Supporting Information

Biological potential of novel methoxy and hydroxy substituted heteroaromatic amides designed as promising antioxidative agents: Synthesis, 3D-QSAR analysis and biological activity

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1. General methods for synthesis

General method for the synthesis of cyano substituted *N*-2-benzimidazolyl methoxybenzamides (21-22)

To a solution of corresponding benzoyl chlorides **2-3** in dry toluene, a solution of 2-amino-5(6)-cyanobenzimidazole **18** in dry toluene was added dropwise, followed by the addition of Et_3N. The reaction mixture was refluxed for several hours. After cooling the solution was concentrated and the obtained solid was filtered off and recrystallized from appropriate solvent.

N-(5(6)-Cyanobenzimidazol-2-yl)-2,4-dimethoxybenzamide (**21**): This compound was prepared using above described method from 2,4-dimethoxybenzoyl chloride **2** (0.19 g, 0.95 mmol) and 2-amino-5(6)-cyanobenzimidazole **18** (0.15 g, 0.95 mmol) in dry toluene (10 mL) followed by the addition of Et_3N (0.19 mL, 1.33 mmol) after refluxing for 36 hours and recrystalization from methanol/acetone to obtain 0.11 g (36%) of light yellow powder; mp 236--239 °C; ^{1}H NMR (300 MHz, DMSO-d_6) (δ/ppm): 12.71 (brs, 1H, $NH_{benzimidazole}$), 11.07 (s, 1H, NH_{amide}), 7.90 (d, J = 8.49 Hz, 1H, H_{arom}), 7.89 (s, 1H, H_{arom}), 7.62 (d, J = 8.25 Hz, 1H, H_{arom}), 7.51 (dd, J_1 = 8.27 Hz, J_2 = 1.54 Hz, 1H, H_{arom}), 6.77 (d, J = 2.01 Hz, 1H, H_{arom}), 6.74 (dd, J_1 = 8.64 Hz, J_2 = 2.25 Hz, 1H, H_{arom}), 4.01 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3); ^{13}C NMR (150 MHz, DMSO-d_6) (δ/ppm): 164.8, 164.4, 159.7, 133.0, 113.6, 107.0, 99.2 (2C), 57.0, 56.2; Anal. Calcd. for $C_{19}H_{20}N_5O_3$: C, 63.52; H, 4.20; N, 17.22. Found: C, 63.35; H, 4.38; N, 17.38.

N-(5(6)-Cyanobenzimidazol-2-yl)-3,4,5-trimethoxybenzamide (**22**): This compound was prepared using above described method from 3,4,5-trimethoxybenzoyl chloride **3** (0.50 g, 2.18 mmol) and 2-amino-5(6)-cyanobenzimidazole **18** (0.35 g, 2.18 mmol) in dry toluene (40 mL) followed by the addition of Et_3N (0.19 mL, 1.33 mmol) after refluxing for 24 hours and recrystalization from methanol to obtain 0.55 g (72%) of white powder; mp 247--250 °C; ^{1}H NMR (300 MHz, DMSO-d_6) (δ/ppm): 12.75 (brs, 1H, $NH_{benzimidazole}$), 12.26 (s, 1H, NH_{amide}), 7.94 (d, J = 0.80 Hz, 1H,H_{arom}), 7.65 (d, J = 8.28 Hz, 1H, H_{arom}), 7.54 (dd, J_1 = 8.27 Hz, J_2 = 1.55 Hz, 1H, H_{arom}), 7.51 (s, 2H, H_{arom}), 3.90 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3); ^{13}C NMR (150 MHz, DMSO-d_6) (δ/ppm): 166.2, 153.2 (2C), 150.3, 141.7, 133.7, 127.8, 125.6, 120.7 (2C), 107.1, 106.3 (3C), 103.4, 60.6, 56.9 (2C); Anal. Calcd. for $C_{18}H_{16}N_4O_4$: C, 61.54; H, 4.76; N, 15.68. Found: C, 61.36; H, 4.58; N, 15.90.

General method for the synthesis of amidino substituted *N*-2-benzimidazolyl methoxybenzamides (38, 39, 43 and 44)

A stirred suspension of 5(6)-cyanobenzimidazol-2-yl substituted methoxybenzamides **21-22** in absolute ethanol was cooled in an ice-salt bath and was saturated with dry HCl gas. The reaction mixture was maintained at room temperature until nitrile band disappeared (monitored by IR analysis at 2200 cm^{-1}). The corresponding imidate was filtered off, washed with dry diethyl ether and dried under reduced pressure.

Amidines. The corresponding imidate was suspended in absolute ethanol and dry NH_3 was bubbled into the suspension. The mixture was stirred at room temperature for 2 days. The crude product was then filtered off and washed with diethyl ether.

Imidazolynilamidines. Ethylenediamine (EDA) was added to a suspension of the corresponding imidate in absolute ethanol (5 mL) and the mixture was stirred at reflux for 24 h. The crude product was then filtered off and washed with diethyl ether.

N-(5(6)-Amidinobenzimidazol-2-yl)-2,4-dimethoxybenzamide hydrochloride (**38**): This compound was prepared using above described method. A solution of **21** (0.18 g, 0.54 mmol) in absolute ethanol (20 mL) was saturated with dry HCl gas and stirred for 10 days. The crude imidate was filtered off, suspended in absolute ethanol (20 mL) and dry NH_3 was bubbled into the suspension. The mixture was worked up as it is described to give 0.05 g (25%) of light yelow powder; mp 259–-262 °C; ^{1}H NMR (300 MHz, DMSO-d_6) (δ/ppm): 11.08 (s, 1H, NH_{amide}), 9.33 (s, 2H, $NH_{amidine}$), 9.17 (s, 2H, $NH_{amidine}$), 8.01 (s, 1H, H_{arom}), 7.92 (d, J = 8.55 Hz, 1H, H_{arom}), 7.64 (d, J = 2.55 Hz, 1H, H_{arom}), 6.78–-6.73 (m, 2H, H_{arom}), 4.03 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3); ^{13}C NMR (150 MHz, DMSO-d_6) (δ/ppm): 166.3, 164.3, 163.8, 159.2, 148.6, 132.6 (2C), 121.4, 120.6, 120.5, 113.0, 106.6, 98.7 (2C), 56.6, 55.8; Anal. Calcd. for $C_{17}H_{18}ClN_5O_3$: C, 54.61; H, 4.65; N, 18.78. Found: C, 54.33; H, 4.83; N, 18.64.

N-(5(6)-Amidinobenzimidazol-2-yl)-3,4,5-trimethoxybenzamide hydrochloride (**39**): This compound was prepared using above described method. A solution of **22** (0.20 g, 0.57 mmol) in absolute ethanol (15 mL) was saturated with dry HCl gas and stirred for 6 days. The crude imidate was filtered off, suspended in absolute ethanol (20 mL) and dry NH_3 was bubbled into the suspension. The mixture was worked up as it is described to give 0.16 g (69%) of white powder; mp 224–-226 °C; ^{1}H NMR (300 MHz, DMSO-d_6) (δ/ppm): 12.76 (s, 1H, $NH_{benzimidazole}$), 12.21 (s, 1H, NH_{amide}), 9.28 (s, 2H, $NH_{amidine}$), 9.02 (s, 2H, $NH_{amidine}$), 8.02 (1H,

s, H_{arom}), 7.68 (d, J = 8.34 Hz, 1H, H_{arom}), 7.63 (d, J = 8.46 Hz, 1H, H_{arom}), 7.53 (s, 2H, H_{arom}), 3.91 (s, 6H, OCH_3), 3.77 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, DMSO-d_6) (δ/ppm): not enough soluble; Anal. Calcd. for $C_{18}H_{20}ClN_5O_2$: C, 53.48; H, 4.75; N, 17.44. Found: C, 53.27; H, 4.97; N, 17.26.

N-[5(6)-2-(Imidazolinyl)benzimidazol-2-yl]-2,4-dimethoxybenzamide hydrochloride (**43**): This compound was prepared using above described method. A solution of **21** (0.18 g, 0.54 mmol) in absolute ethanol (20 mL) was saturated with dry HCl gas and stirred for 10 days. The crude imidate was filtered off, suspended in absolute ethanol (20 mL) and EDA (0.107 ml, 1.61 mmol) was added into the suspension. The mixture was worked up as it is described to give 0.09 g (41%) of light yelow powder; mp 273--275 °C; ^{1}H NMR (300 MHz, DMSO-d_6) (δ/ppm): 11.41 (s, 1H, NH_{amide}), 10.79 (s, 2H, $NH_{amidine}$), 8.29 (s, 1H, H_{arom}), 7.97 (d, J = 8.49 Hz, 1H, H_{arom}), 7.95 (dd, J_1 = 8.46 Hz, J_2 =1.50 Hz, 1H, H_{arom}), 7.78 (d, J = 8.49 Hz, 1H, H_{arom}), 6.82 – 6.76 (m, 2H, H_{arom}), 4.02 (s, 3H, OCH_3), 4.01 (s, 4H, CH_2), 3.90 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, DMSO-d_6) (δ/ppm): 164.9, 163.3, 159.6, 147.3, 133.1, 123.4, 116.3, 115.0, 114.1, 111.8, 107.0, 98.7, 56.8, 55.6, 44.2 (2C); Anal. Calcd. for $C_{19}H_{20}ClN_5O_3$: C, 56.95; H, 5.21; N, 17.24. Found: C, 56.79; H, 5.02; N, 17.43.

N-[5(6)-2-(Imidazolinyl)benzimidazol-2-yl]-3,4,5-trimethoxybenzamide hydrochloride (**44**): This compound was prepared using above described method. A solution of **21** (0.20 g, 0.57 mmol) in absolute ethanol (15 mL) was saturated with dry HCl gas and stirred for 6 days. The crude imidate was filtered off, suspended in absolute ethanol (20 mL) and EDA (0.128 ml, 1.92 mmol) was added into the suspension. The mixture was worked up as it is described to give 0.15 g (61%) of white powder; mp 277--279 °C; ^{1}H NMR (300 MHz, DMSO-d_6) (δ/ppm): 11.46 (s, 1H, NH_{amide}), 9.96 (s, 2H, $NH_{amidine}$), 7.96 (d, J = 1.08 Hz, 1H, H_{arom}), 7.65 (dd, J_1 = 8.37 Hz, J_2 = 1.53 Hz, 1H,H_{arom}), 7.51 (s, 2H, H_{arom}), 7.48 (d, J = 8.43 Hz, 1H, H_{arom}), 3.89 (s, 6H, OCH_3), 3.76 (s, 3H, OCH_3), 3.66 (s, 4H, CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) (δ/ppm): 167.2, 165.3, 153.2, 150.4, 141.9, 138.6, 136.4, 128.9, 121.7, 115.2, 114.3, 113.7, 111.1, 106.9 (2C), 60.6, 56.7 (2C), 48.4 (2C); Anal. Calcd. for $C_{20}H_{22}ClN_5O_4$: C, 55.85; H, 5.30; N, 16.38. Found: C, 55.62; H, 5.13; N, 16.22.

2. NMR spectrum of novel compounds

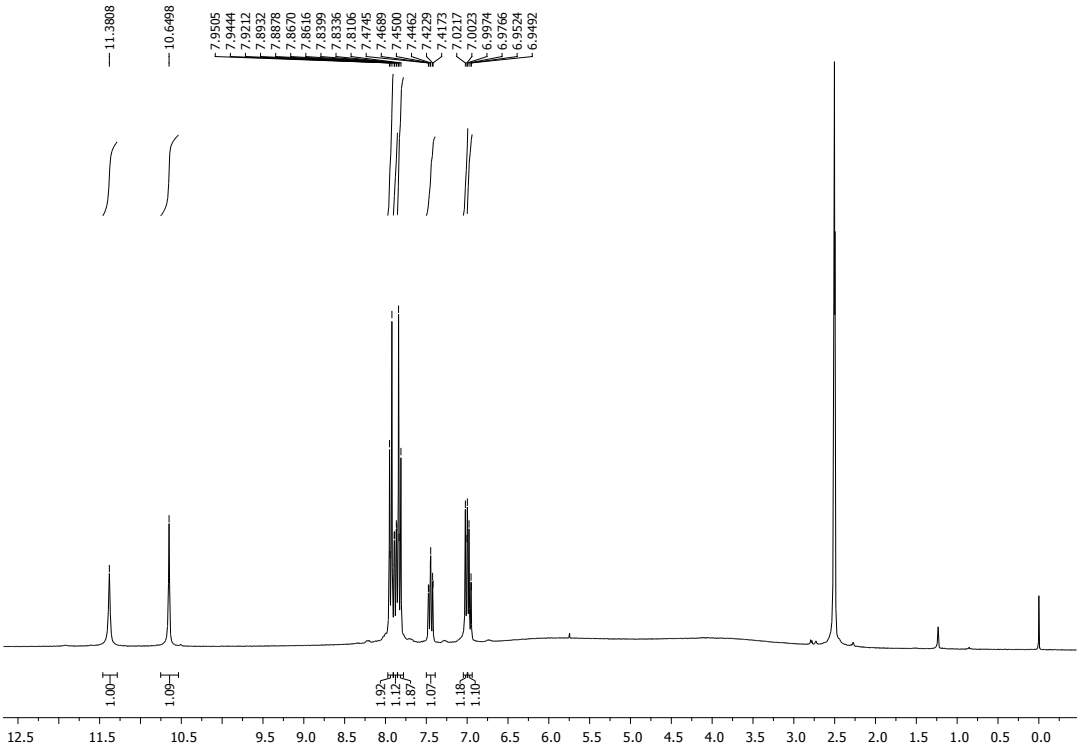


Figure S1. ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of ***2-hydroxy-N-(4-cyanophenyl)benzamide 7***.

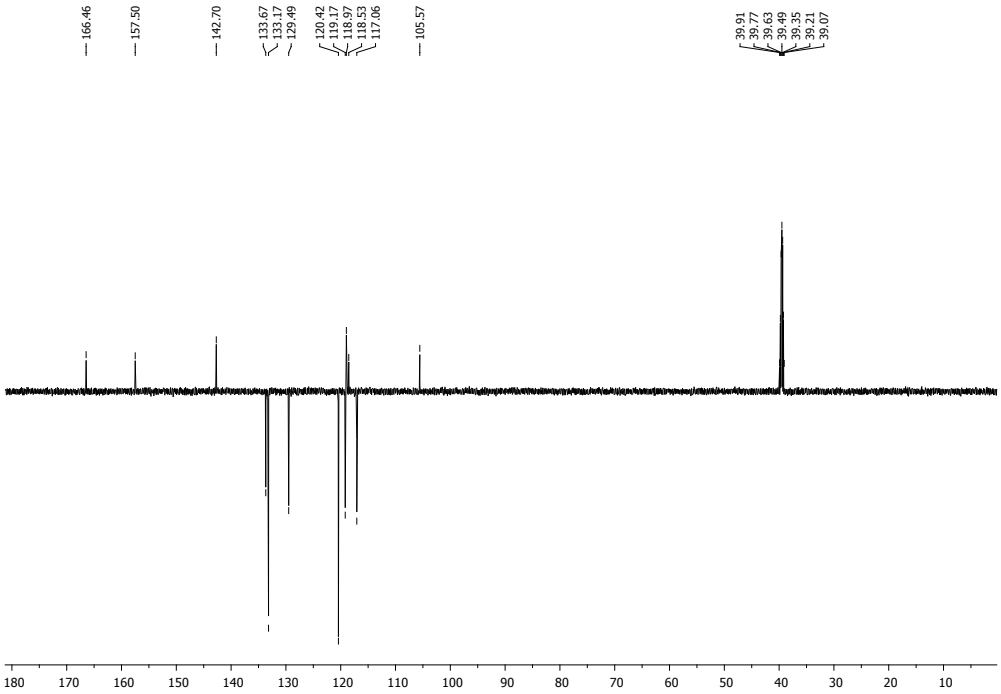


Figure S2. ^{13}C NMR spectrum (DMSO-d_6, 150 MHz) of ***2-hydroxy-N-(4-cyanophenyl)benzamide 7***.

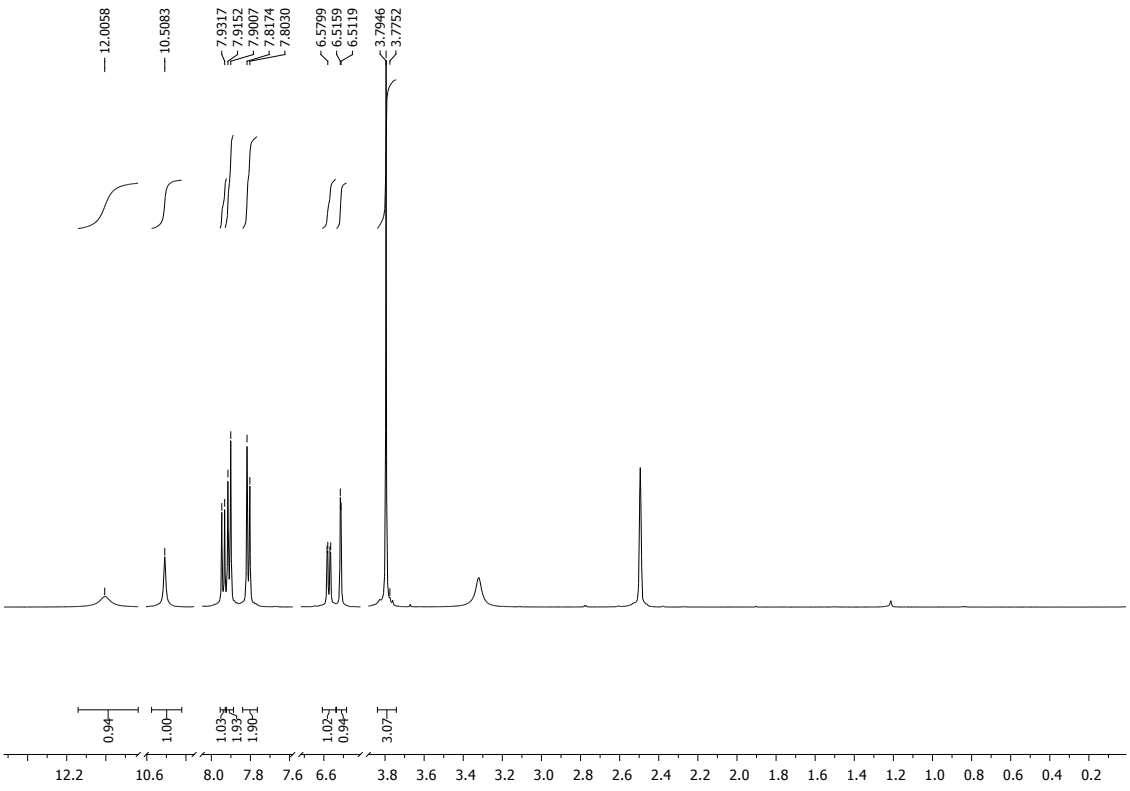


Figure S3. ^{1}H NMR spectrum (DMSO-d_6, 600 MHz) of ***2-hydroxy-4-methoxy-N-(4-cyanophenyl)benzamide 8***.

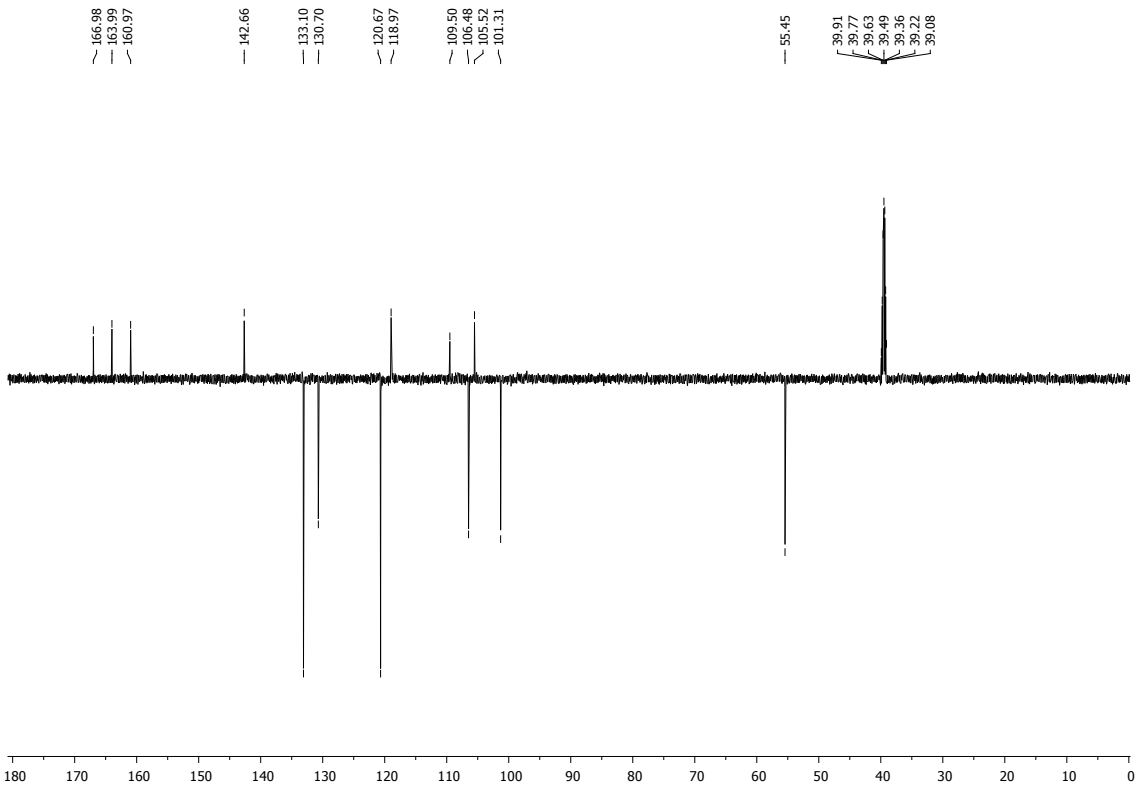


Figure S4. ^{13}C NMR spectrum (DMSO-d_6, 150 MHz) of ***2-hydroxy-4-methoxy-N-(4-cyanophenyl)benzamide 8***.

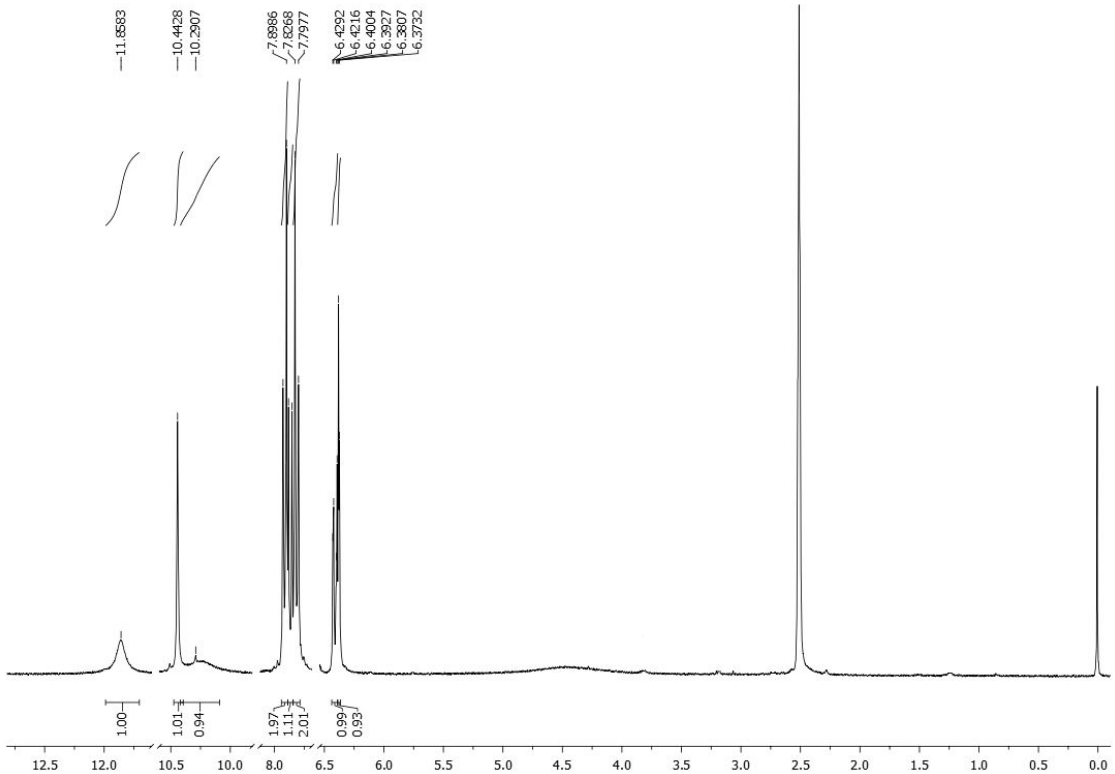


Figure S5. ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of ***2,4-dihydroxy-N-(4-cyanophenyl)benzamide 9***.

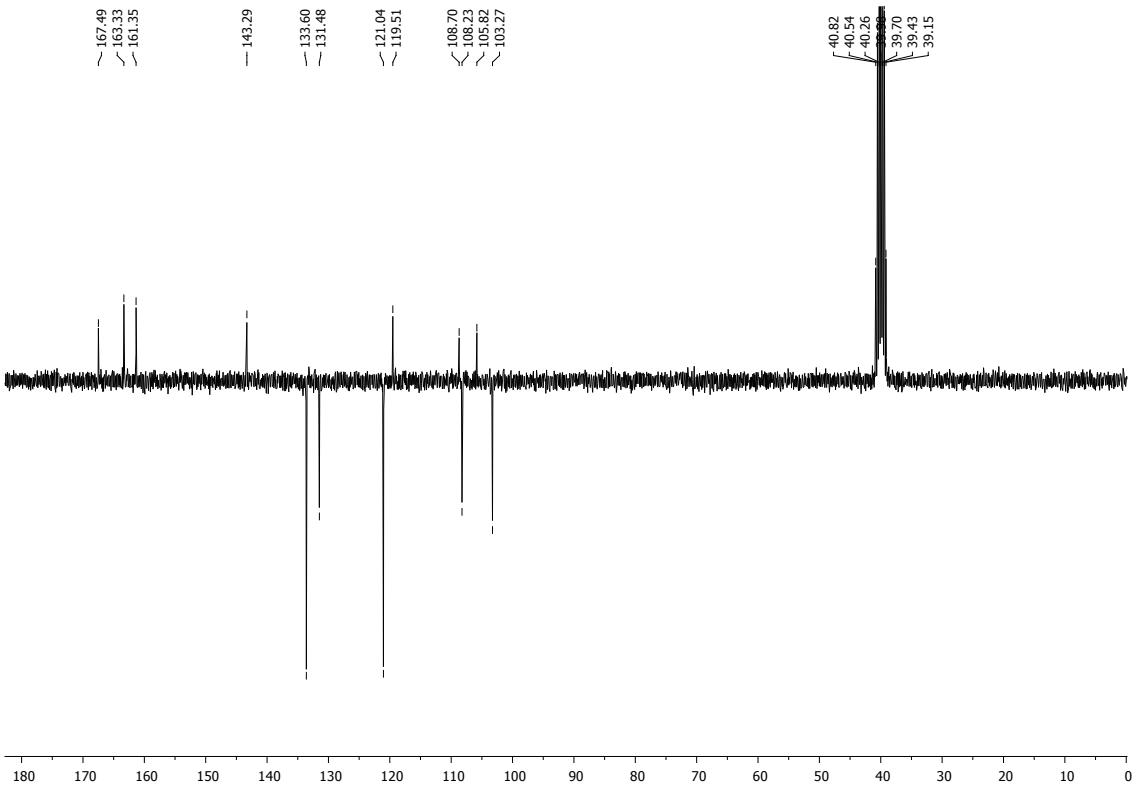


Figure S6. ^{13}C NMR spectrum (DMSO-d_6, 75 MHz) of ***2,4-dihydroxy-N-(4-cyanophenyl)benzamide 9***.

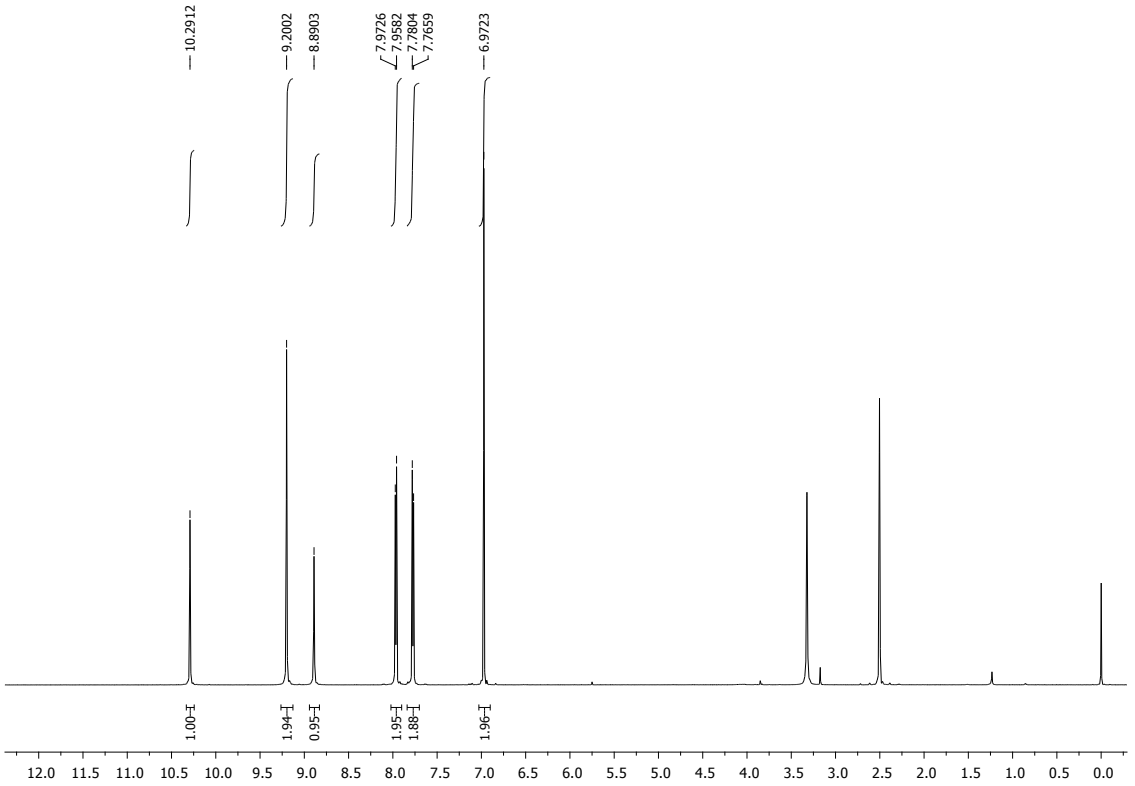


Figure S7. ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of ***3,4,5-trihydroxy-N-(4-cyanophenyl)benzamide 10***.

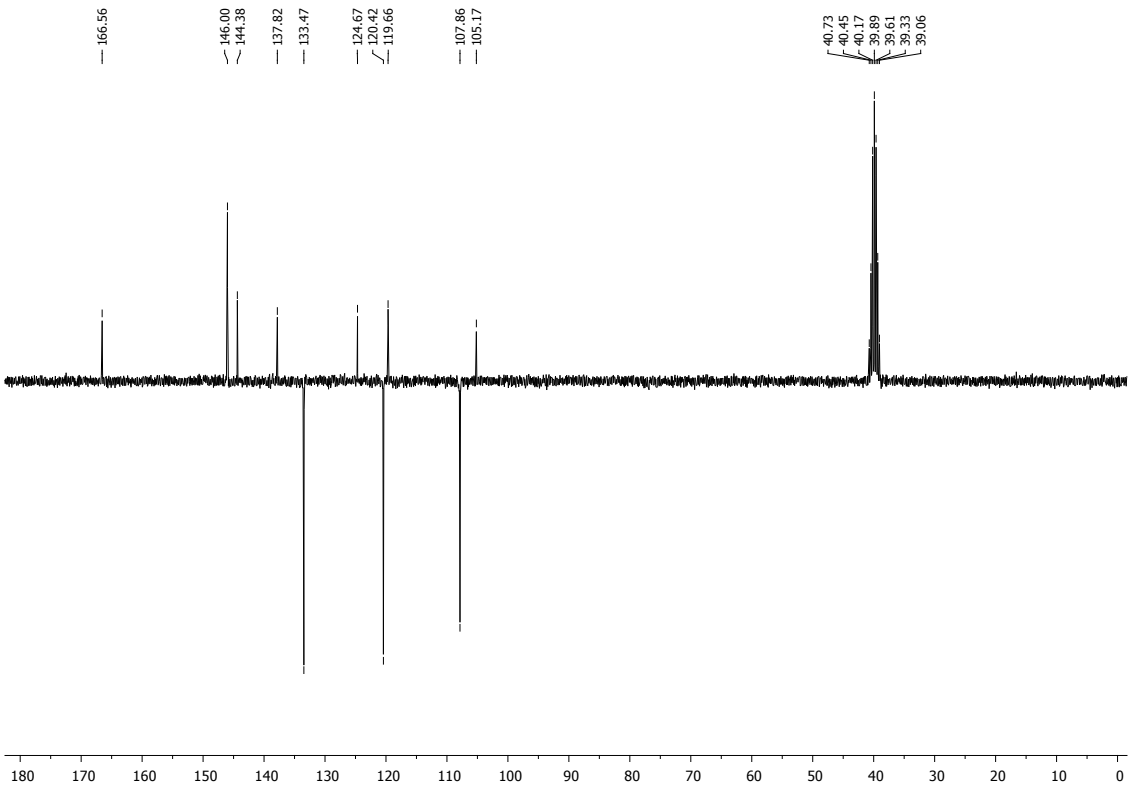


Figure S8. ^{13}C NMR spectrum (DMSO-d_6, 75 MHz) of ***3,4,5-trihydroxy-N-(4-cyanophenyl)benzamide 10***.

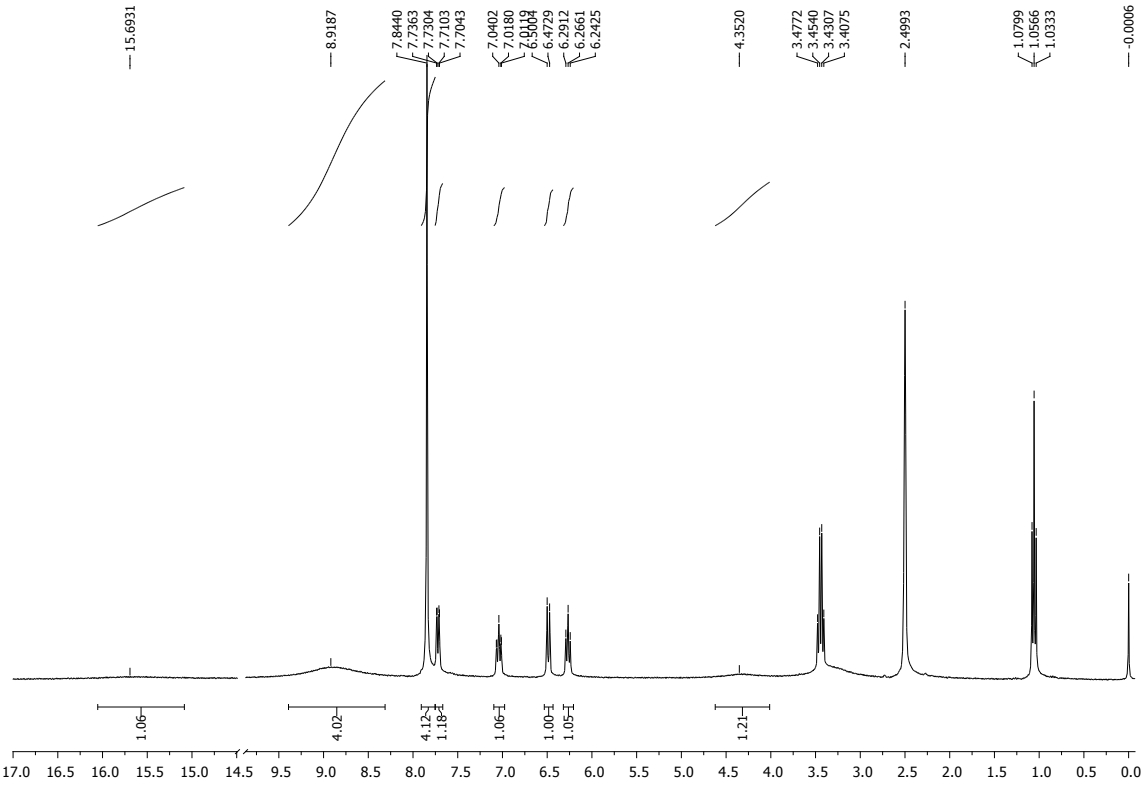


Figure S9. ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of ***2-hydroxy-N-(4-amidinophenyl)benzamide 11***.

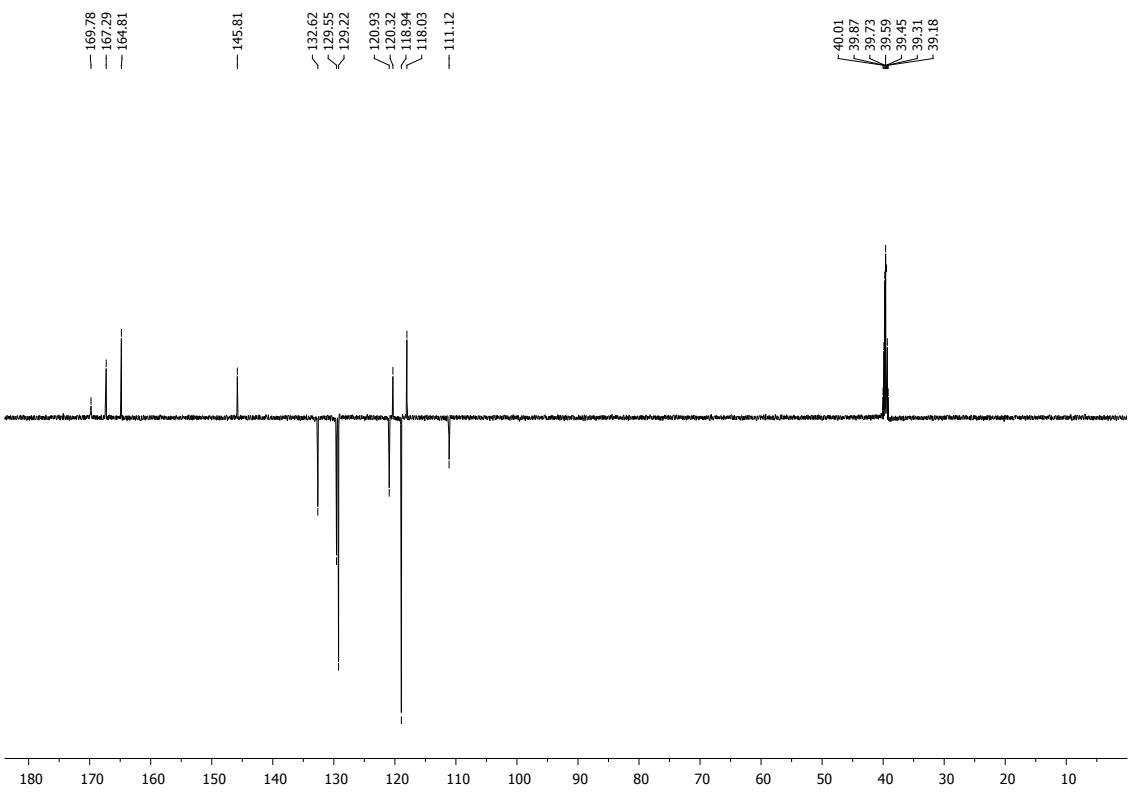


Figure S10. ^{13}C NMR spectrum (DMSO-d_6, 150 MHz) of ***2-hydroxy-N-(4-amidinophenyl)benzamide 11***.

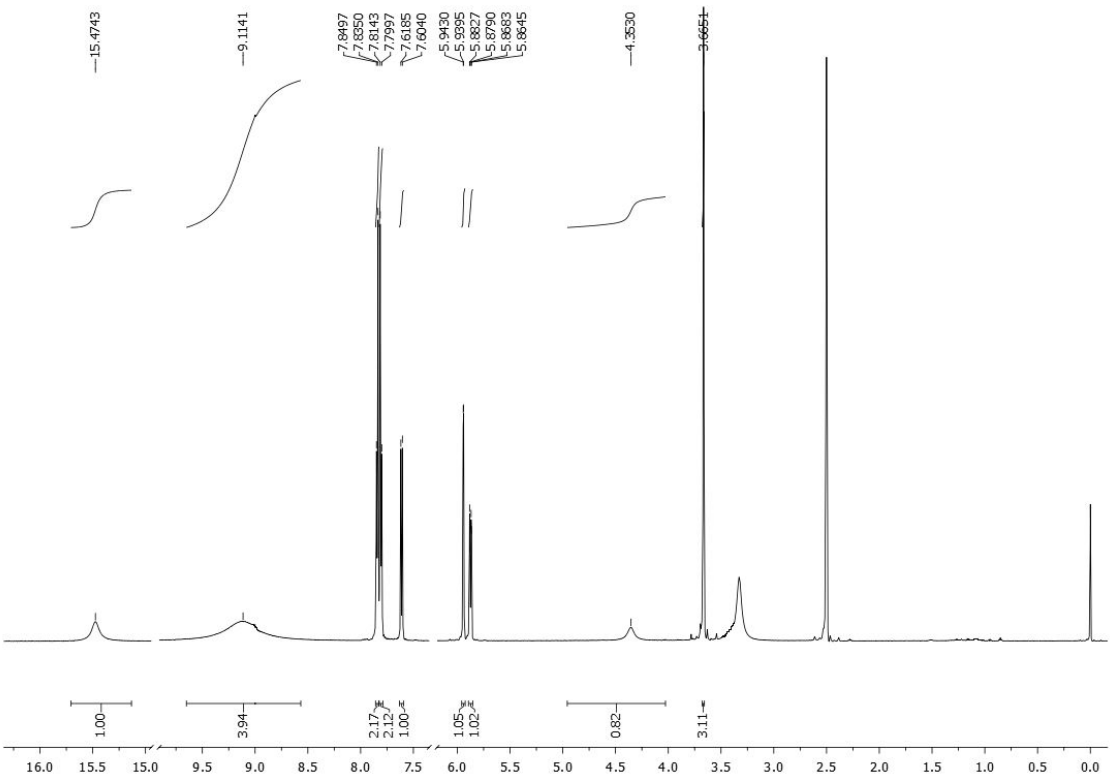


Figure S11. ^{1}H NMR spectrum (DMSO-d_6, 600 MHz) of ***2-hydroxy-4-methoxy-N-(4-amidinophenyl)benzamide 12***.

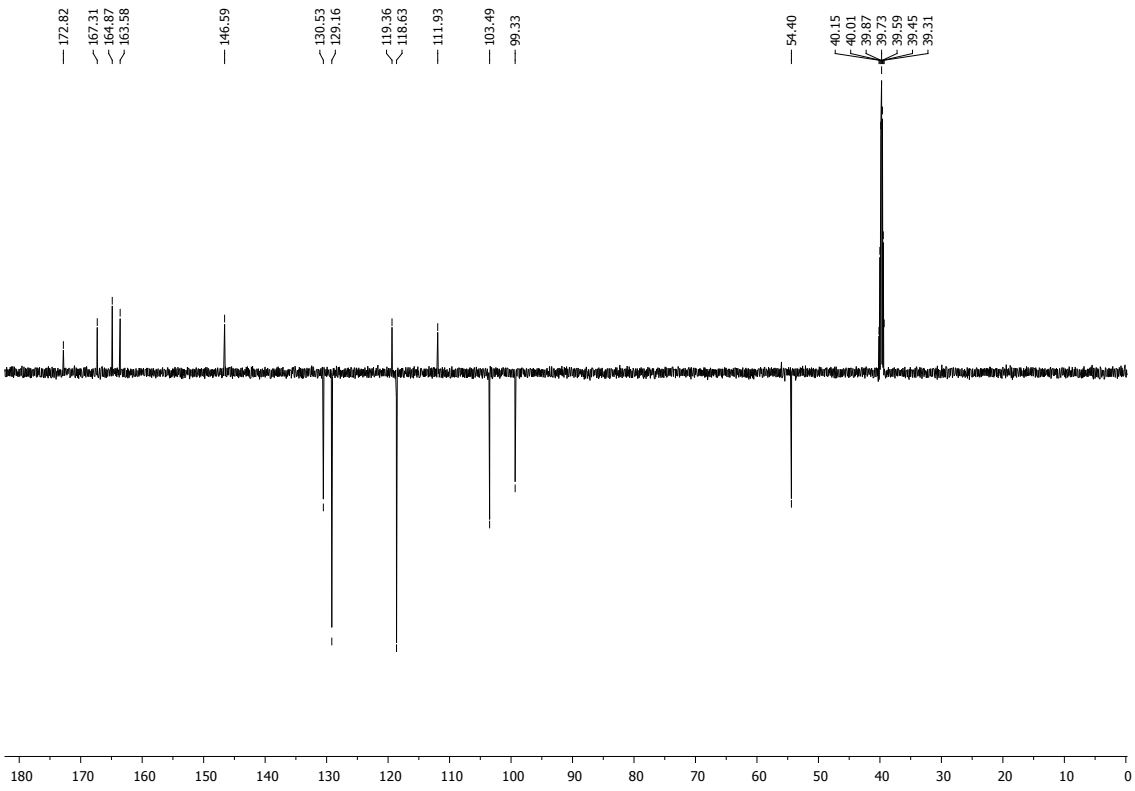


Figure S12. ^{13}C NMR spectrum (DMSO-d_6, 150 MHz) of ***2-hydroxy-4-methoxy-N-(4-amidinophenyl)benzamide 12***.

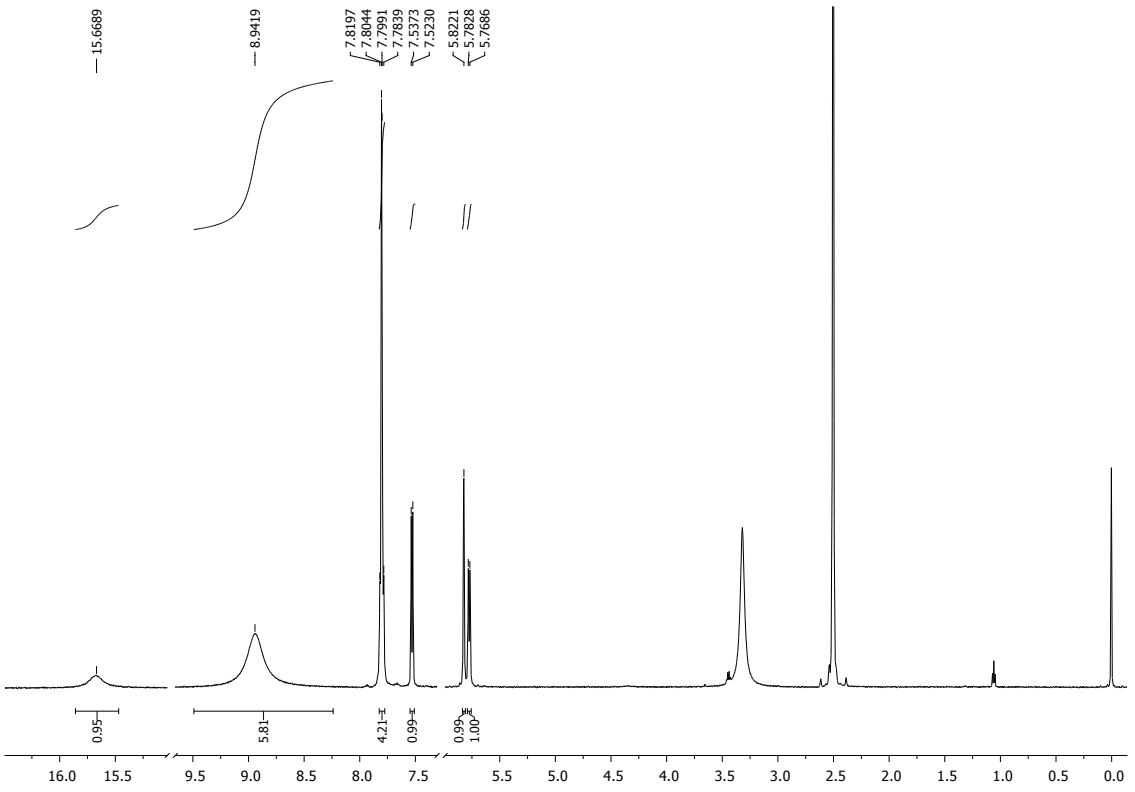


Figure S13. ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of ***2,4-dihydroxy-N-(4-amidinophenyl)benzamide 13***.

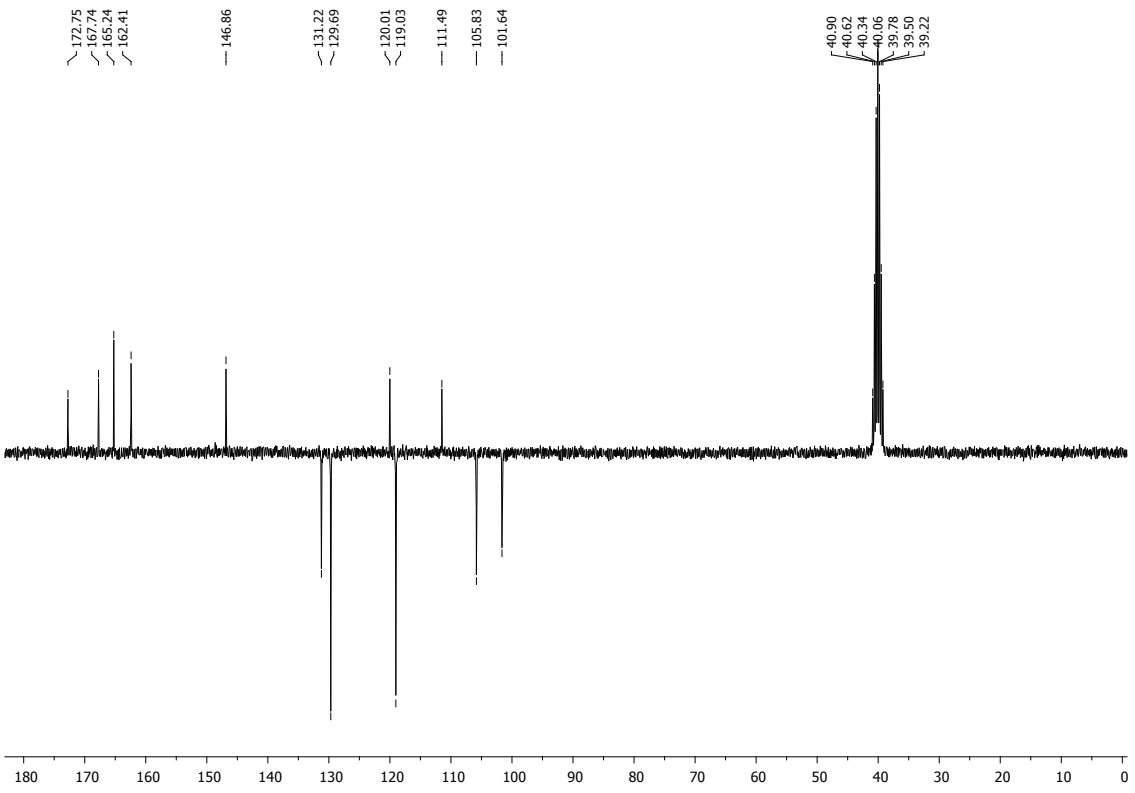


Figure S14. ^{13}C NMR spectrum (DMSO-d_6, 75 MHz) of ***2,4-dihydroxy-N-(4-amidinophenyl)benzamide 13***.

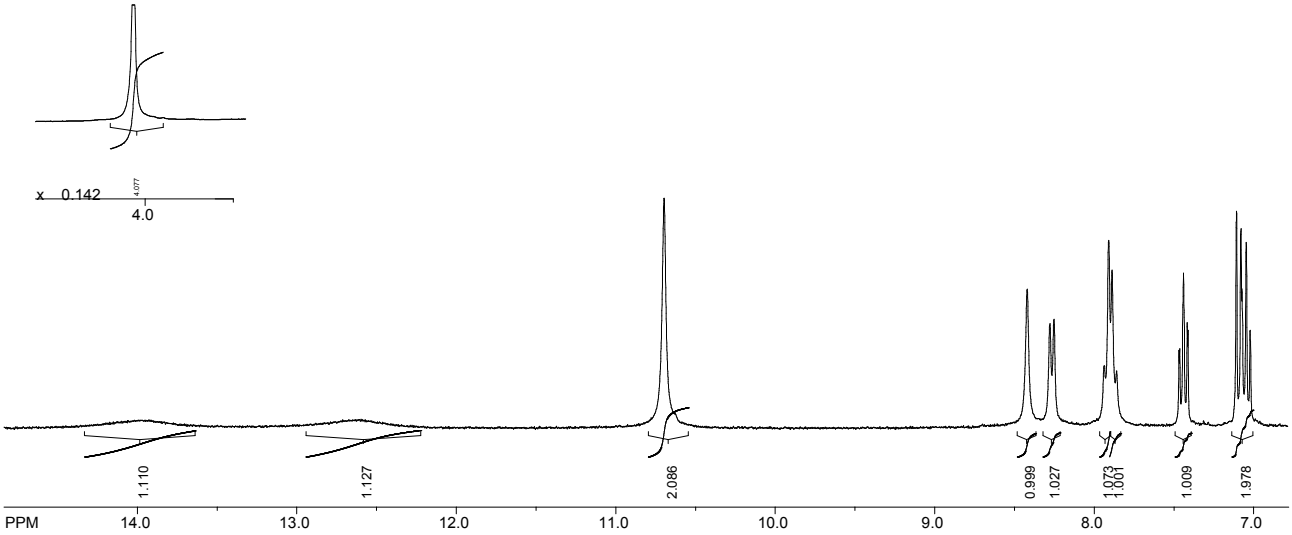


Figure S15. ^{1}H NMR spectrum (DMSO-d_6, 600 MHz) of ***2-hydroxy-N-[4-(imidazolin-2-yl)phenyl]benzamide 14***.

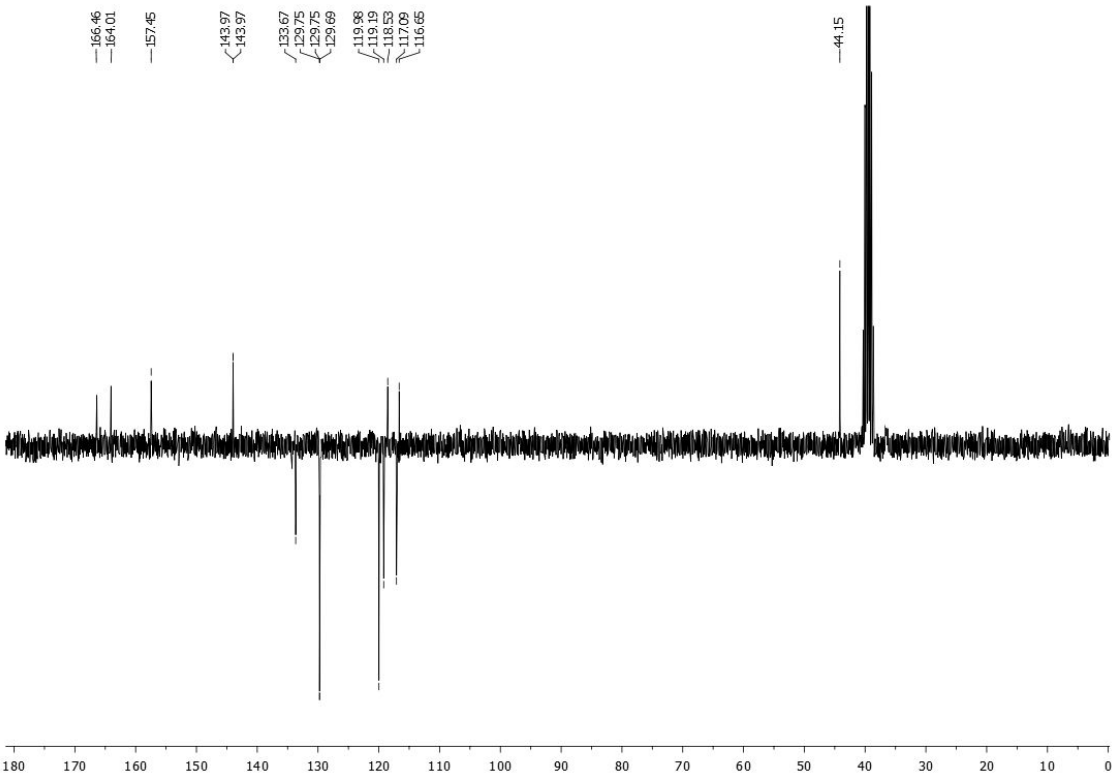


Figure S16. ^{13}C NMR spectrum (DMSO-d_6, 75 MHz) of ***2-hydroxy-N-[4-(imidazolin-2-yl)phenyl]benzamide 14***.

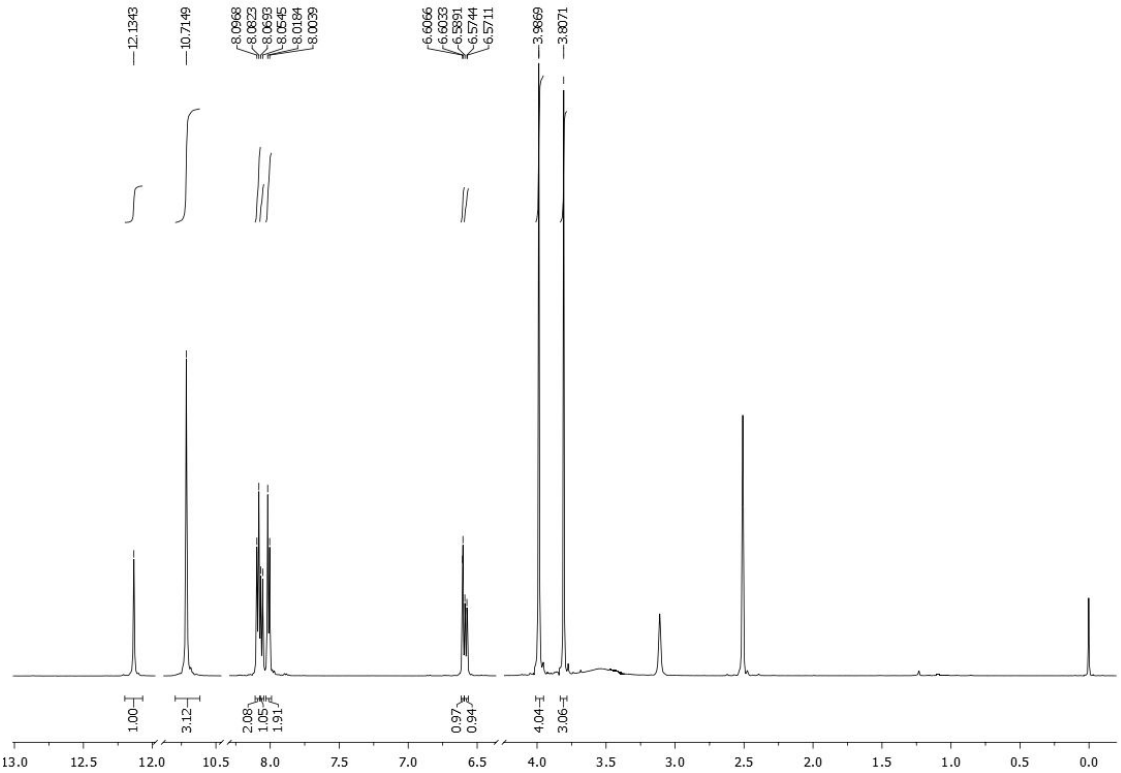


Figure S17. ^{1}H NMR spectrum (DMSO-d_6, 600 MHz) of ***2-hydroxy-4-methoxy-N-[4-(imidazolin-2-yl)phenyl]benzamide 15***.

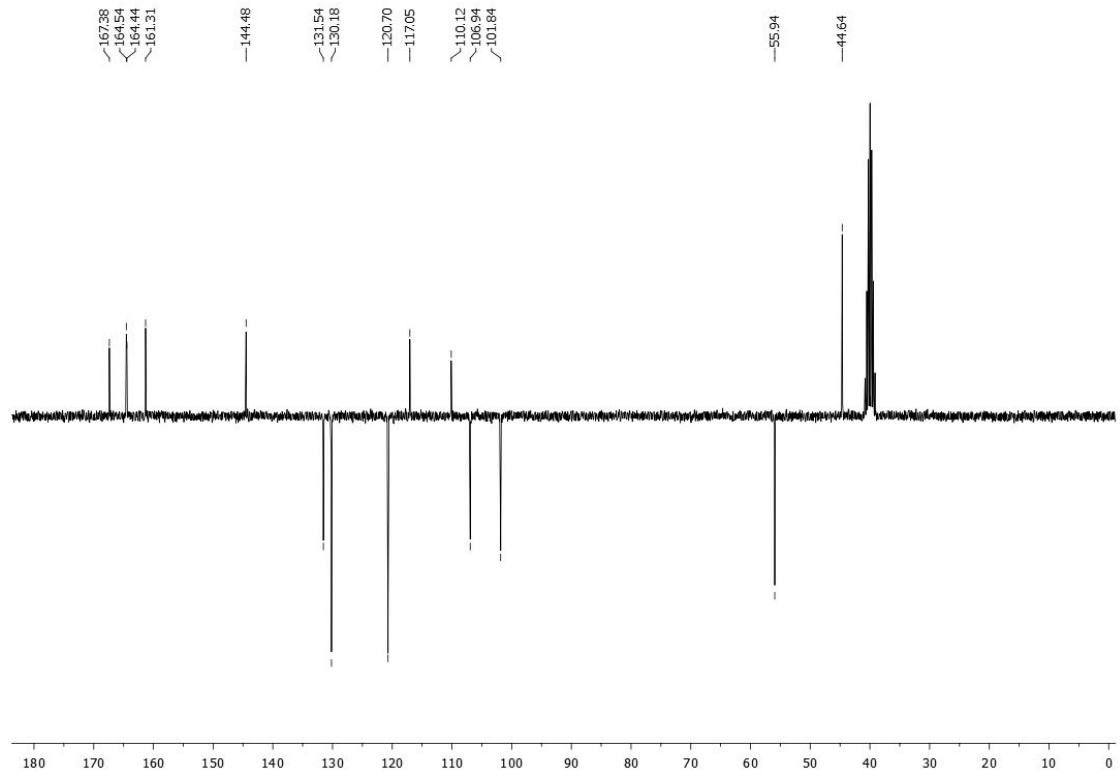


Figure S18. ^{13}C NMR spectrum (DMSO-d_6, 75 MHz) of ***2-hydroxy-4-methoxy-N-[4-(imidazolin-2-yl)phenyl]benzamide 15***.

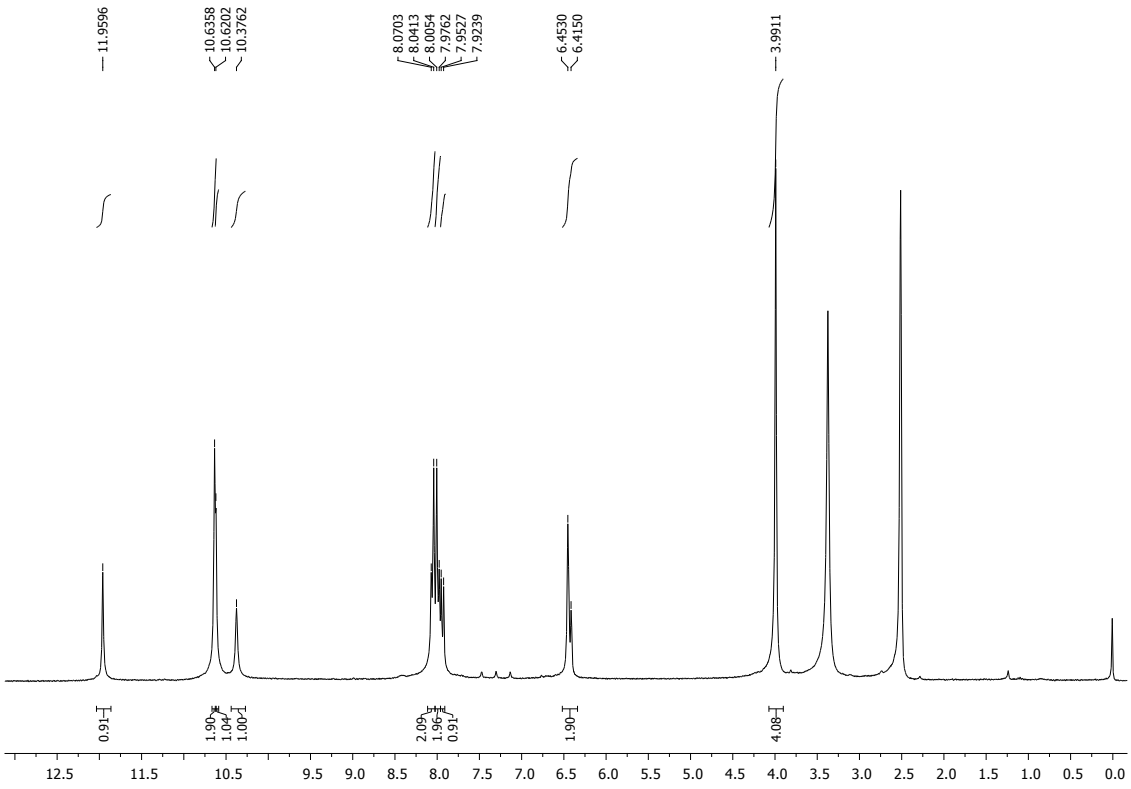


Figure S19. ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of ***2,4-dihydroxy-N-[4-(imidazolin-2-yl)phenyl]benzamide 16***.

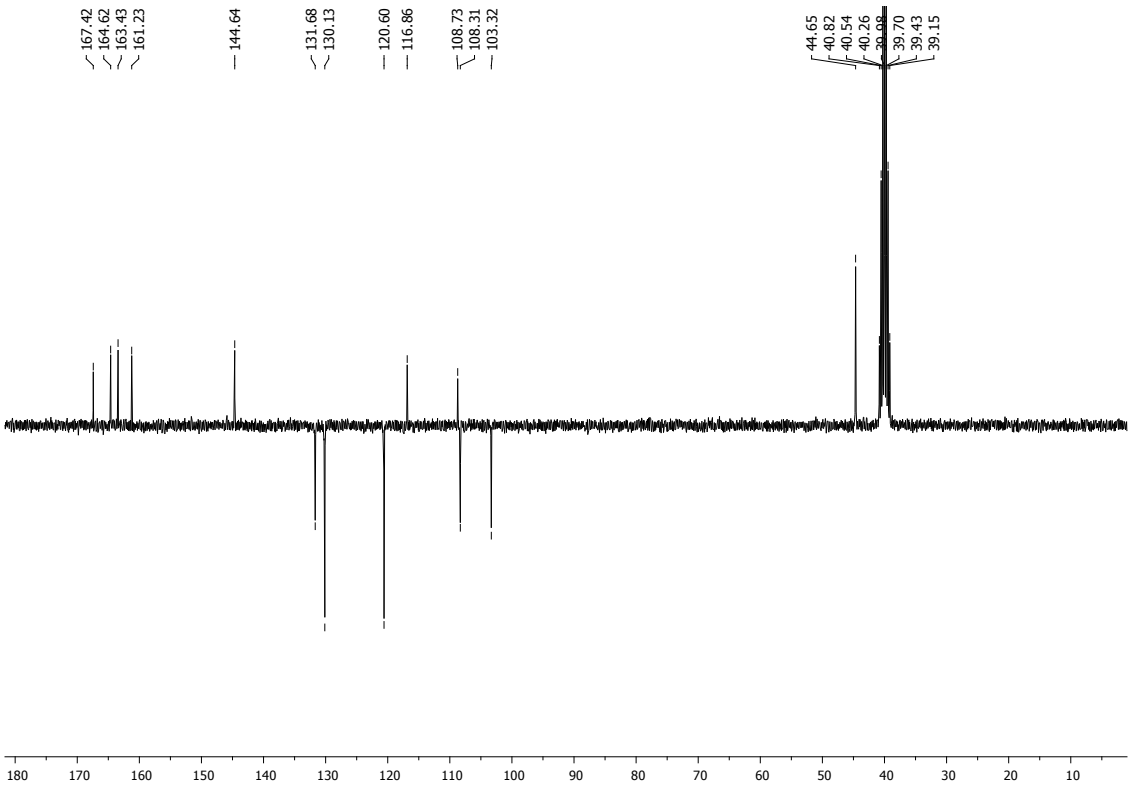


Figure S20. ^{13}C NMR spectrum (DMSO-d_6, 75 MHz) of ***2,4-dihydroxy-N-[4-(imidazolin-2-yl)phenyl]benzamide 16*.**

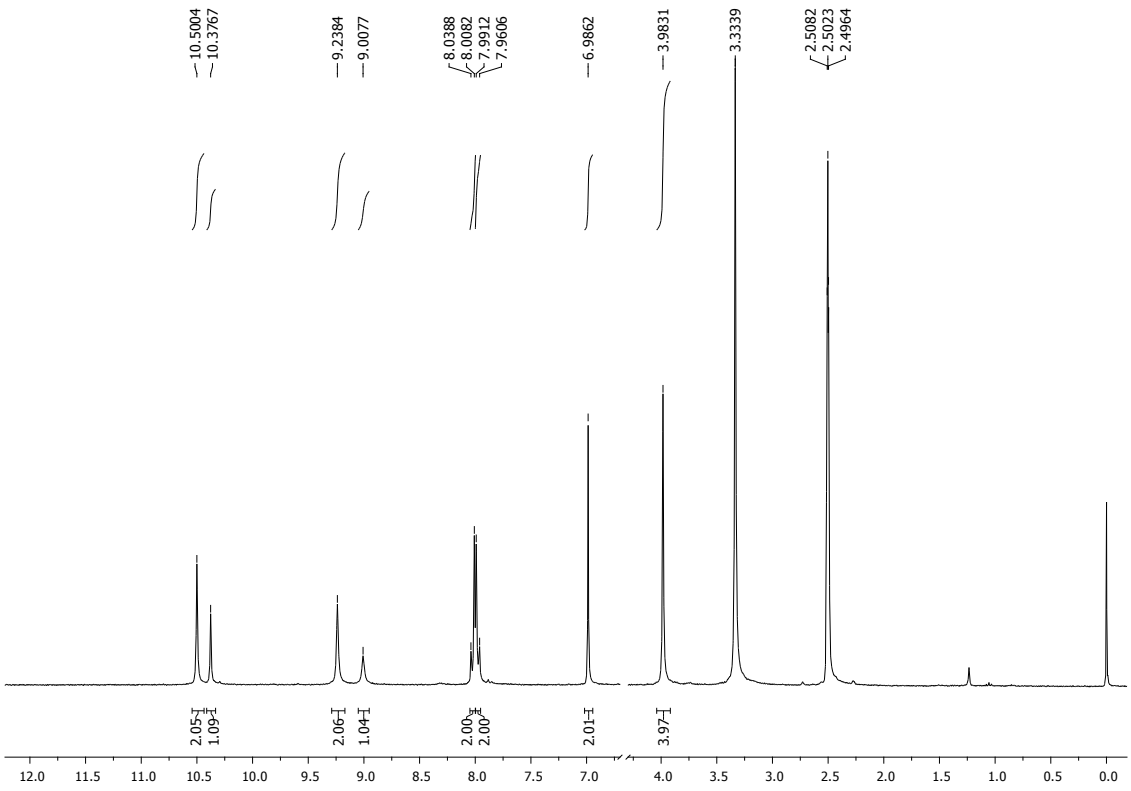


Figure S21. [1]H NMR spectrum (DMSO-d_6, 300 MHz) of ***3,4,5-trihydroxy-N-[4-(imidazolin-2-yl)phenyl]benzamide 17*** .

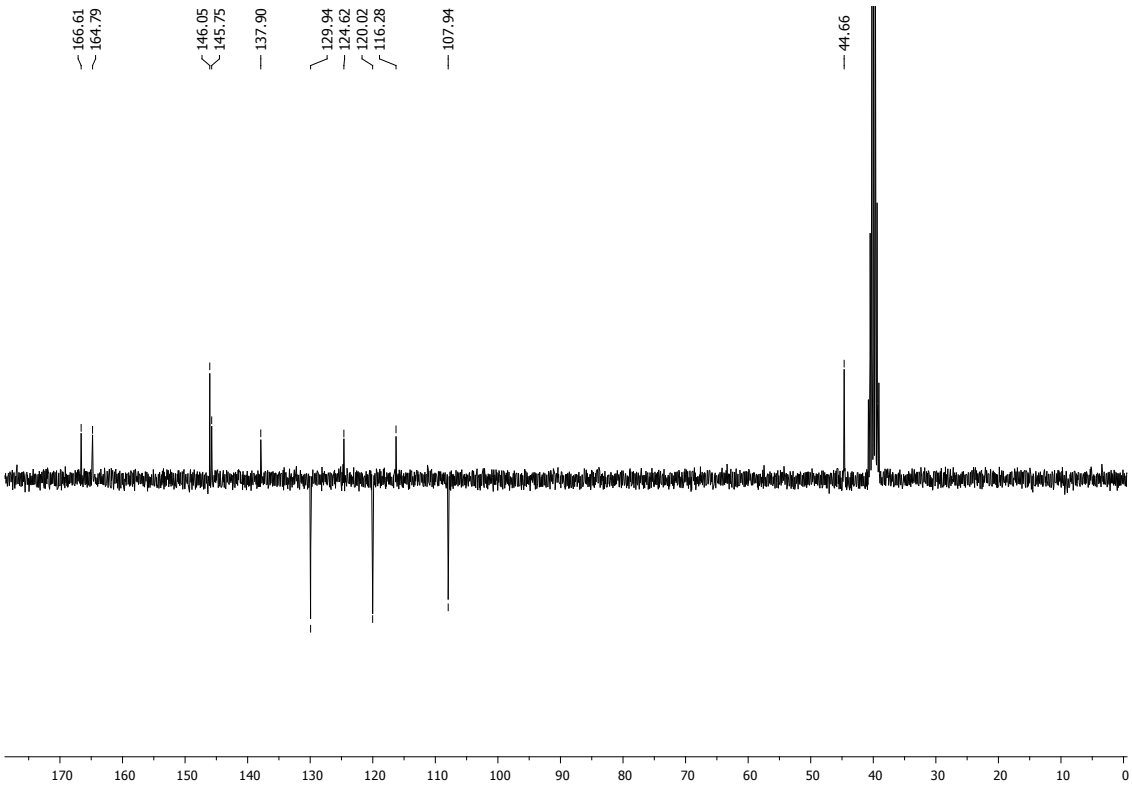


Figure S22. [13]C NMR spectrum (DMSO-d_6, 75 MHz) of ***3,4,5-trihydroxy-N-[4-(imidazolin-2-yl)phenyl]benzamide 17***.

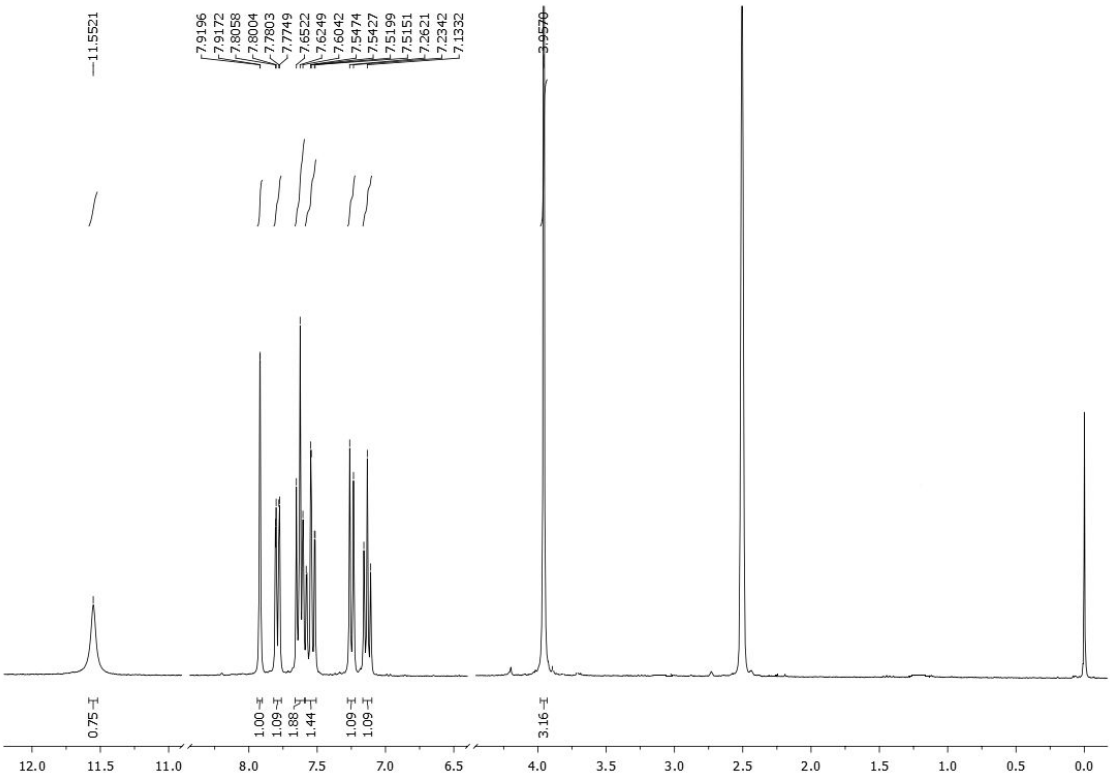


Figure S23. ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of ***2-methoxy-N-[5(6)-cyano-1H-benzimidazol-2-yl]benzamide 20***.

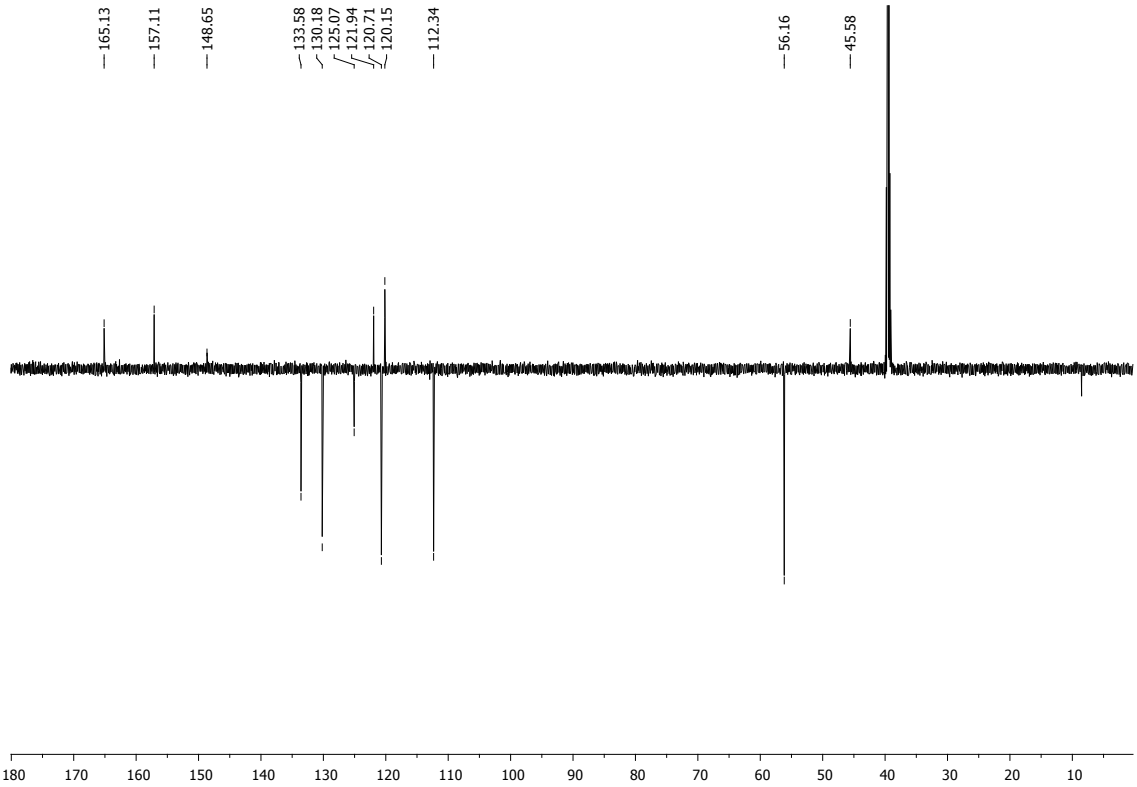


Figure S24. ^{13}C NMR spectrum (DMSO-d_6, 150 MHz) of ***2-methoxy-N-[5(6)-cyano-1H-benzimidazol-2-yl]benzamide 20***.

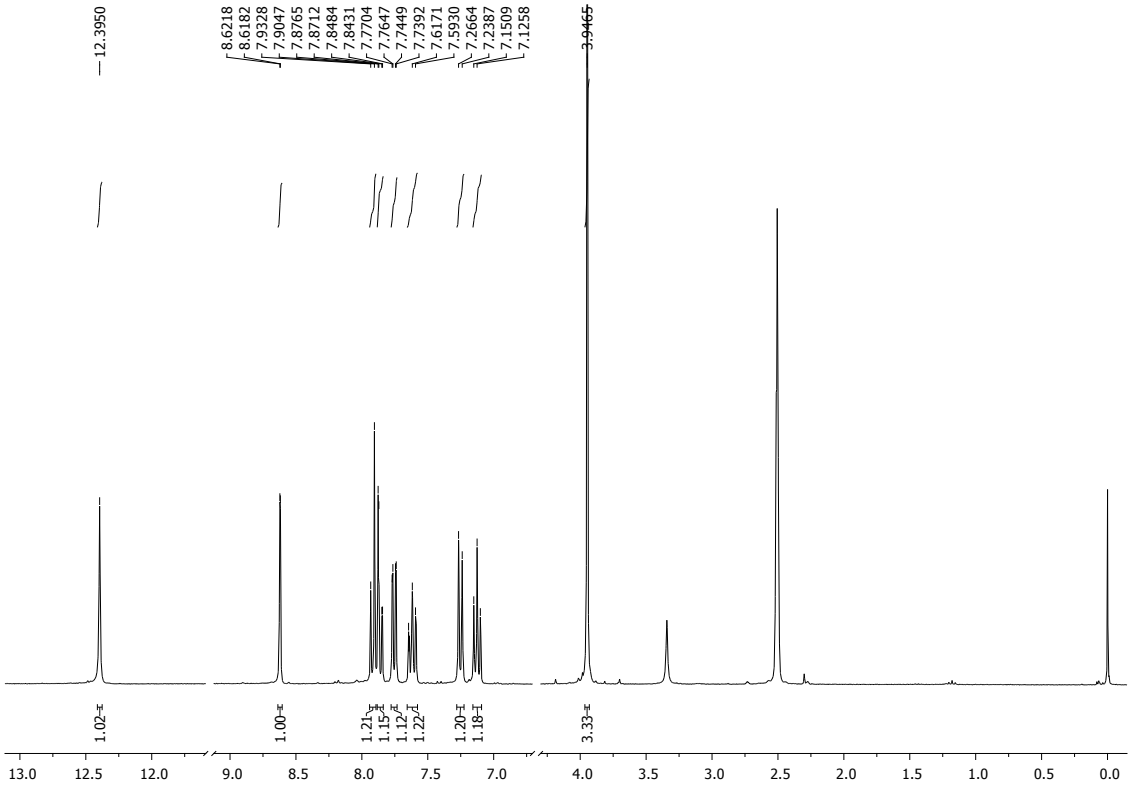


Figure S25. ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of ***2-Methoxy-N-(6-cyanobenzothiazol-2-yl)benzamide 23***.

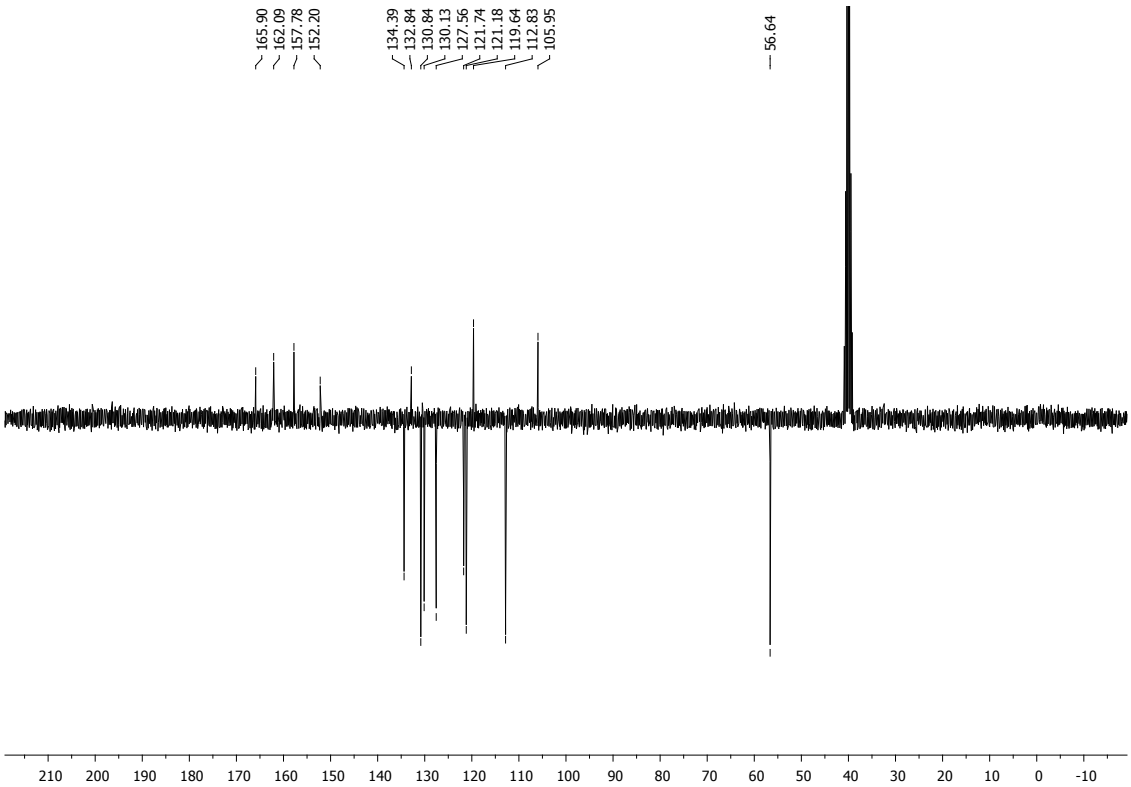


Figure S26. ^{13}C NMR spectrum (DMSO-d_6, 75 MHz) of ***2-Methoxy-N-(6-cyanobenzothiazol-2-yl)benzamide 23***.

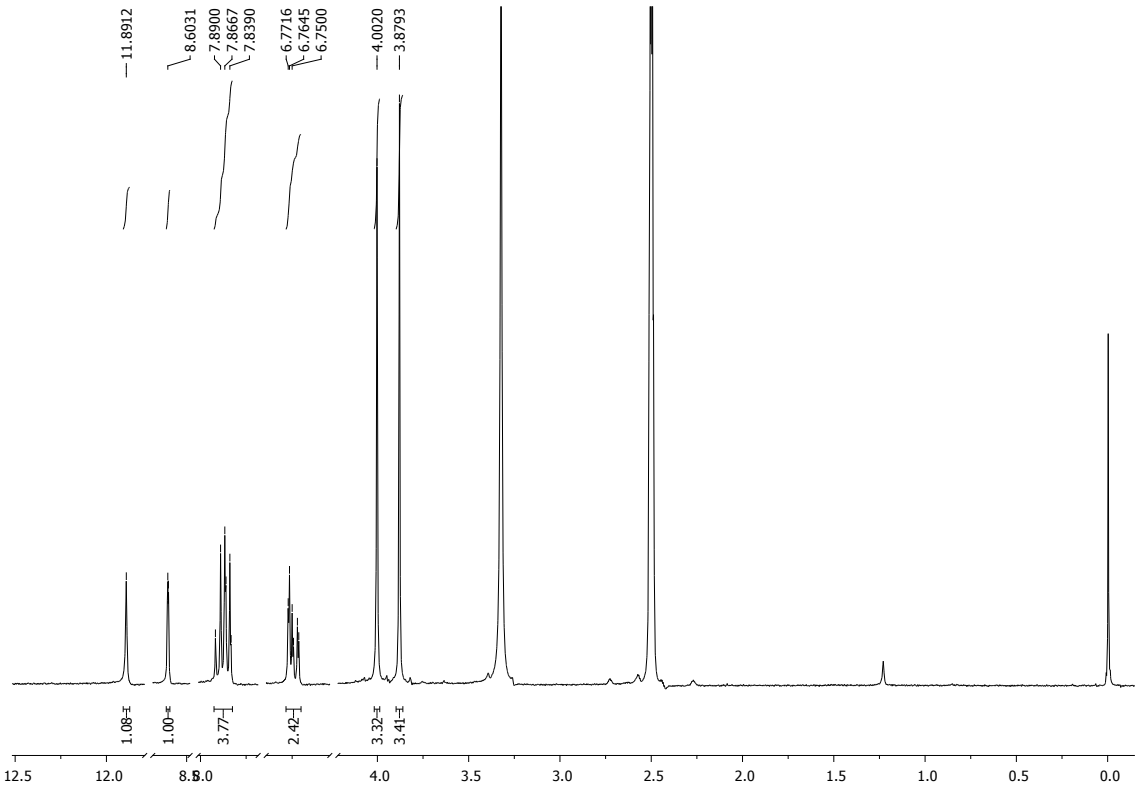


Figure S27. ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of ***2,4-dimethoxy-N-(6-cyanobenzothiazol-2-yl)benzamide 24***.

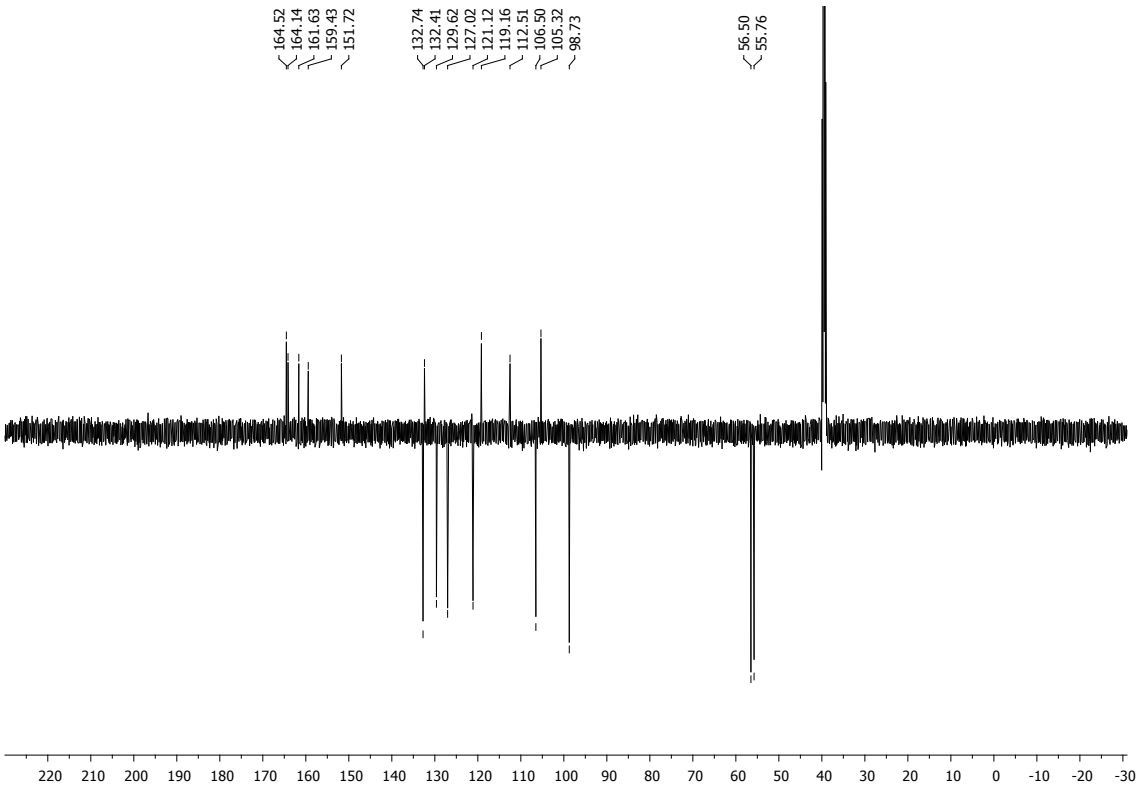


Figure S28. ^{13}C NMR spectrum (DMSO-d_6, 150 MHz) of ***2,4-dimethoxy-N-(6-cyanobenzothiazol-2-yl)benzamide 24***.

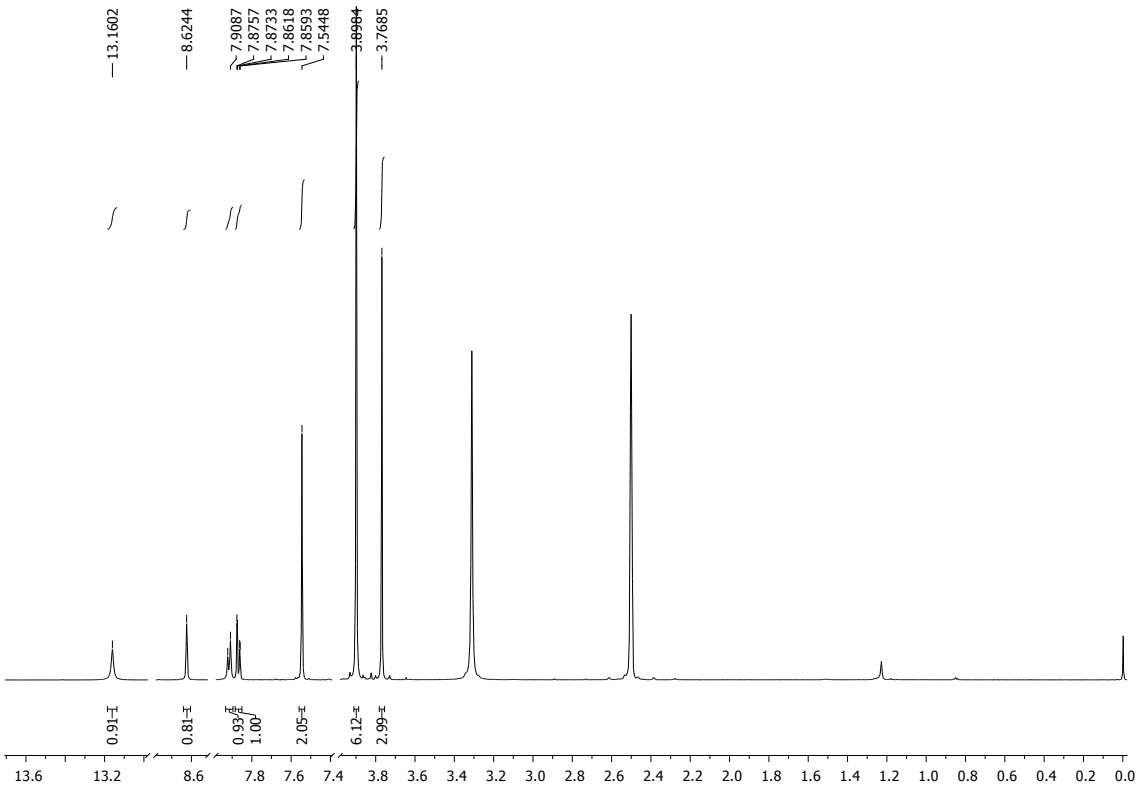


Figure S29. ^{1}H NMR spectrum (DMSO-d_6, 600 MHz) of ***3,4,5-trimethoxy-N-(6-cyanobenzothiazol-2-yl)benzamide 25***.

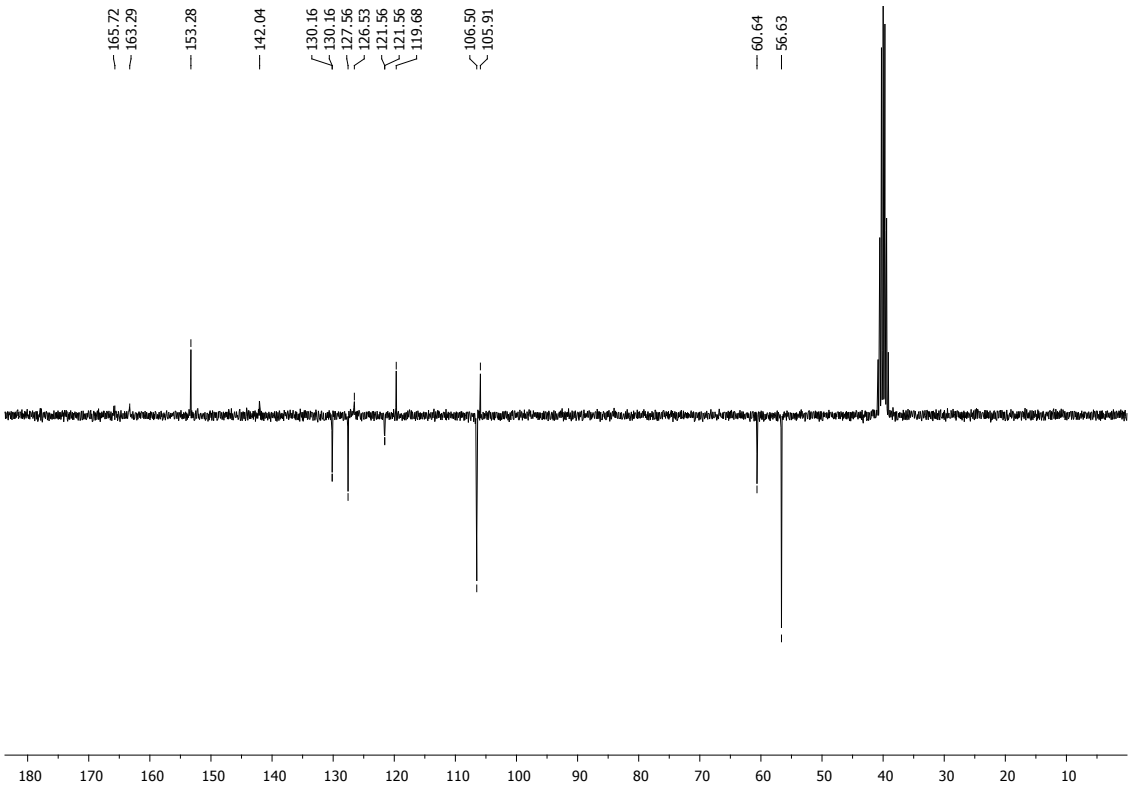


Figure S30. ^{13}C NMR spectrum (DMSO-d_6, 75 MHz) of ***3,4,5-trimethoxy-N-(6-cyanobenzothiazol-2-yl)benzamide 25***.

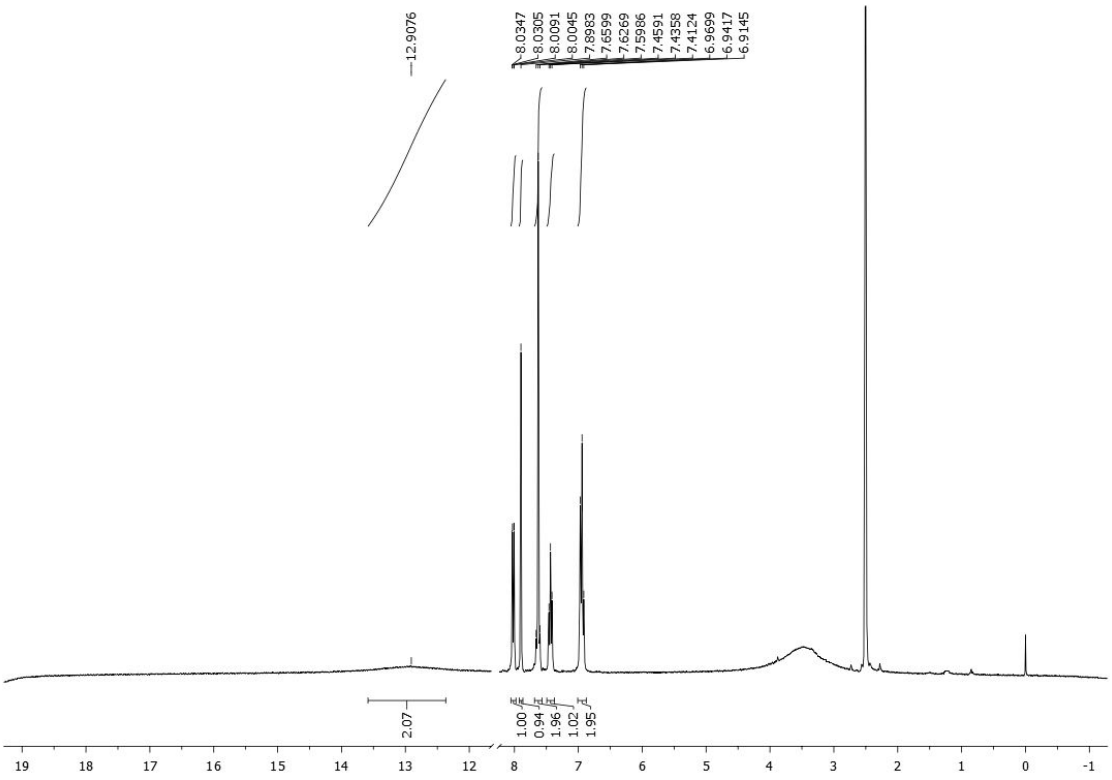


Figure S31. ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of ***2-hydroxy-N-[5(6)-cyano-1H-benzimidazol-2-yl]benzamide 26***.

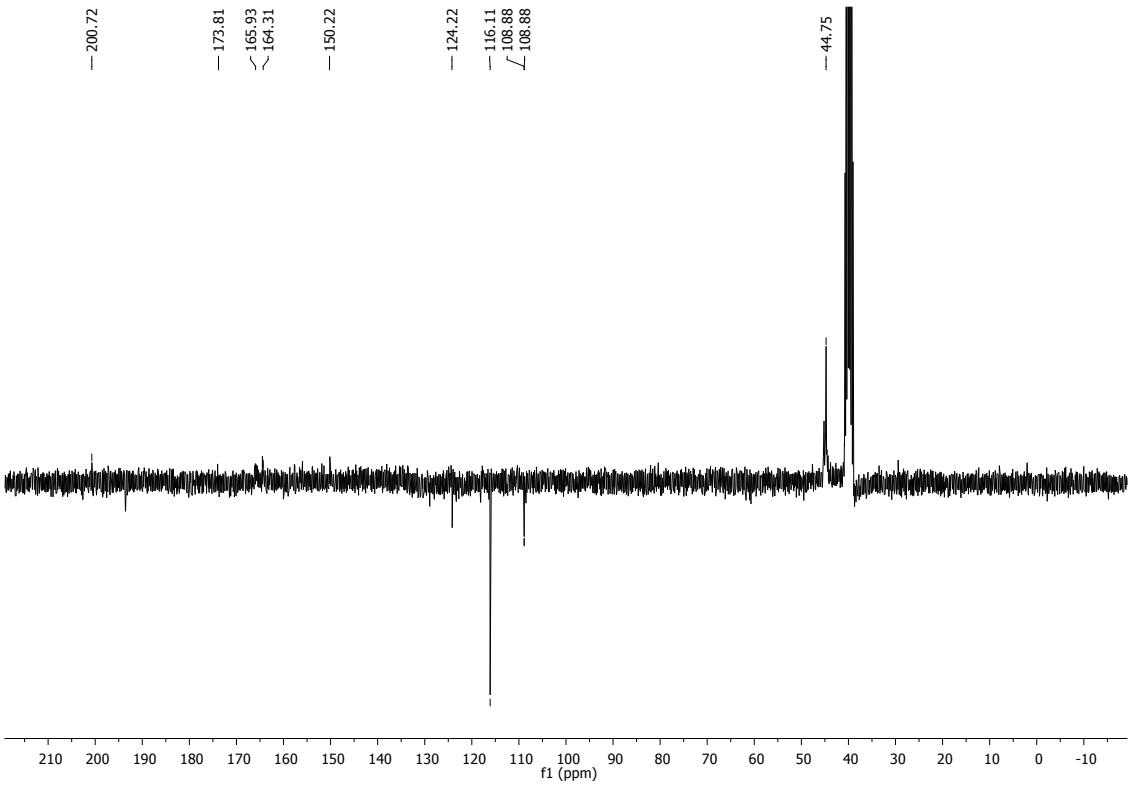


Figure S32. ^{13}C NMR spectrum (DMSO-d_6, 75 MHz) of ***2-hydroxy-N-[5(6)-cyano-1H-benzimidazol-2-yl]benzamide 26***.

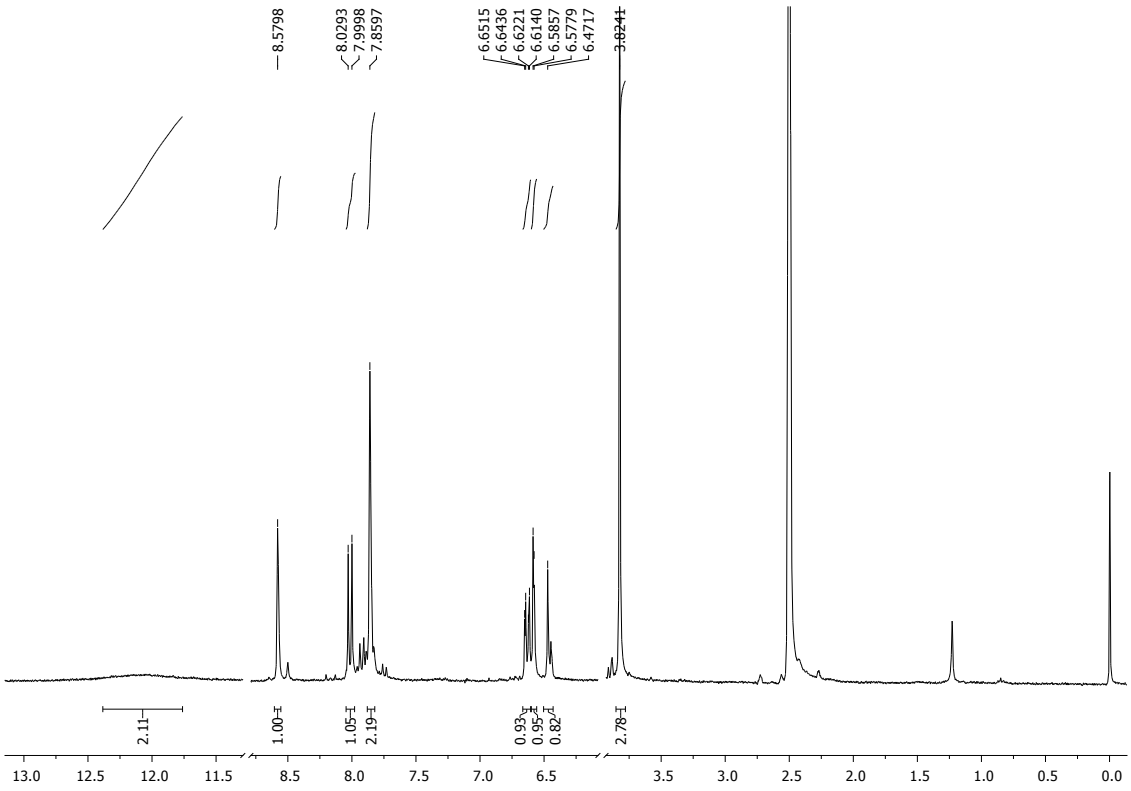


Figure S33. ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of ***2-hydroxy-4-methoxy-N-[5(6)-cyano-1H-benzimidazol-2-yl]benzamide 27***.

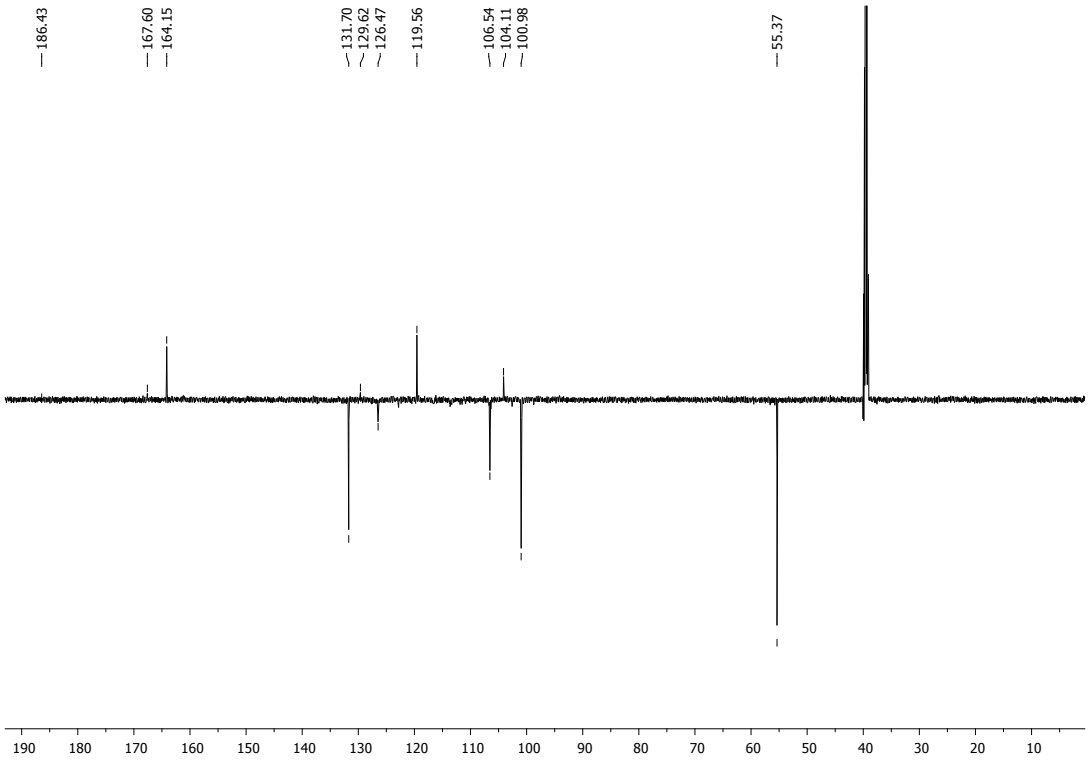


Figure S34. ^{13}C NMR spectrum (DMSO-d_6, 150 MHz) of ***2-hydroxy-4-methoxy-N-[5(6)-cyano-1H-benzimidazol-2-yl]benzamide 27***.

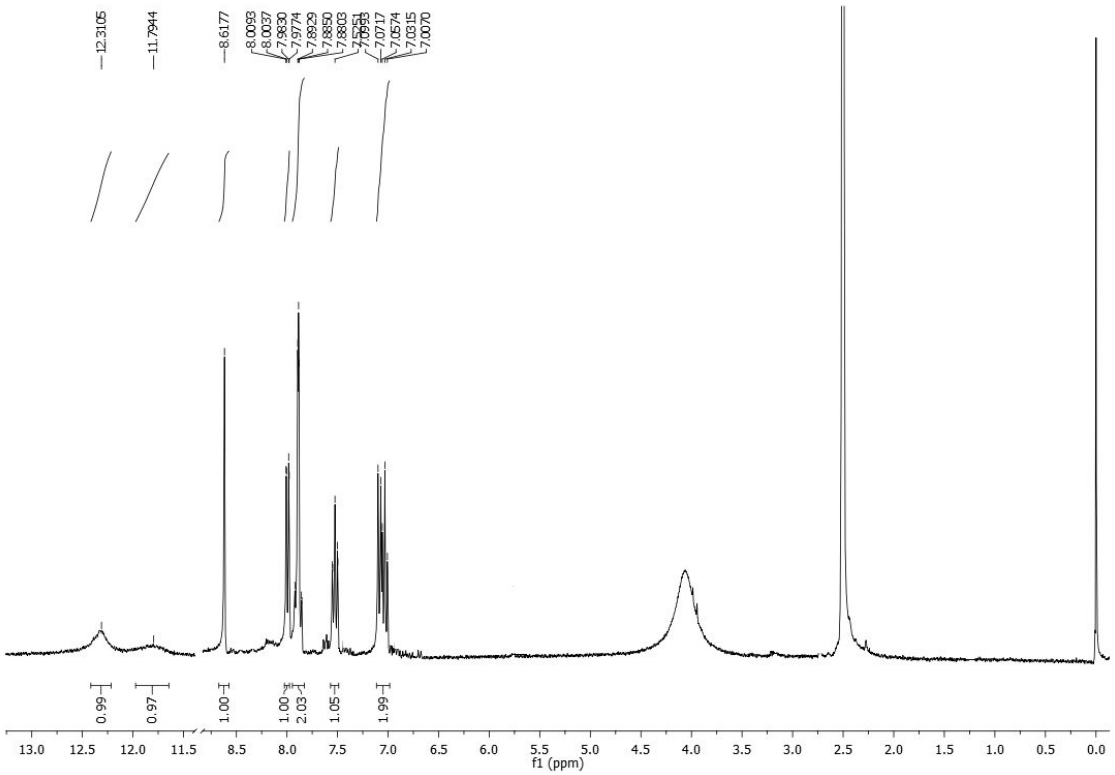


Figure S35. ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of ***2-hydroxy-N-(6-cyanobenzothiazol-2-yl)benzamide 28***.

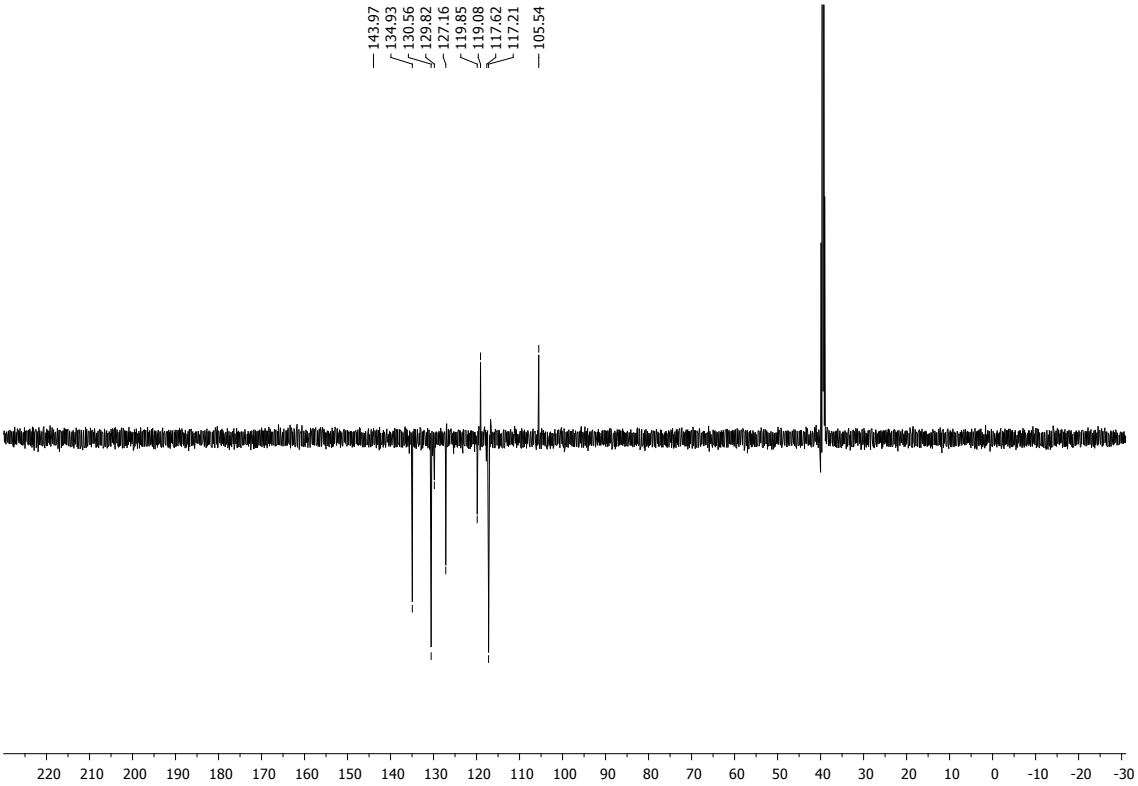


Figure S36. ^{13}C NMR spectrum (DMSO-d_6, 150 MHz) of ***2-hydroxy-N-(6-cyanobenzothiazol-2-yl)benzamide 28***.

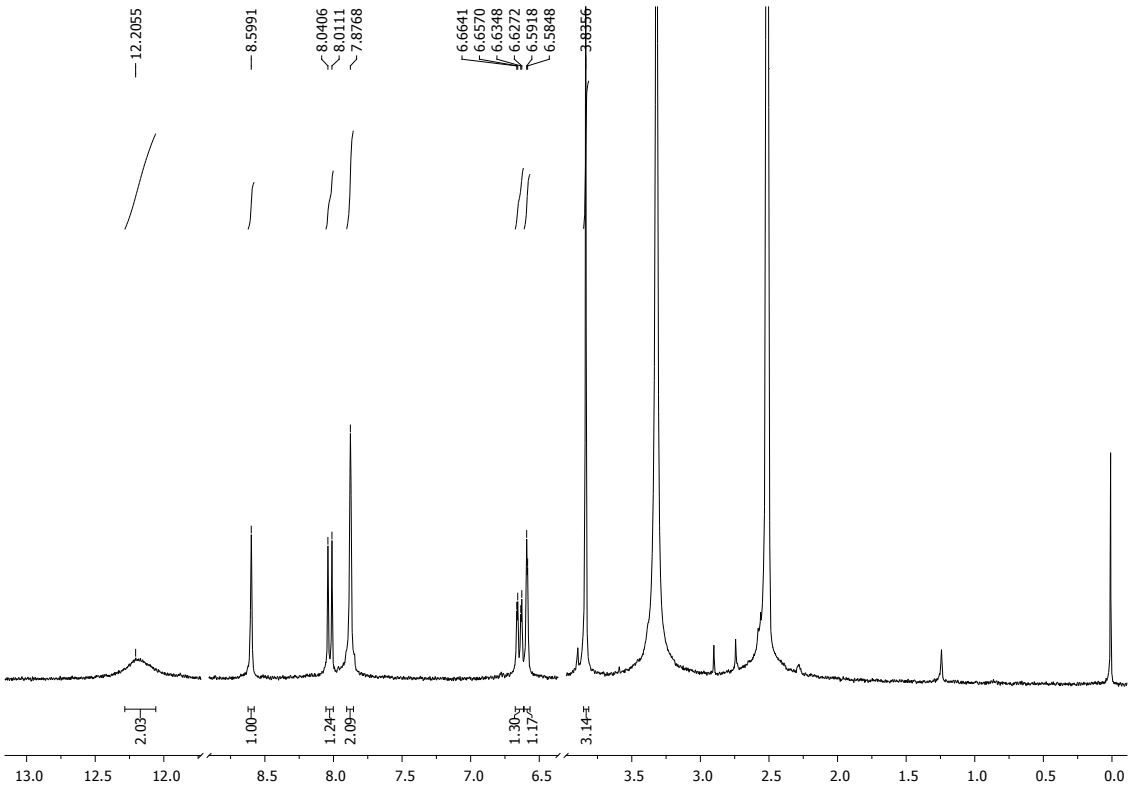


Figure S37. ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of ***2-hydroxy-4-methoxy-N-(6-cyanobenzothiazol-2-yl)benzamide 29***.

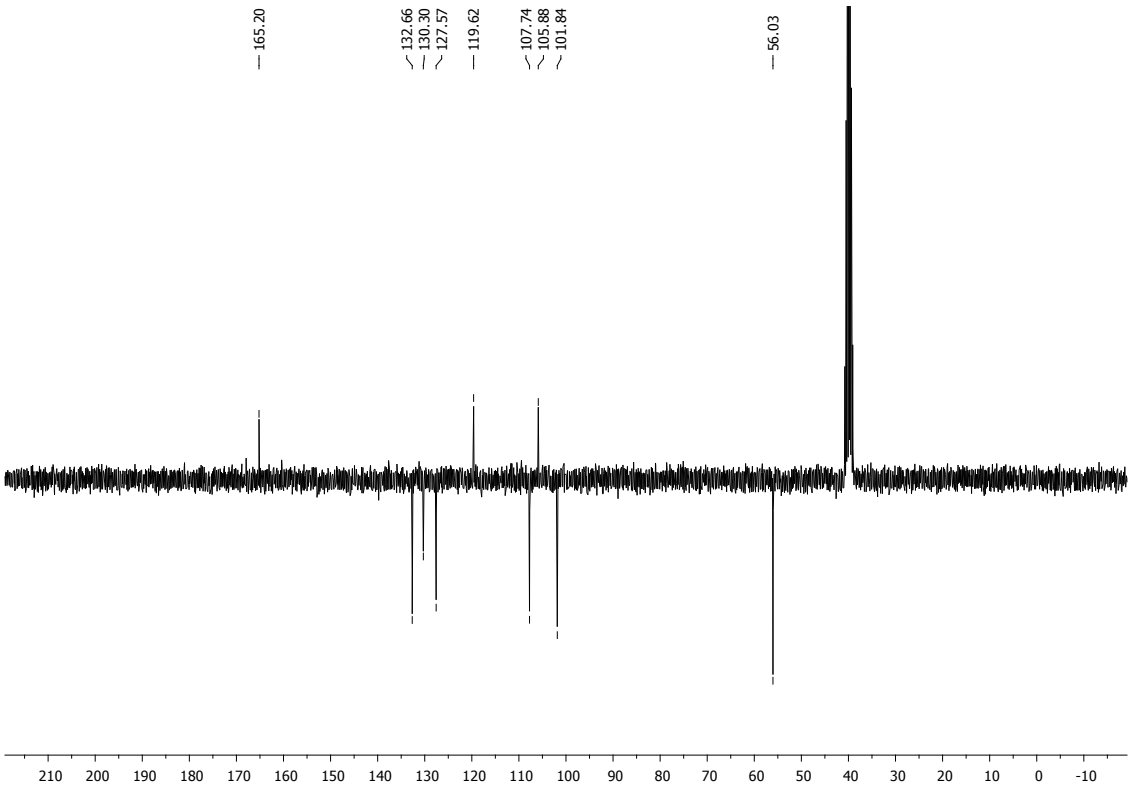


Figure S38. ^{13}C NMR spectrum (DMSO-d_6, 75 MHz) of ***2-hydroxy-4-methoxy-N-(6-cyanobenzothiazol-2-yl)benzamide 29***.

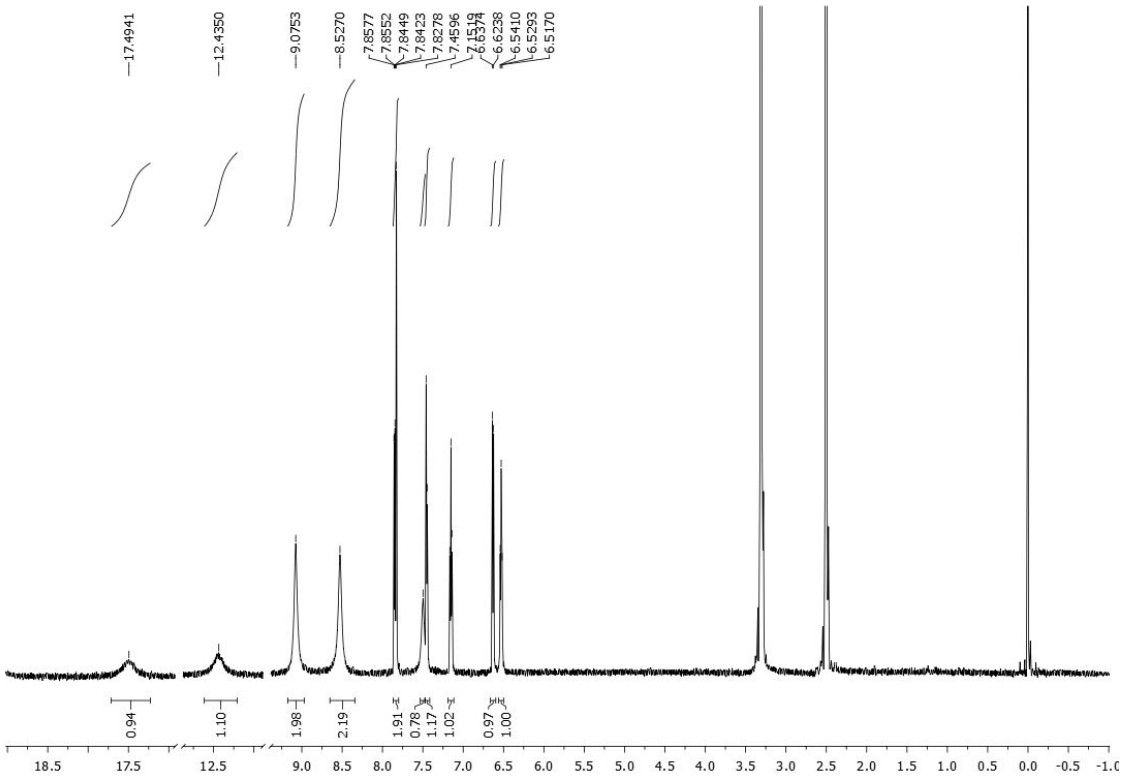


Figure S39. ^{1}H NMR spectrum (DMSO-d_6, 600 MHz) of ***2-hydroxy-N-[5(6)-amidino-1H-benzimidazol-2-yl]benzamide 30***.

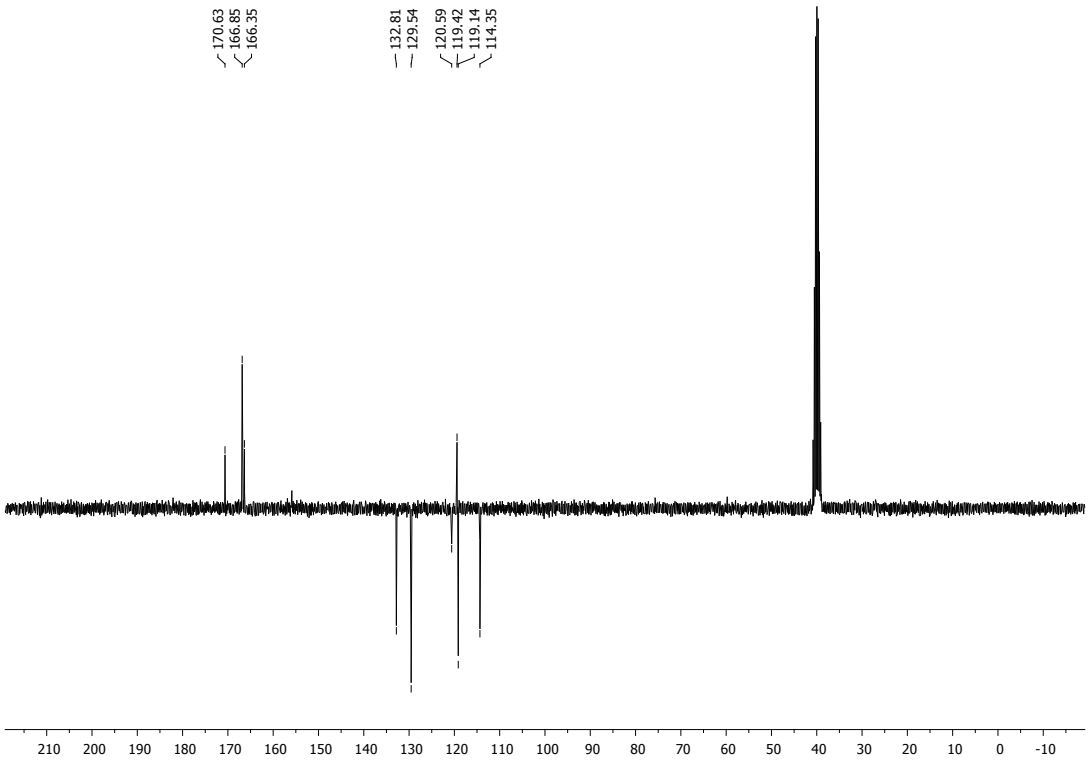


Figure S40. ^{13}C NMR spectrum (DMSO-d_6, 150 MHz) of ***2-hydroxy-N-[5(6)-amidino-1H-benzimidazol-2-yl]benzamide 30***.

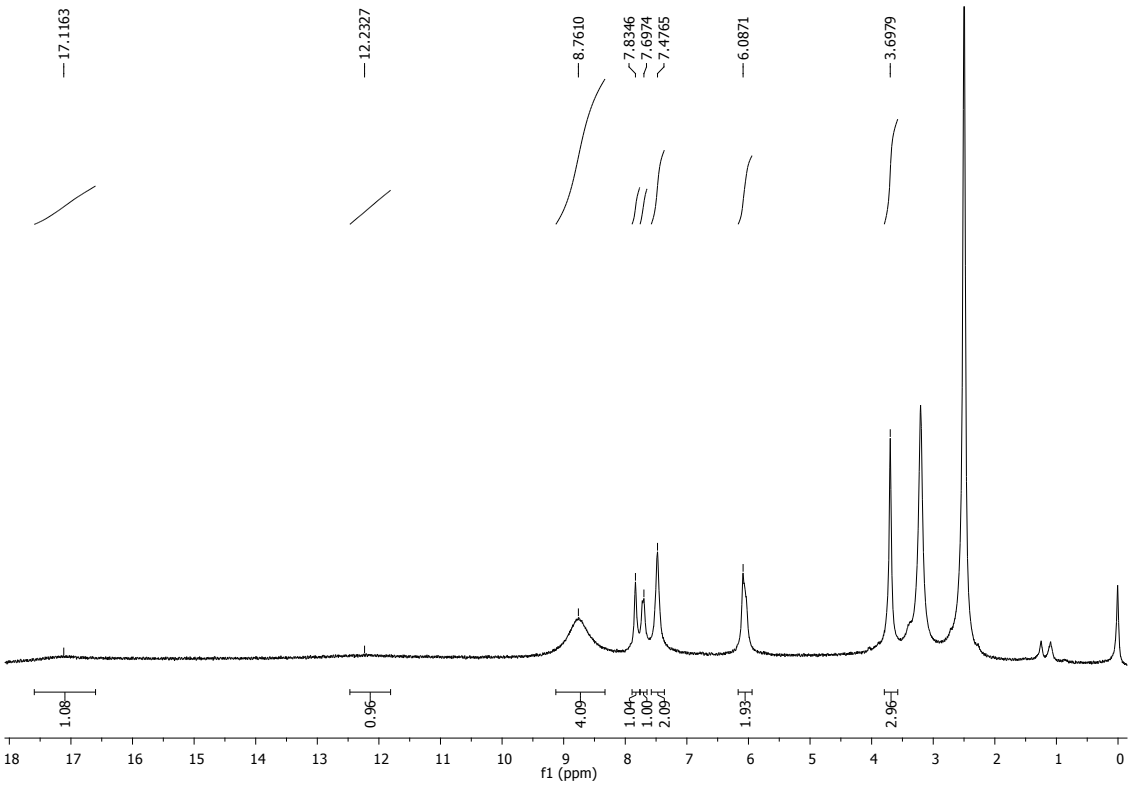


Figure S41. ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of ***2-hydroxy-4-methoxy -N-[5(6)-amidino-1H-benzimidazol-2-yl]benzamide 31***.

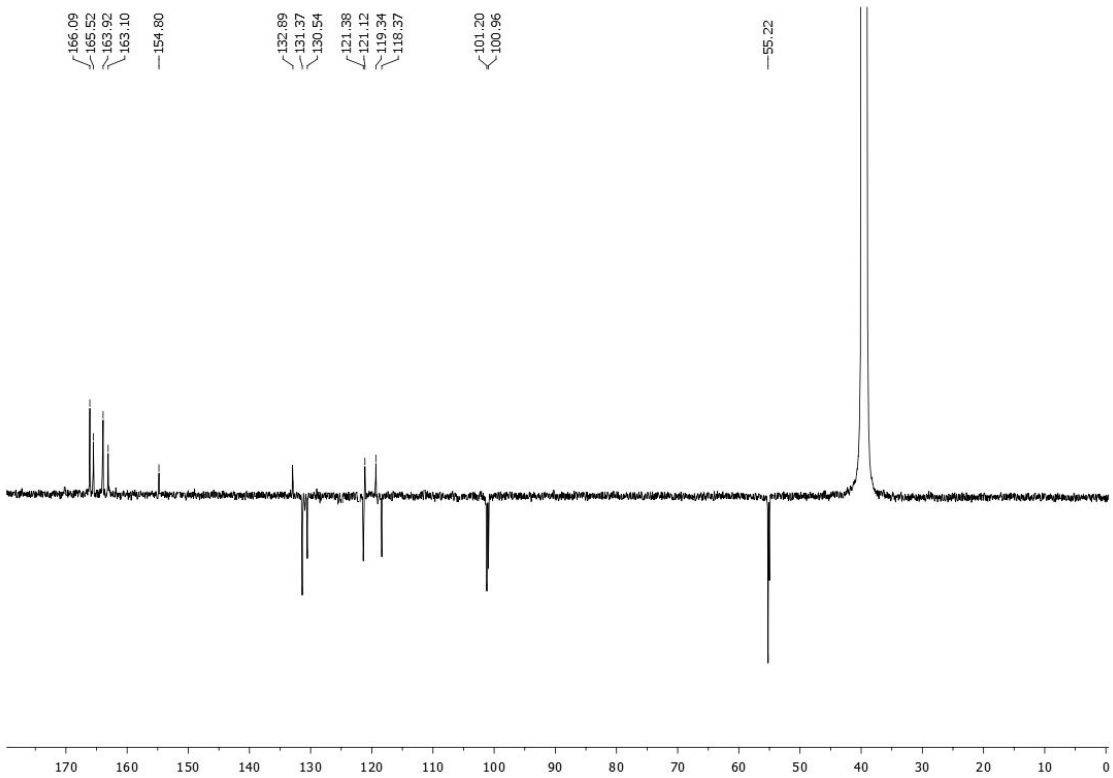


Figure S42. ^{13}C NMR spectrum (DMSO-d_6, 150 MHz) of ***2-hydroxy-4-methoxy -N-[5(6)-amidino-1H-benzimidazol-2-yl]benzamide 31***.

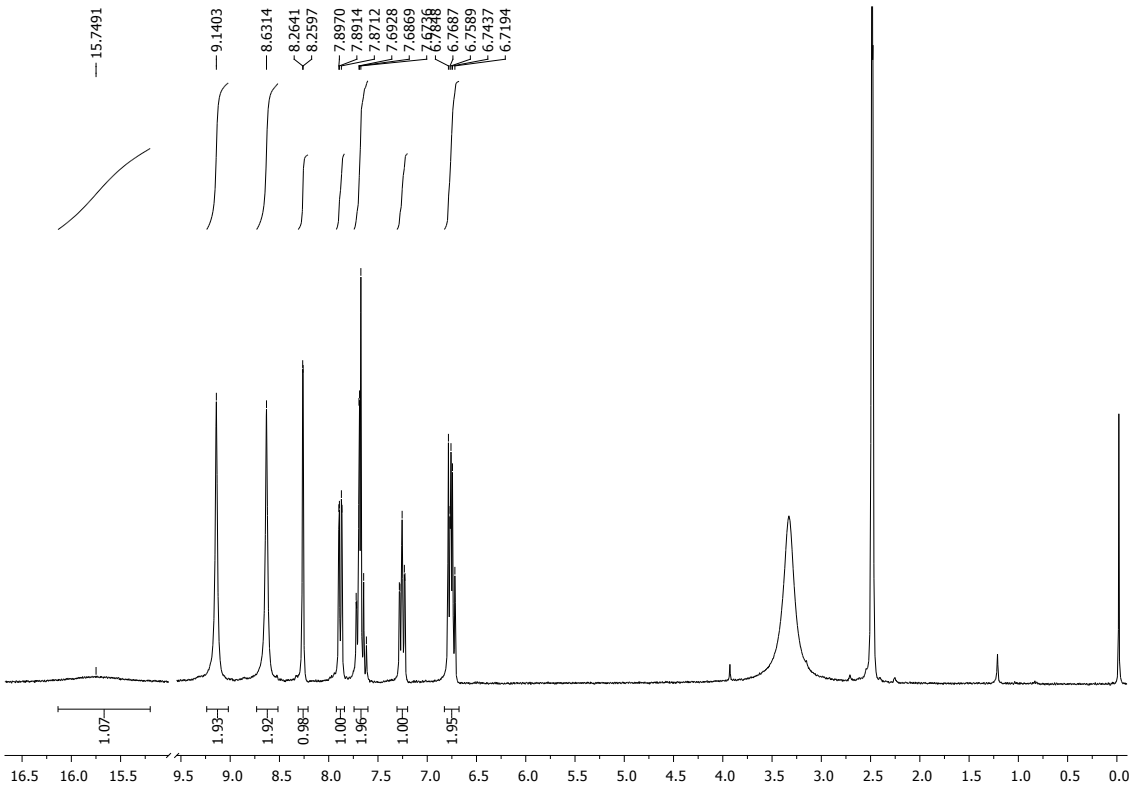


Figure S43. ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of ***2-hydroxy-N-(6-amidinobenzothiazol-2-yl)benzamide 32***.

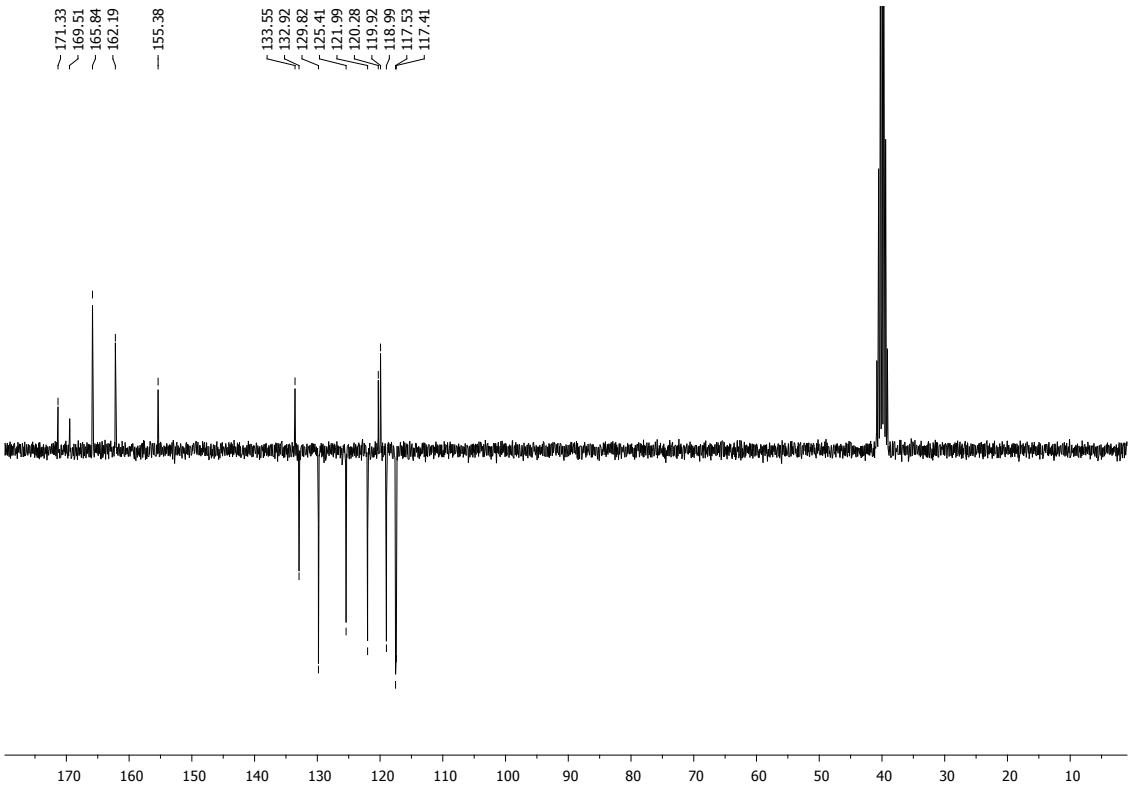


Figure S44. ^{13}C NMR spectrum (DMSO-d_6, 75 MHz) of ***2-hydroxy-N-(6-amidinobenzothiazol-2-yl)benzamide 32***.

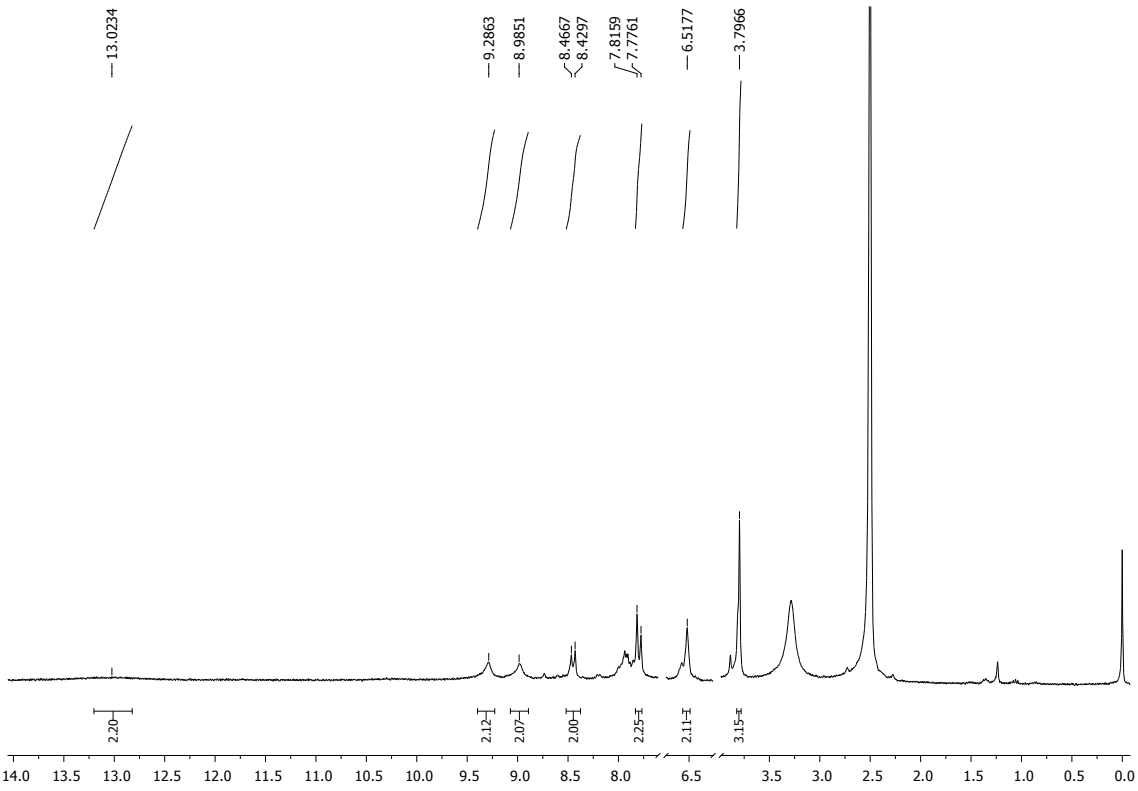


Figure S45. ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of ***2-hydroxy-4-methoxy-N-(6-amidinobenzothiazol-2-yl)benzamide 33***.

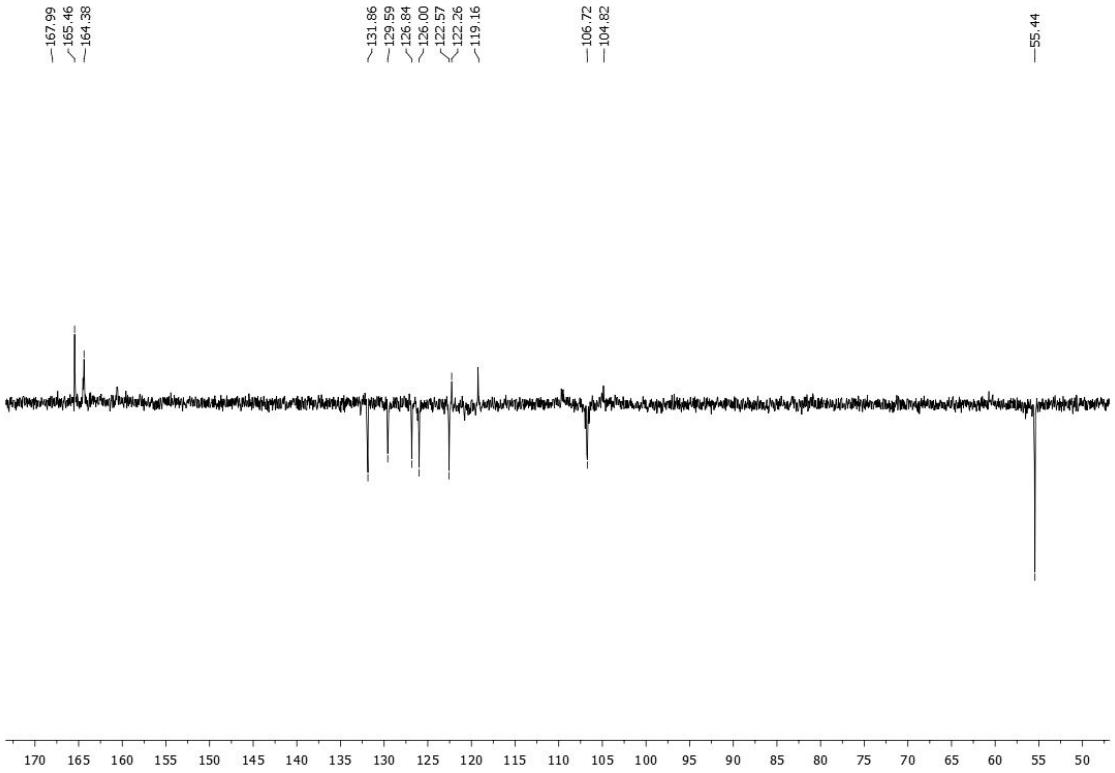


Figure S46. ^{13}C NMR spectrum (DMSO-d_6, 150 MHz) of ***2-hydroxy-4-methoxy-N-(6-amidinobenzothiazol-2-yl)benzamide 33***.

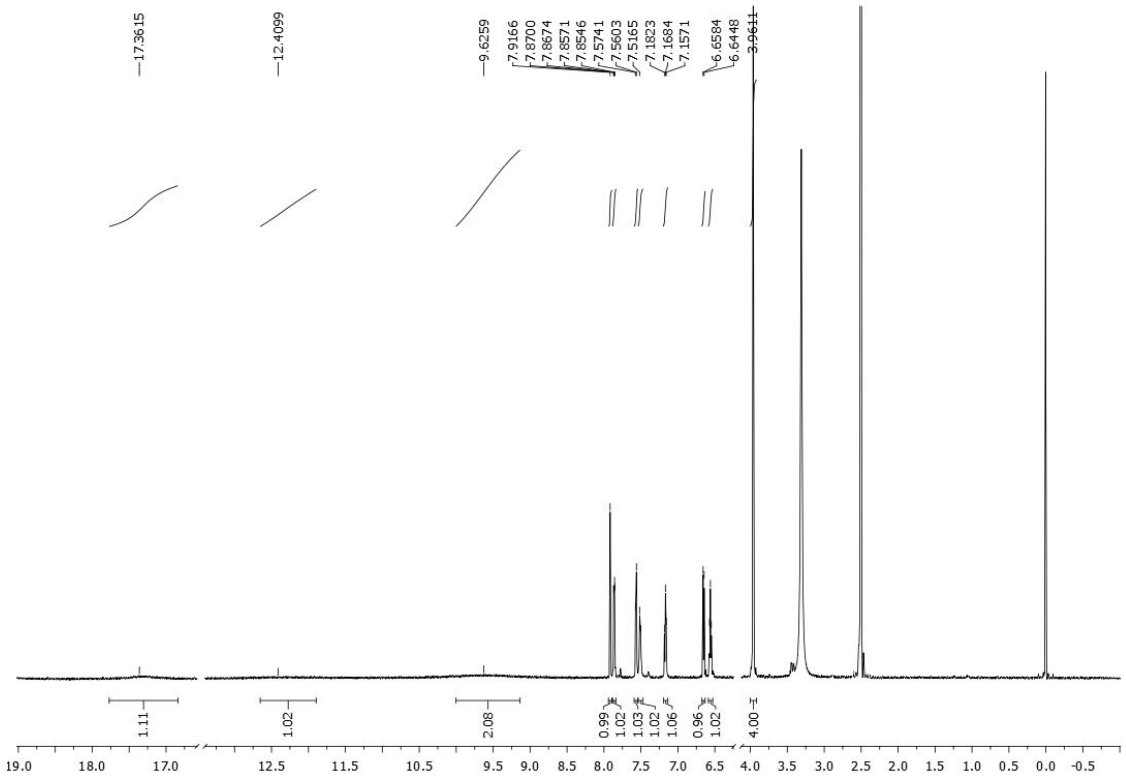


Figure S47. [1]H NMR spectrum (DMSO-d_6, 600 MHz) of ***2-hydroxy-N-[5(6)-(imidazolinil-2-yl)-1H-benzimidazol-2-yl]benzamide 34***.

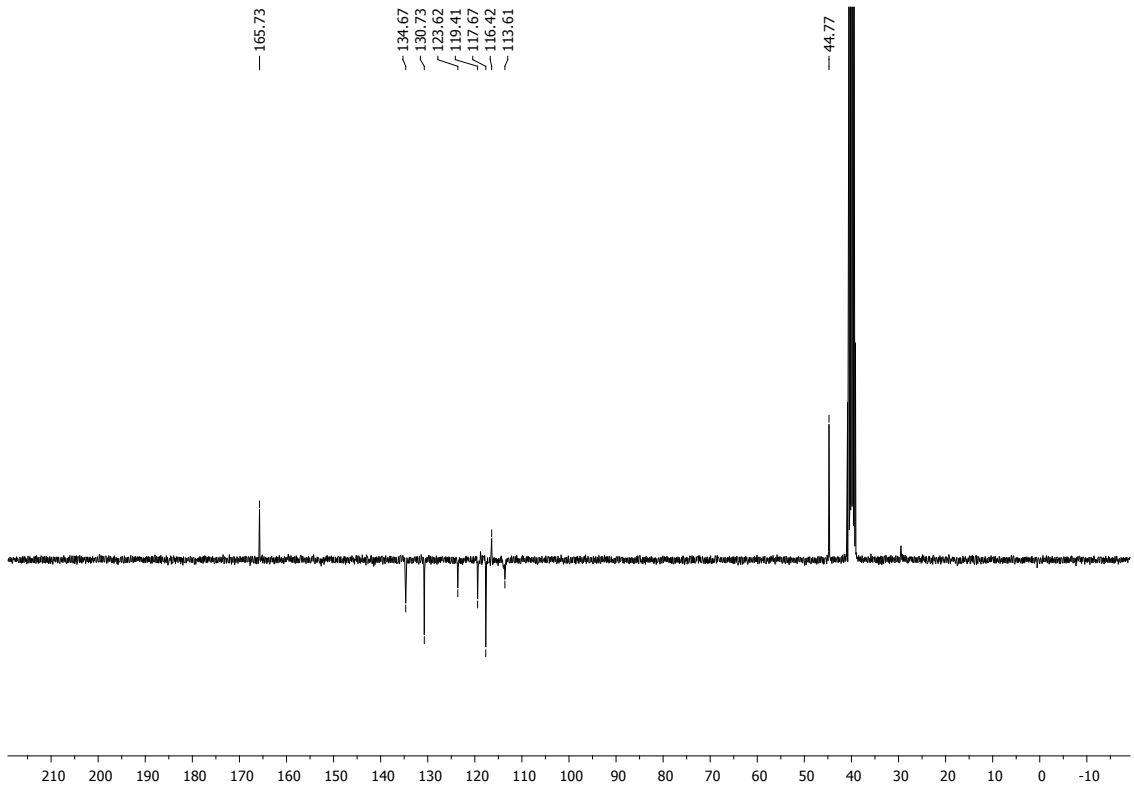


Figure S48. [13]C NMR spectrum (DMSO-d_6, 75 MHz) of ***2-hydroxy-N-[5(6)-(imidazolinil-2-yl)-1H-benzimidazol-2-yl]benzamide 34***.

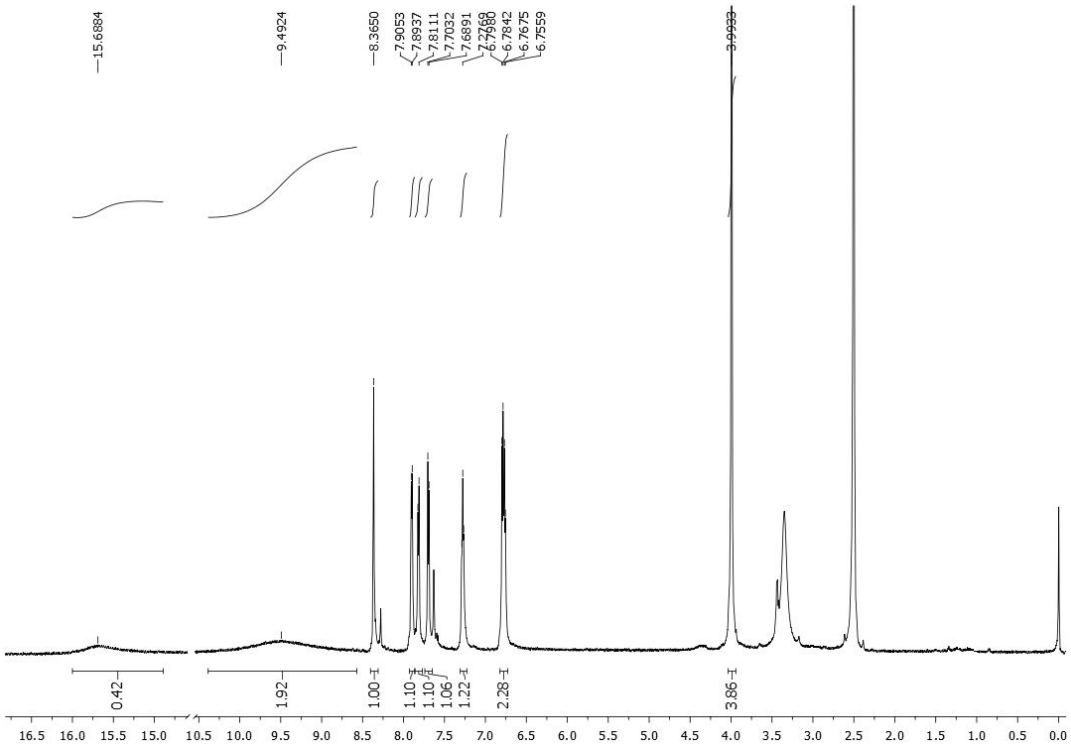


Figure S49. ^{1}H NMR spectrum (DMSO-d_6, 600 MHz) of ***2-hydroxy-N-[6-(imidazolinil-2-yl)benzothiazol-2-yl]benzamide 36***.

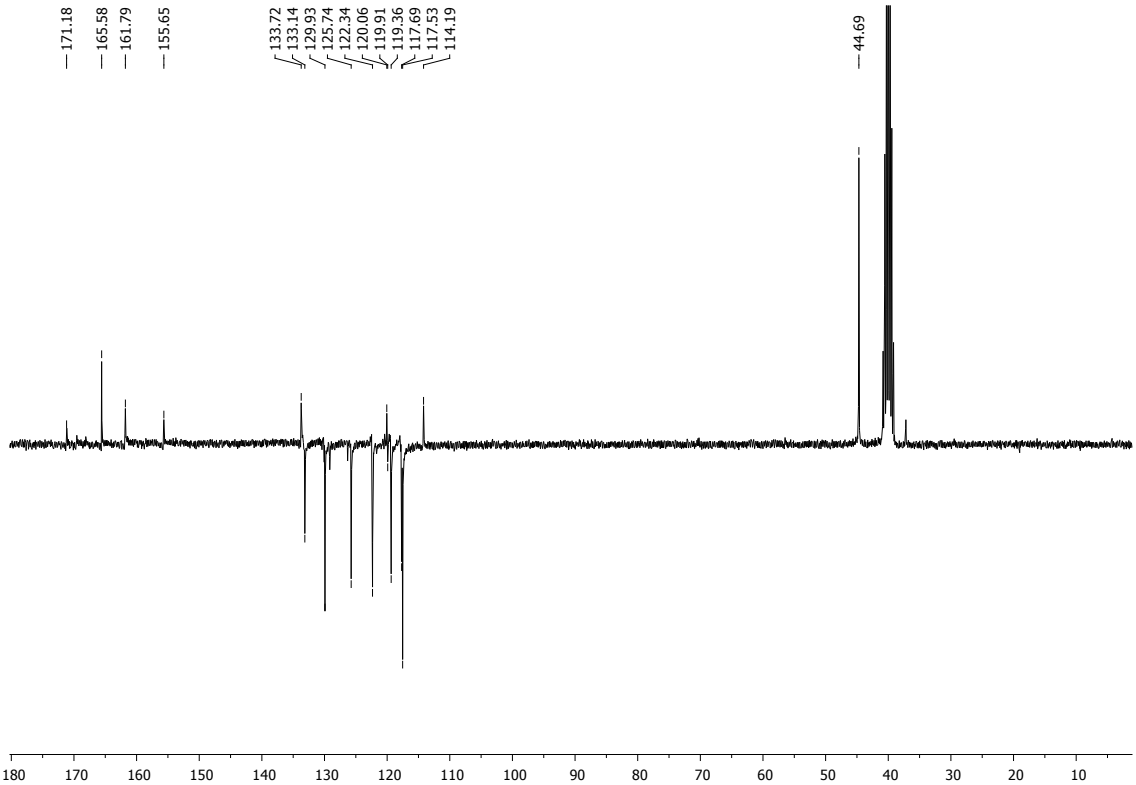


Figure S50. ^{13}C NMR spectrum (DMSO-d_6, 75 MHz) of ***2-hydroxy-N-[6-(imidazolinil-2-yl)benzothiazol-2-yl]benzamide 36***.

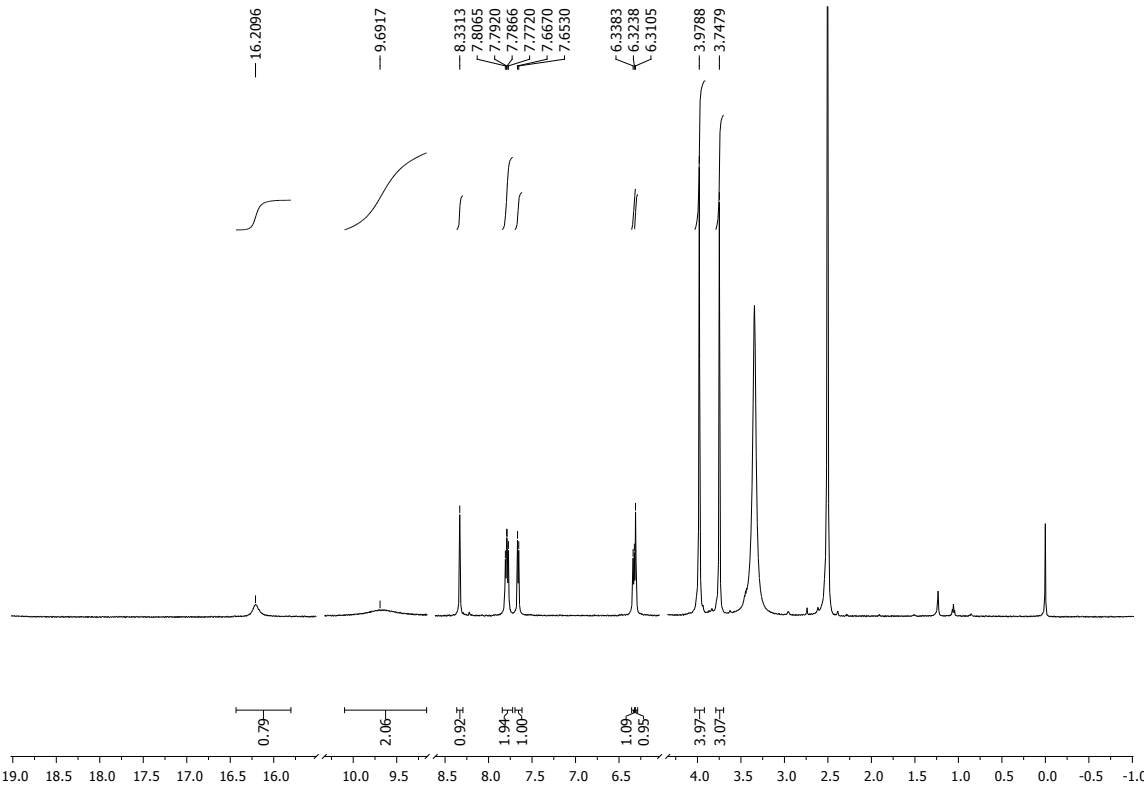


Figure S51. ^{1}H NMR spectrum (DMSO-d_6, 600 MHz) of ***2-hydroxy-4-methoxy-N-[6-(imidazolinil-2-yl)benzothiazol-2-yl]benzamide 37***.

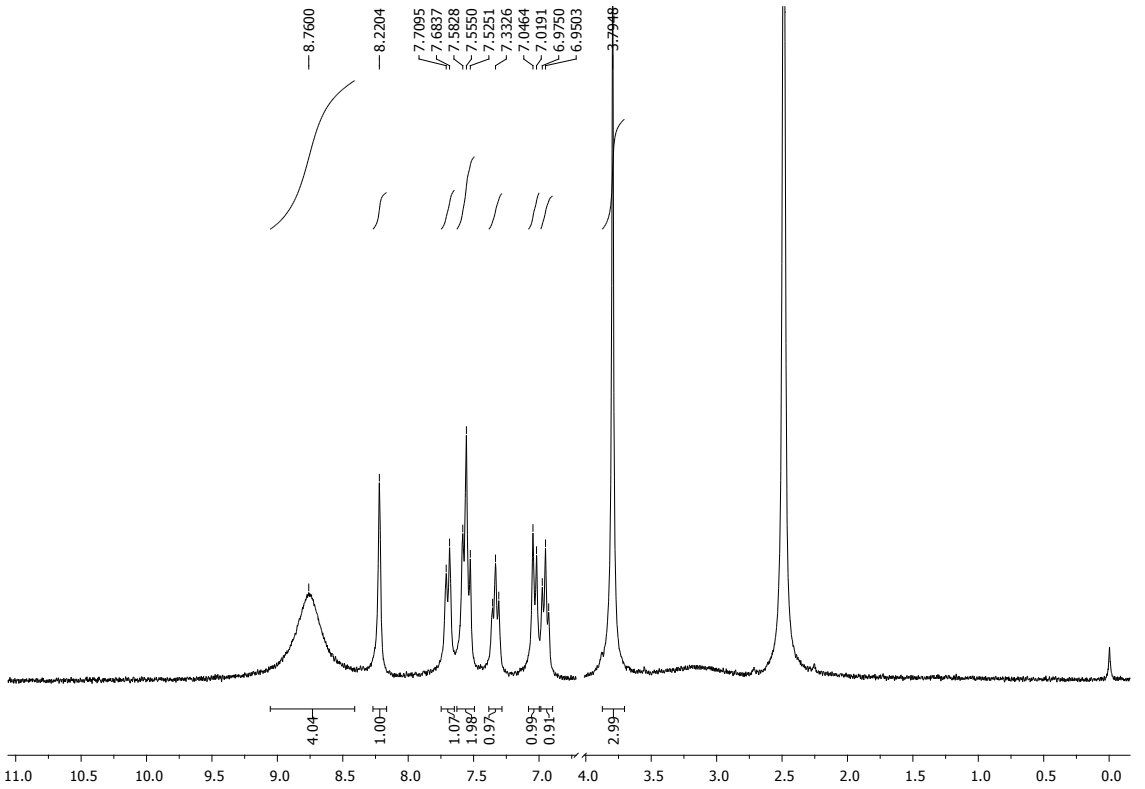


Figure S52. ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of ***2-methoxy-N-(6-amidinobenzothiazol-2-yl)benzamide 40***.

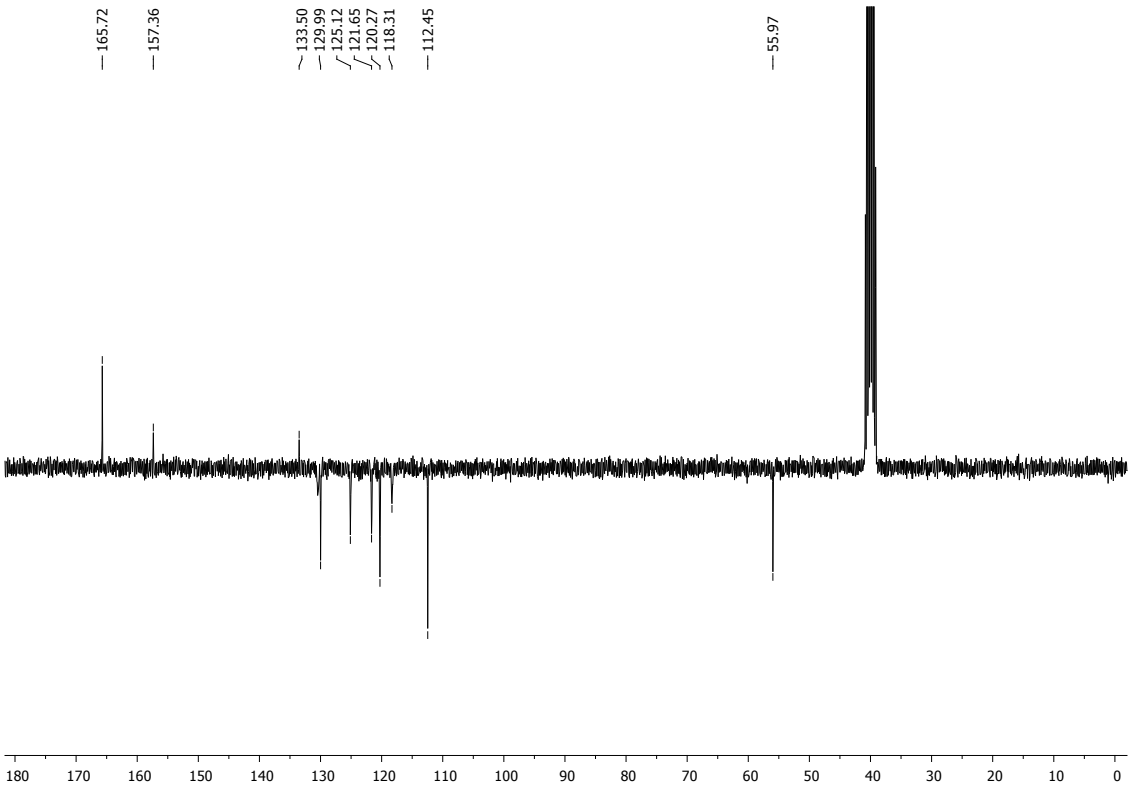


Figure S53. ^{13}C NMR spectrum (DMSO-d_6, 75 MHz) of ***2-methoxy-N-(6-amidinobenzothiazol-2-yl)benzamide 40***.

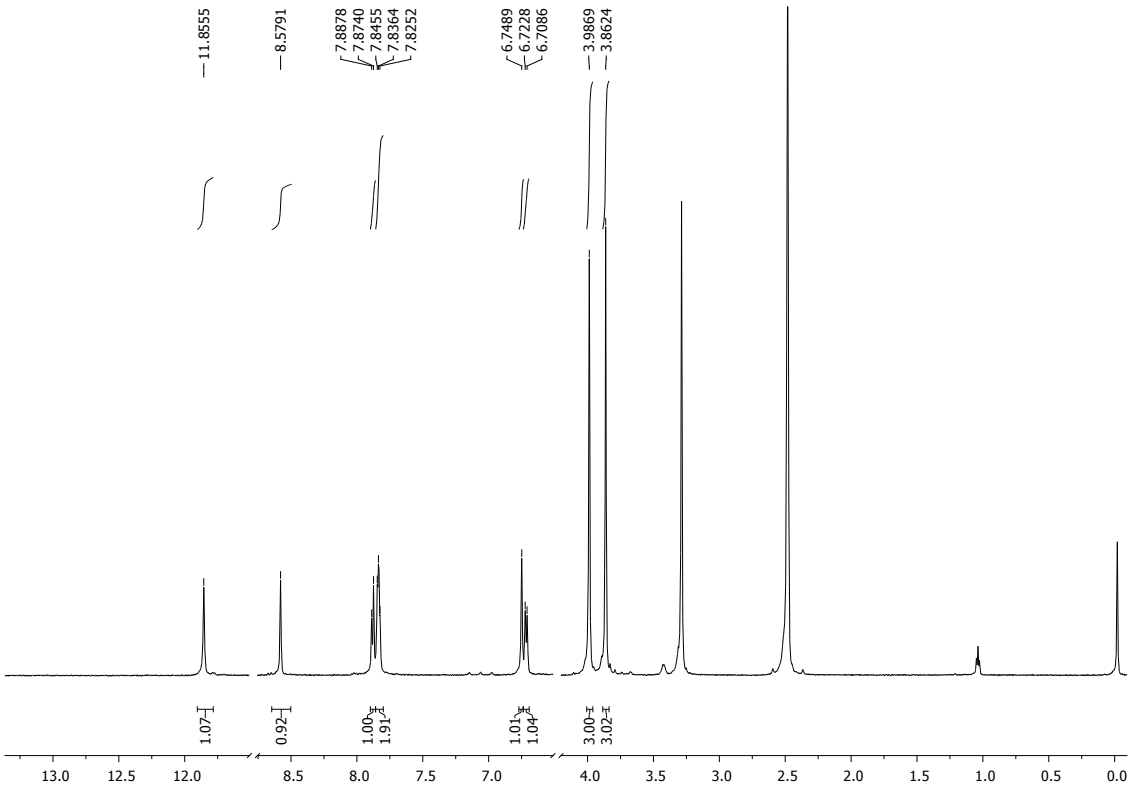


Figure S54. ^{1}H NMR spectrum (DMSO-d_6, 600 MHz) of ***2,4-dimethoxy-N-(6-amidinobenzothiazol-2-yl)benzamide*** **41.**

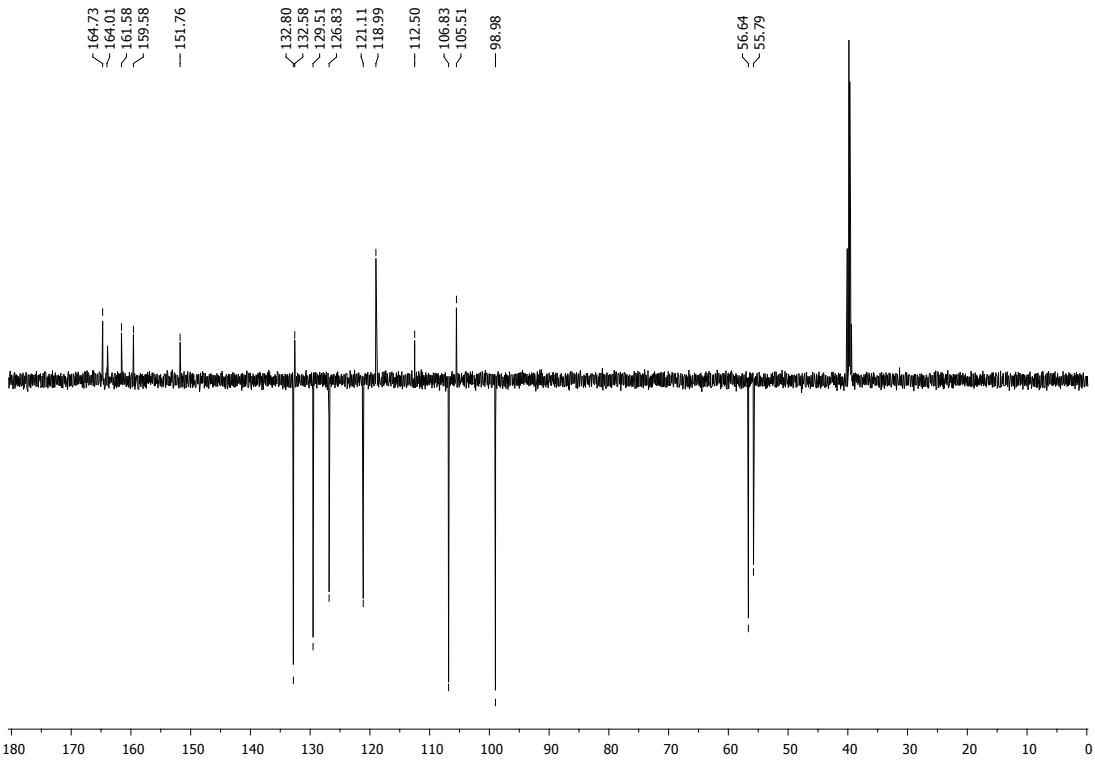


Figure S55. ^{13}C NMR spectrum (DMSO-d_6, 150 MHz) of ***2,4-dimethoxy-N-(6-amidinobenzothiazol-2-yl)benzamide*** **41.**

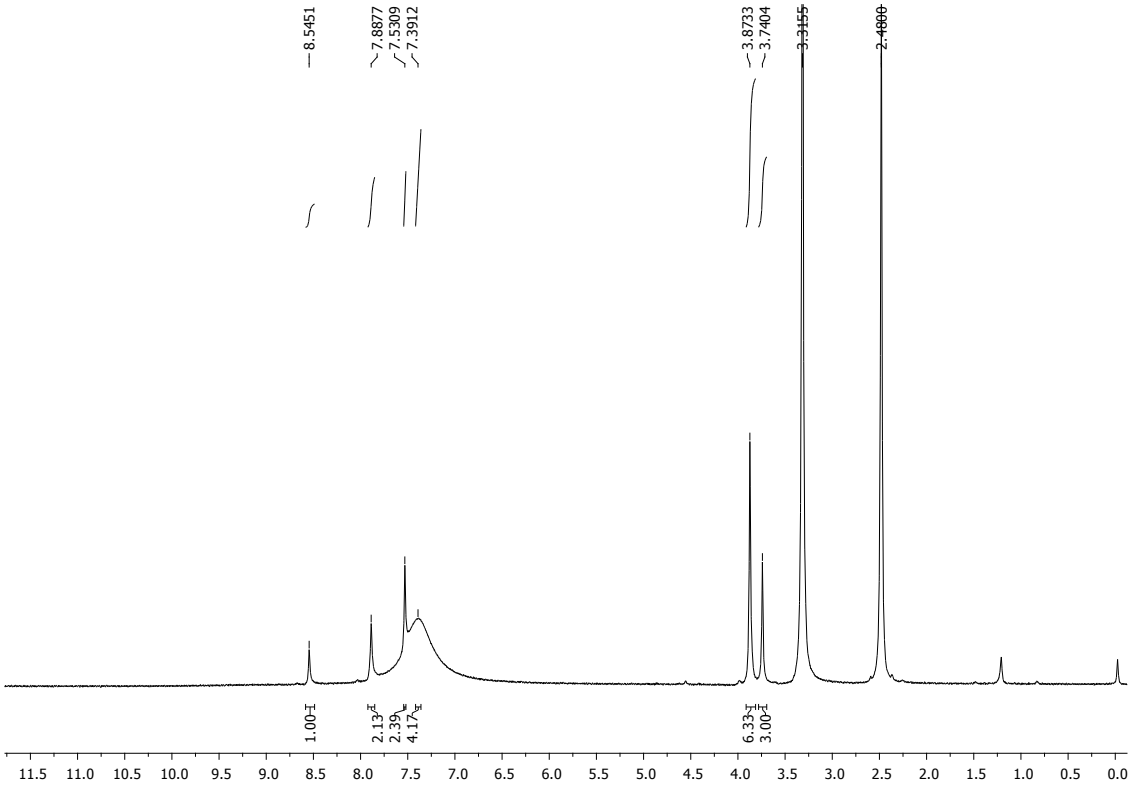


Figure S56. ^{1}H NMR spectrum (DMSO-d_6, 600 MHz) of ***3,4,5-trimethoxy-N-(6-amidinobenzothiazol-2-yl)benzamide 42***.

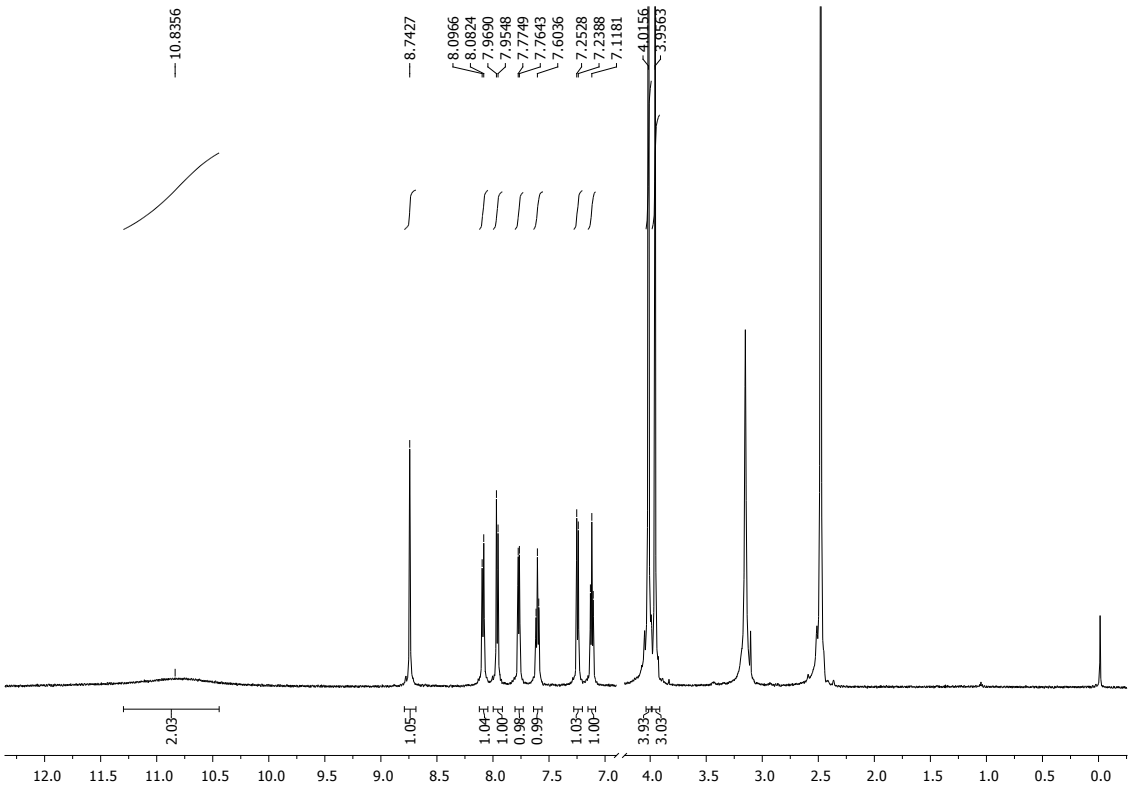


Figure S57. ^{1}H NMR spectrum (DMSO-d_6, 600 MHz) of ***2-methoxy-N-[6-(imidazolinil-2-yl)benzothiazol-2-yl]benzamide 45***.

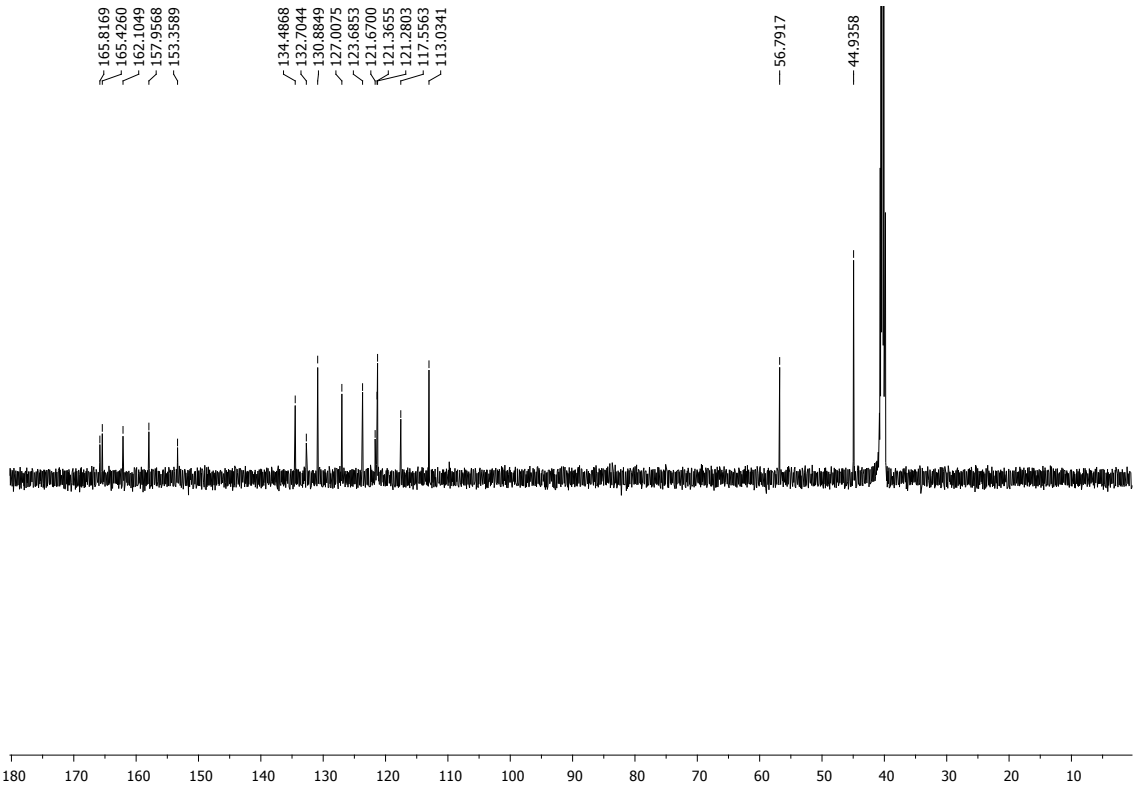


Figure S58. ^{13}C NMR spectrum (DMSO-d_6, 150 MHz) of ***2-methoxy-N-[6-(imidazolinil-2-yl)benzothiazol-2-yl]benzamide 45***.

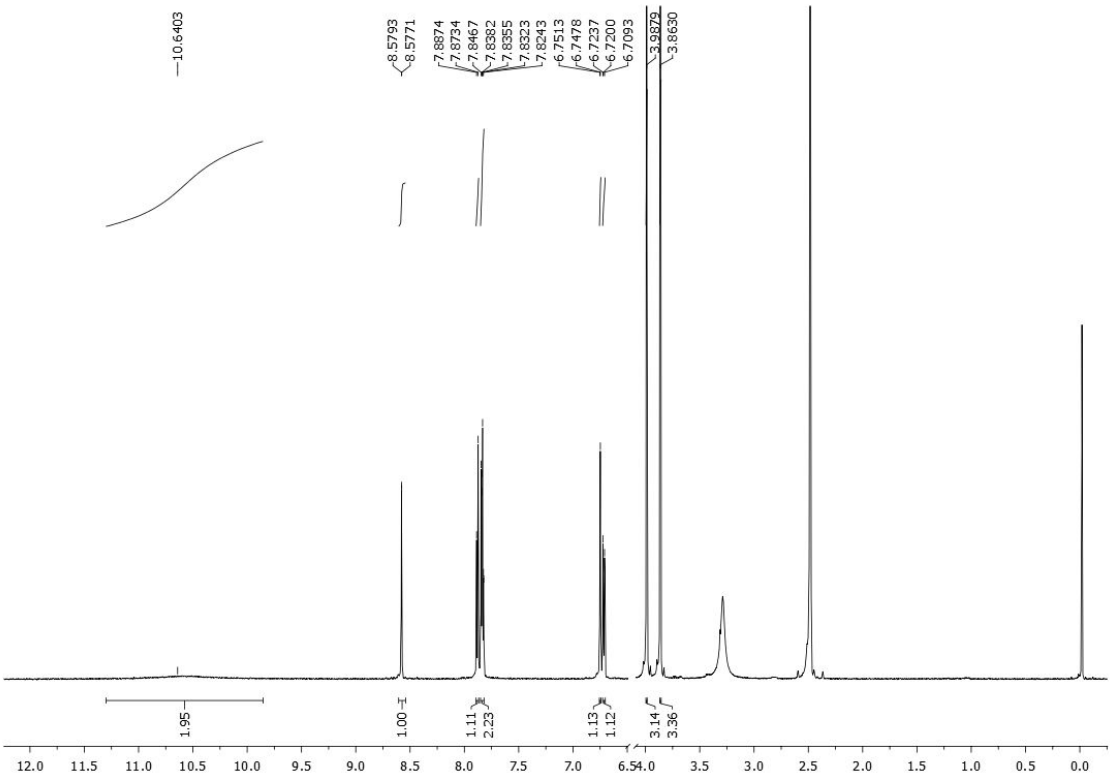


Figure S59. ^{1}H NMR spectrum (DMSO-d_6, 600 MHz) of ***2,4-dimethoxy-N-[6-(imidazolinil-2-yl)benzothiazol-2-yl]benzamide 46***.

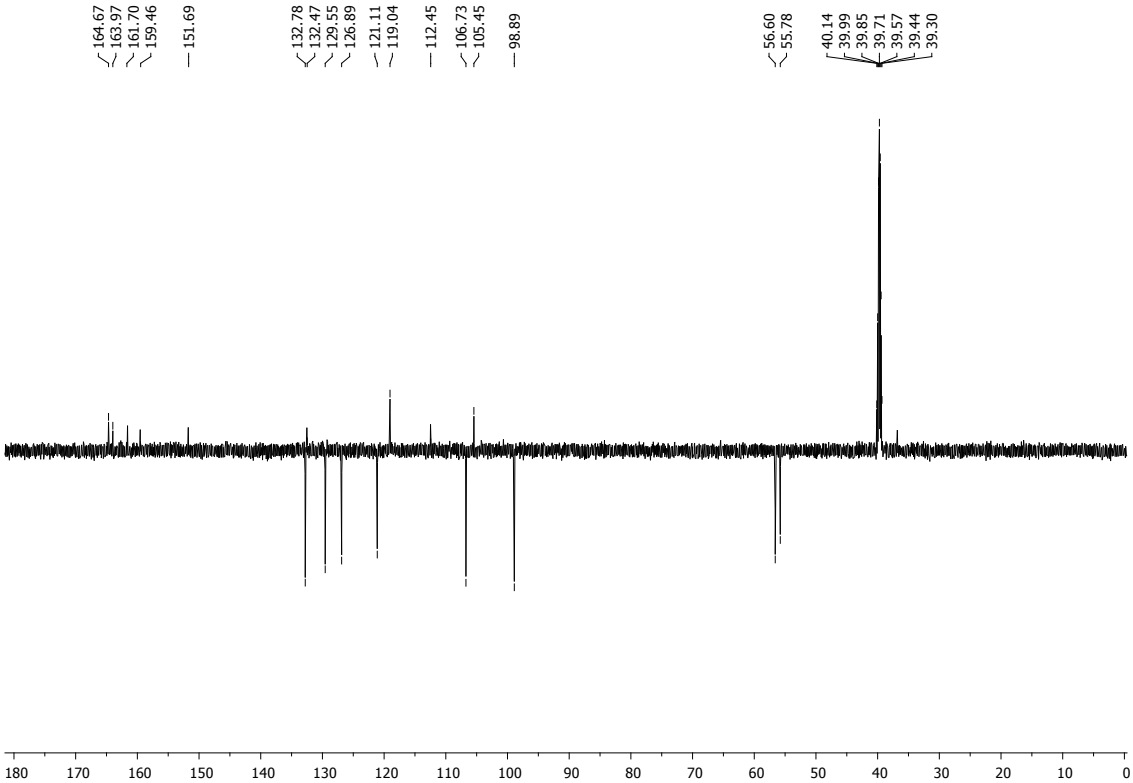


Figure S60. ^{13}C NMR spectrum (DMSO-d_6, 150 MHz) of ***2,4-dimethoxy-N-[6-(imidazolinil-2-yl)benzothiazol-2-yl]benzamide 46***.

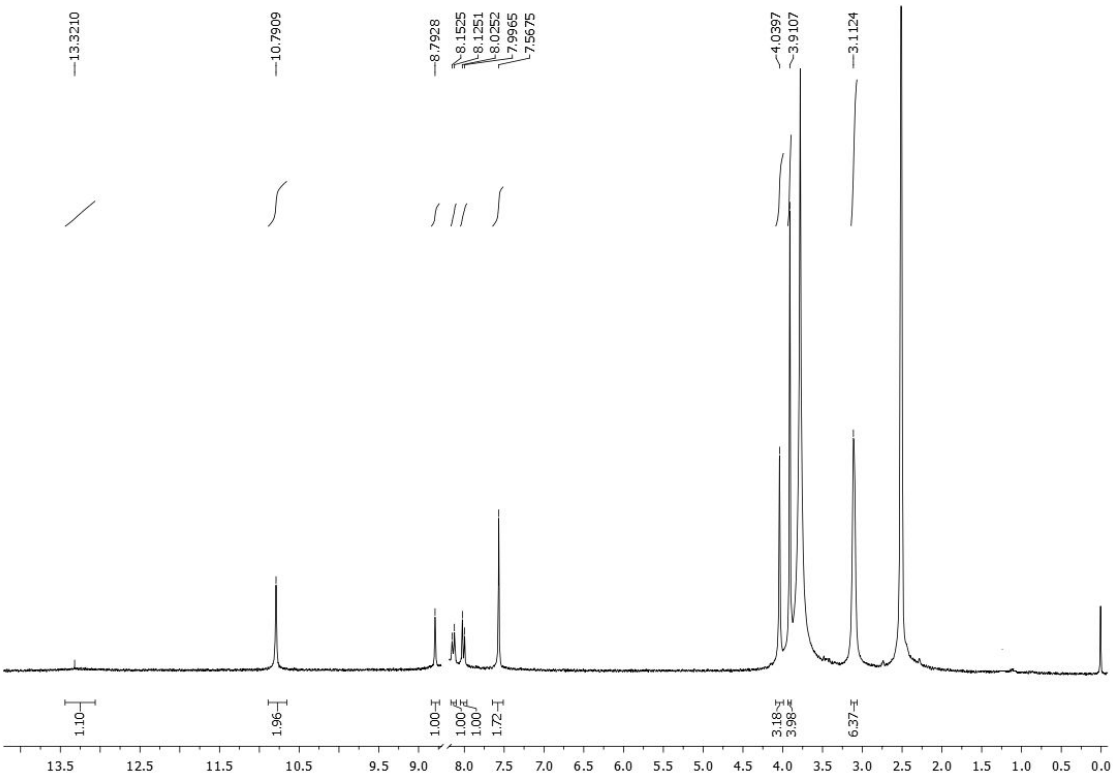


Figure S61. ^{1}H NMR spectrum (DMSO-d_6, 600 MHz) of ***3,4,5-trimethoxy-N-[6-(imidazolin-2-yl)benzothiazol-2-yl]benzamide 47***.

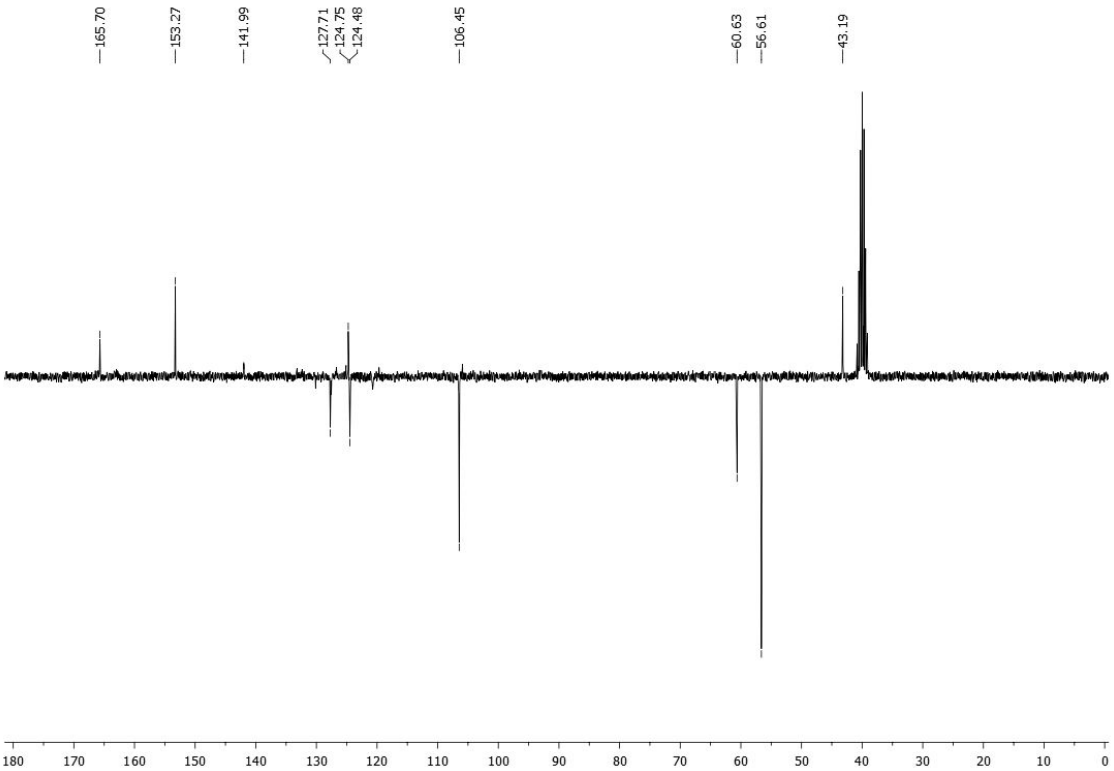


Figure S62. ^{13}C NMR spectrum (DMSO-d_6, 150 MHz) of ***3,4,5-trimethoxy-N-[6-(imidazolin-2-yl)benzothiazol-2-yl]benzamide 47***.

3. Pharmacology

Antiproliferative activity

Compounds and solution

Stock solutions of the compounds were prepared in dimethyl sulfoxide (DMSO) at a concentration of 10 mM and afterwards diluted with a nutrient medijum (RPMI 1640), supplemented with L-glutamine (3 mM), streptomycin (100 mg/ml) and penicillin (100 IU/ml), 10% heat inactivated (56 °C) fetal bovine serum (FBS) and 25 mM Hepes, adjusted to pH 7.2 by bicarbonate solution and applied to target cells to various final concentration ranging from 0 to 100 mM.

Cell growing

HeLa cells were grown in RPMI1640 medium supplemented with L-glutamine, streptomycin- penicillin and 10% fetal bovine serum (FBS). HeLa cells were seeded (2000 cells per well) into 96-well flat-bottomed microtiter plates and 24 h later, after the cell adherence, five different concentrations of investigated compounds were added to the wells. Only nutrient medium was added to the cells in the control wells with corresponding concentrations of DMSO. The nutrient medium with corresponding concentrations of compounds, but void of cells, was used as the blank. After 72 h of cultures incubation, the effect of the prepared compounds on cancer cell survival was determined by the microculture tetrazolium test (MTT) according to Mosmann[1,2].

Determination of cell survival

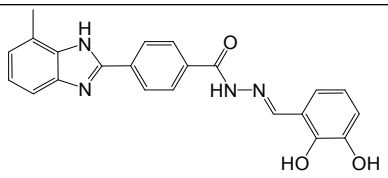
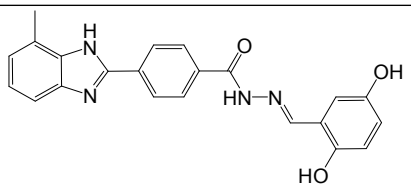
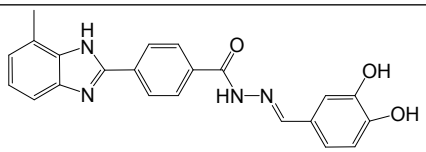
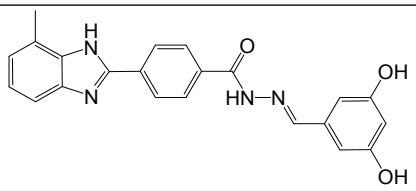
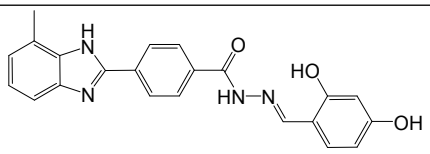
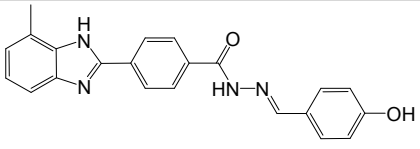
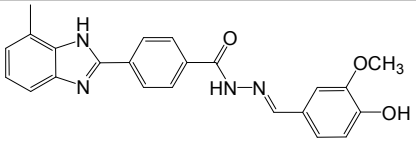
Briefly, 20 mL of MTT solution (5 mg/mL phosphate-buffered saline) was added to each well. Samples were incubated for a further 4 h at 37°C in a humidified atmosphere of 95% air/5% CO2 (v/v). Then, 100 mL of 100 g/L sodium dodecyl sulfate were added to the extract, resulting in formation of insoluble formazan by conversion of the MTT dye by viable cells. The number of viable cells in each well was proportional to the intensity of the absorbance of light, which was read in an enzyme-linked immunosorbent assay (ELISA) plate reader at 570 nm. The absorbance (A) at 570 nm was measured 24 h later. To determine cell survival (%), A of the sample with cells grown in the presence of various concentrations of the investigated compounds was divided by the control optical density (A of control cells grown only in nutrient medium) and multiplied by 100. It was implied that A of the blank was always subtracted from A of the corresponding sample with target cells. IC50 was defined as the concentration of an agent inhibiting cell survival by 50% compared with a vehicle-treated control. As positive controls, cis-diamminedichloroplatinum (cisplatin) was used.

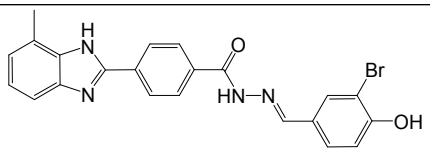
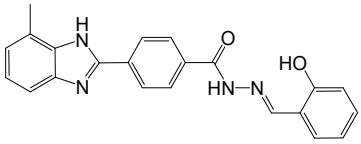
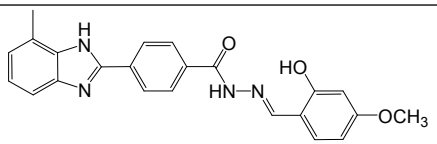
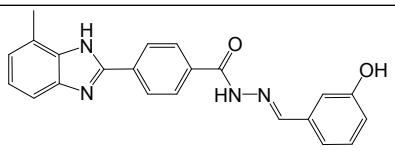
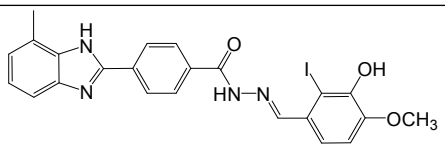
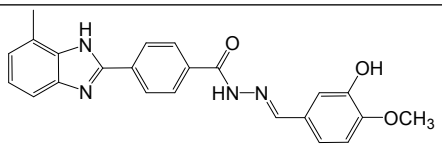
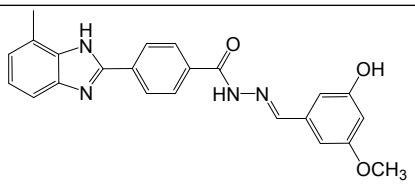
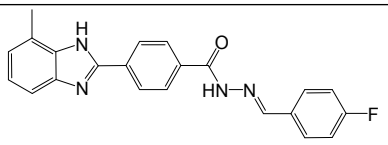
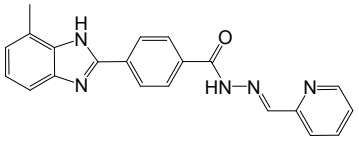
4. Determination of antimicrobial activity

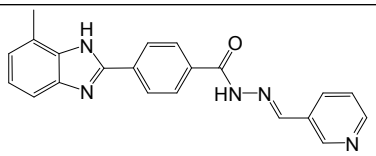
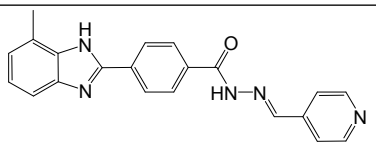
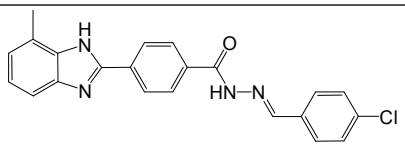
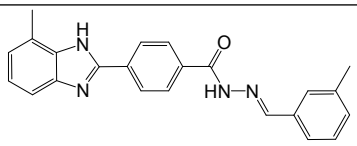
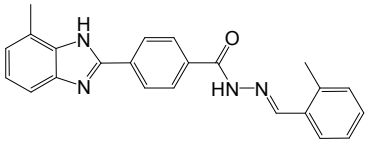
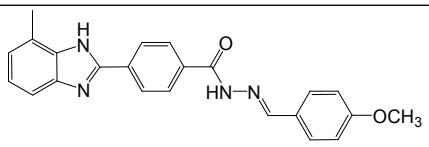
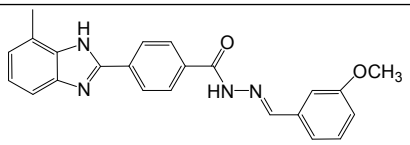
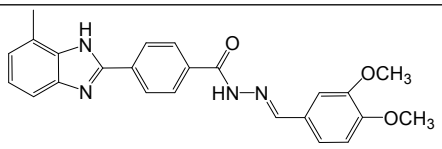
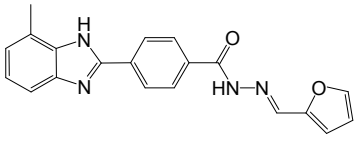
The antimicrobial activity was determinated using four different strains of the Gram-positive bacteria: *Staphylococcus aureus* (ATCC 6538), *Micrococcus luteus* (ATCC 4698), *Clostridium sporogenes* (ATCC 19404), *Bacillus subtilis* (ATCC 6633), four different strains of the Gram-negative bacteria: *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 10031), *Proteus hauseri* (ATCC 13315), *Pseudomonas aeruginosa* (ATCC 9027) and three strains of the fungi: *Candida albicans* (ATCC 10231), *Sacharomyces cerevisiae* (ATCC 9763) and *Aspergillus brasiliensis* (ATCC 16404). Antimicrobial activity was determinated by broth microdulution method.[3] 100 μL of fresh Mueller-Hinton broth (for bacteria) and Sabouraund dextrose broth (for fungi) were poured into all wells. Stock solutions of the compounds were prepared in dimethyl sulfoxide (concentration 10 mg/mL) and 100 μL of the compound stock solution were added to each well of the first column. Amikacin served as positive controls for bacteria, while nystatin served as positive controls for fungi. After the double dilution of the compounds, in each well 10 μL of bacterial suspension (10^6 cells) and 10 μL of fungi (10^5 spores) were added. The microtiter plates were incubated at 37 °C for 24 h for bacteria or at 28 °C for 48 h for fungi. The lowest concentration of compound that inhibited visible growth of a microorganism (MIC) was determined under a binocular microscope. When there is no microbial growth, the solution in the well remains clear while turbidity indicates the growth of microorganism.

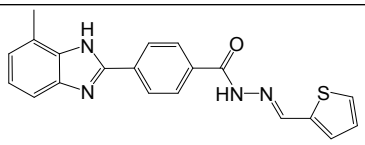
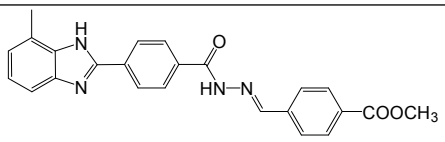
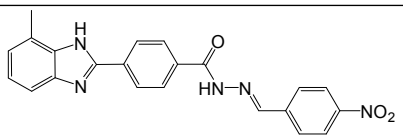
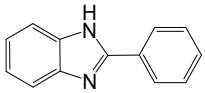
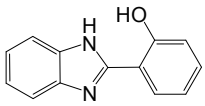
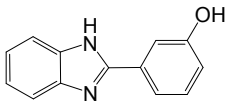
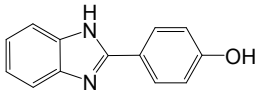
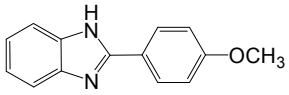
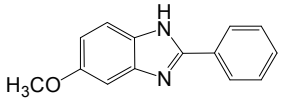
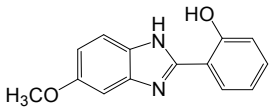
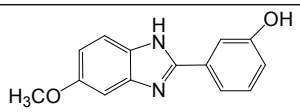
5. 3D-QSAR dataset

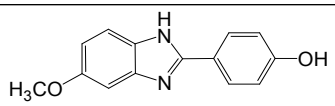
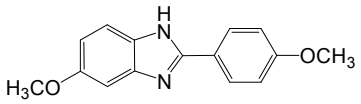
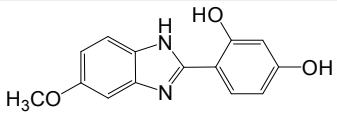
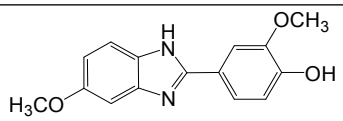
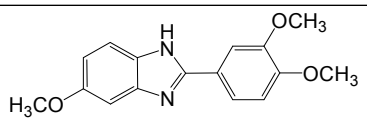
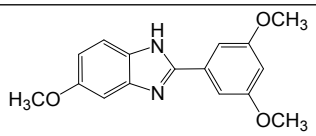
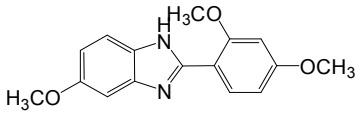
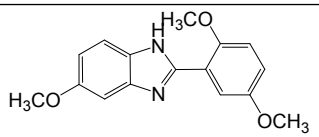
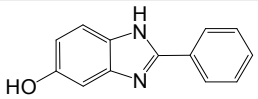
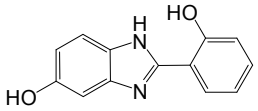
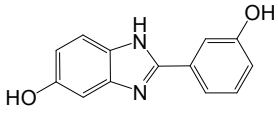
Table S1. Structures and experimental activities of the dataset compounds used to generate 3D-QSAR models.[3,4]

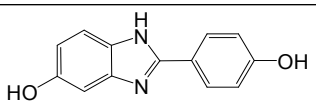
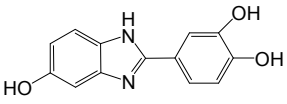
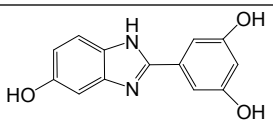
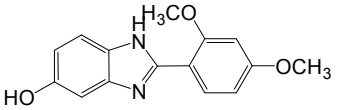
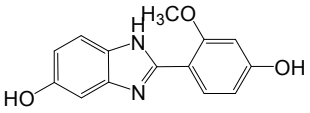
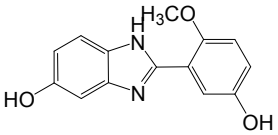
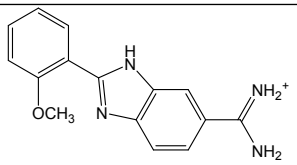
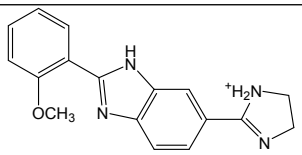
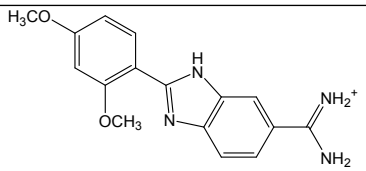
	Compound	DPPH Radical Scavenging	Superoxide Anion Assay
O-1		12.05 ± 0.45	82 ± 1.2
O-2		18.52 ± 0.60	86.12 ± 1.5
O-3		14.20 ± 0.55	81.02 ± 1.7
O-4		17.30 ± 0.62	90.01 ± 2.1
O-5		27.45 ± 1.02	92.12 ± 3.2
O-6		29.01 ± 1.10	101.12 ± 3.5
O-7		26.15 ± 1.05	104.12 ± 3.8

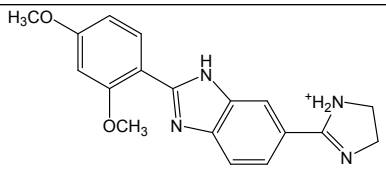
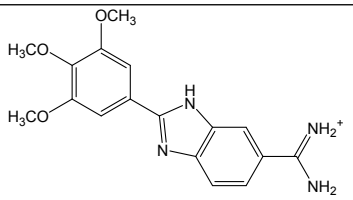
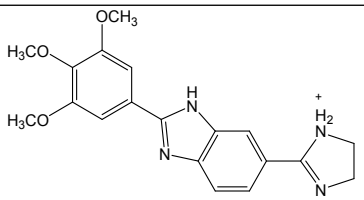
O-8		25.23 ± 0.94	190.10 ± 3.9
O-9		54.12 ± 2.12	208.99 ± 5.41
O-10		47.5 ± 1.85	201.99 ± 5.41
O-11		60.23 ± 2.45	211.99 ± 5.55
O-12		40.12 ± 1.58	210.10 ± 4.41
O-13		49.12 ± 1.82	222.01 ± 5.41
O-14		74.21 ± 2.65	215.30 ± 6.41
O-15		90.21 ± 3.12	320.30 ± 6.15
O-16		94.31 ± 3.21	350.30 ± 6.70

O-17		105 ± 3.85	310.30 ± 6.41
O-18		88.41 ± 3.18	340.15 ± 6.41
O-19		120.21 ± 3.60	360.30 ± 7.50
O-20		150.01 ± 4.12	345.30 ± 7.10
O-21		158.12 ± 4.10	340.30 ± 6.88
O-22		140.21 ± 3.82	340.15 ± 6.41
O-23		145.12 ± 4.01	345.30 ± 6.92
O-24		120.12 ± 3.84	330.30 ± 6.20
O-25		180.12 ± 4.50	310.30 ± 6.30

O-26		110.12 ± 3.48	320.15 ± 6.41
O-27		160.12 ± 4.15	322.30 ± 6.52
O-28		138.45 ± 3.84	312.30 ± 6.10
O-29		>200	>200
O-30		>200	122 ± 17
O-31		>200	>200
O-32		>200	>200
O-33		>200	>200
O-34		>200	>200
O-35		>200	87 ± 13
O-36		>200	>200

O-37		>200	>200
O-38		>200	>200
O-39		129 ± 7	189 ± 15
O-40		>200	>200
O-41		>200	>200
O-42		>200	>200
O-43		>200	>200
O-44		>200	>200
O-45		42 ± 2	>200
O-46		30 ± 3	169 ± 11
O-47		40 ± 2	>200

O-48		34 ± 2	>200
O-49		33 ± 1	>200
O-50		26 ± 2	>200
O-51		32 ± 1	>200
O-52		27 ± 2	>200
O-53		23 ± 3	>200
O-54		136.8	–
O-55		151.6	–
O-56		186.5	–
O-57		171.7	–

			
O-58		51.33	–
O-59		23.7	–

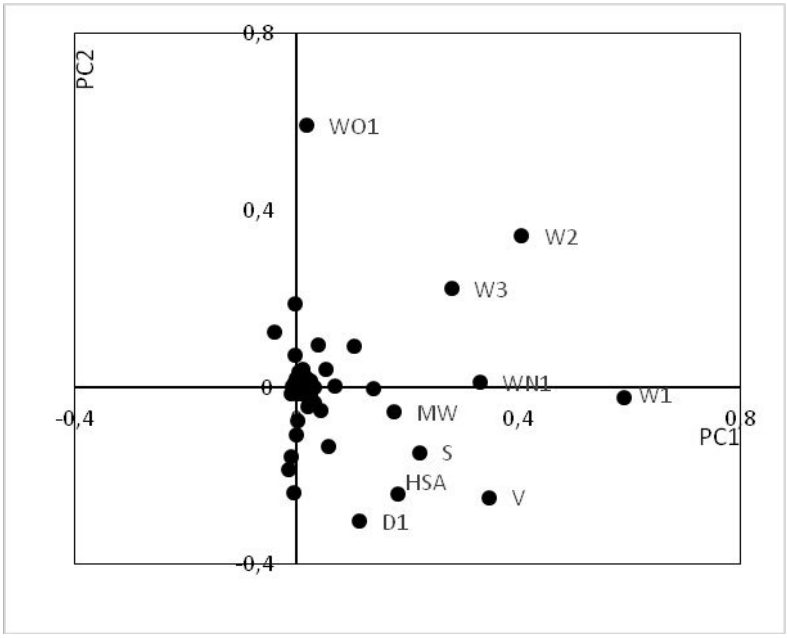
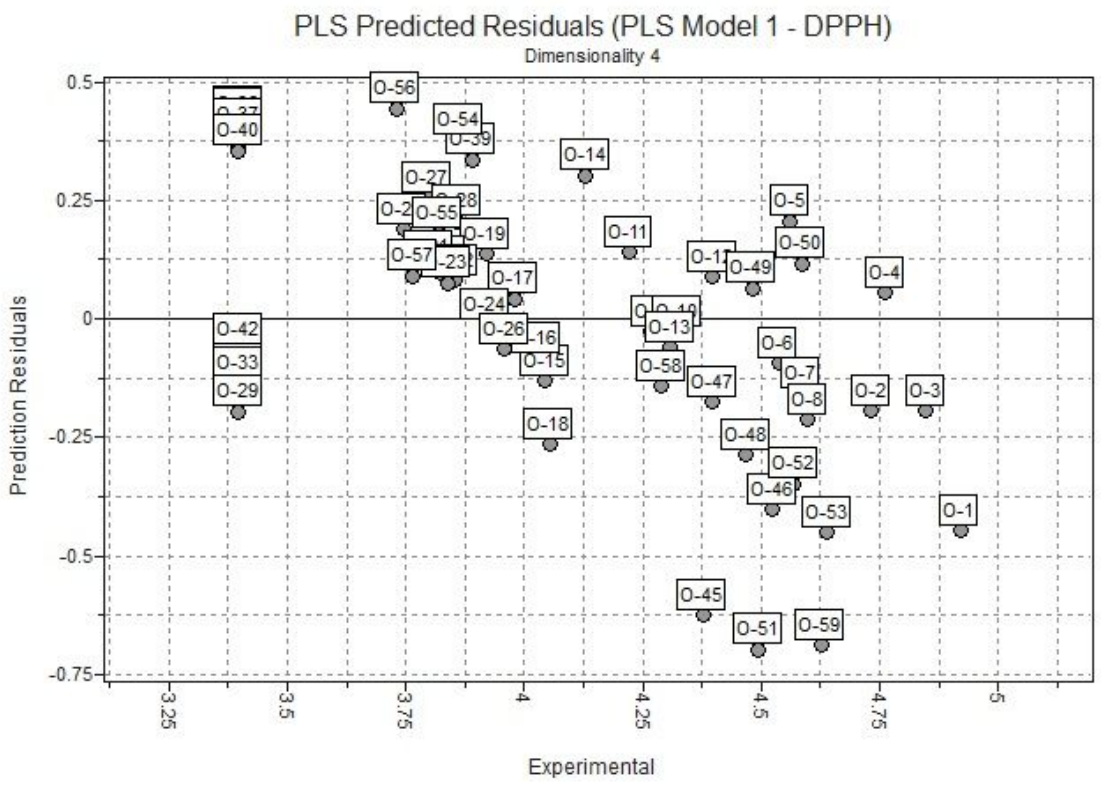


Figure S63. PCA loadings plot. Molecular descriptors with the highest contributions to the first two principal components are labelled.

A)



B)

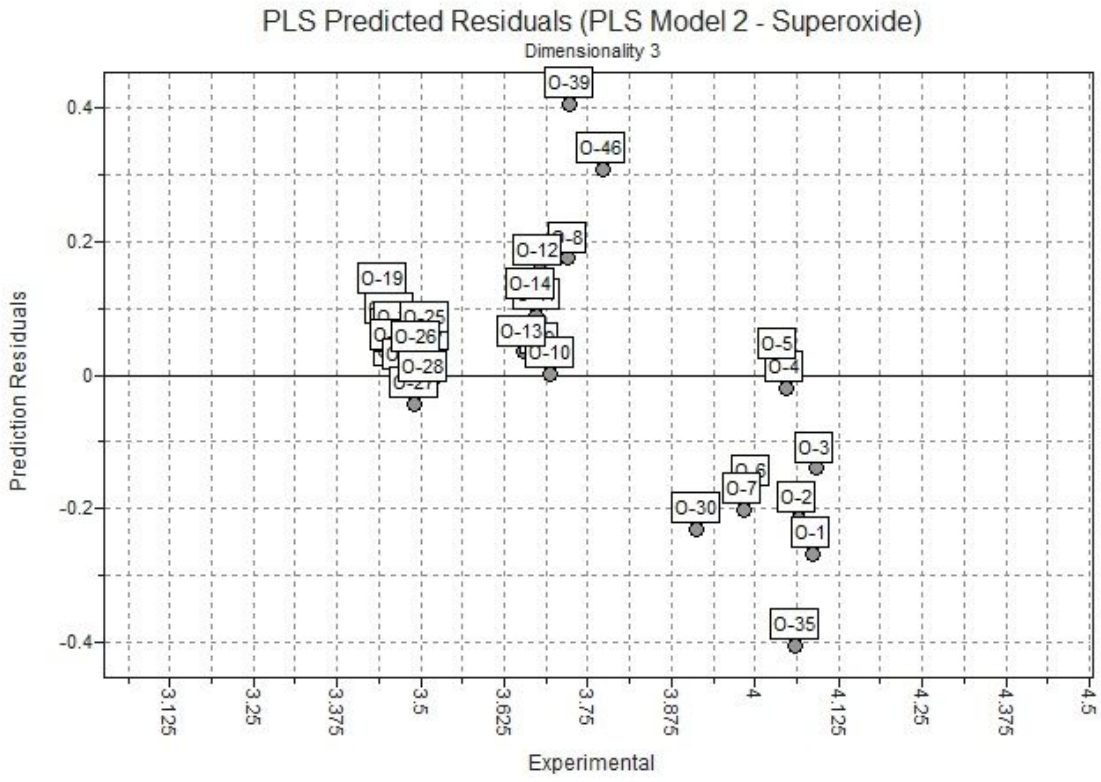


Figure S64. Predicted residuals of**:** A) model **1** and B) model **2**. Name of the compounds are given in Table 1S.

6. Determination of antioxidative activity

Free-radical scavenging antioxidant assay (DPPH method)

Free-radical scavenging antioxidant activity was assayed using a protocol for the determination of radical scavenging activity.[6] Compounds were dissolved in pure DMSO and were diluted into ten different concentrations. Commercially available free radical DPPH was dissolved in methanol at a concentration of 6.58 x 10^{-5} M. Into a 96-well microplate, 140 µL of DPPH solution was loaded and 10 µL DMSO solution of the tested compounds was added, or pure DMSO (10 µL) as the control. The microplate was incubated for 30 min at 298 K in the dark and the absorbance was measured at 517 nm. All the measurements were carried out in triplicate. The scavenging activity of the compounds was calculated using the eq. (1):

$$\text{Scavenging activity (\%)} = (A_{control} - A_{sample})/A_{control} \times 100$$

where A_{sample} and $A_{control}$ refer to the absorbances at 517 nm of DPPH in the sample and control solutions, respectively. IC_{50} values were calculated from the plotted graph of scavenging activity against the concentrations of the samples. IC_{50} is defined as the total antioxidant concentration necessary to decrease the amount of the initial DPPH radical by 50%. IC_{50} was calculated for all compounds based on the percentage of DPPH radicals scavenged. Ascorbic acid was used as the reference compound (positive control) with concentrations 50 to 500 mg mL^{-1}.

Superoxide test

Superoxide radicals are generated in PMS-NADH systems by oxidation of NADH and assayed by the reduction of NBT.[7] In these experiments in microplate, the superoxide radicals were generated in 234 µL phosphate buffer (100 mM, pH 7.4) containing 6 µL of NBT (3.18 mM) solution, 6 µL NADH (12.72 mM) solution and 2.5 µL sample solution of fraction (25 – 500 mg/ml) in methanol. The reaction started by adding 1.5 µL of PMS solution (1.28 mM) to the mixture. The reaction mixture was incubated at 25 °C for 5 min, the color change was monitored at 560 nm by spectrophotometer (ThermoScientific Appliscan, SkanIt version 2.3) against blank samples using ascorbic acid as a control. Decreased absorbance of the reaction mixture indicated the increasing of superoxide anion scavenging activity. All the measurements were carried out in triplicate. The percentage inhibition of superoxide anion generation was calculated using the following formula:

% inhibition = $[(A_0-A_1)/A_0] \times 100$

where A_0 was the absorbance of the control , and A_1 was the absorbance in the presence of fraction or standards.

7. References

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