2, 121.8, 118.4, 111.7, 110.2, 109.0, 105.5, 104.9, 97.0, 96.4, 44.6, 44.6, 42.6, 31.2, 29.6, 26.4, 22.5, 14.2, 13.0, 12.9; Anal. Calcd. for C₂₄H₃₂N₄O: C, 73.43; H, 8.22; N, 14.27; O, 4.08. Found: C, 73.37; H, 8.09; N, 14.15; O, 4.02%.

(E)-2-((4-(diethylamino)-2-hydroxybenzylidene)amino)-1-hexyl-1H-benzo[d]imidazole-6-carbonitrile **45**

¹³C NMR (151 MHz, DMSO-*d*₆) (δ/ppm): 164.3, 153.9, 141.7, 138.3, 125.2, 122.6, 120.6, 111.5, 109.1, 105.9 (2C), 104.1, 96.9 (2C), 44.7 (2C), 42.9, 31.1, 29.5, 26.3, 22.4, 14.2, 13.0; Anal. Calcd. for C₂₅H₃₁N₅O: C, 71.91; H, 7.48; N, 16.77; O, 3.83. Found: C, 71.94; H, 7.42; N, 16.71; O, 3.88%.

4.2. Biology

4.2.1. Antiviral activity

HEL 299 (ATCC CCL-137; human lung fibroblast), Huh-7 (CLS – 300156; human hepatoblastoma), and MDCK (Madin-Darby canine kidney cells; a kind gift from M. Matrosovich, Marburg, Germany) were maintained in Dulbecco's Modified Eagle Medium (DMEM, Gibco Life Technologies) supplemented with 8% heat-inactivated fetal bovine serum (HyClone, GE Healthcare Life Sciences), 0.075% sodium bicarbonate (Gibco Life Technologies) and 1mM sodium pyruvate (Gibco Life Technologies), and maintained at 37°C under 5% CO₂. Antiviral assays towards herpes simplex virus-1 (HSV-1 KOS), human coronavirus (HCoV-229E and -OC43) and respiratory syncytial virus A in HEL 299 cell cultures, sindbis virus, yellow fever virus, Zika virus and human coronavirus (HCoV-NL63) in Huh-7 cell cultures and influenza A/H1N1 (A/Ned/378/05), influenza A/H3N2 (A/HK/7/87), influenza B (B/Ned/537/05) in MDCK cell cultures were performed. On the day of the infection, growth medium was aspirated and replaced by serial dilutions of the test compounds. The virus was then added to each well, diluted to obtain a viral input of 100 CCID₅₀ (CCID₅₀ being the virus dose that is able to infect 50% of the cell cultures). Mock-treated cultures receiving solely the test compounds were included, to determine the cytotoxicity. After 3 to 7 days of incubation, the virus-induced cytopathogenic effect was measured colorimetrically by the formazan-based MTS cell viability assay (CellTiter 96 AQueous One Solution Cell Proliferation Assay from Promega, Madison, WI), and the antiviral activity was expressed as the 50% effective concentration (EC₅₀). In parallel, the 50% cytotoxic concentration (CC₅₀) was derived from the mock-infected cells. The activities were compared with the activities of reference antiviral drugs: remdesivir, ribavirin, zanamivir, rimantadine and brivudine (BVDU).

4.2.2. Antibacterial activity

4.2.2.1. Materials

In addition to the synthesized compounds, standard antibiotics ampicillin, ceftazidime, ciprofloxacin and meropenem from USP were tested. Selected bacterial strains were gram negative *E. coli*, *K. pneumoniae*, *A. baumannii* and *P. aeruginosa* and gram positive *S. aureus*, *S. pneumoniae* and *E. faecalis*. Synthesized compounds were prepared as 10 mM DMSO solutions and tested in a final concentration range of 100 - 0.2 µM.ⁱ

Standard antibiotics were prepared as 5 mg/mL DMSO solutions and tested in a final concentration range of 64 – 0.125 µg/mL.

4.2.2.2. Methods

Broth microdilution testing was performed according to CLSI (Clinical Laboratory Standards Institute) guidelines. MIC (minimal inhibitory concentration) value was defined as the last tested concentration of compound at which there is no visible growth of bacteria. Inoculums for each microorganism were prepared using the direct colony suspension method where broth solutions that achieved turbidity equivalent to 0.5 McFarland standard were additionally diluted 100x with Ca adjusted MH media (Becton Dickinson). All test plates were incubated for 16 - 24h at 37 °C. MIC values for reference antibiotics against quality control strains were used for confirming the validity of the screen according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically M07, 11th edition, 2018. and Clinical and Laboratory Standards Institute (CLSI) guidelines. Performance standards for antimicrobial susceptibility testing M100, 28th edition, 2018.

4.2.3 Cell culture and reference compounds

Human cancer cells used in this manuscript, namely Capan-1, HCT-116, NCI-H460, LN-229, HL-60, K-562 and Z-138 were acquired from the American Type Culture Collection (ATCC, Manassas, VA, USA), while the DND-41 cell line was purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ Leibniz-Institut, Germany). Culture media were purchased from Gibco Life Technologies, USA, and supplemented with 10% fetal bovine serum (HyClone, GE Healthcare Life Sciences, USA). Vincristine and *docetaxel*, which were used as reference inhibitors, were purchased from Selleckchem (Munich, Germany). Stock solutions were prepared in DMSO.

4.2.4 Proliferation assays

Adherent cell lines LN-229, HCT-116, NCI-H460 and Capan-1 cells were seeded at a density between 500 and 1500 cells per well, in 384-well tissue culture plates (Greiner). After overnight incubation, cells were treated with seven different concentrations of the test compounds, ranging from 100 to 0.006 μ M. Suspension cell lines HL-60, K-562, Z-138 and DND-41 were seeded at densities ranging from 2500 to 5500 cells per well in 384-well culture plates containing the test compounds at the same concentration points.

Cells were incubated for 72 hours with compounds and were then analysed using the CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS) reagent (Promega) according to the manufacturer's instructions. Absorbance of the samples was measured at 490 nm using a SpectraMax Plus 384 (Molecular Devices), and OD values were used to calculate the 50% inhibitory concentration (IC_{50}). Compounds were tested in two independent experiments.

Figure S1. ^1H NMR spectrum (DMSO- d_6 , 400 MHz) of *N*-hexyl-2-nitroaniline **7**

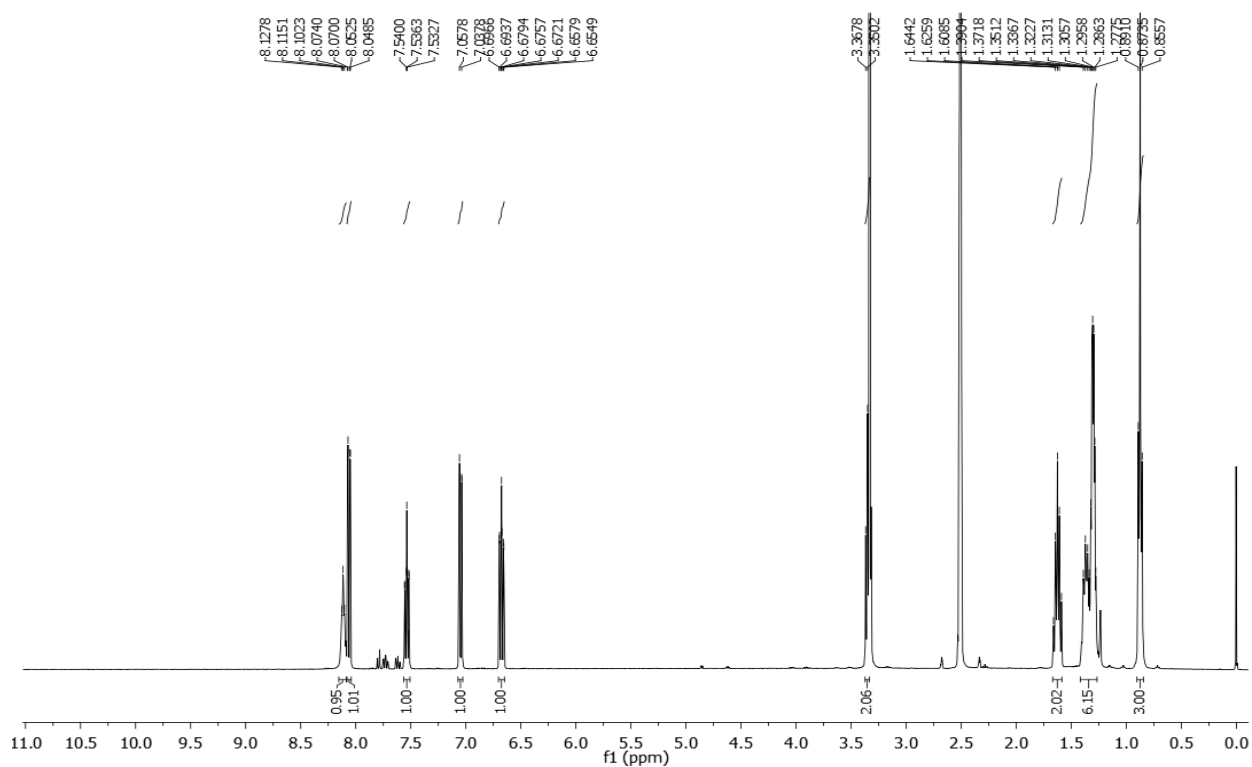


Figure S2. ^{13}C NMR spectrum (DMSO- d_6 , 151 MHz) of *N*-hexyl-2-nitroaniline **7**

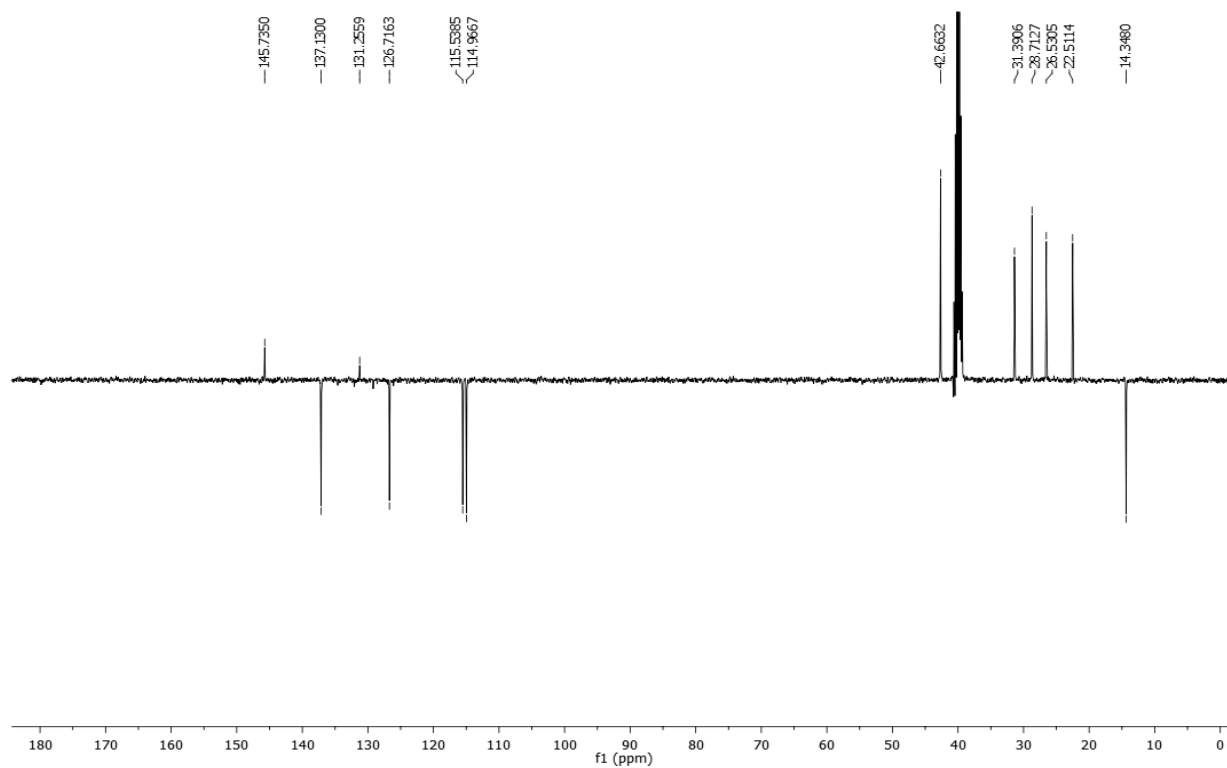


Figure S3. ^1H NMR spectrum (DMSO- d_6 , 400 MHz) of 3-*N*-(hexylamino)-4-nitrobenzonitrile **8**

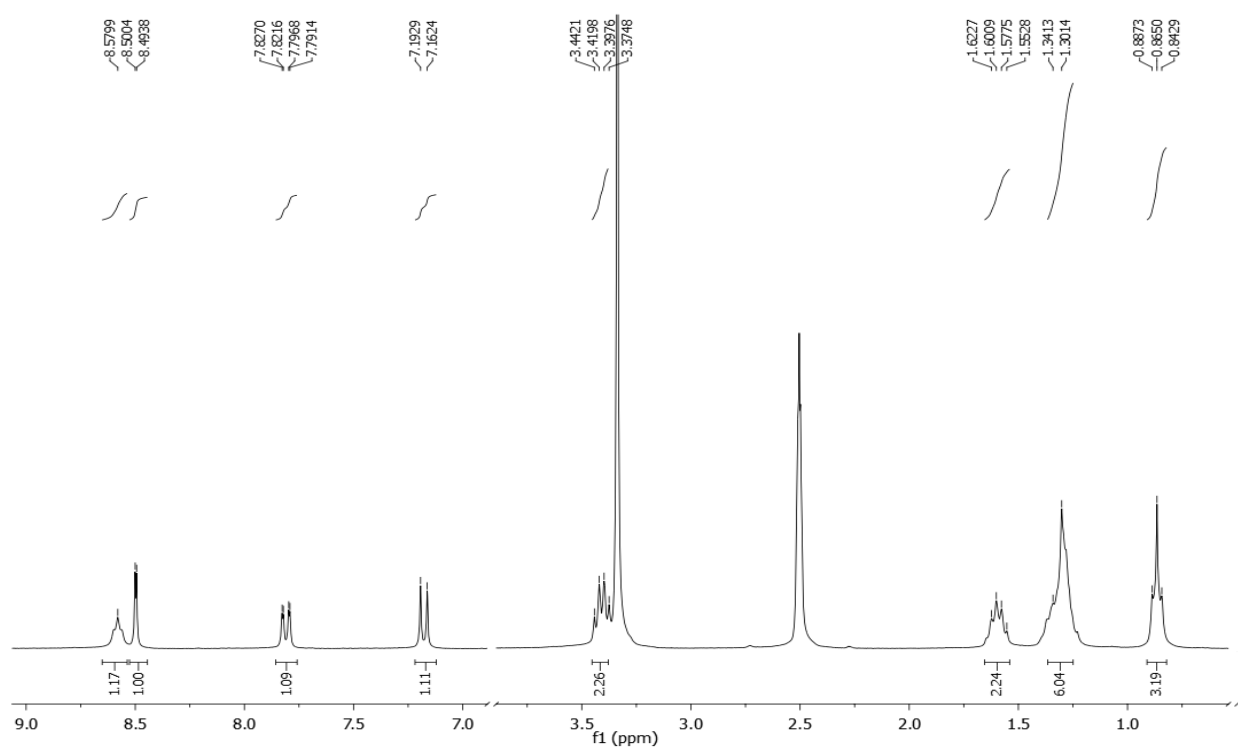


Figure S4. ^{13}C NMR spectrum (DMSO- d_6 , 75 MHz) of 3-*N*-(hexylamino)-4-nitrobenzonitrile **8**

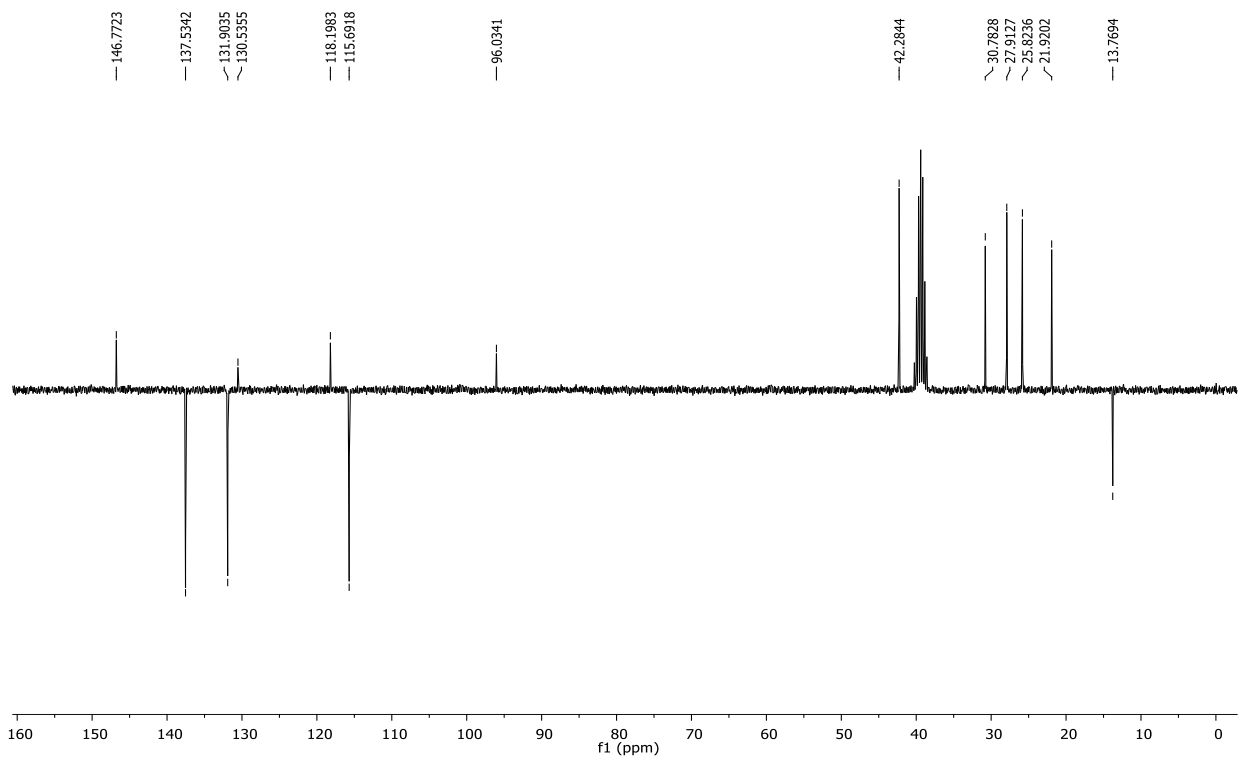


Figure S5. ^1H NMR spectrum (DMSO- d_6 , 600 MHz) of *N*¹-hexylbenzene-1,2-diamine **10**

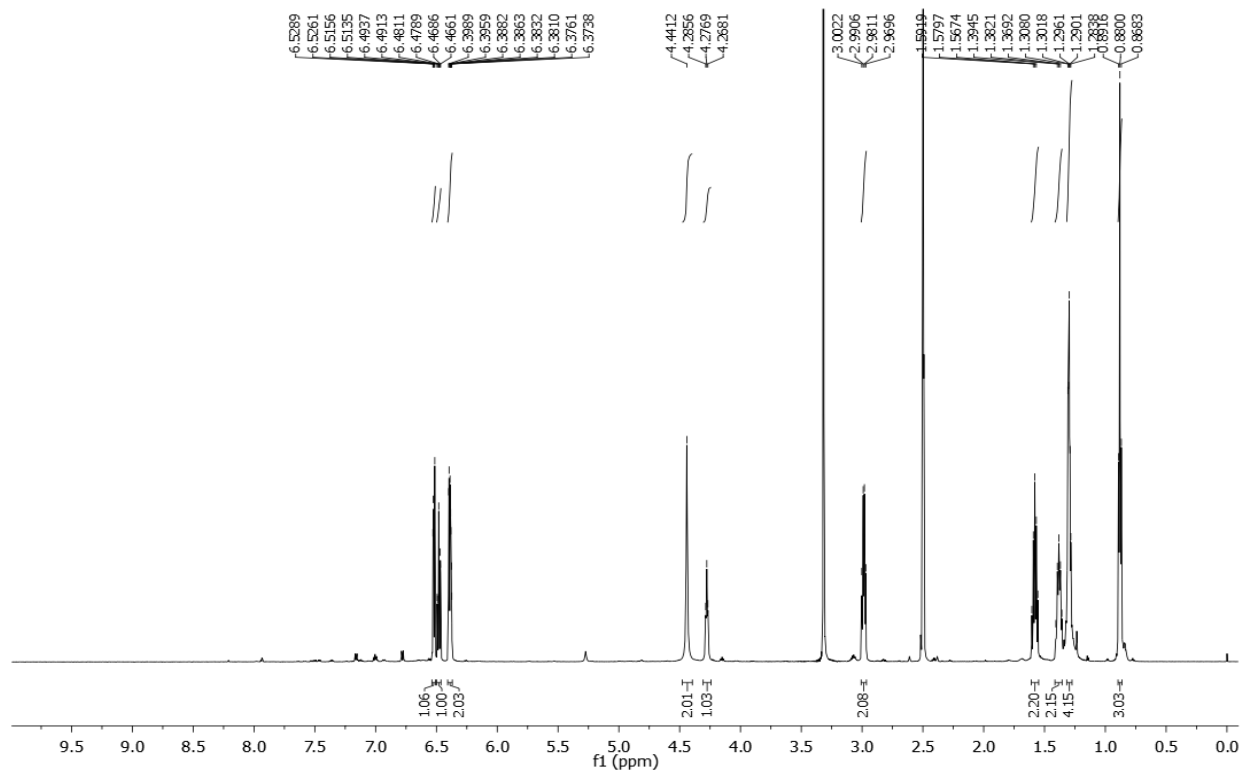


Figure S6. ¹³C NMR spectrum (DMSO-*d*₆, 151 MHz) of *N*¹-hexylbenzene-1,2-diamine 10

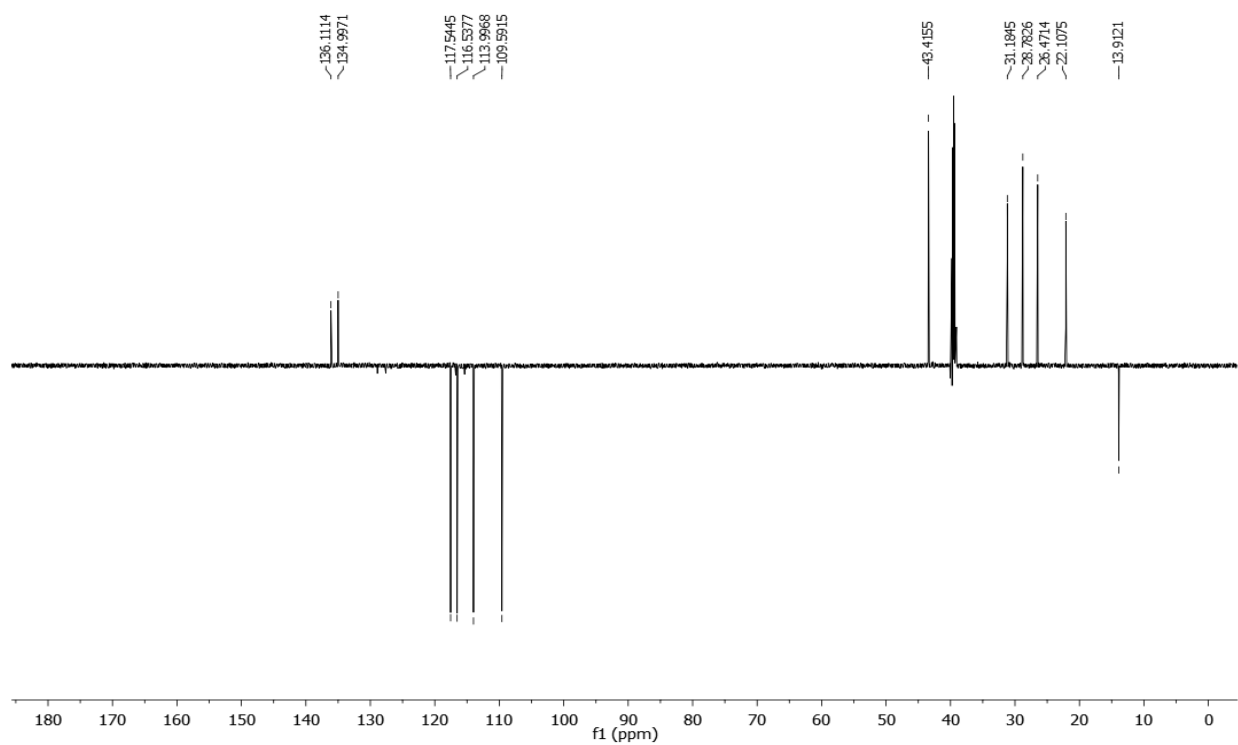


Figure S7. ^1H NMR spectrum (DMSO- d_6 , 600 MHz) of *3-amino-4-N-(hexylamino)benzonitrile 16*

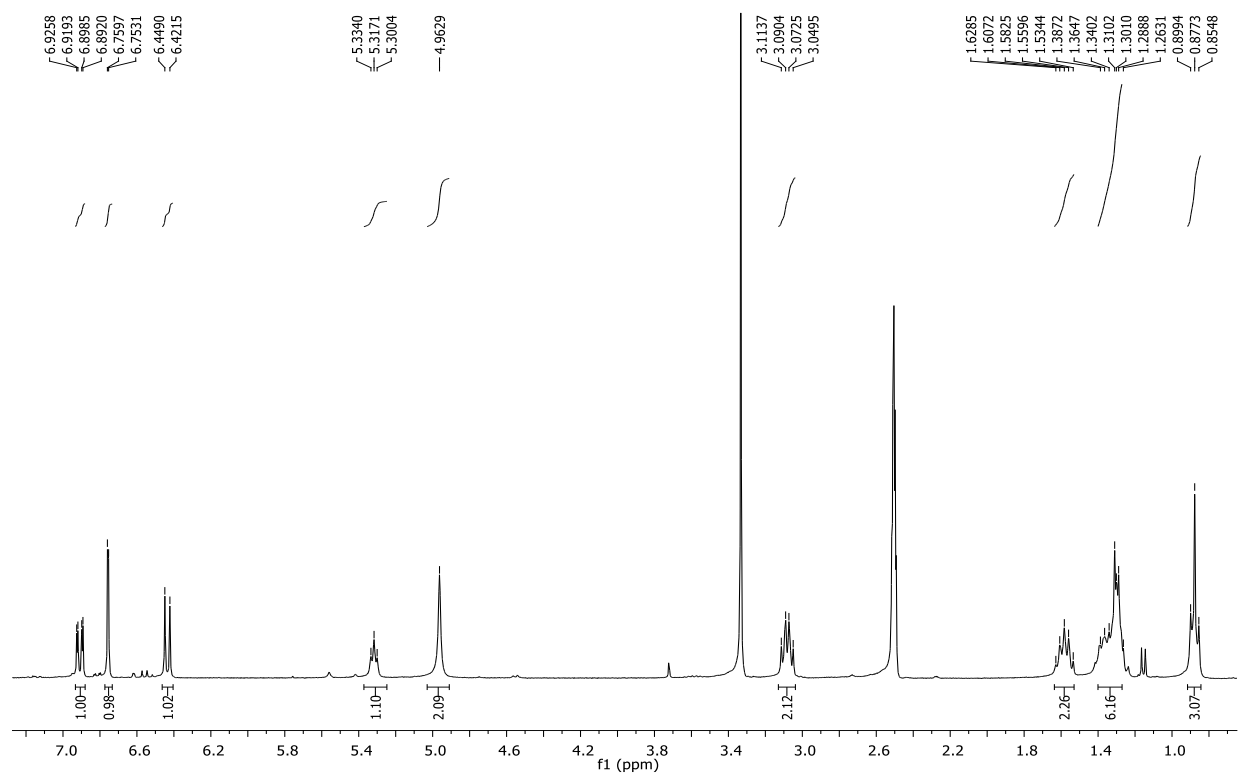


Figure S8. ^{13}C NMR spectrum (DMSO- d_6 , 75 MHz) of *3-amino-4-N-(hexylamino)benzonitrile 16*

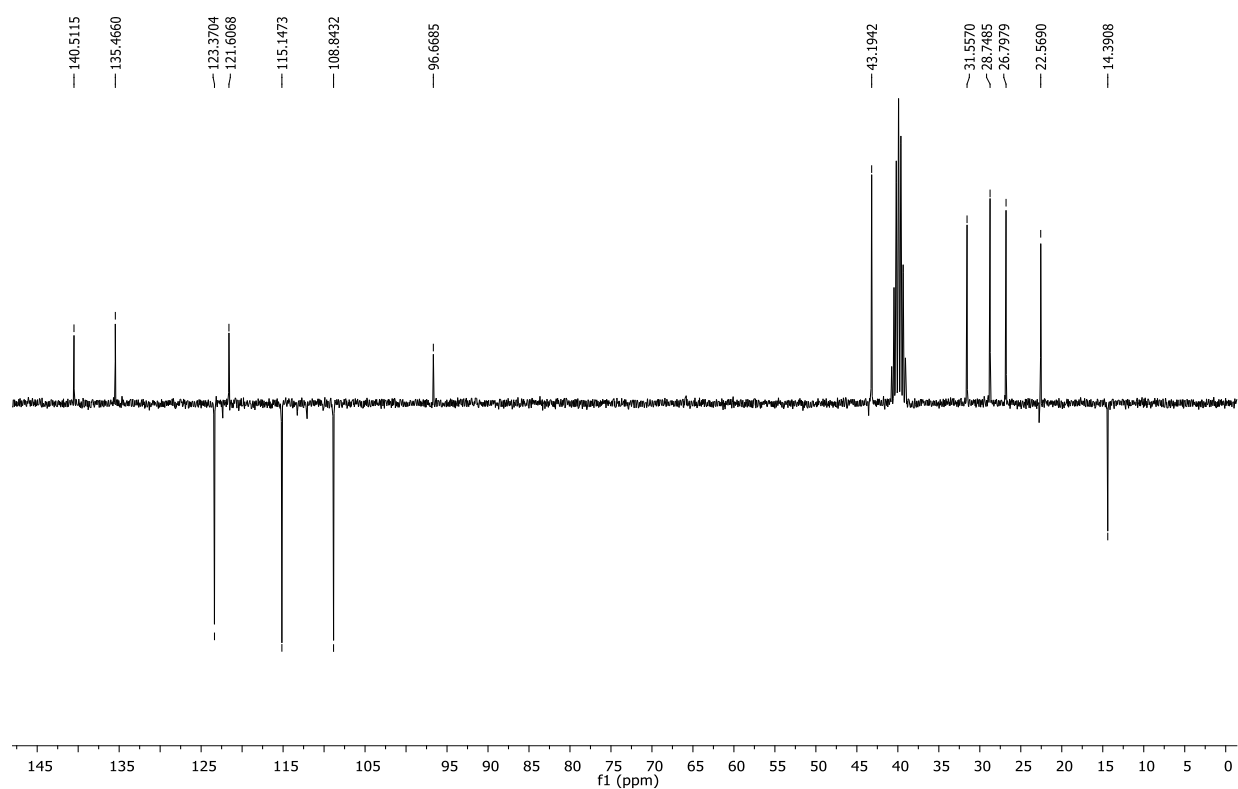


Figure S9. ^1H NMR spectrum (DMSO- d_6 , 600 MHz) of *2-amino-1-hexylbenzimidazole 23*

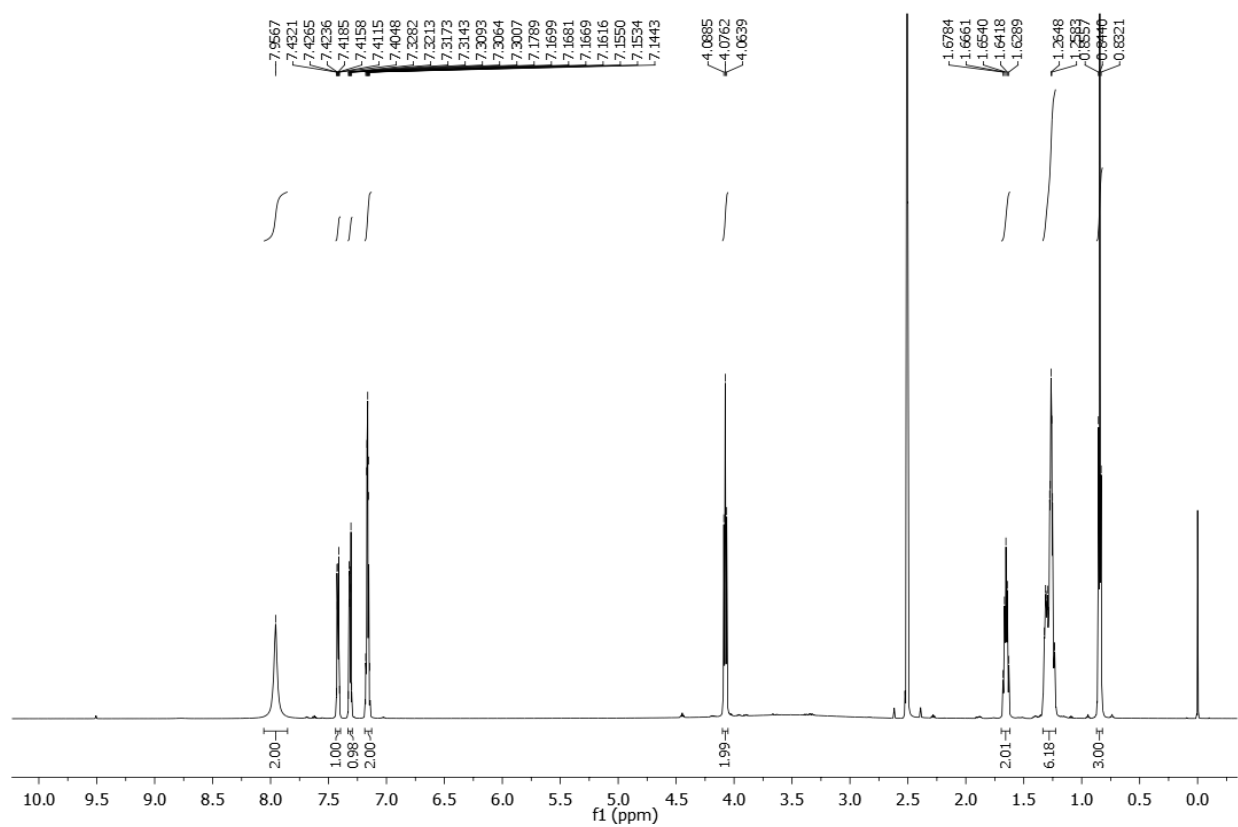


Figure S10. ^{13}C NMR spectrum (DMSO- d_6 , 151 MHz) of *2-amino-1-hexylbenzimidazole 23*

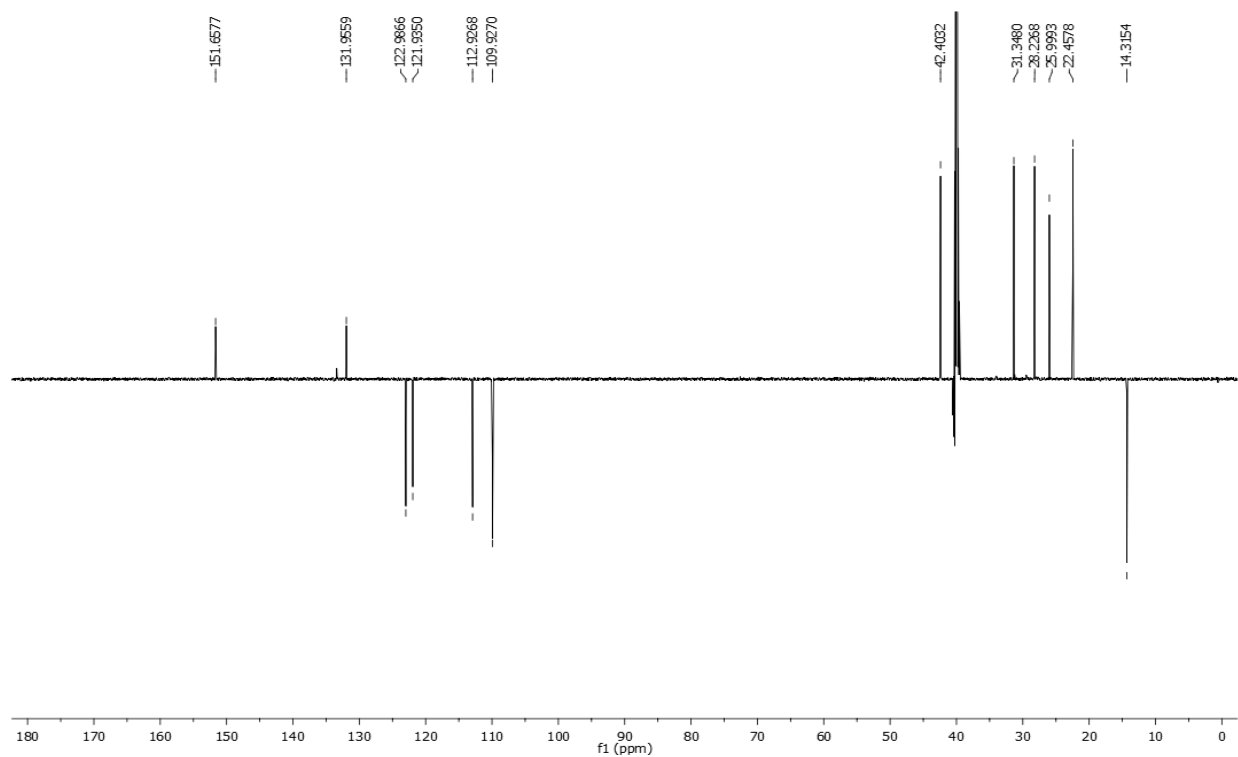


Figure S11. ^1H NMR spectrum (DMSO- d_6 , 600 MHz) of **2-amino-6-cyano-1-hexylbenzimidazole** **24**

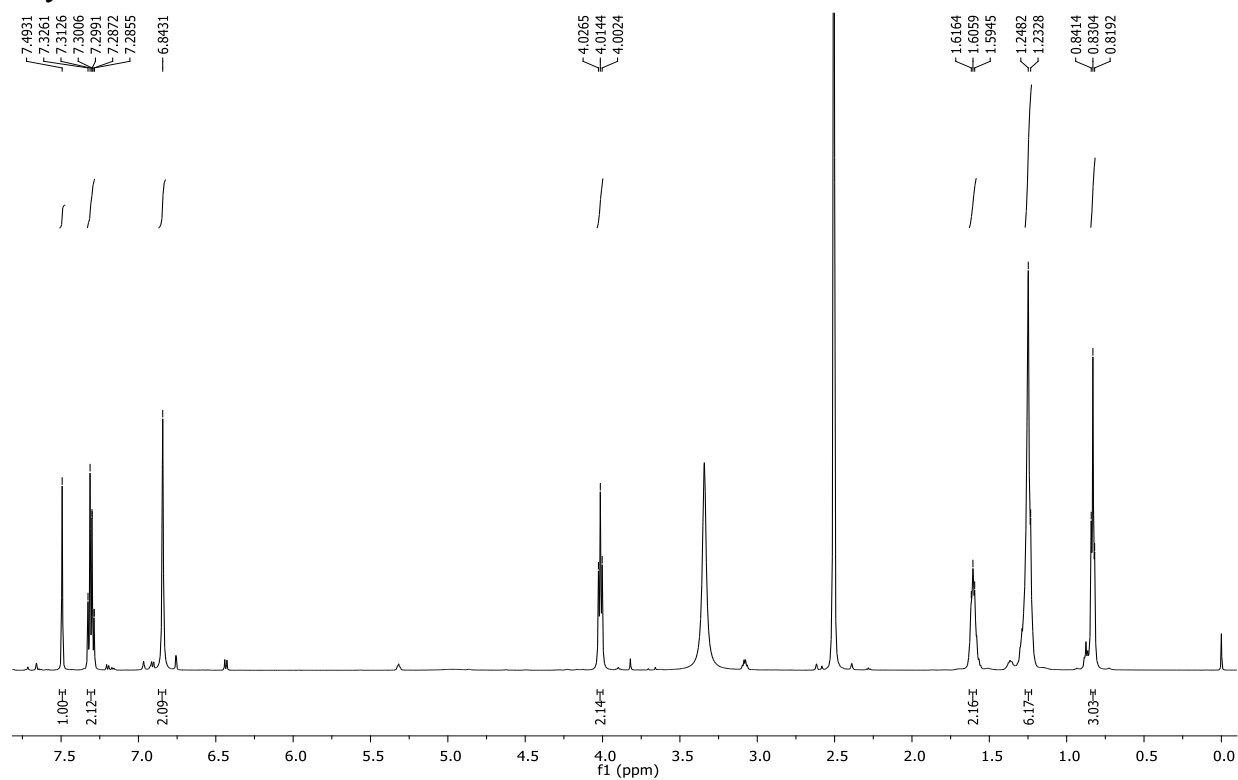


Figure S12. ^{13}C NMR spectrum (DMSO- d_6 , 151 MHz) of **2-amino-6-cyano-1-hexylbenzimidazole** **24**

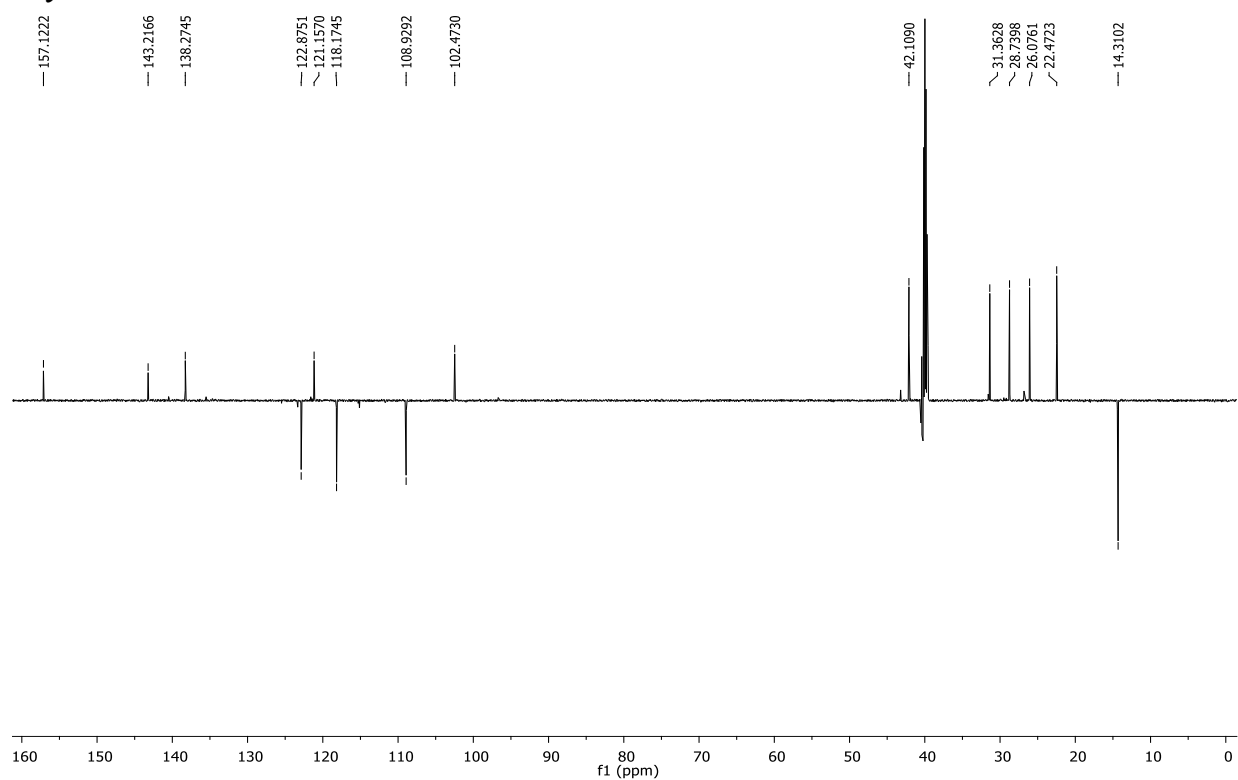


Figure S13. ^1H NMR spectrum (DMSO- d_6 , 600 MHz) of *(E)*-4-(((1-isobutyl-1*H*-benzo[d]imidazol-2-yl)imino)methyl)-*N,N*-dimethylaniline **28**

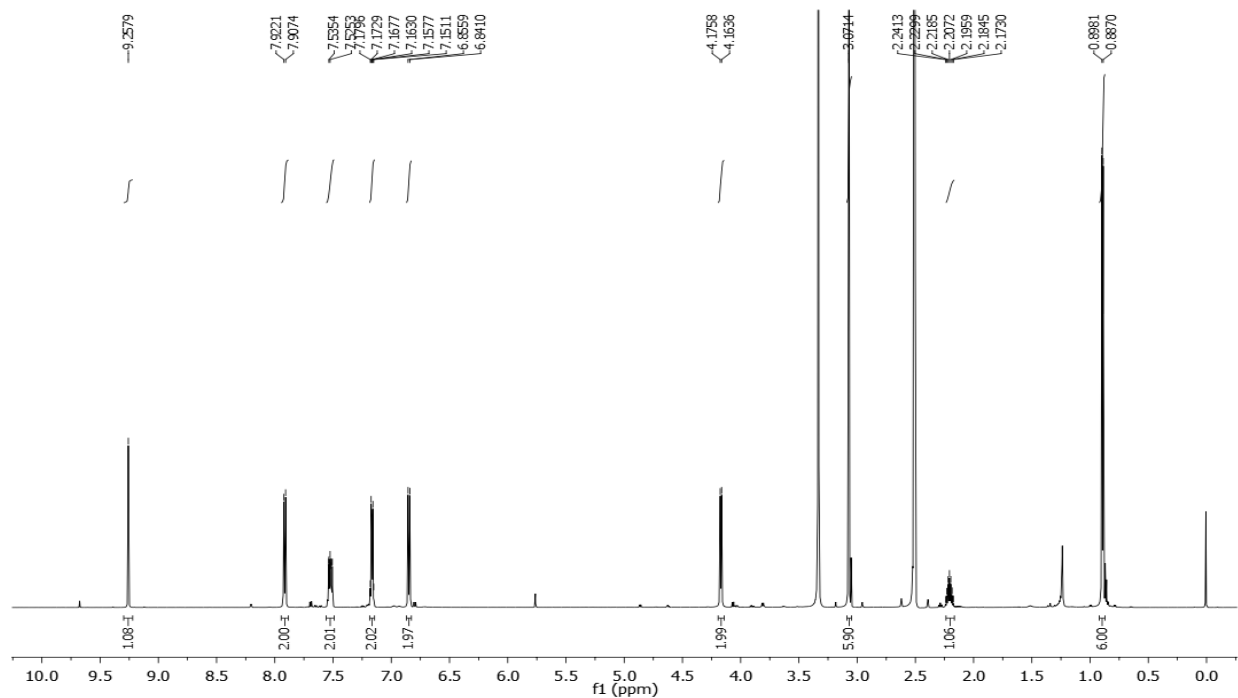


Figure S14. ^{13}C NMR spectrum (DMSO- d_6 , 151 MHz) of *(E)*-4-(((1-isobutyl-1*H*-benzo[d]imidazol-2-yl)imino)methyl)-*N,N*-dimethylaniline **28**

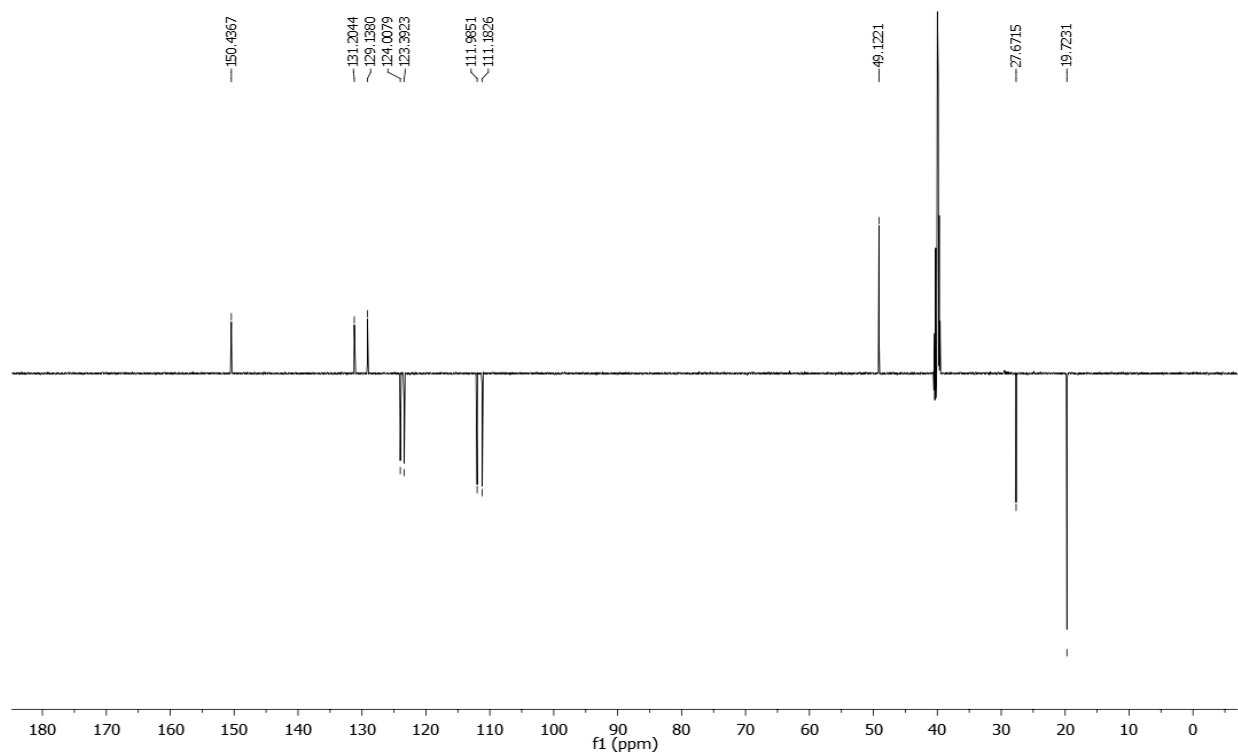


Figure S15. ^1H NMR spectrum (DMSO- d_6 , 400 MHz) of *E(Z)*-2-((4-(dimethylamino)benzylidene)amino)-1-isobutyl-1H-benzo[d]imidazole-6-carbonitrile **29**

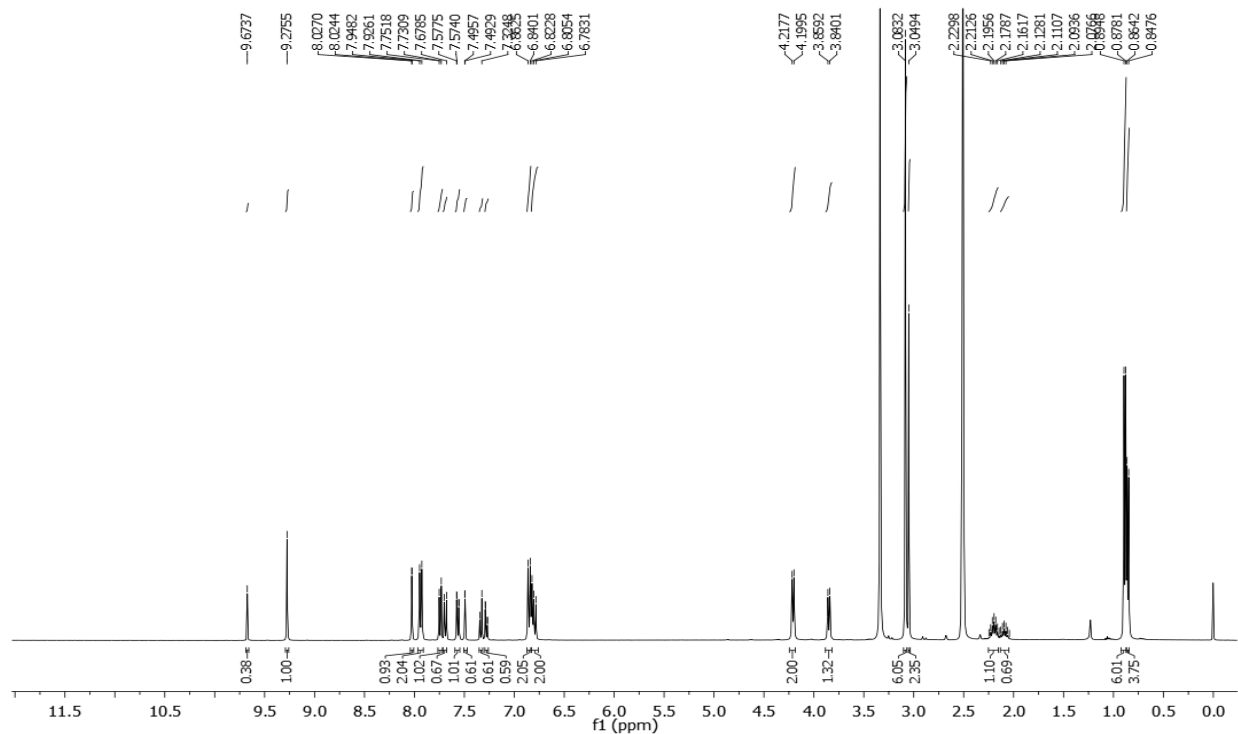


Figure S16. ^{13}C NMR spectrum (DMSO- d_6 , 100 MHz) of *E(Z)*-2-((4-(dimethylamino)benzylidene)amino)-1-isobutyl-1H-benzo[d]imidazole-6-carbonitrile **29**

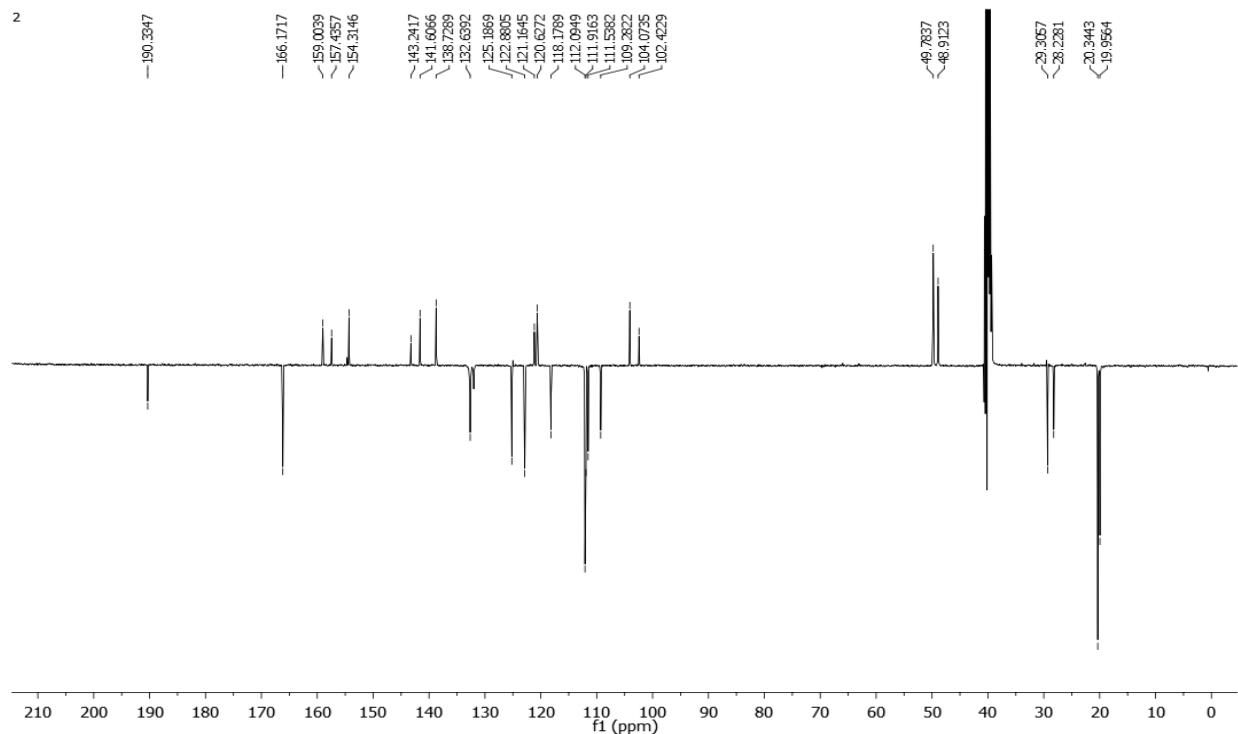


Figure S17. ^1H NMR spectrum (DMSO- d_6 , 300 MHz) of (*E*)-5-(diethylamino)-2-(((1-isobutyl-1*H*-benzo[d]imidazol-2-yl)imino)methyl)phenol 30

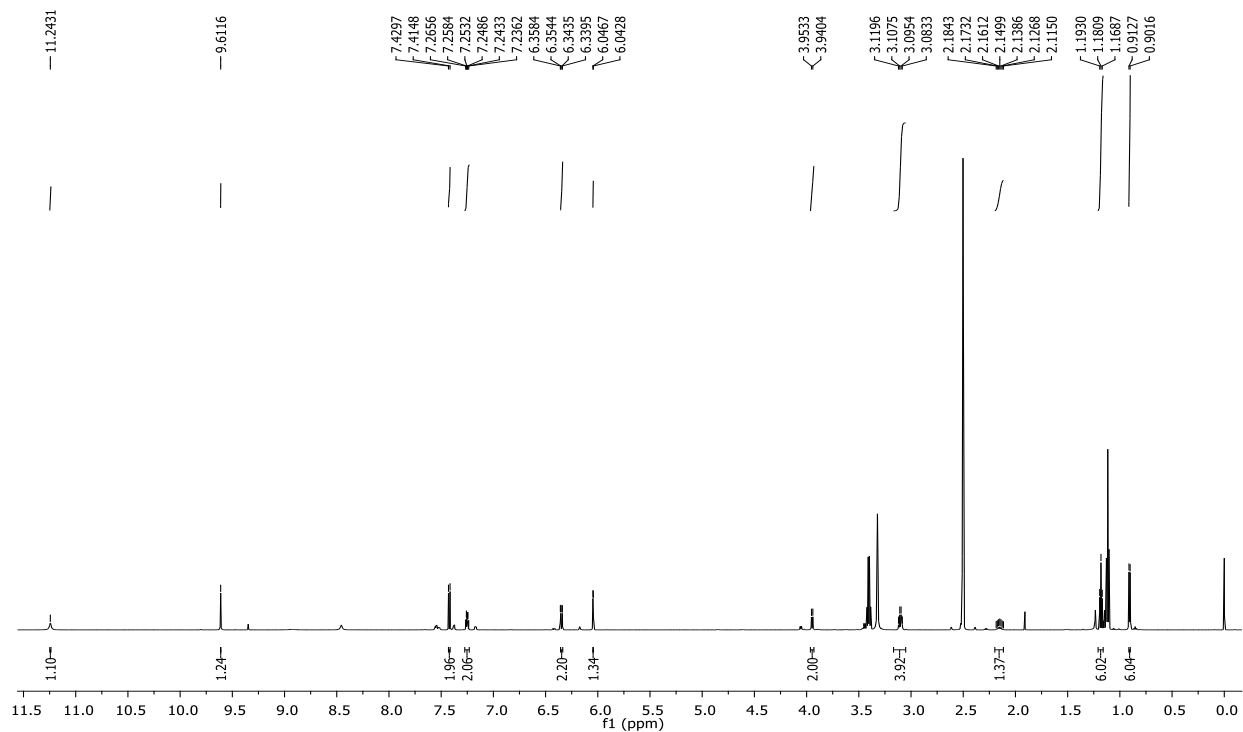


Figure S18. ^{13}C NMR spectrum (DMSO- d_6 , MHz) of (*E*)-5-(diethylamino)-2-(((1-isobutyl-1*H*-benzo[d]imidazol-2-yl)imino)methyl)phenol 30

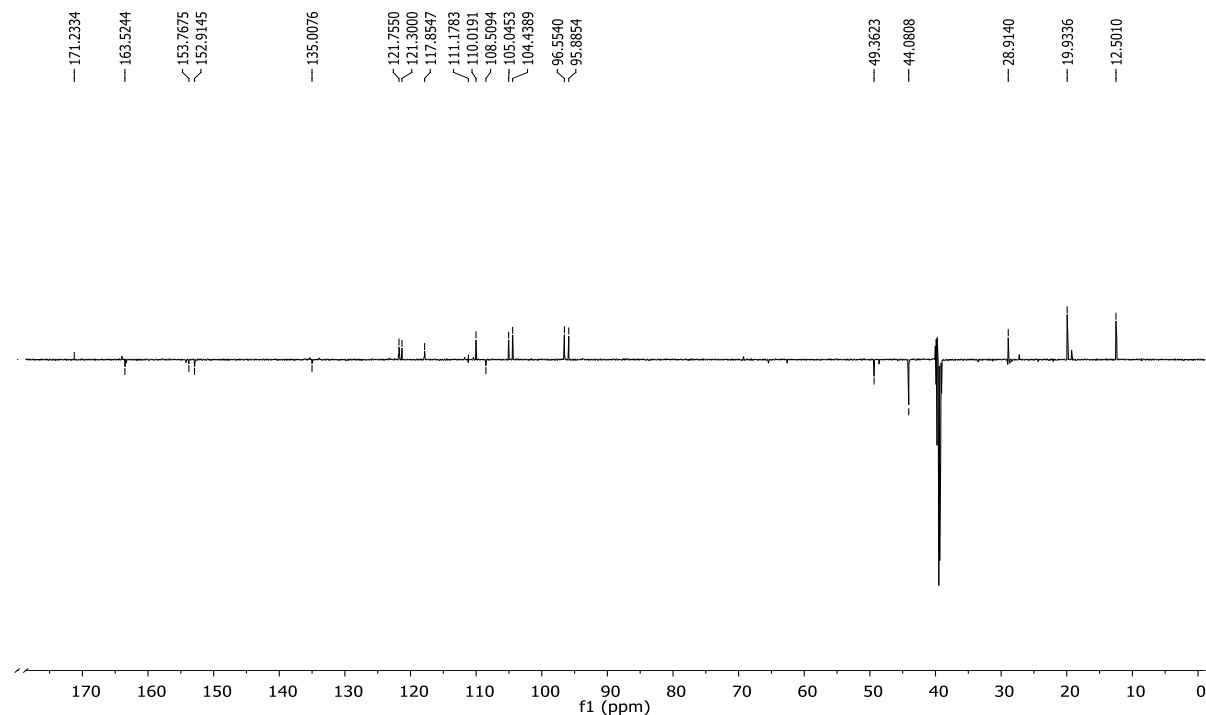


Figure S19. ^1H NMR spectrum ($\text{DMSO-}d_6$, 600 MHz) of *(E)*-2-((4-(diethylamino)-2-hydroxybenzylidene)amino)-1-isobutyl-1*H*-benzo[d]imidazole-6-carbonitrile **31**

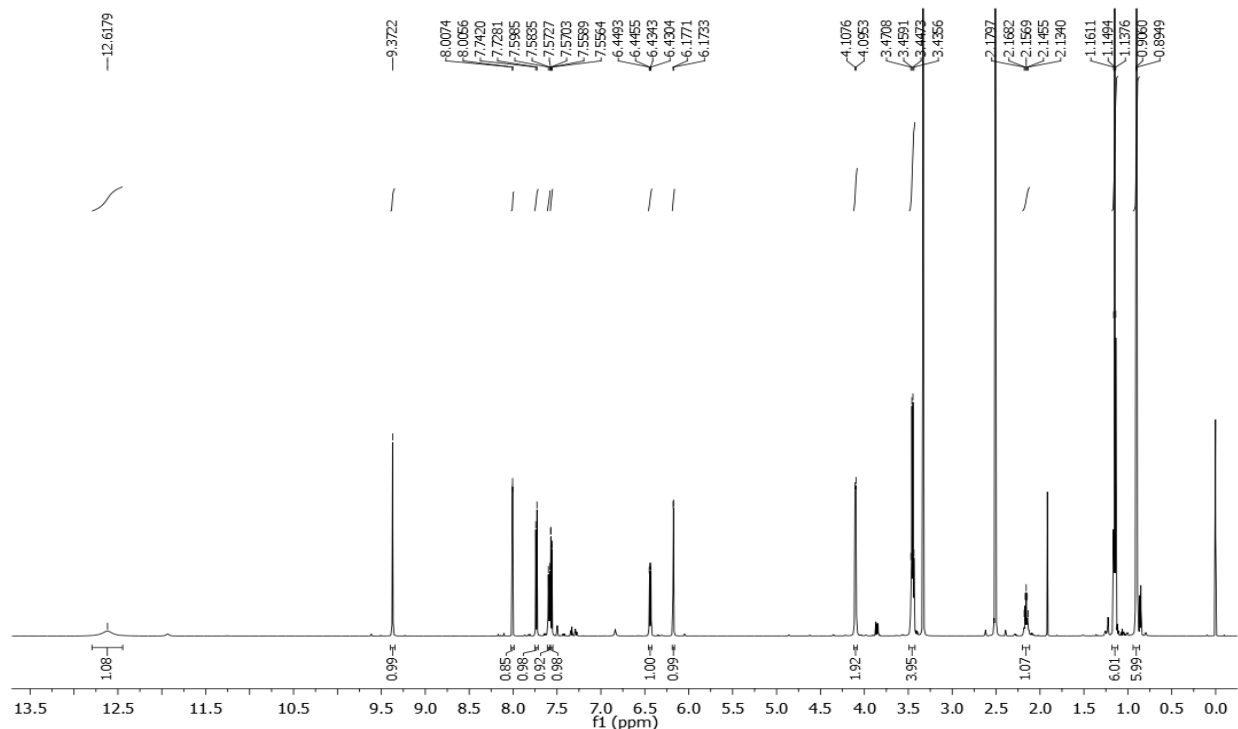


Figure S20. ^{13}C NMR spectrum ($\text{DMSO-}d_6$, 151 MHz) of *(E)*-2-((4-(diethylamino)-2-hydroxybenzylidene)amino)-1-isobutyl-1*H*-benzo[d]imidazole-6-carbonitrile **31**

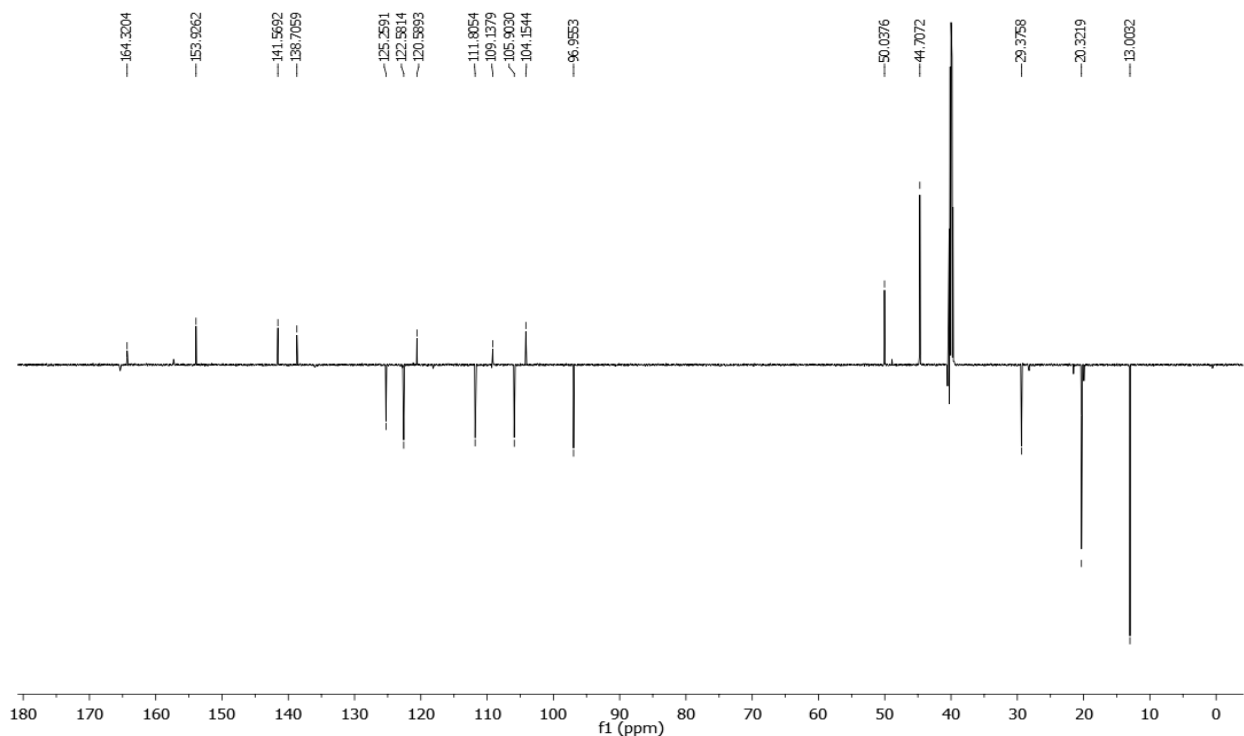
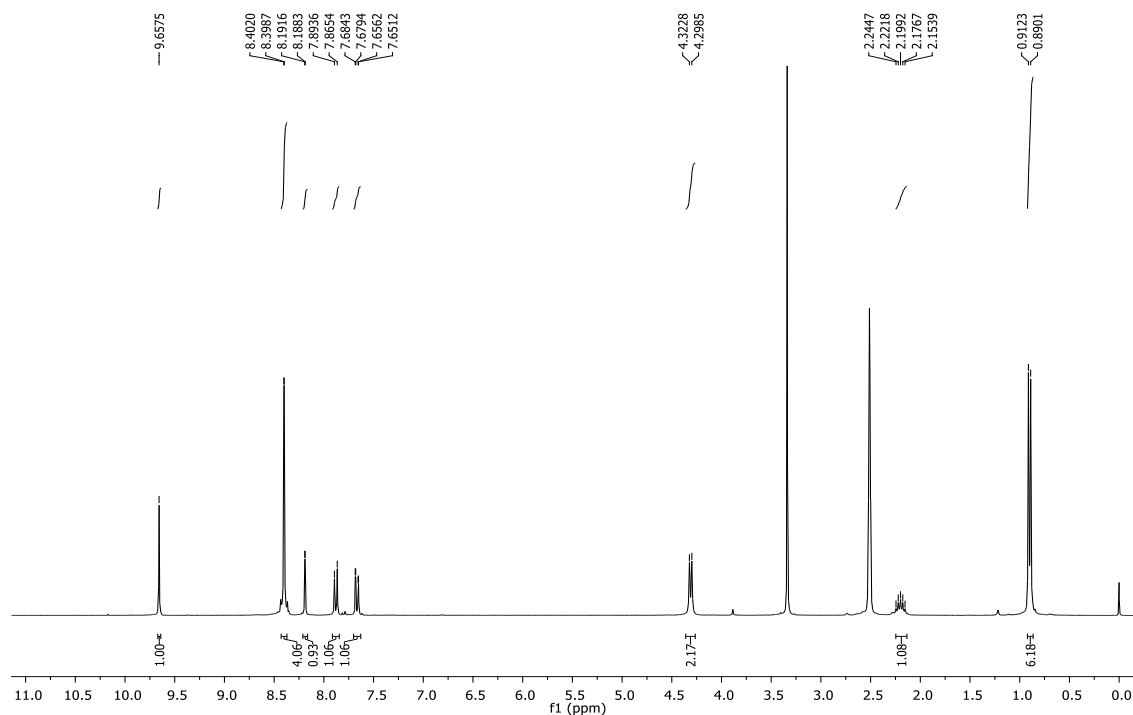


Figure S21. ^1H NMR spectrum (DMSO- d_6 , 300 MHz) of *(E)*-1-isobutyl-2-((4-nitrobenzylidene)amino)-1H-benzo[d]imidazole-6-carbonitrile 32



Figure

S22. ^{13}C NMR spectrum (DMSO- d_6 , 151 MHz) of *(E)*-1-isobutyl-2-((4-nitrobenzylidene)amino)-1H-benzo[d]imidazole-6-carbonitrile 32

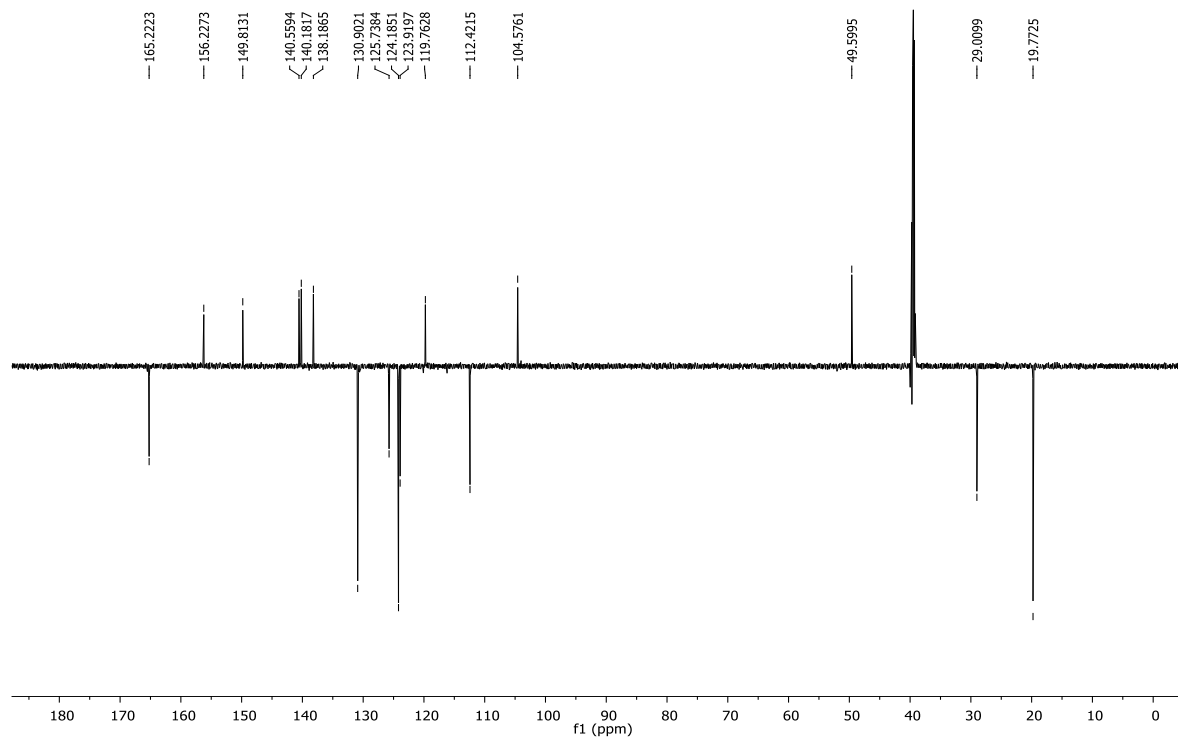


Figure S23. ^1H NMR spectrum (DMSO- d_6 , 600 MHz) of (*E*)-*N,N*-dimethyl-4-(((1-methyl-1*H*-benzo[d]imidazol-2-yl)imino)methyl)aniline **33**

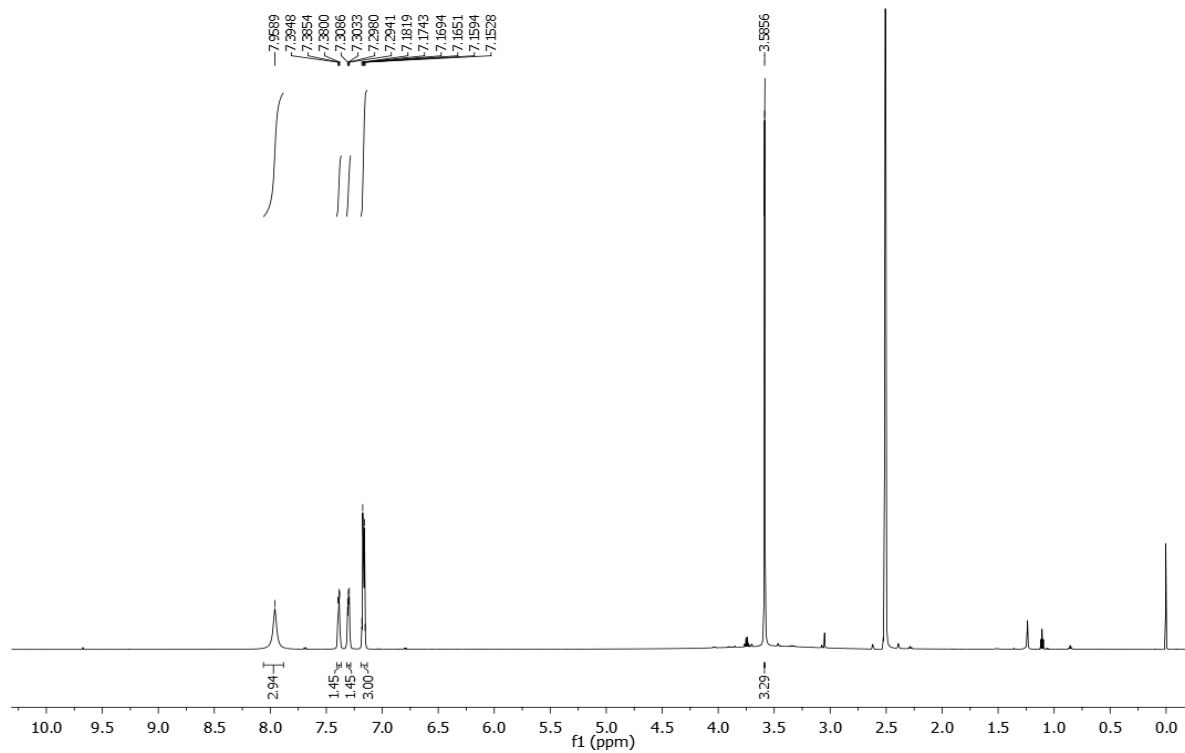


Figure S24. ^{13}C NMR spectrum (DMSO- d_6 , MHz) of (*E*)-*N,N*-dimethyl-4-(((1-methyl-1*H*-benzo[d]imidazol-2-yl)imino)methyl)aniline **33**

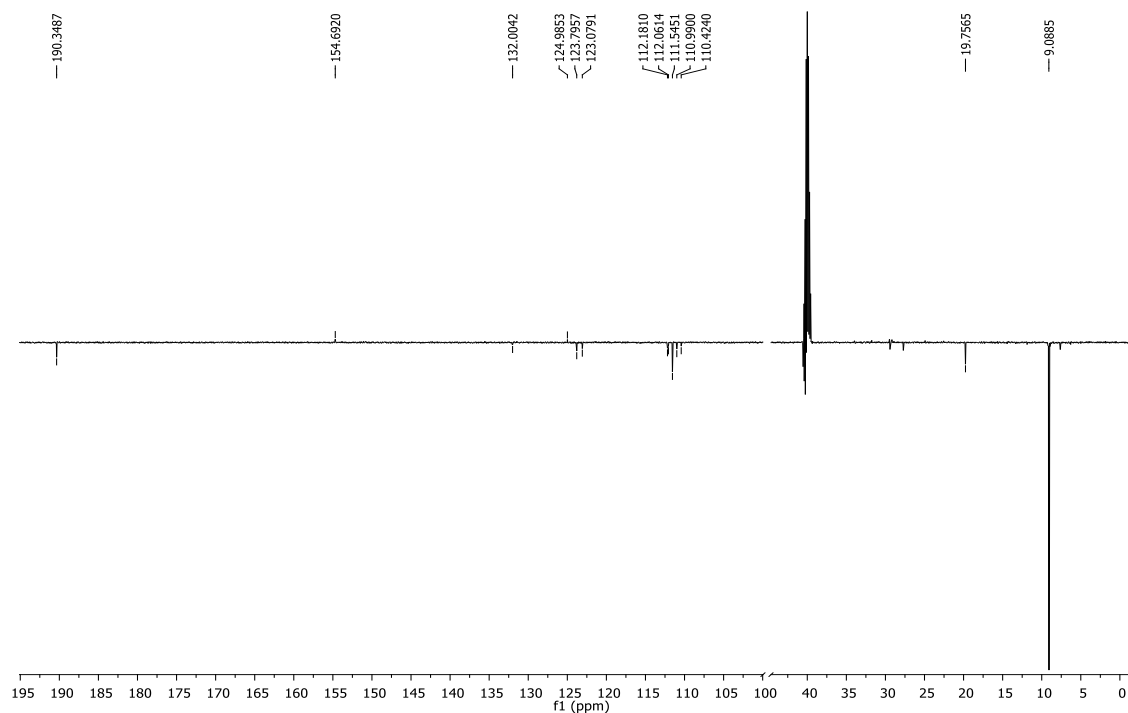


Figure S25. ^1H NMR spectrum (DMSO- d_6 , 400 MHz) of *(E)*-2-((4-(dimethylamino)benzylidene)amino)-1-methyl-1H-benzo[d]imidazole-6-carbonitrile **34**

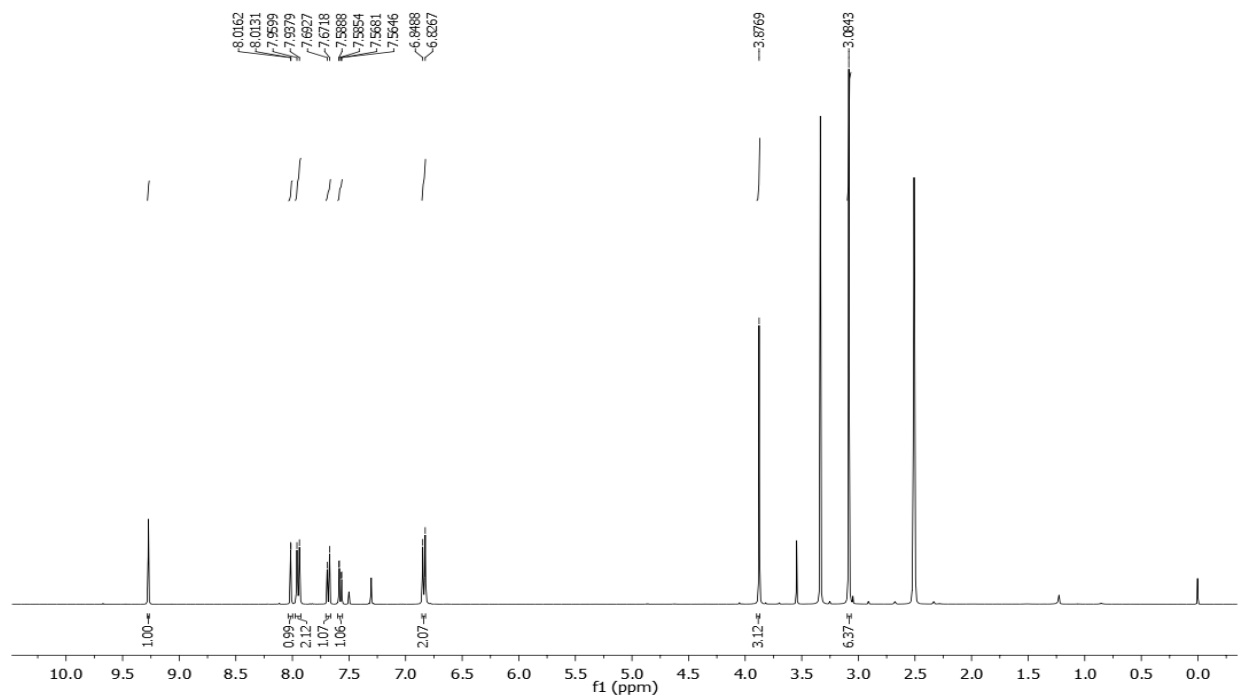


Figure S26. ^{13}C NMR spectrum (DMSO- d_6 , 100 MHz) of *(E)*-2-((4-(dimethylamino)benzylidene)amino)-1-methyl-1H-benzo[d]imidazole-6-carbonitrile **34**

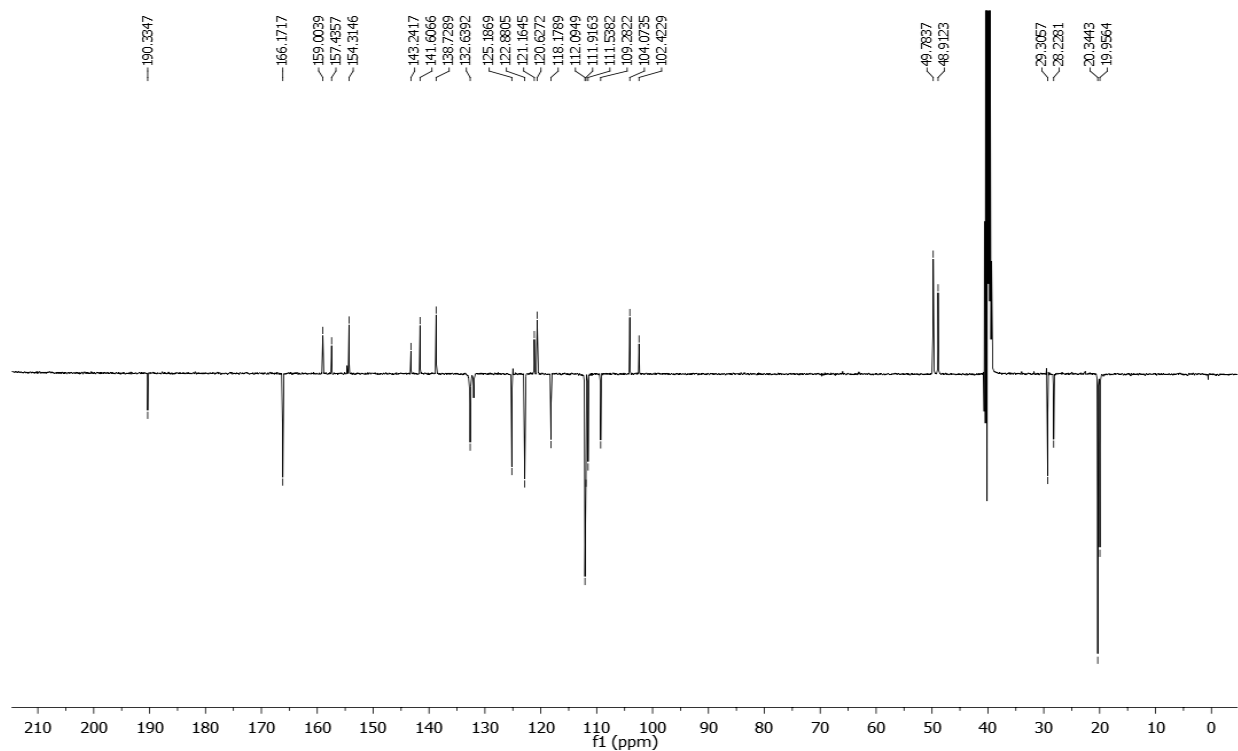


Figure S27. ^1H NMR spectrum (DMSO- d_6 , 600 MHz) of *(E)*-5-(diethylamino)-2-(((1-methyl-1*H*-benzo[d]imidazol-2-yl)imino)methyl)phenol 35

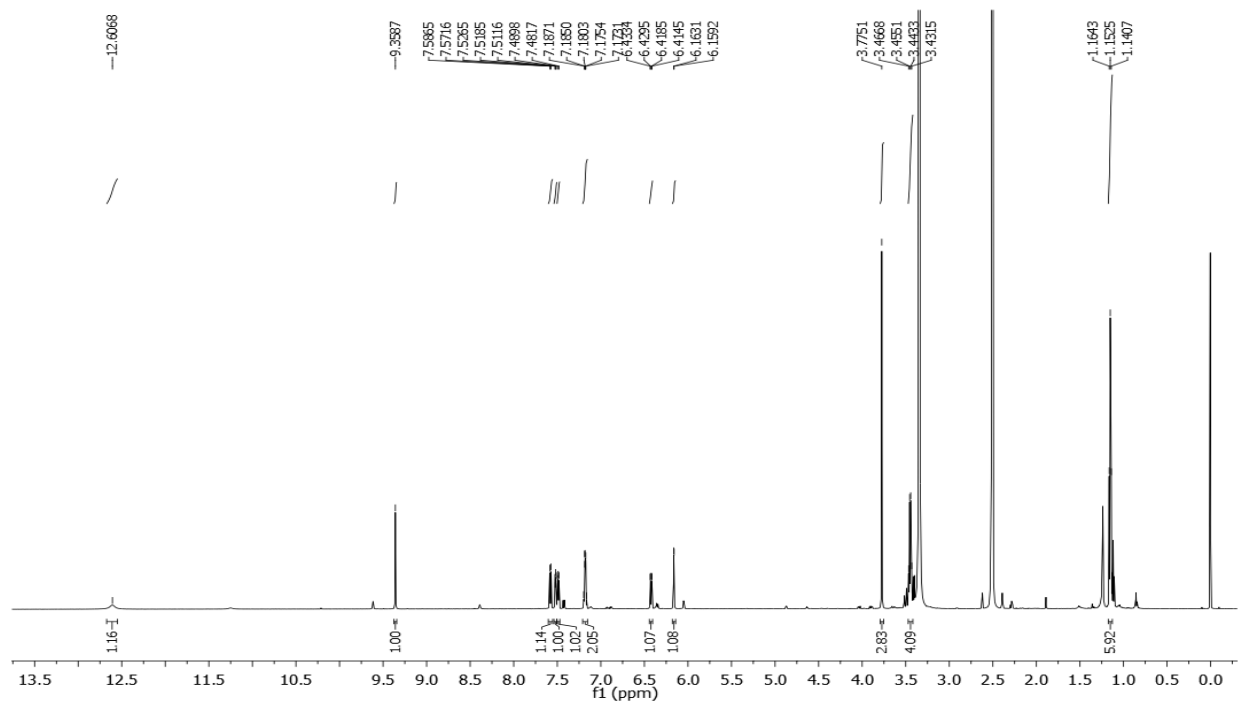


Figure S28. ^{13}C NMR spectrum (DMSO- d_6 , 151 MHz) of *(E)*-5-(diethylamino)-2-(((1-methyl-1*H*-benzo[d]imidazol-2-yl)imino)methyl)phenol 35

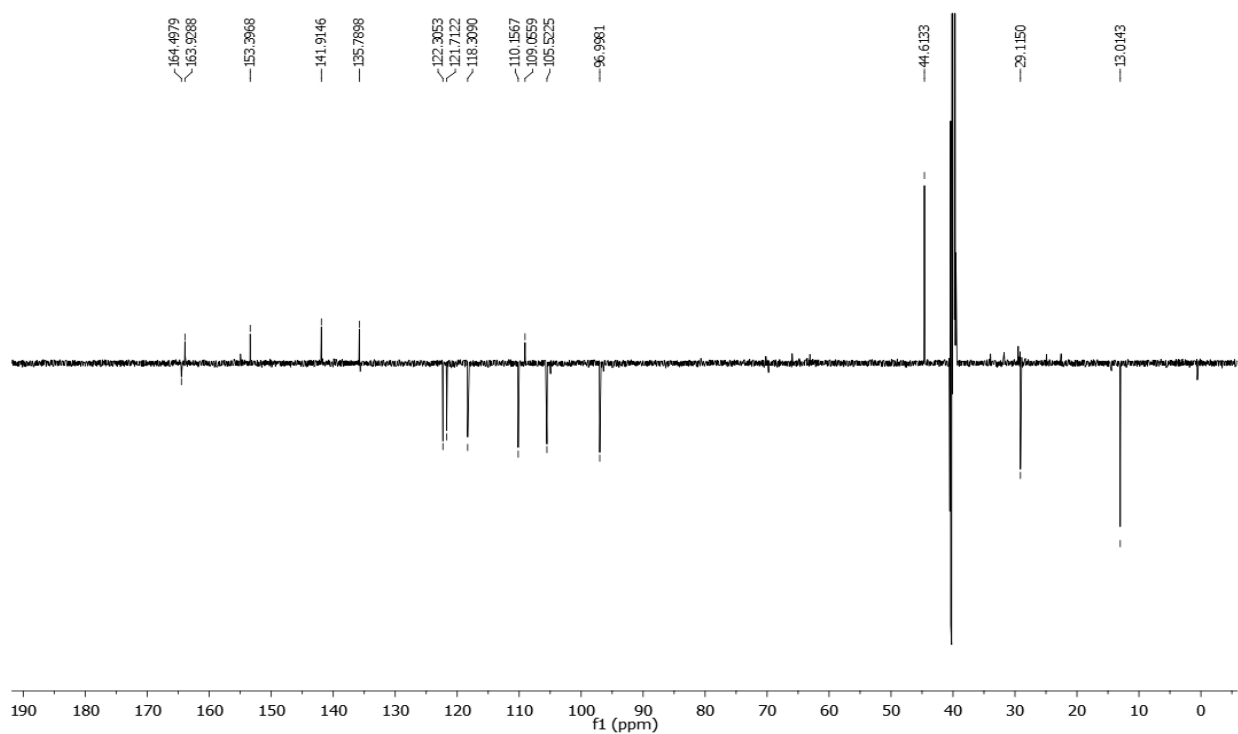


Figure S29. ^1H NMR spectrum (DMSO- d_6 , 400 MHz) of (*E*)-2-((4-(diethylamino)-2-hydroxybenzylidene)amino)-1-methyl-1*H*-benzo[*d*]imidazole-6-carbonitrile **36**

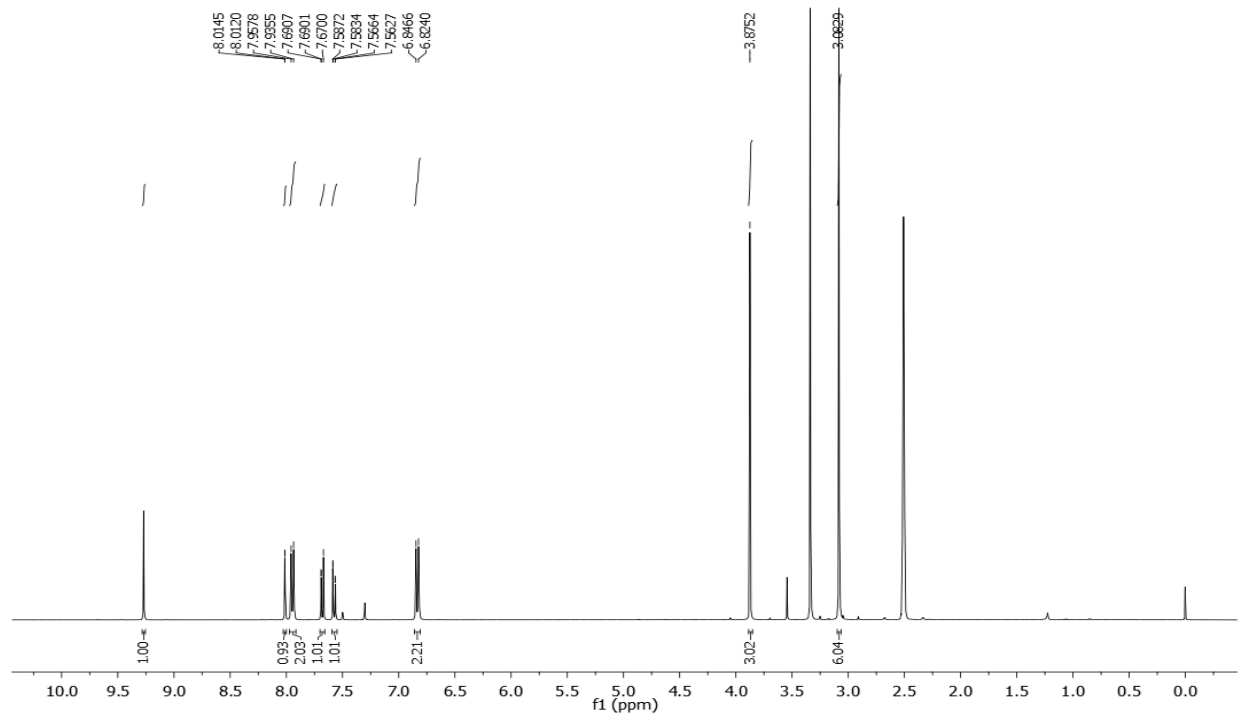


Figure S30. ^{13}C NMR spectrum (DMSO- d_6 , 100 MHz) of (*E*)-2-((4-(diethylamino)-2-hydroxybenzylidene)amino)-1-methyl-1*H*-benzo[*d*]imidazole-6-carbonitrile **36**

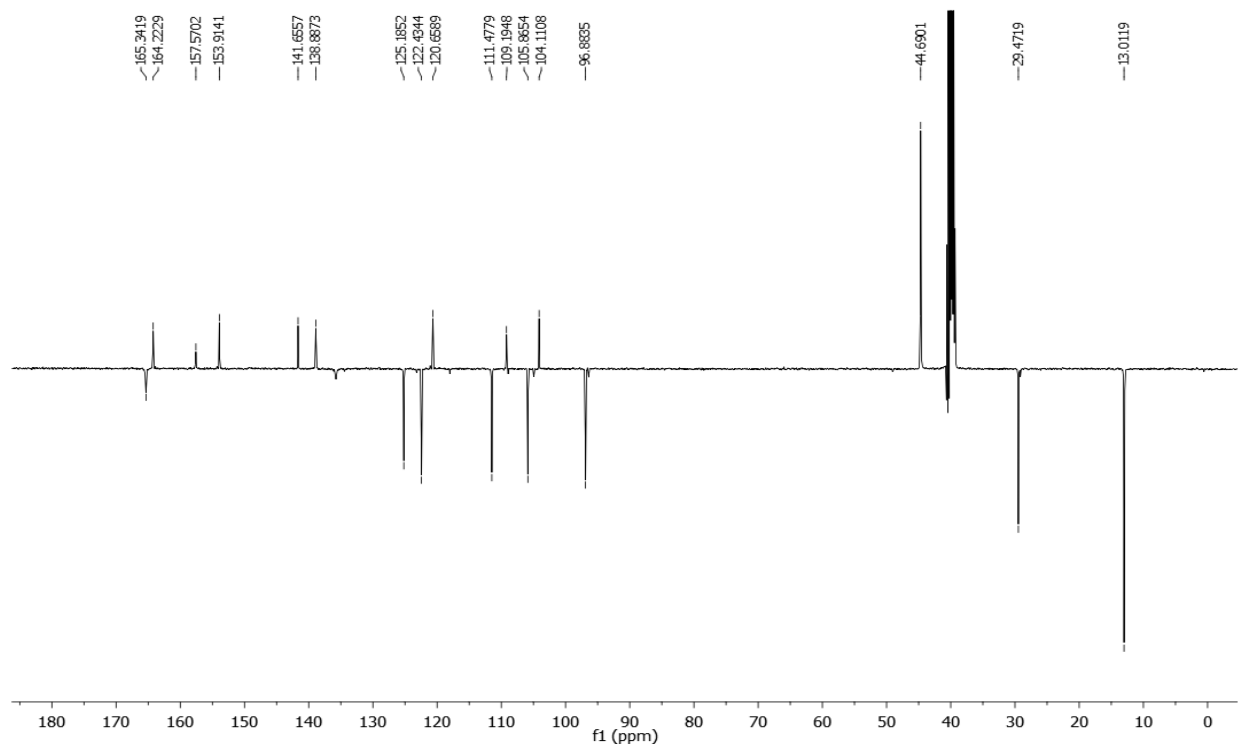


Figure S31. ^1H NMR spectrum (DMSO- d_6 , 400 MHz) of *(E)*-1-methyl-2-((4-nitrobenzylidene)amino)-1H-benzo[d]imidazole-6-carbonitrile **37**

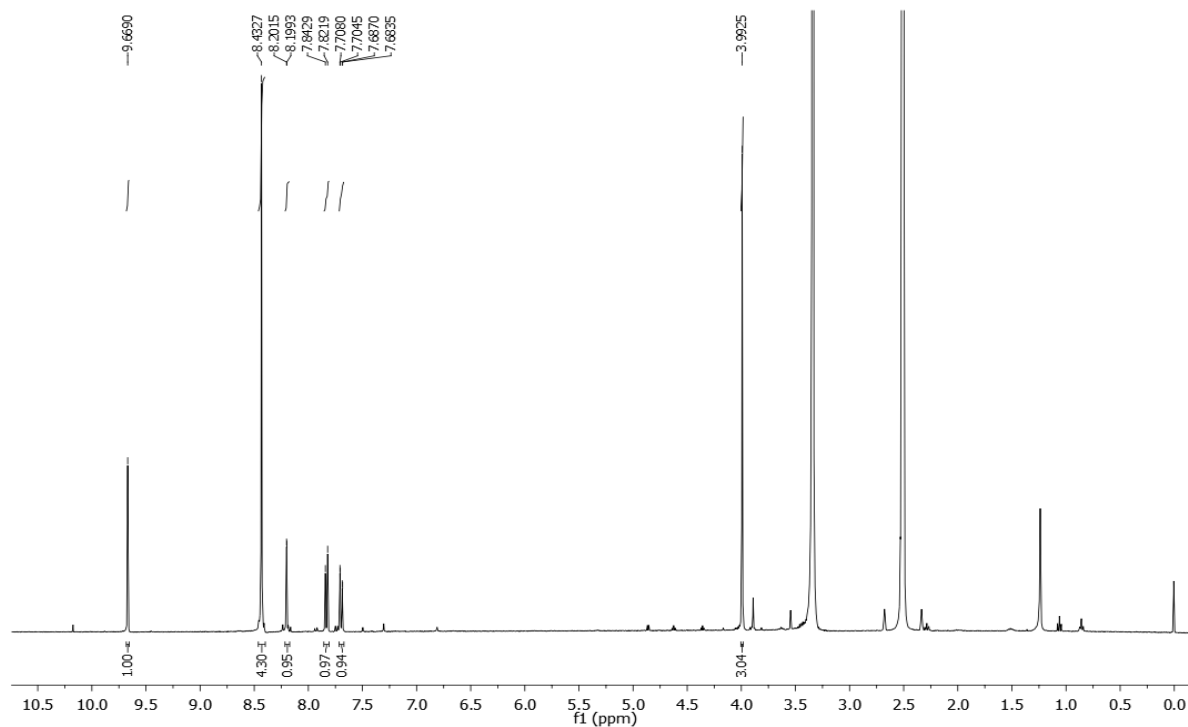


Figure S32. ^{13}C NMR spectrum (DMSO- d_6 , MHz) of *(E)*-1-methyl-2-((4-nitrobenzylidene)amino)-1H-benzo[d]imidazole-6-carbonitrile **37**

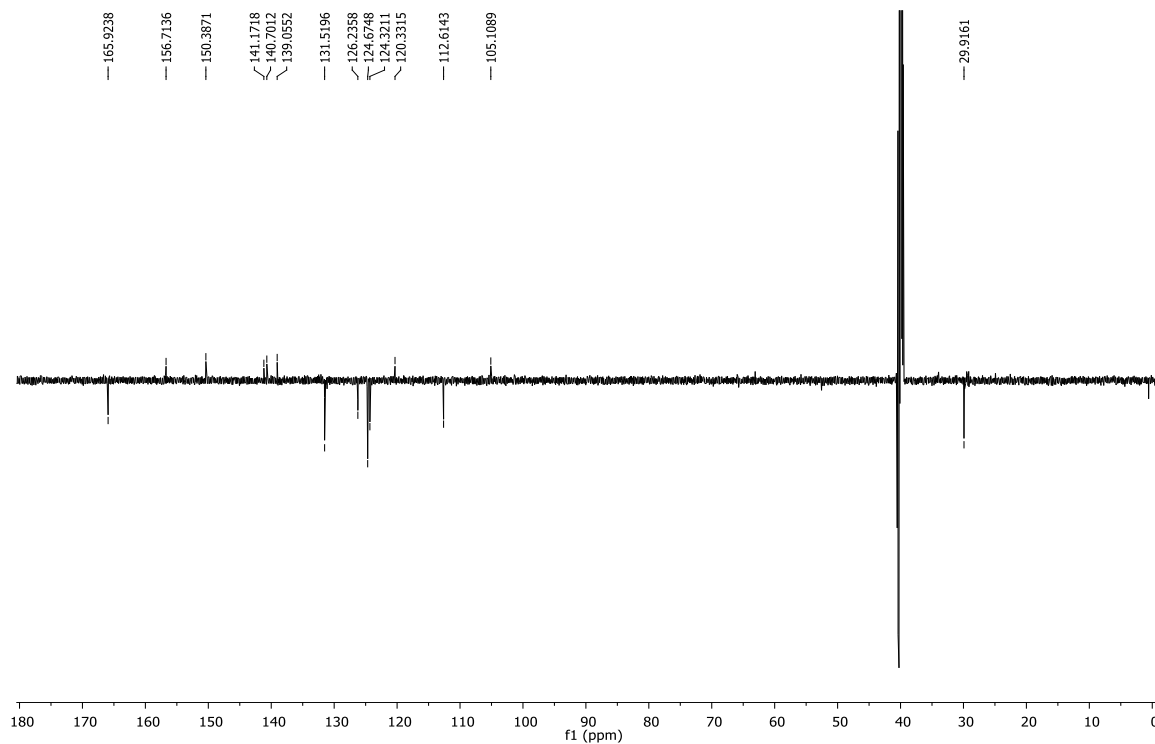


Figure S33. ^1H NMR spectrum (DMSO- d_6 , 400 MHz) of *(E)*-*N,N*-dimethyl-4-(((1-phenyl-1*H*-benzo[d]imidazol-2-yl)imino)methyl)aniline

38

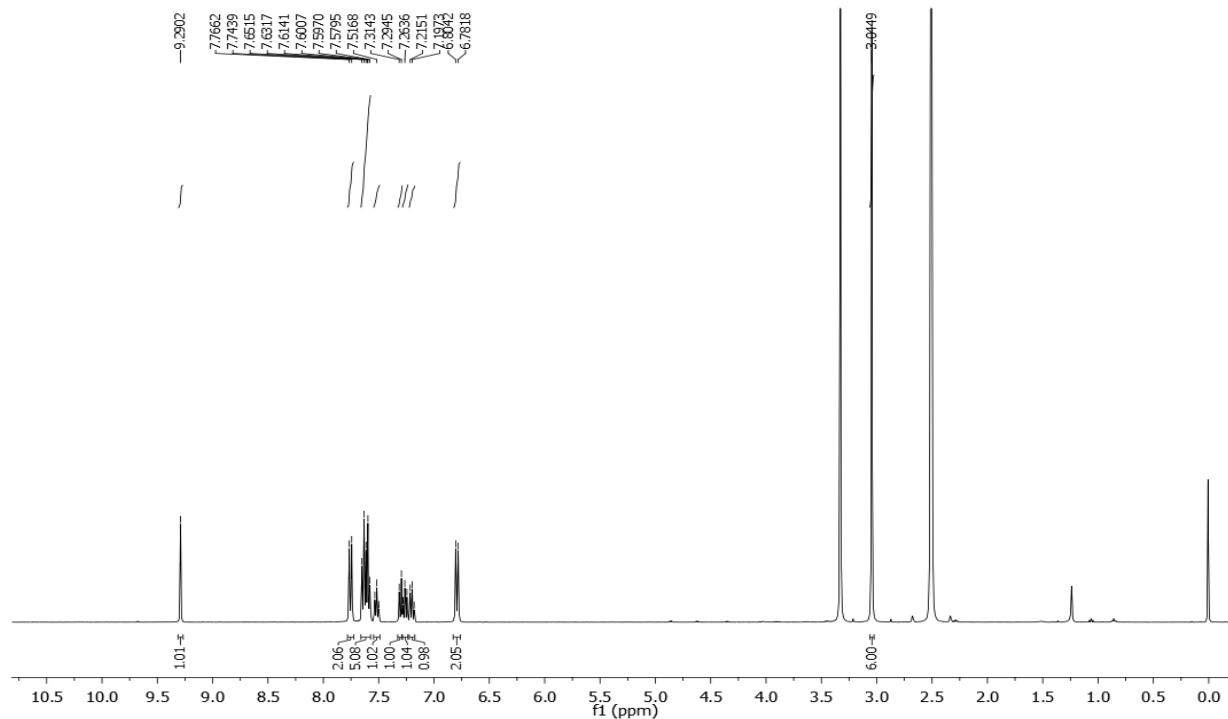


Figure S34. ^{13}C NMR spectrum (DMSO- d_6 , 151 MHz) of *(E)*-*N,N*-dimethyl-4-(((1-phenyl-1*H*-benzo[d]imidazol-2-yl)imino)methyl)aniline

38

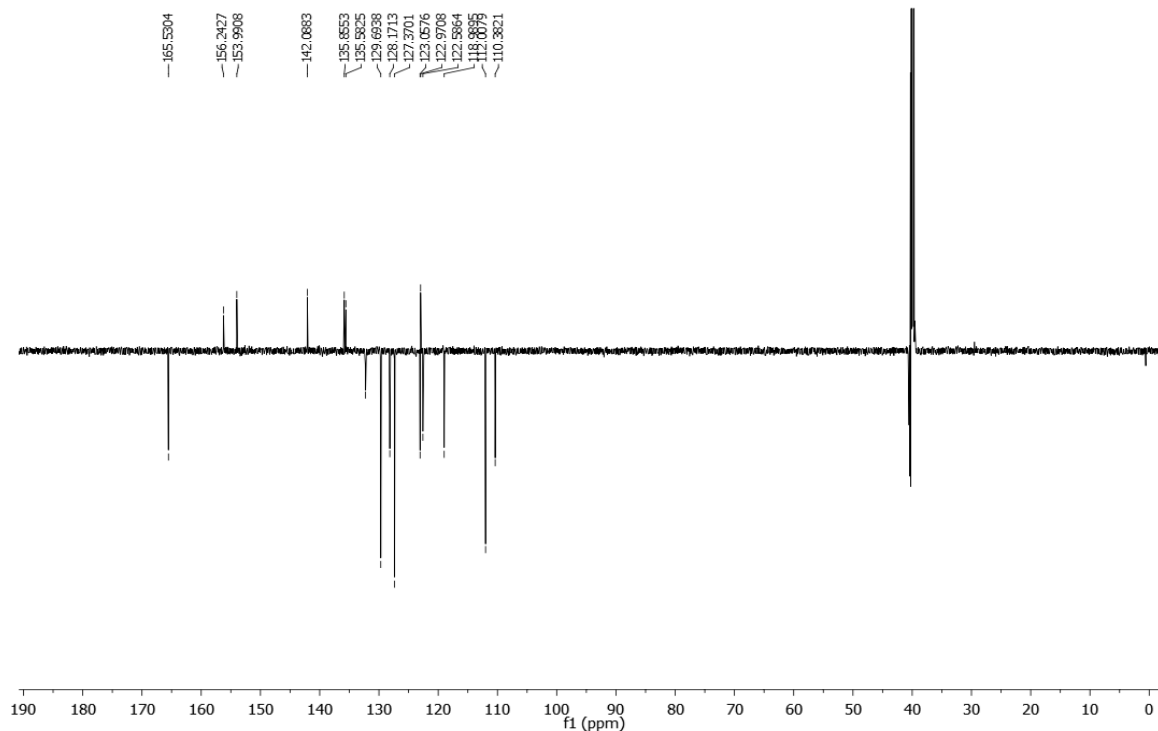


Figure S35. ^1H NMR spectrum (DMSO- d_6 , 600 MHz) of *(E)*-2-((4-(dimethylamino)benzylidene)amino)-1-phenyl-1H-benzo[d]imidazole-6-carbonitrile **39**

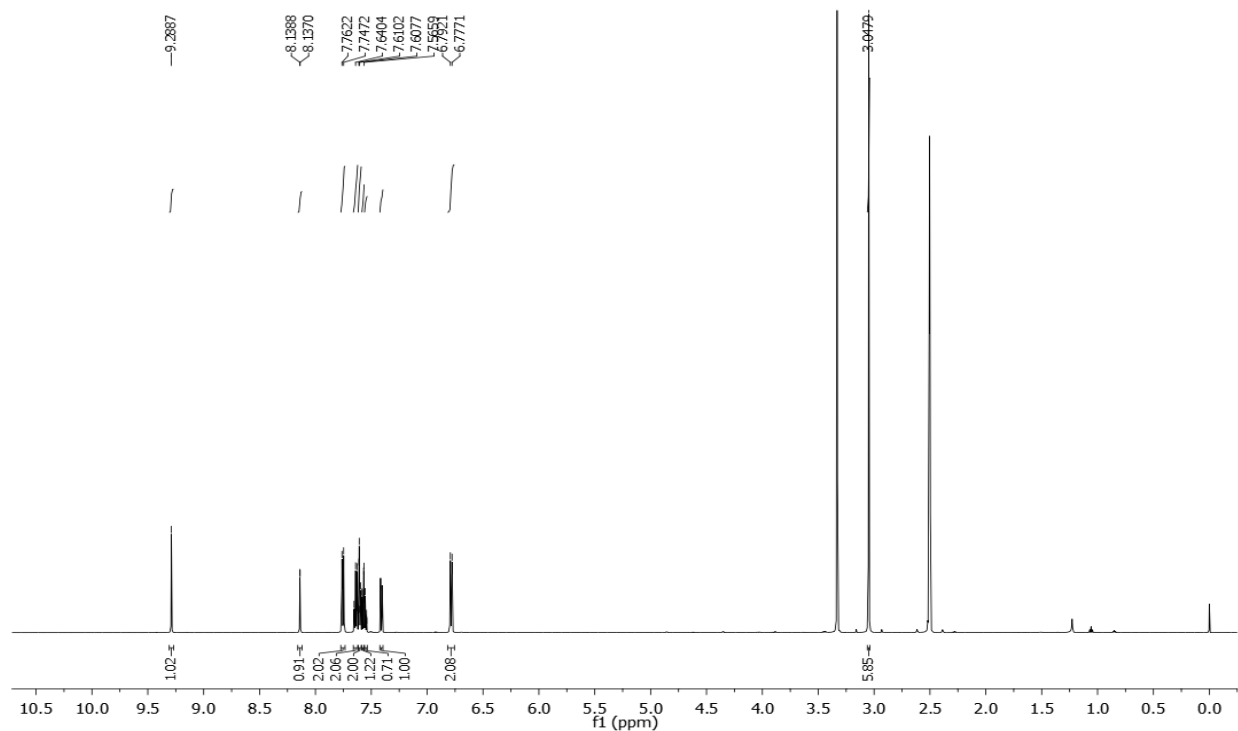


Figure S36. ^{13}C NMR spectrum (DMSO- d_6 , 151 MHz) of *(E)*-2-((4-(dimethylamino)benzylidene)amino)-1-phenyl-1H-benzo[d]imidazole-6-carbonitrile **39**

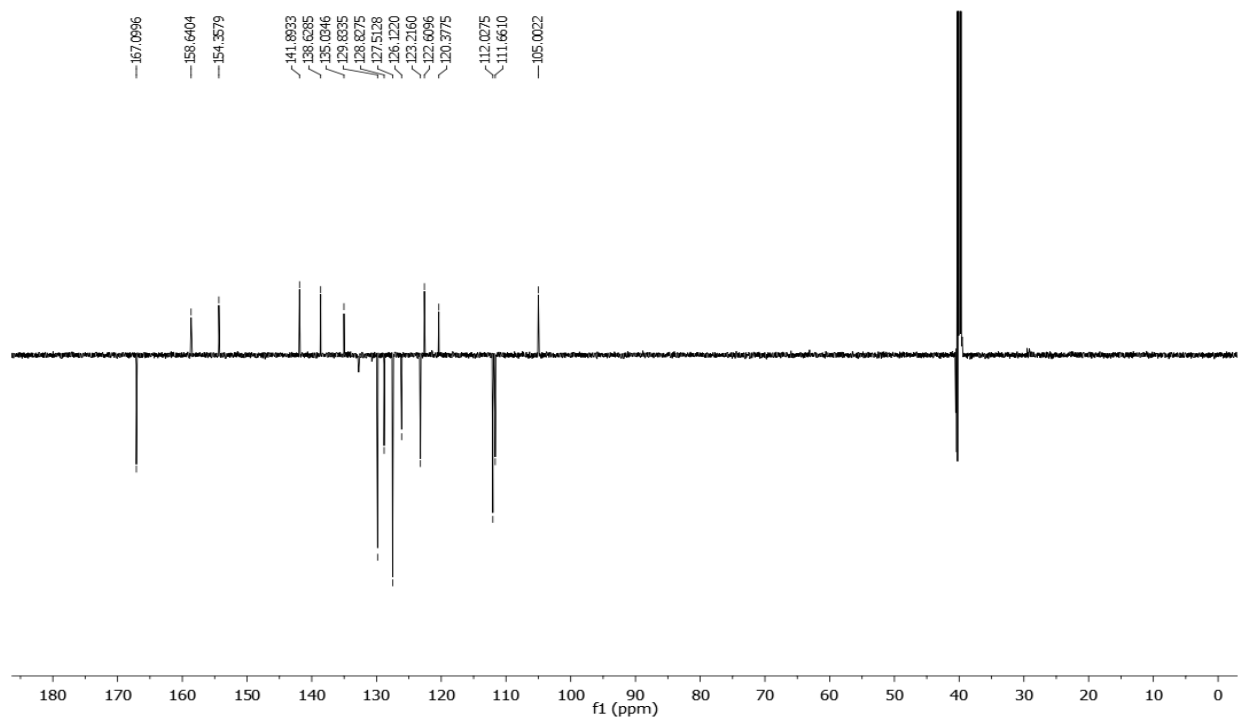


Figure S37. ^1H NMR spectrum (DMSO- d_6 , 400 MHz) of *(E)*-5-(diethylamino)-2-(((1-phenyl-1H-benzo[d]imidazol-2-yl)imino)methyl)phenol **40**

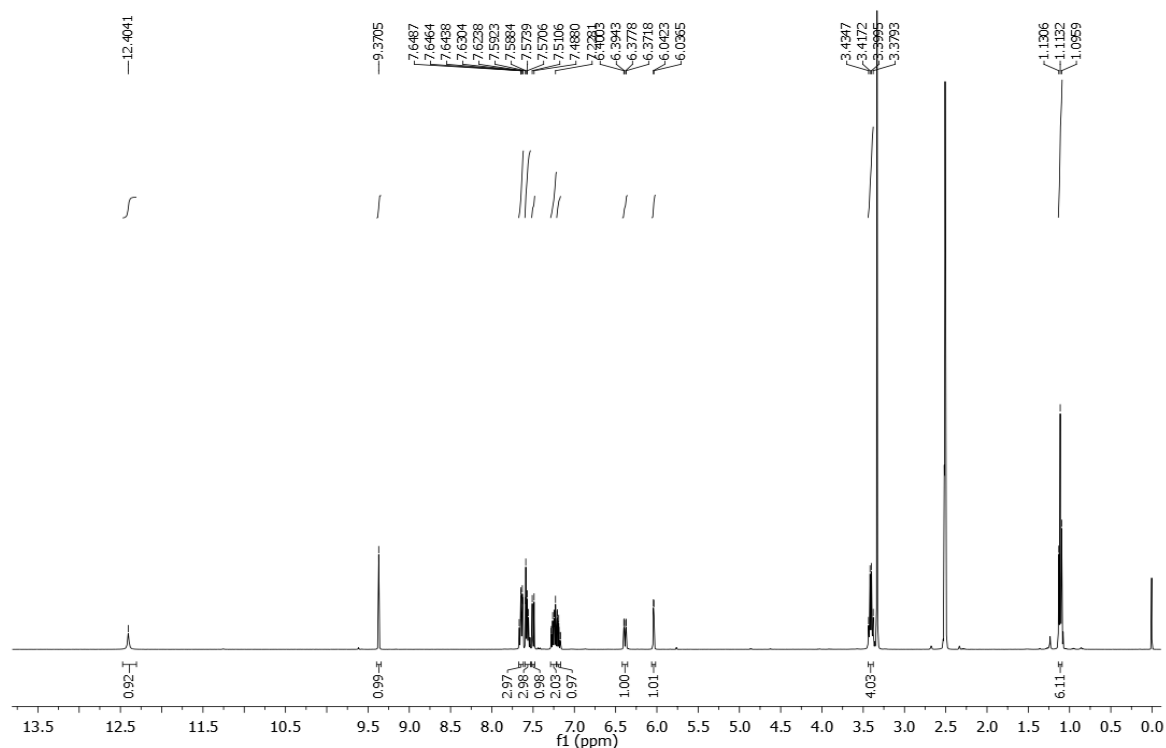


Figure S38. ^{13}C NMR spectrum (DMSO- d_6 , 75 MHz) of *(E)*-5-(diethylamino)-2-(((1-phenyl-1H-benzo[d]imidazol-2-yl)imino)methyl)phenol **40**

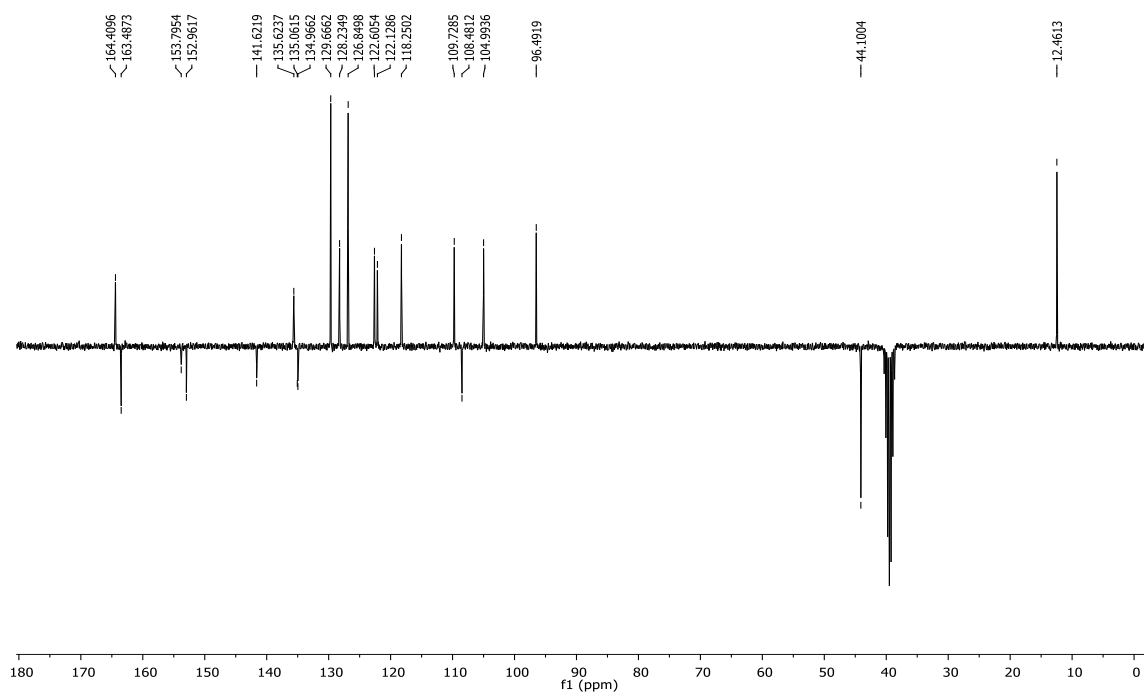


Figure S39. ^1H NMR spectrum (DMSO- d_6 , 400 MHz) of (*E*)-2-((4-(diethylamino)-2-hydroxybenzylidene)amino)-1-phenyl-1*H*-benzo[d]imidazole-6-carbonitrile **41**

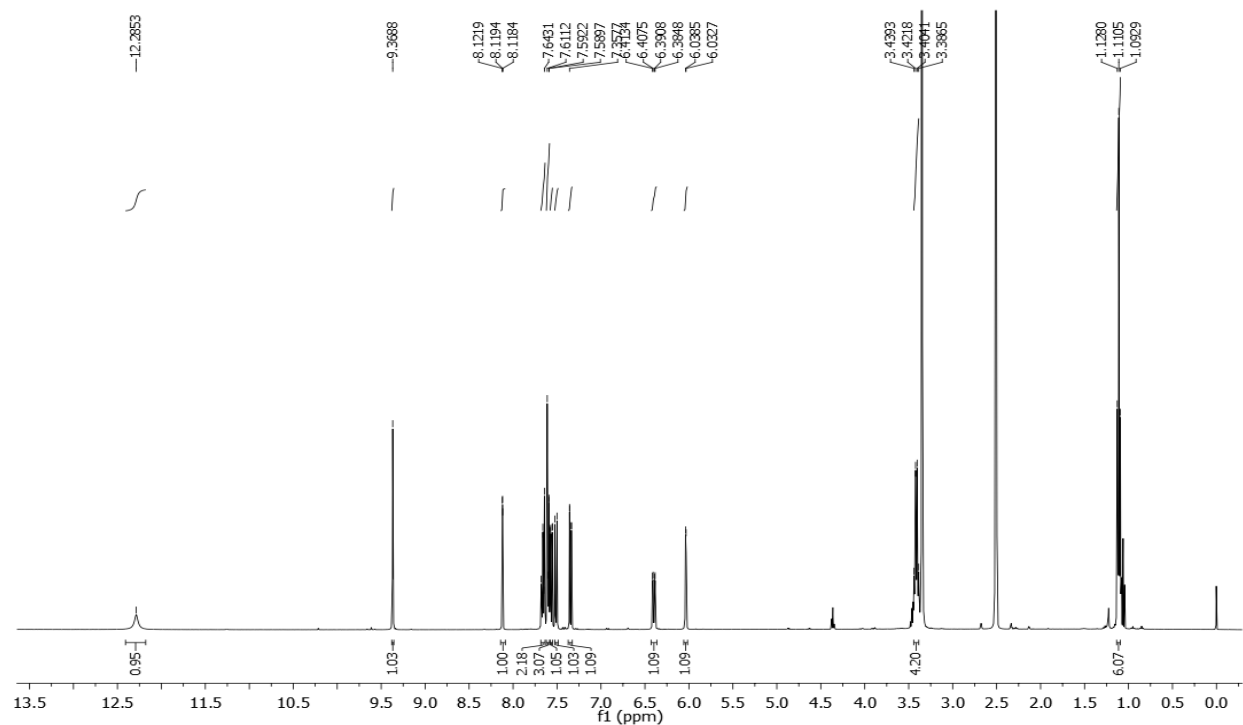


Figure S40. ^{13}C NMR spectrum (DMSO- d_6 , 100 MHz) of (*E*)-2-((4-(diethylamino)-2-hydroxybenzylidene)amino)-1-phenyl-1*H*-benzo[d]imidazole-6-carbonitrile **41**

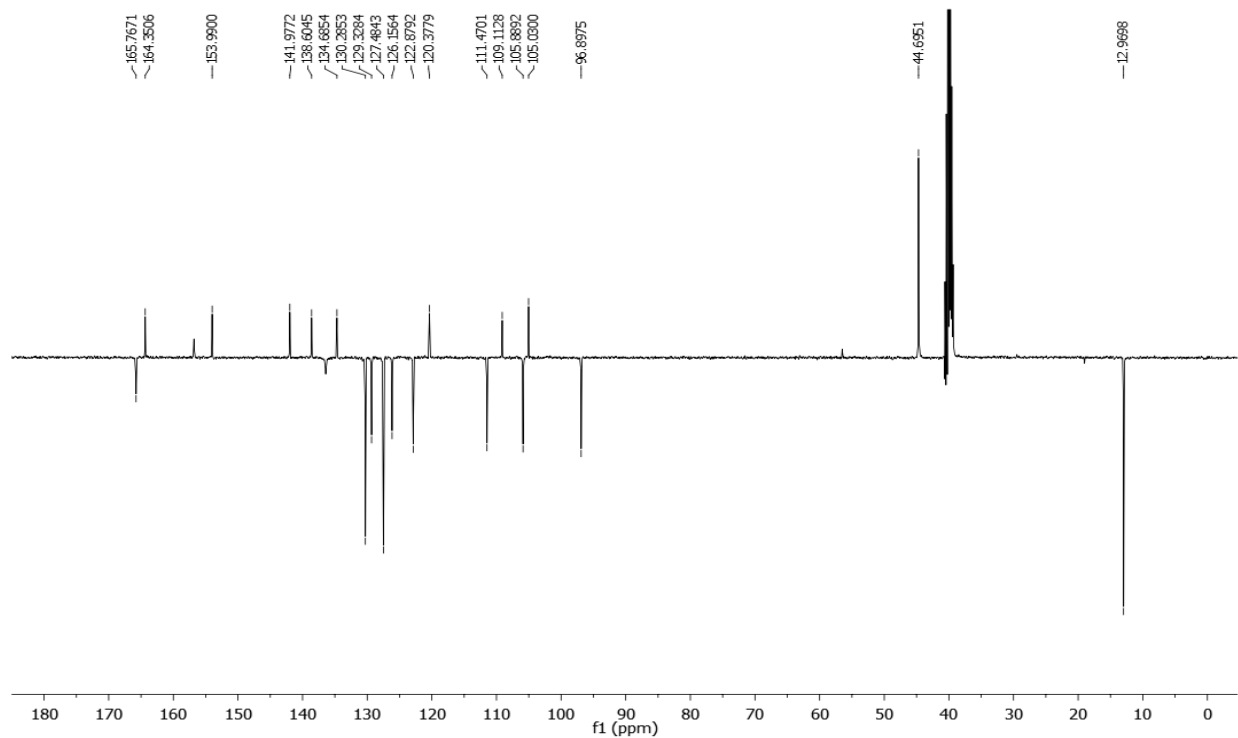


Figure S43. ^1H NMR spectrum (DMSO- d_6 , 300 MHz) of *(E)*-2-((4-(dimethylamino)benzylidene)amino)-1-hexyl-1H-benzo[d]imidazole-6-carbonitrile **43**

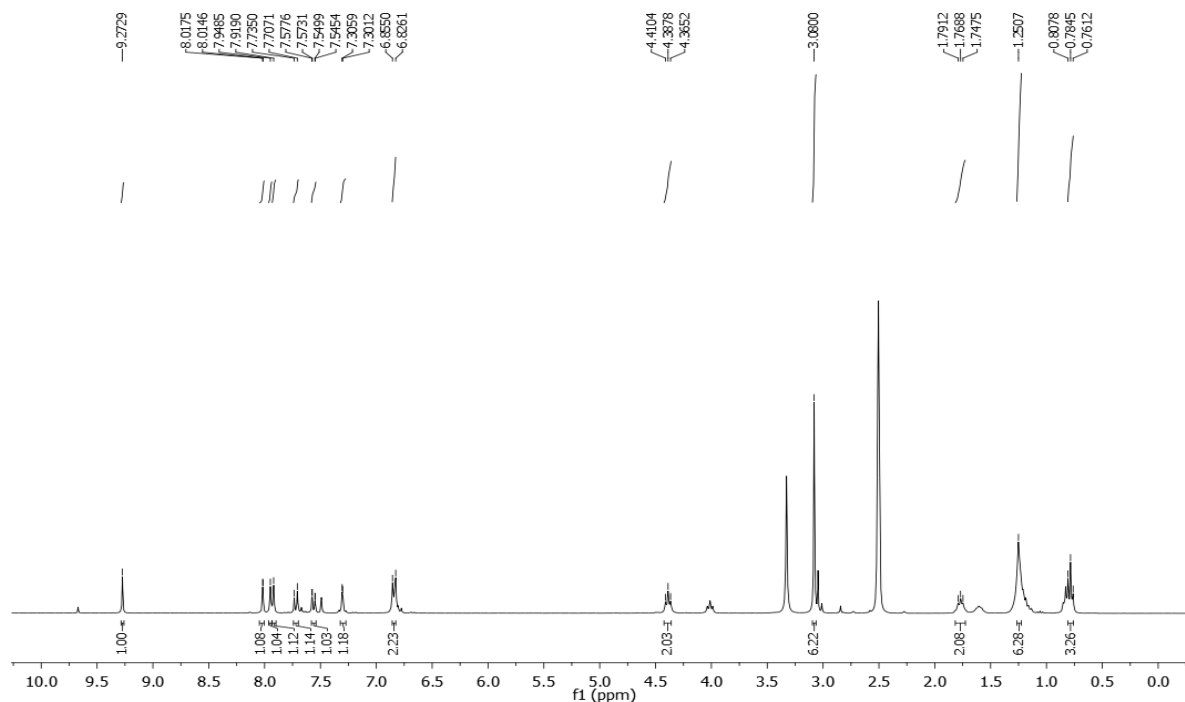


Figure S44. ^{13}C NMR spectrum (DMSO- d_6 , 100 MHz) of *(E)*-2-((4-(dimethylamino)benzylidene)amino)-1-hexyl-1H-benzo[d]imidazole-6-carbonitrile **43**

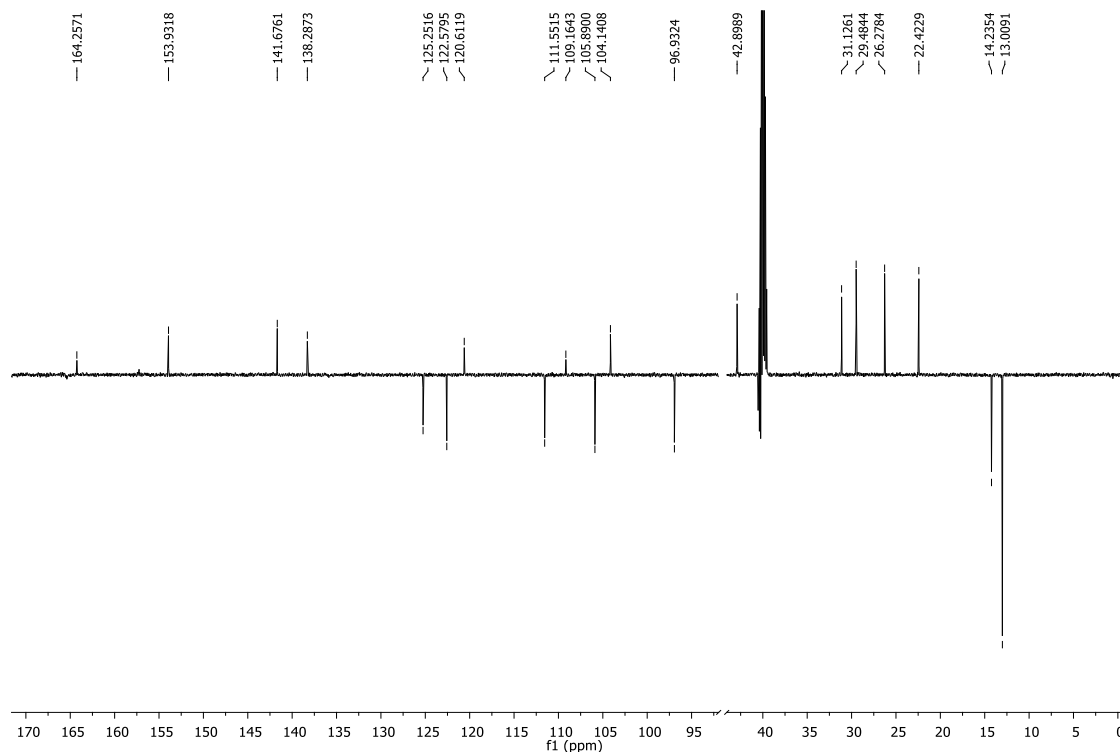


Figure S45. ^1H NMR spectrum (DMSO- d_6 , 600 MHz) of *(E)*-5-(diethylamino)-2-(((1-hexyl-1*H*-benzo[d]imidazol-2-yl)imino)methyl)phenol **44**

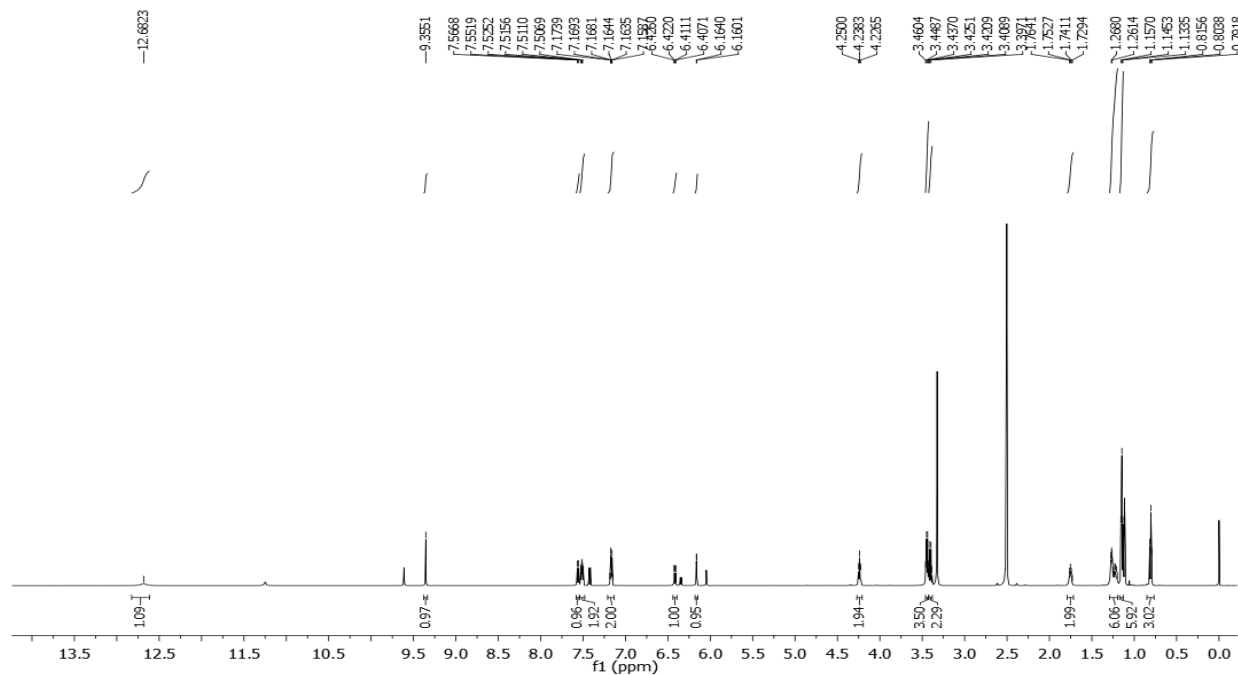


Figure S46. ^{13}C NMR spectrum (DMSO- d_6 , 100 MHz) of *(E)*-5-(diethylamino)-2-(((1-hexyl-1*H*-benzo[d]imidazol-2-yl)imino)methyl)phenol **44**

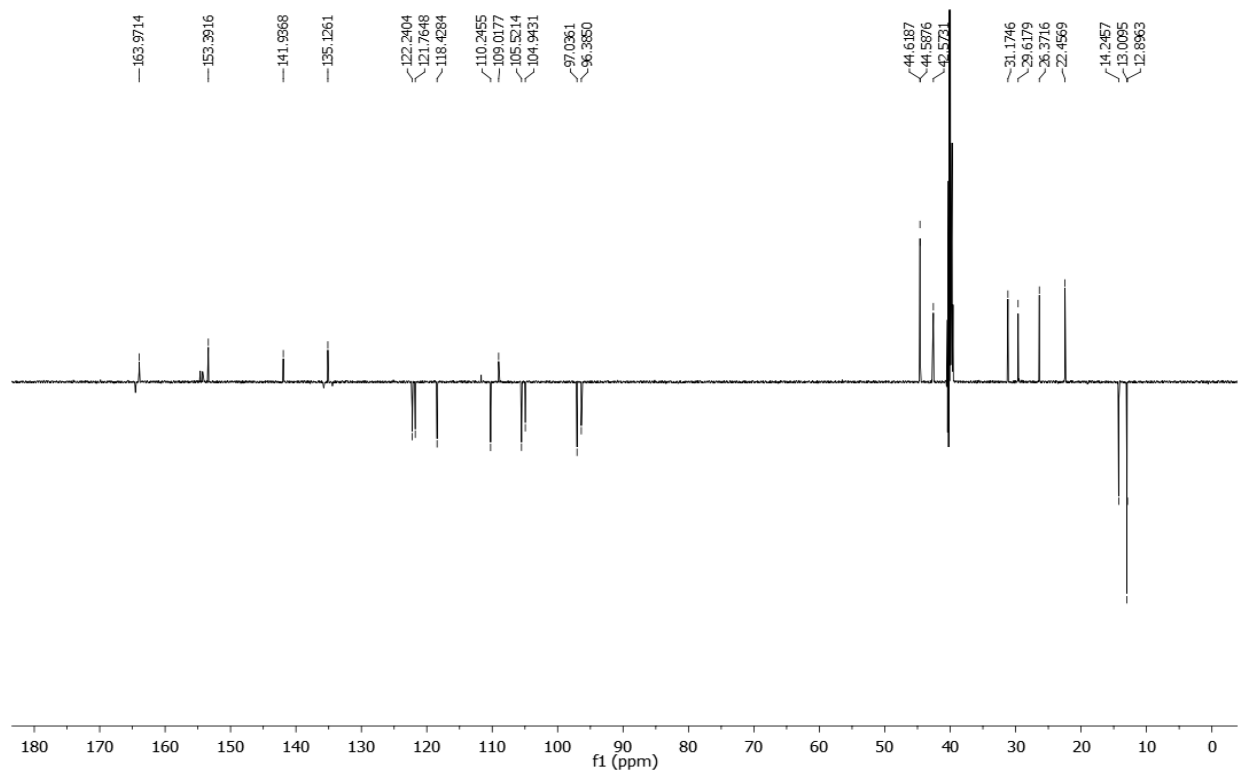


Figure S47. ^1H NMR spectrum (DMSO- d_6 , 600 MHz) of *(E)*-2-((4-(diethylamino)-2-hydroxybenzylidene)amino)-1-hexyl-1H-benzo[d]imidazole-6-carbonitrile **45**

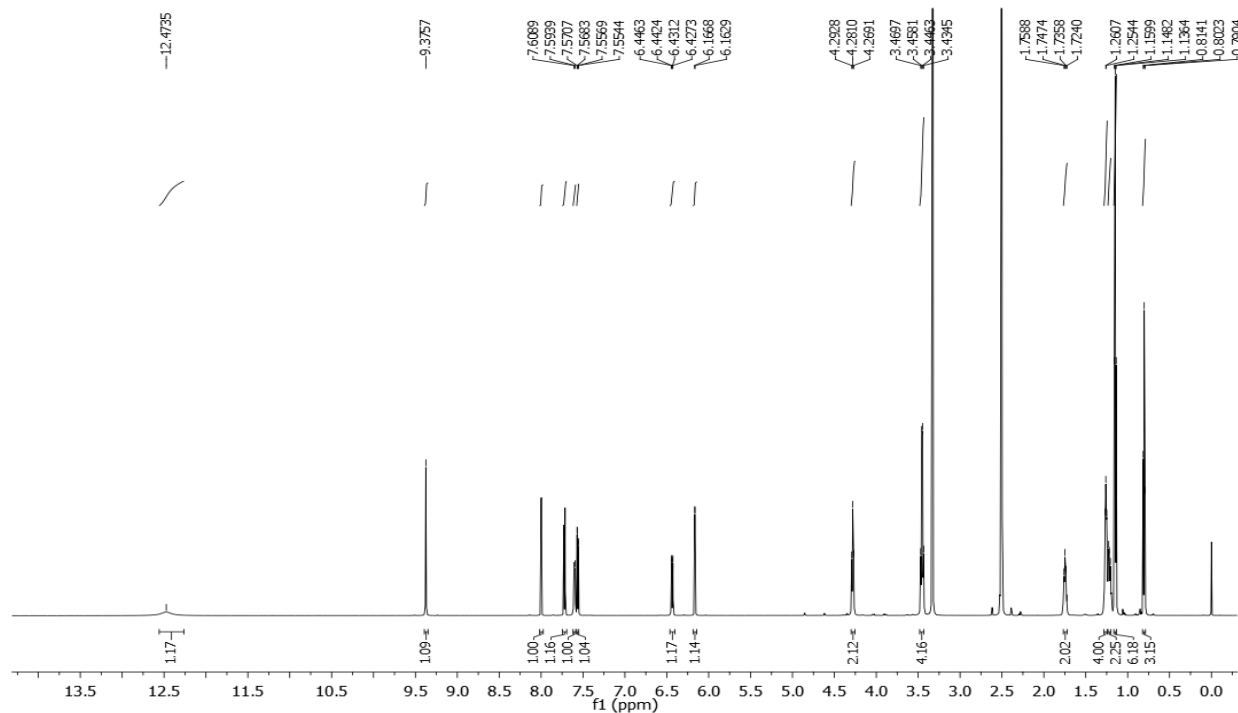
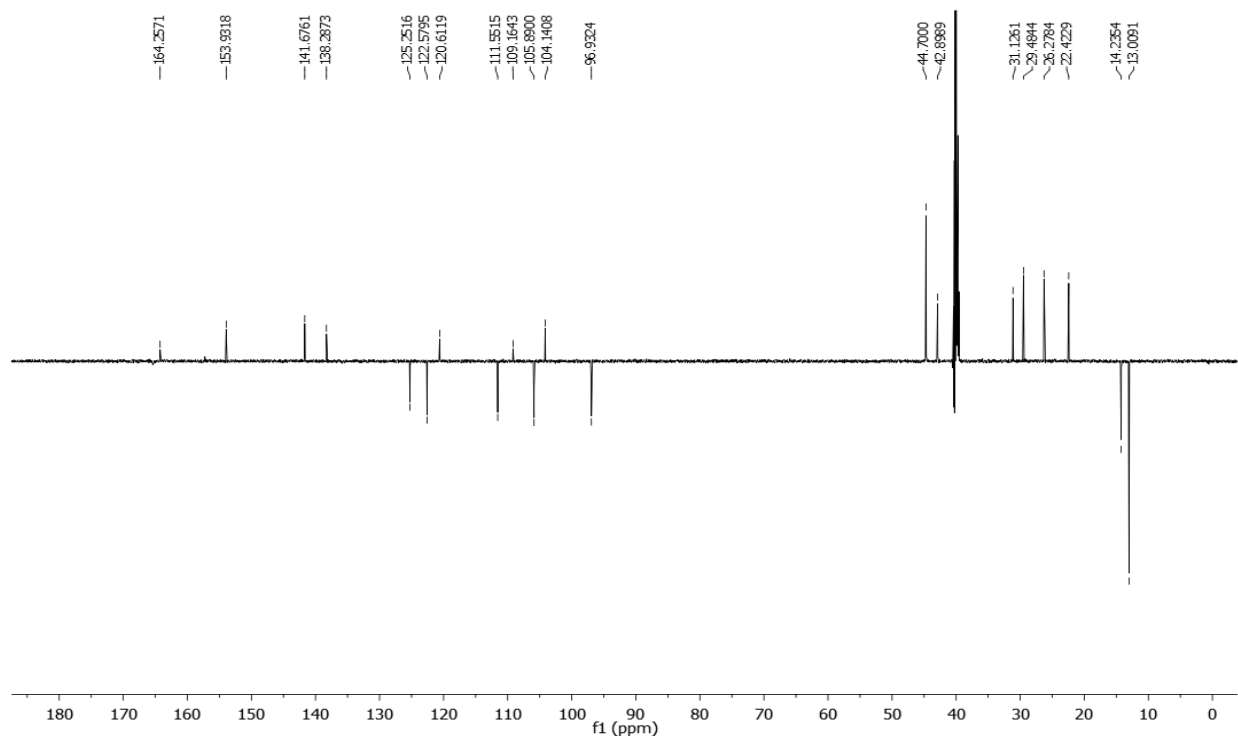


Figure S48. ^{13}C NMR spectrum (DMSO- d_6 , 151 MHz) of *(E)*-2-((4-(diethylamino)-2-hydroxybenzylidene)amino)-1-hexyl-1H-benzo[d]imidazole-6-carbonitrile **45**



ⁱ Grgičević, I.; Mikulandra, I.; Bukvić, M.; Banjanac, M.; Radovanović, V.; Habinovec, I.; Bertoša, B.; Novak, P. Discovery of macrozones, new antimicrobial thiosemicarbazone-based azithromycin conjugates: design, synthesis and in vitro biological evaluation. *Int.J. Antimicrob. Agents* **2020**, *56*, 106147, 5.