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Evaluation of Antimicrobial Effect against Some Microorganisms and Apoptotic Activity against Candida Species of New Vanillin Derivatives

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Abstract

Eleven vanillin derivatives was synthesized, characterized successfully and their antibacterial and anticandidal properties were investigated on seven bacterial species and four candida species. These bacterial species are *Staphylococcus aureus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Bacillus subtilis* and candidal species are *Candida albicans*, *Candida glabrata*, *Candida krusei* and *Candida parapsilosis*. Most of the synthesized compounds were showed good activity against studied microorganisms compared with Chloramphenicol. Compounds 2c, 2d and 2k were exhibited remarkable antibacterial activities especially on *Escherichia coli*. In addition, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Bacillus subtilis* were found to be the most susceptible species amongst the investigated microorganisms.

Keywords: Antimicrobial, Schiff Base, Vanillin.

1. Introduction

Designing new effective anti-bacterial agents have been the most important issue due to the improving bacterial resistance because of high antibiotic consumption [1-3]. The synthesis of natural products and their derivatives is evolving an interesting research area [4, 5]. Amongst these natural products, vanillin (4-hydroxy-3-methoxy benzaldehyde) is one of the most challenging molecule due to possesses several biological properties including; anti-microbial, anti-candidal, anti-oxidant, anti-carcinogenic, anti-angiogenic, anti-mutagenic etc. Zhu and Sun et al. [6] were synthesized two vanillin derivatives and characterized their structures mainly with single crystal x-ray technique and tested their antibacterial activities on *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* by MTT assay. The synthesized compounds were found to be effective on studied microorganisms. Naik and Harini et al. [7] were investigated the antimicrobial activity of some piperidin-4-one oxime esters carrying vanillin part on some bacteria and fungi, including *Candida albicans* MTCC 3017, and the results showed that, compounds having -Br and -NO_2 groups found to be have moderate growth inhibitory activities. Devasagayam and Kamat et al. [8] were investigated the anti-oxidant activity of natural vanillin on rat liver mitochondria against oxidative damage. According to the results, 2.5 mmol/L vanillin concentration found to be have significant protective property from oxidation of proteins and peroxidation of lipids in hepatic mitochondria**.** Hassan and Naz et al. [9] were investigated the binding mechanism of vanillin to calcium/calmodulin-dependent protein kinase IV (CAMKIV), which is associated with different types of cancer and neurodegenerative diseases. From the results, vanillin strongly bonded to the active site of the CAMKIV and it inhibits the proliferation of cancer cells**.** Park and Jung et al. [10] were studied the anti-angiogenic activity of ethyl vanillin (EVA) on chorioallantoic angiogenesis. They found that, the anti-angiogenic activity of EVA depends on its suppressive effect on the nitric oxide production by decreasing the reactive oxygen species**.** King and Mure et al. [11] were studied the anti-mutagenic activity of vanilin against spontaneous gene mutations in mammalian cells. They found that, long term treatment of vanillin with HCT116 cells between 0.5-2.5 mM concentrations, reduced the spontaneous HPRT mutant fraction between 19-73 % values.

Imines are well known type of molecules consist of azomethine (C=N) bonds have numerous application areas [12-14]. Several imine compounds can be synthesized from hydrazides (called hydrazones) and have potential biological activities according to the literature. Zayed and Zayed et al. [15] were synthesized bisaldehyde-hydrazide macrocyclic schiff bases and tested against *Escherichia coli*, *Proteus vulgaris*, *Bacillus subtilis* and *Staphylococcus aureus* bacteria. Synthesized compounds found to be active against studied microorganisms. Ceylan [16] was synthesized several Mannich bases using nicotinic acid hydrazide and compounds were tested for their anti-microbial, antilipase and antiurease activities. According to the study, excellent anti-microbial activities was observed**.** Maja and Milan et al. [17] were obtained different schiff

bases derived from dipicolinic acid and investigated their antioxidant activity. Di-hydrazide compounds showed higher antioxidant activity than mono hydrazides and even better than standards that used in the investigation.

In the light of all these literature information, we were synthesized eleven new hydrazone derivatives from trifluoromethyl benzyl substituted vanillin and tested their antibacterial activities against seven bacterial species including *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Listeria monocytogenes* (ATCC 1911), *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 35218), and *Bacillus subtilis* (NRRL B478) and four candida species including *Candida albicans* (ATCC 90028), *Candida glabrata* (ATCC 90030), *Candida krusei* (ATCC 6258), *and Candida parapsilosis* (ATCC 22019).

2. Materials and Methods

2.1. Chemistry

All of the used chemicals and reagents purchased as analytical grade and used without further purification. HR-MS measurements were recorded on Schimadzu LCMS-IT-TOF spectrometer. Nuclear Magnetic Resonance (^{1}H-NMR and ^{13}C-NMR) measurements were recorded on Bruker DPX FT spectrometer. FT-IR spectra were recorded with Perkin Elmer Spectrum 100 Spectrometer with KBr pellets. Elemental analysis was carried out using Elementar Vario EL III microanalyzer device. Melting points were determined in a Stuart SMP-30 apparatus.

2.2. General synthesis of vanillin derivatives (2a-l)

Twelve new vanillin derivatives were synthesized in two steps. Compounds synthesis procedure was depicted in Figure 1.

2.3. Synthesis of 3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzaldehyde (compound 1)

Compound 1 was synthesized according to the literature [18]**.** Briefly, 4-hydroxy-3-methoxy benzaldehyde (1 eq.) and K_2CO_3 (1.5 eq.) were stirred in 20 mL dimethyl formamide (DMF) for 30 minutes at room temperature. Afterwards, 4-trifluoromethylbenzyl bromide (1 eq.) added drop by drop to the solution, then the solution was kept constant stirring for 12 hours under the same temperature. After the reaction completed, the solution was poured in an ice bath and extracted with dichloromethane three times, then diluted NaOH and brine. Organic phases were collected and dried with $MgSO_4$ and crystallized. Product was directly used in the second step.

2.4. Synthesis of vanillin derivatives (2a-k)

The condensation reaction between compound 1 and hydrazides with different substituted groups resulted in eleven new vanillin derivatives in ethanolic solution.

To the solution of 3-methoxy-4-((4-(trifluoro methyl) benzyl)oxy)benzaldehyde (1 eq., 0.97 mmol) in 10 mL ethanol, the ethanolic solution of substituted hydrazide (1 eq., 0.97 mmol) was added drop by drop over 10 minutes. Then, 0.5 mL conc. acetic acid (CH_3COOH) was added slowly to the final solution then heated to 80 ºC with constant stirring. Precipitation was observed between 5 minutes to 4 hours according to the hydrazide type.

(E)-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl) oxy) benzylidene)benzohydrazide (2a)

M.p.: 224 ºC Yield: 58%. ^{1}H-NMR (400 MHz, *d*^{6}-DMSO): 11.74 (s, 1H), 8.35 (s, 1H), 7.87(d, 2H), 7.75 (d, 2H), 7.65 (d, 2H), 7.52 (m, 3H), 7.36 (s, 1H), 7.17 (d, 1H), 7.08 (d, 1H), 5.24 (s, 2H), 3.83 (s, 3H). ^{13}C-NMR (100 MHz, *d*^{6}-DMSO): 55.99, 69.34, 108.92, 113.58, 122.16, 125.76, 125.79, 125.83, 128.01, 128.07, 128.56, 128.91, 132.09, 132.98, 142.17, 142.15, 148.27, 149.79, 163.43. FT-IR: 3235, 3077, 1645, 1609, 1509 cm^{-1}. Anal. Calc. (%) for $C_{23}H_{19}F_3N_2O_3$: C:64.48; H:4.47; N:6.54, Found: C:64.56; H:4.29; N:6.48, HR-Mass $[M+H]^+$: 429.1423 *m/z.*

(E)-2-chloro-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide(2b)

M.p.: 217 ºC Yield: 49%. ^{1}H-NMR (400 MHz, *d*^{6}-DMSO): 11.81 (s, 1H), 8.34 (s, 1H), 8.05 (s, 1H), 7.87 (d, 2H), 7.76 (t, 2H), 7.65 (d, 2H), 7.48 (t, 1H), 7.36 (s, 1H), 7.16 (d, 1H), 7.08 (d, 1H), 5.24 (s, 2H), 3.82 (s, 3H). ^{13}C-NMR (100 MHz, *d*^{6}-DMSO): 56.02, 69.36, 108.99, 113.61, 122.17, 122.32, 125.75, 125.79, 125.83, 127.23, 127.90, 128.56, 130.54, 131.20, 134.83, 136.15, 142.15, 148.82, 149.82, 149.92, 161.86. FT-IR: 3222, 3048, 1659, 1540, 1514 cm^{-1}. Anal. Calc. (%) for $C_{23}H_{18}ClF_3N_2O_3$: C:59.68; H:3.92; N:6.05, Found: C:59.72; H:3.98; N:6.11, HR-Mass $[M+H]^+$: 463.1026 *m/z.*

(E)-3-bromo-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2c)

M.p.: 185 ºC Yield: 63%. ^{1}H-NMR (400 MHz, *d*^{6}-DMSO): 11.81 (s, 1H), 8.34 (s, 1H), 8.05 (s, 1H), 7.87 (d, 1H), 7.76 (t, 3H), 7.65 (d, 2H), 7.47 (t, 1H), 7.36 (s, 1H), 7.17 (d, 1H), 7.08 (d, 1H), 5.24 (s, 2H), 3.82 (s, 3H). ^{13}C-NMR (100 MHz, *d*^{6}-DMSO): 56.02, 69.36, 108.99, 133.61, 122.17, 122.32, 125.75, 125.79, 125.83, 127.23, 127.90, 128.56, 130.54, 131.20, 134.83, 136.15, 142.15, 148.82, 149.82, 149.92, 161.86. FT-IR: 3228, 3064, 1659, 1509, 1326 cm^{-1}. Anal. Calc. (%) for $C_{23}H_{18}BrF_3N_2O_3$: C:54.45; H:3.58; N:5.52, Found: C:54.26; H:3.48; N:5.68. HR-Mass $[M+H]^+$: 507.0519 *m/z.*

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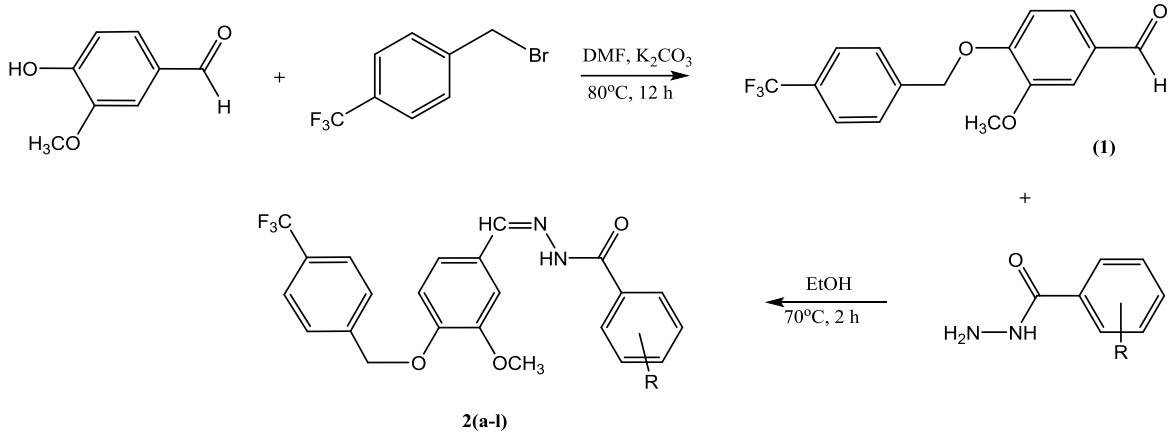


R= **a)** -H **b)** -Cl (2-chloro) **c)** -Br (3-bromo) **d)** -OH (3-hydroxy) **e)** -NO_2 (4-nitro) **f)** -CH_3 (4-methyl) **g)** -$C(CH_3)_3$ (4-tBu) **h)** -F (4-fluoro) **i)** -Br (4-bromo) **j)** -Cl (4-chloro) **k)** -OCH_3 (3-methoxy)

Figure 1. The synthesis procedure of compounds 2a-k.

(E)-3-hydroxy-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2d)

M.p.: 255 ºC Yield: 57%. ^{1}H-NMR (400 MHz, *d*^{6}-DMSO): 11.65 (8s, 1H), 9.74 (s, 1H), 8.33 (s, 1H), 7.75 (d, 2H), 7.65 (d, 2H), 7.34 (s, 2H), 7.26 (d, 2H), 7.14 (d, 1H), 7.07 (d, 1H), 6.93 (s, 1H), 5.24 (s, 2H), 3.82 (s, 3H). ^{13}C-NMR (100 MHz, *d*^{6}-DMSO): 55.99, 69.34, 108.90, 113.58, 114.90, 118.47, 119.01, 122.11, 125.80, 125.84, 128.12, 128.56, 128.65, 129.95, 135.38, 142.18, 148.11, 149.74, 149.78, 157.82, 163.43. FT-IR: 3135, 2955, 1649, 1504, 1322 cm^{-1}. Anal. Calc. (%) for $C_{23}H_{19}F_3N_2O_4$: C:62.16; H:4.31; N:6.30. Found: C:62.09; H:4.28; N:6.42. HR-Mass [M+H]$^+$: 445.1359 *m/z.*

(E)-N'-(3-methoxy-4-((4-(trifluoromethyl)benzyl)oxy) benzylidene)-4-nitrobenzohydrazide (2e)

M.p.: 205 ºC Yield: 61%. ^{1}H-NMR (400 MHz, *d*^{6}-DMSO): 12.02 (s, 1H), 8.34 (d, 3H), 8.10 (d, 2H), 7.75 (d, 2H), 7.65 (d, 2H), 7.37 (s, 1H), 7.20 (d, 1H), 7.09 (d, 1H), 5.25 (s, 2H), 3.83 (s, 3H). ^{13}C-NMR (100 MHz, *d*^{6}-DMSO): 56.02, 69.34, 109.02, 113.58, 122.45, 124.10, 125.80, 125.84, 127.75, 128.57, 128.98, 129.56, 139.65, 142.13, 149.39, 149.63, 149.81, 150.02, 161.77. FT-IR: 3231, 3074, 1652, 1514, 1327 cm^{-1}. Anal. Calc. (%) for $C_{23}H_{18}F_3N_3O_5$: C:58.35; H:3.83; N:8.88. Found: C:58.27; H:3.88; N:9.01. HR-Mass [M+H]$^+$: 474.1279 *m/z.*

(E)-N'-(3-methoxy-4-((4-(trifluoromethyl)benzyl) oxy)benzylidene)-4-methylbenzohydrazide (2f)

M.p.: 234 ºC Yield: 64%. ^{1}H-NMR (400 MHz, *d*^{6}-DMSO): 11.66 (s, 1H), 8.35 (s, 1H), 7.77 (m, 4H), 7.65 (d, 2H), 7.35 (s, 1H), 7.29 (d, 2H), 7.15 (d, 1H), 7.07 (d, 1H), 5.24 (s, 2H), 3.82 (s, 3H), 2.34 (s, 3H). ^{13}C-NMR (100 MHz, *d*^{6}-DMSO): 21.46, 55.99, 69.34, 108.90, 113.59, 122.09, 125.80, 125.83, 128.02, 128.14, 128.55, 128.64, 129.42, 131.08, 142.12, 142.17, 148.00, 149.73, 149.79, 163.23. FT-IR: 3222, 3045, 1645, 1509, 1328 cm^{-1}. Anal. Calc. (%) for $C_{24}H_{21}F_3N_2O_3$: C:65.15; H:4.78; N:6.33. Found: C:65.18; H:4.62; N:6.40. HR-Mass [M+H]$^+$: 443.1571 *m/z.*

(E)-4-(tert-butyl)-N'-(3-methoxy-4-((4-(trifluoro methyl)benzyl)oxy)benzylidene)benzohydrazide (2g)

M.p.: 180 ºC Yield: 59%. ^{1}H-NMR (400 MHz, *d*^{6}-DMSO): 11.67 (s, 1H), 8.35 (s, 1H), 7.81 (d, 2H), 7.74 (d, 2H), 7.65 (d, 2H), 7.50 (d, 2H), 7.36 (s, 1H), 7.14 (d, 1H), 7.07 (d, 1H), 5.24 (s, 2H), 3.82 (s, 3H), 1.28 (s, 9H). ^{13}C-NMR (100 MHz, *d*^{6}-DMSO): 31.34, 35.12, 55.98, 69.36, 108.95, 113.60, 122.07, 125.67, 125.78, 126.01, 127.88, 128.15, 128.54, 128.66, 131.22, 142.16, 148.03, 149.74, 149.79, 154.95, 163.37. FT-IR: 3112, 3045, 1647, 1516, 1336 cm^{-1}. Anal. Calc. (%) for $C_{27}H_{27}F_3N_2O_3$: C:66.93; H:5.62; N:5.78. Found: C:67.08; H:5.67; N:5.71. HR-Mass [M+H]$^+$: 485.2044 *m/z.*

(E)-4-fluoro-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2h)

M.p.: 188 ºC Yield: 48%. ^{1}H-NMR (400 MHz, *d*^{6}-DMSO): 11.76 (s, 1H), 8.34 (s, 1H), 7.95 (m, 2H), 7.74 (d, 2H), 7.65 (d, 2H), 7.33 (m, 3H), 7.17 (d, 1H), 7.07 (d, 1H), 5.24 (s, 2H), 3.82 (s, 3H). ^{13}C-NMR (100 MHz, *d*^{6}-DMSO): 55.99, 69.35, 108.95, 113.58, 115.78, 115.99, 122.18, 125.74, 125.78, 125.82, 125.86, 128.01, 128.54, 130.67, 130.76, 142.15, 148.37, 149.79, 162.35. FT-IR: 3241, 3087, 1649, 1505, 1328 cm^{-1}. Anal. Calc. (%) for $C_{23}H_{18}F_4N_2O_3$: C:61.88; H:4.06; N:6.28. Found: C:61.96; H:3.98; N:6.35. HR-Mass [M+H]$^+$: 447.1323 *m/z.*

(E)-4-bromo-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2i)

M.p.: 218 ºC Yield: 42%. ^{1}H-NMR (400 MHz, *d*^{6}-DMSO): 11.80 (s, 1H), 8.34 (s, 1H), 7.83 (d, 3H), 7.73 (t, 2H), 7.65 (d, 3H), 7.36 (s, 1H), 7.17 (d, 1H), 7.08 (d, 1H), 5.24 (s, 2H), 3.82 (s, 3H). ^{13}C-NMR (100 MHz, *d*^{6}-DMSO): 56.01, 69.36, 108.98, 113.59, 122.26, 125.16, 125.80, 125.84, 127.95, 128.56, 128.98, 130.12, 131.94,

133.02, 142.15, 148.63, 149.80, 149,87, 162.46. FT-IR: 3225, 3067, 1645, 1513, 1327 cm^{-1}. Anal. Calc. (%) for $C_{23}H_{18}BrF_3N_2O_3$: C:54.45; H:3.58; N:5.52. Found: C:54.38; H:3.69; N:5.67. HR-Mass $[M+H]^+$: 507.0522 *m/z.*

(E)-4-chloro-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2j)

M.p.: 209 ºC Yield: 61%. ^{1}H-NMR (400 MHz, d^6-DMSO): 11.80 (s, 1H), 8.35 (s, 1H), 7.89 (t, 2H), 7.75 (d, 2H), 7.65 (d, 2H), 7.57 (d, 2H), 7.35 (s, 1H), 7.18 (d, 1H), 7.07 (d, 1H), 5.24 (s, 2H), 3.83 (s, 3H). ^{13}C-NMR (100 MHz, d^6-DMSO): 56.00, 69.35, 108.96, 113.58, 122.24, 125.79, 125.83, 127.96, 128.56, 128.66, 129.00, 129.95, 132.67, 136.90, 142.15, 148.60, 149.80, 149.86, 162.33. FT-IR: 3228, 3071, 1645, 1512, 1328 cm^{-1}. Anal. Calc. (%) for $C_{23}H_{18}ClF_3N_2O_3$: C:59.68; H:3.92; N:6.05. Found: C:59.78; H:4.05; N:6.15. HR-Mass $[M+H]^+$: 463.1020 *m/z.*

(E)-3-methoxy-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2k)

M.p.: 175 ºC Yield: 53%. ^{1}H-NMR (400 MHz, d^6-DMSO): 11.69 (s, 1H), 8.35 (s, 1H), 7.75 (d, 2H), 7.65 (d, 2H), 7.41 (m, 4H), 7.14 (m, 3H), 5.24 (s, 2H), 3.82 (s, 3H), 3.80 (s, 3H). ^{13}C-NMR (100 MHz, d^6-DMSO): 55.77, 56.00, 69.35, 108.96, 113.26, 113.60, 117.78, 120.21, 122.15, 125.80, 125.84, 128.05, 128.56, 128.65, 128.97, 130.10, 135.36, 142.16, 148.38, 149.79, 159.62, 163.15. FT-IR: 3219, 3065, 1637, 1540, 1327 cm^{-1}. Anal. Calc. (%) for $C_{24}H_{21}F_3N_2O_4$: C:62.88; H:4.62; N:6.11. Found: C:62.99; H:4.59; N:6.15. HR-Mass $[M+H]^+$: 459.1526 *m/z.*

2.5. Microorganisms

The following organisms were used in this study: *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Listeria monocytogenes* (ATCC 1911), *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 35218), and *Bacillus subtilis* (NRRL B478). The bacteria were in liquid Nutrient Broth (Merck) for fresh pure cultures. For Candidal apoptosis test, *Candida albicans* (ATCC 90028), *Candida glabrata* (ATCC 90030), *Candida krusei* (ATCC 6258) *and Candida parapsilosis* (ATCC 22019) were grown in Saboraud Dextrose Broth (Merck).

2.6. Inoculum

To obtain accurate and reproducible results, the bacterial cell number used in susceptibility tests is very important. The optimum final inoculum size recommended as $5x10^5$ colony-forming units per mL. For that reason, all inocula were set 0.5 McFarland standard with McFarland Tube Densitometer for accurate and reproducible results.

2.7. Microdilution Broth Assays

Vanillin derivatives dissolved in DMSO and concentrations were prepared on ranging from 800 to 1.56 µL/mL. The prepared concentrations were distributed in duplicate 100 µL for each well on the 96-well plate. After that, fresh pure bacterial and Candida cultures which set 0.5 McFarland standard in Mueller Hinton Broth (Sigma-Aldrich) and Saboraud Dextose Broth (Merck) were added on concentrations for 100 µL. with adding the bacterial or Candida cultures, final concentrations for Vanillin derivatives were ranging from 800 to 1.56 µL/mL. At the end of this process, all plates were incubated for 24 hours.

2.8. Candidal Apoptosis Tests

Following the MIC value were determined by incubation with four different Candida strains and the flow cytometer apoptosis test were performed with Annexin V-PI to evaluate whether these values were causing apoptosis. For this, Candida strains and compounds were incubated in 96-well plates for 24 hours at the indicated concentrations. At the end of the incubation period, centrifuged and depleted cultures were incubated in the dark with 5 µL Annexin V and 5 µL PI for 20 min. After incubation, cultures were washed once with PBS. Then, $1x10^5$ cells were counted in flow cytometry (Accuri C6-BD) device and percentage of apoptotic, necrotic Candida were determined as percentage and were shown in a table [19].

3. Results and Discussion

3.1. Characterization of the compounds

The condensation reaction between aryl substituted hydrazides and trifluoromethylbenzyl substituted vanillin resulted in eleven different target compounds. Obtained yields of compounds were found to be between 42-65 %. All compounds were successfully characterized with ^{1}H-NMR, ^{13}C-NMR, FT-IR, Elemental Analysis and HR-Mass techniques. Spectroscopic data is given in experimental section.

From the FT-IR spectra, compounds carbonyl group (C=O) frequencies was observed between 1637-1659 cm^{-1}. Azomethine (-CH=N-) group frequencies was observed between 1609-1504 cm^{-1}. Peaks was observed between 3254-2955 cm^{-1} are related to aromatic (C-H) vibrations.

In the ^{1}H-NMR spectra of compounds, singlet signals which was observed around 12.02-11.65 ppm are related to the -NH- protons of hydrazide moiety. Peaks which was observed between 8.35-8.22 ppm are related to azomethine group (-CH=N-) proton as a singlet. Aromatic protons (C-H) which were appeared in the region of 8.10-7.07 ppm as doublet, triplet or multiplets. Methylene ($-CH_2-$) protons singlet peaks observed at 5.24 ppm. Methoxy part ($-OCH_3$) singlet proton peaks appeared around 3.82 ppm. Additionally, hydroxyl group (-OH) peak appeared at 9.74 ppm, methoxy group ($-OCH_3$) peak appeared at 3.83 ppm, *tert*-butyl ($-CCH_3$) proton peak appeared at 1.28 ppm and methyl proton ($-CH_3$) peak appeared at 2.34 ppm are related with compounds 2d, 2k, 2g and 2f respectively. Aromatic and

aliphatic ^{13}C-NMR signals found to be in agreement with suggested structures for compounds 2a-k.

From the mass spectra, peaks observed between 507.0522-429.1423 *m/z* values supported the expected structures of $[M+H]^+$ molecular ions of synthesized compounds.

Elemental analysis values of the compounds were found to be acceptable ranges.

3.2. Antimicrobial Activity Results

The antimicrobial activities of synthesized compounds were tested on microorganisms including *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Listeria monocytogenes* (ATCC 1911), *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 35218), and *Bacillus subtilis* (NRRL B478) and on four Candida species including *Candida albicans*, *Candida glabrata*, *Candida krusei*, and *Candida parapsilosis*. The results are given in Table 1.

Table 1. Antibacterial activity results of compounds 2a-k (µL/mL).

Microorganisms & Chemicals	2a	2b	2c	2d	2e	2f	2g	2h	2i	2j	2k	Cont.*
Staphylococcus aureus (ATCC 25923)	400	400	400	400	400	400	400	400	400	400	400	100
Enterococcus faecalis (ATCC 29212)	400	400	400	400	400	400	400	400	400	400	400	200
Listeria monocytogenes (ATCC 1911)	400	400	400	400	400	400	400	400	400	400	400	100
Klebsiella pneumoniae (ATCC 700603)	200	200	200	200	200	200	200	200	200	200	200	100
Pseudomonas aeruginosa (ATCC 27853)	200	200	200	200	200	200	200	200	200	200	200	100
Escherichia coli (ATCC 35218)	800	400	**100**	**100**	400	200	200	200	200	200	**100**	100
Bacillus subtilis (NRRL B478)	200	200	200	200	200	200	200	200	400	400	400	100
*****Chloramphenicol was used as a positive control.												

According to the antibacterial activity results, obtained from minimum inhibitory concentration tests (MIC), many of the compounds found to be active against the identified microorganisms compared to Chloramphenicol. Compounds 2c, 2d and 2k exhibited remarkable antibacterial activity especially on *Escherichia coli* (ATCC 35218). These compounds (2c, 2d and 2k) showed equal activity with standard drug (chloramphenicol). In addition to this, *E. coli* (ATCC 35218); *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 27853) and *Bacillus subtilis* were determined as the most susceptible species among the investigated microorganisms to the studied compounds.

Table 2. Anticandidal activity results of compounds 2a-k (µL/mL).

Microorganisms & Chemicals	2a	2b	2c	2d	2e	2f	2g	2h	2i	2j	2k	Control*
Candida albicans (ATCC 90028)	200	200	200	200	200	200	200	200	200	200	200	100
Candida glabrata (ATCC 90030)	200	200	200	200	200	200	200	200	200	200	200	100
Candida krusei (ATCC 6258)	200	200	**100**	200	200	200	200	200	**100**	**100**	200	25
Candida parapsilosis (ATCC 22019)	200	200	200	200	200	200	200	200	200	200	200	25

The anticandidal activity results (Table 2) which was obtained from minimum inhibitory concentration tests (MIC), the activity values of many derivatives found to be around 200 µg/mL against to Candida species including *Candida albicans* (ATCC 90028), *Candida glabrata* (ATCC 90030), *Candida krusei* (ATCC 6258), and *Candida parapsilosis* (ATCC 22019). Additionally, the activity values of compounds 2c, 2i, and 2j were observed higher than the other compounds as 100 µg/mL. Ketoconazole was used as positive control for this assay.

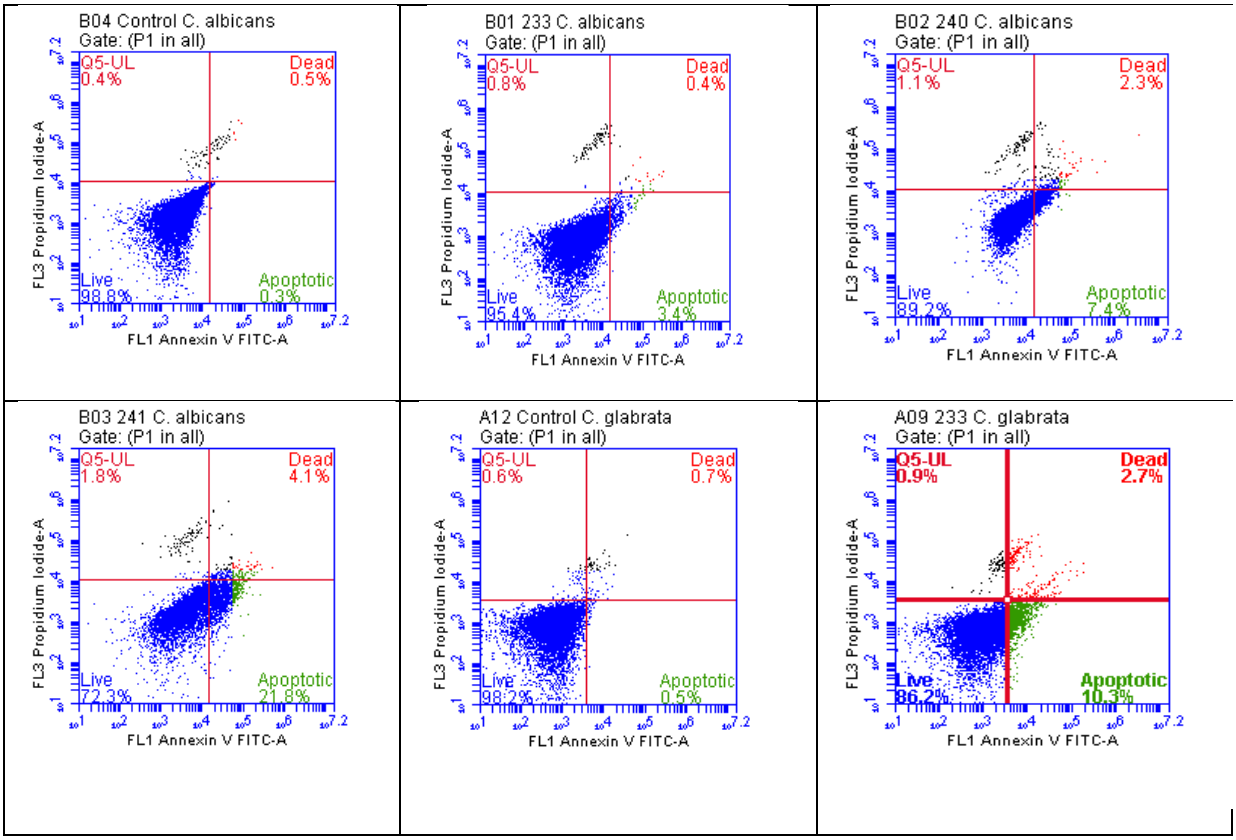
3.3. Candidal Apoptotic Effect Tests

The apoptotic effect tests were investigated with compounds 2c, 2i and 2j, which were found to be the most effective derivatives from the anticandidal tests. From the subject compounds (2c, 2i, and 2j), apoptotic rates of analogues on *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis* were found to be 3.8; 13.0; 91.5; 1.0 for analogue 2c, 9.7, 76.0, 92.2, 1,9 for analogue 2i and 25.9, 59.4, 94.0, 12.2 for

analogue 2j, respectively. The results are given in Table 3 and Figure 2.

Table 3. Apoptotic effect results of compounds 2a-k (µL/mL)

	Control			**Compound 2c**		
	Live (%)	Dead (%)	Apoptosis (%)	Live (%)	Dead (%)	Apoptosis (%)
Candida albicans (ATCC 90028)	98.8	0.4	0.8	95.4	0.8	3.8
Candida glabrata (ATCC 90030)	98.2	0.6	1.2	86.2	0.9	13.0
Candida krusei (ATCC 6258)	96.4	0.4	3.2	8.1	0.4	**91.5**
Candida parapsilosis (ATCC 22019)	97.8	1.6	0.6	97.8	1.5	1.0
	Compound 2i			**Compound 2j**		
	Live (%)	Dead (%)	Apoptosis (%)	Live (%)	Dead (%)	Apoptosis %)
Candida albicans (ATCC 90028)	89.2	1.1	9.7	72.3	1.8	**25.9**
Candida glabrata (ATCC 90030)	22.4	1.5	**76.0**	37.9	2.7	**59.4**
Candida krusei (ATCC 6258)	7.0	0.7	**92.2**	5.6	0.4	**94**
Candida parapsilosis (ATCC 22019)	87.0	11.1	1.9	85.7	2.1	12.2



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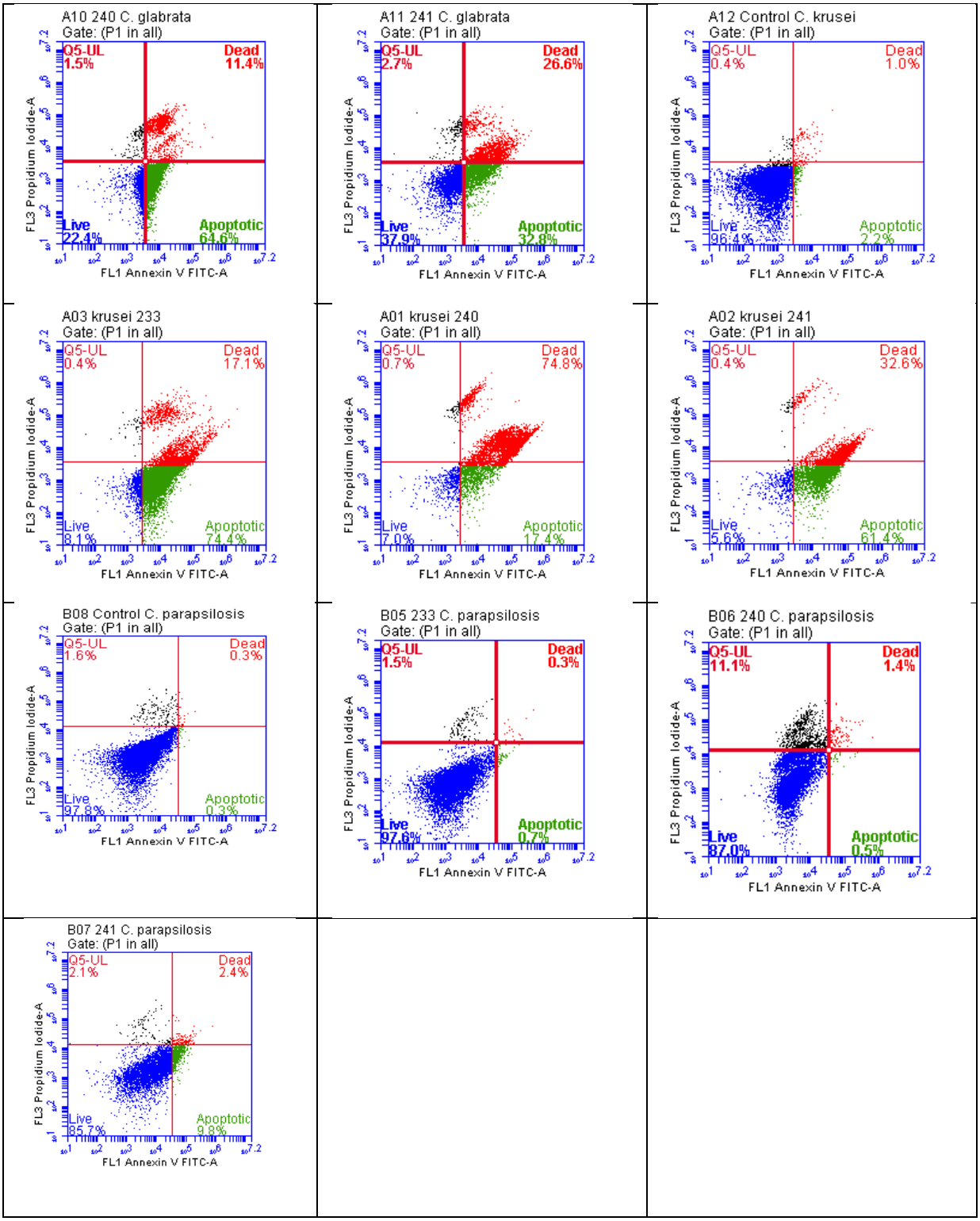

Figure 2. Candidal apoptotic rates of compounds (µL/mL) (233=2c, 240=2i, 241=2j).

The MIC value for the kanamycin against *Escherichia coli* was 3.9 µg/mL, whereas the counter-synthesized derivatives maintained the MIC values of 1.6-5.7 µg/mL. Some Schiff bases showed only *Bacillus subtilis* susceptibility (MIC: 1.8 µg/mL). Some of the synthesized Schiff bases derivatives were found to be effective against *Staphylococcus aureus* and MIC values had found between 3.1 and 1.6 µg/mL, respectively. Isatin derived Schiff bases have also been reported to provide antibacterial activity. In our study, some of synthesized derivatives which named compound 2c, 2d and 2k exhibited remarkable antibacterial activity against *Escherichia coli* (ATCC 35218). MIC values of these compounds (2c, 2d and 2k) found to be 100 µg/mL and the obtained results are in agreement with the standard drug.

4. Conclusions

In summary, the synthesis and characterization of eleven vanillin-derived hydrazones were reported successfully. The characterization steps were conducted using ^{1}H-NMR, ^{13}C-NMR, FT-IR, Elemental Analysis and Mass-spectroscopy techniques. Biological assessments for synthesized compounds were conducted using selected microorganisms. The studies indicated that, compounds 2c, 2d, and 2k were showed effectivity on *Escherichia coli* and also compounds 2c, 2i, and 2j possess strong anti-candidal activities on tested species. The most sensitive strain was found to be *Candida krusei* (ATCC 6258). The minimum MIC value was found to be 100 µg/mL with using compounds 2c, 2d, and 2k on *Escherichia coli* (ATCC 35218). According to our knowledge, besides, some vanillin derivatives have been found to be effective as antibacterial and antifungal agents in some studies [20], the anti-candidal activitiy of schiff base derived vanillin compounds have not been previously reported. Therefore, this study may be considered to be a new contribution to the literature in this regard.

Conflict of Interest

Authors declare no conflict of interest.

Acknowledgements

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Author's Contributions

Hakan Ünver: Drafted and wrote the manuscript, performed the chemical synthesis and characterization of the compounds and result analysis.

Zerrin Cantürk: Drafted and wrote the manuscript, performed the biological experiments and result analysis.

M. Güçlü Özarda: Drafted and wrote the manuscript, assisted in biological experiments and result analysis.

References

1. Hoiby, N, Bjarnsholt, T, Givskov, M, Molin, S, Ciofu, O. 2010. Antibiotic resistance of bacterial biofilms. *International Journal of Antimicrobial Agents;* 35: 322-332.

2. Wright, GD. 2005. Bacterial resistance to antibiotics: Enzymatic degradation and modification. *Advanced Drug Delivery Reviews;* 57: 1451-1470.

3. Fisher, JF, Meroueh, SO, Mobashery, S. 2011. Bacterial resistance to β-lactam antibiotics: compelling opportunism compelling opportunity, *Chemical Reviews;* 105: 395−424.

4. Kuete, V, Alibert-Franco, S, Eyong, KO, Ngameni, B, Folefoc, GN, Nguemeving, JR. 2011. Antibacterial activity of some natural products against bacteria expressing a multidrug resistant phenotype, *International Journal of Antimicrobial Agents;* 37: 156-161.

5. Stavri, M, Piddock, LJV, Gibbons, S. 2007. Bacterial efflux pump inhibitors from natural resources. *Journal of Antimicrobial Chemotheraphy.* 59(6): 1247-1260.

6. Sun, J, Yin, Y, Sheng, GH, Yang, ZB, Zhu, HL. 2013. Synthesis, molecular modelling and structural characterization of vanillin derivatives as antimicrobial agents. *Journal of Molecular Structure;* 1039: 214-218.

7. Harini, ST, Kumar, HV, Rangaswamy, J, Naik, N. 2012. Synthesis, antioxidant and antimicrobial activity of novel vanillin derived piperidin-4-one oxime esters: Preponderant role of the phenyl ester substituents on the piperidin-4-one oxime core. *Bioorganic & Medicinal Chemistry Letters;* 22: 7588-7592.

8. Kamat, JP, Ghosh, A, Devasagayam, TPA. 2000. Vanillin as an antioxidant in rat liver mitochondria: Inhibition of protein oxidation and lipid peroxidation induced by photosensitization, *Molecular and Cellular Biochemistry;* 209:47-53.

9. Naz, H, Tarique, M, Khan, P, Luqman, S, Ahamad, S, Islam, A, Ahmad, F, Hassan, MI. 2018. Evidence of vanillin binding to CAMKIV explains the anti-cancer mechanism in human hepatic carcinoma and neuroblastoma cells. *Molecular and Cellular Biochemistry;* 438: 35-45.

10. Jung, HJ, Song, YS, Kim, K, Lim, CJ, Park, EH. 2010. Assessment of the anti-angiogenic, anti-inflammatory and anti-nociceptive properties of ethyl vanillin. *Archives of Pharmacal Research;* 33:309-316.

11. King, AA, Shaughnessy, DT, Mure, K. 2007. Antimutagenicity of cinnamaldehyde and vanillin in human cells: Global gene expression and possible role of DNA damage and repair. *Mutation Research;* 616: 60-69.

12. Segura, JL, Mancheno, MJ, Zamora, F. 2016. Covalent organic frameworks based on Schiff-base chemistry: synthesis, properties and potential applications. *Chemical Society Reviews;* 45: 5635-5671.

13. Vitago, PA, Tamburini, S. 2004. The challenge of cyclic and acyclic schiff bases and related derivatives. *Coordination Chemistry Reviews;* 248: 1717-2128.

14. Guerriero, P, Vigato, PA, Fenton, DE, Hellier, PC. 1992. Synthesis and Application of Macrocyclic and Macroactclic Schiff bases. *Acta Chemica Scandinavica;* 46: 1025-1046.

15. Zayed, EM, Zayed, MA. 2015. Synthesis of novel schiff's bases of highly potential biological activities and their structure investigation. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy;* 143: 81-90.

16. Ceylan, S. 2016. Synthesis and biological evaluation of new Mannich and Schiff bases containing 1,2,3-triazole and 1,3,4-oxadiazole nucleus. *Medicinal Chemistry Research;* 25: 1958-1970.

17. Maja, M, Milan, C, Zrinusic, Z. 2012. Synthesis and antioxidant evaluation of Schiff bases derived from 2,6-pyridinedicarboxylic acid, *Letters in Organic Chemistry;* 9: 401-410.

18. Gomez, RC, Witter, SK, Hieke, M. 2014. Vanillin-derived antiproliferative compounds influence Plk1 activity, *Bioorganic and Medicinal Chemistry Letters;* 24: 5063-5069.

19. Engeland, M, Luc, JW, Frans, CS, Chris, PM. 1998. Annexin V-Affinity Assay: A Review on an Apoptosis Detection System Based on Phosphatidylserine Exposure. *Cytometry;* 31: 1-9.

20. Faria, NCG, Kim, JH, Goncalves, LAP. 2011. Enhanced activity of antifungal drugs using natural phenolics against yeast strains of Candida and Cryptococcus. *Letters in Applied Microbiology;* 52: 5.

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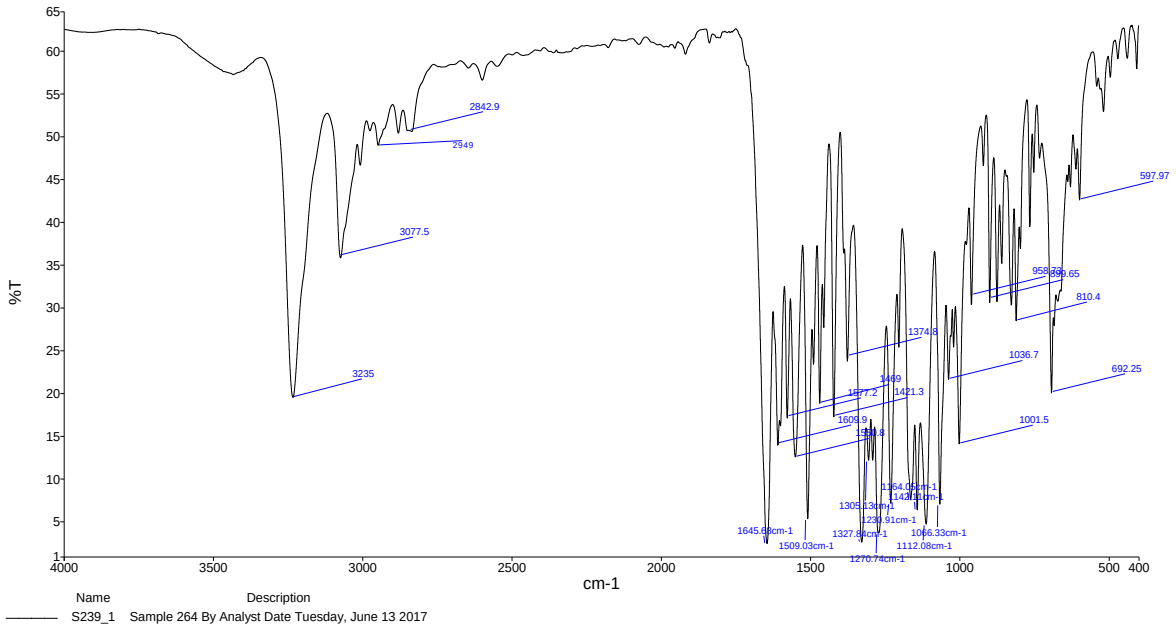

Figure S1. FT-IR spectra of (E)-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2a)

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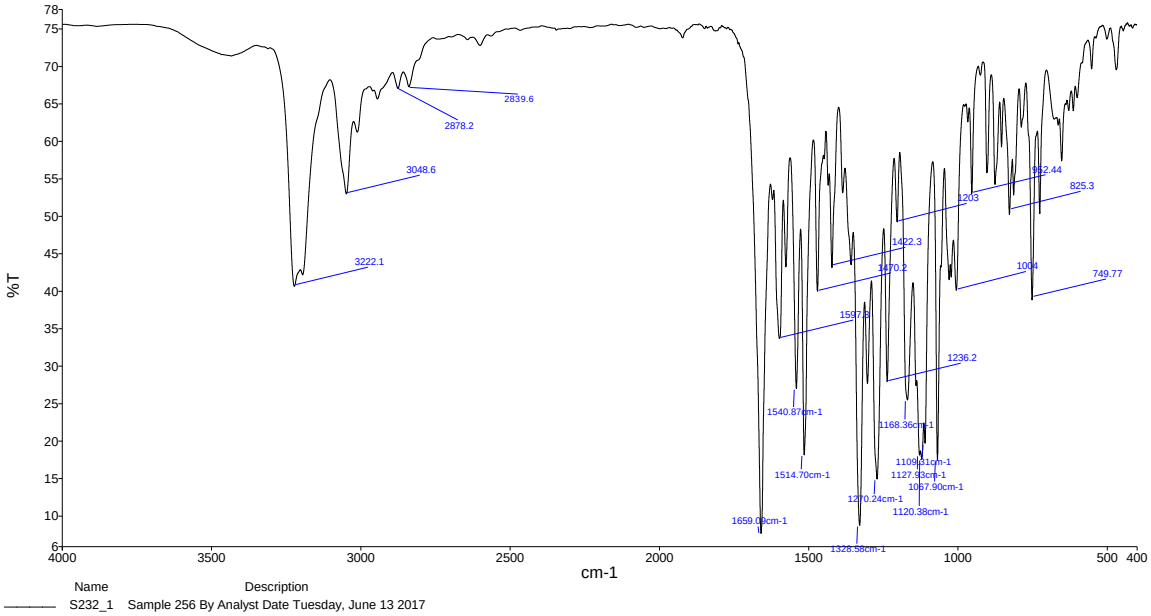

Figure S2. FT-IR spectra of (E)-2-chloro-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2b)

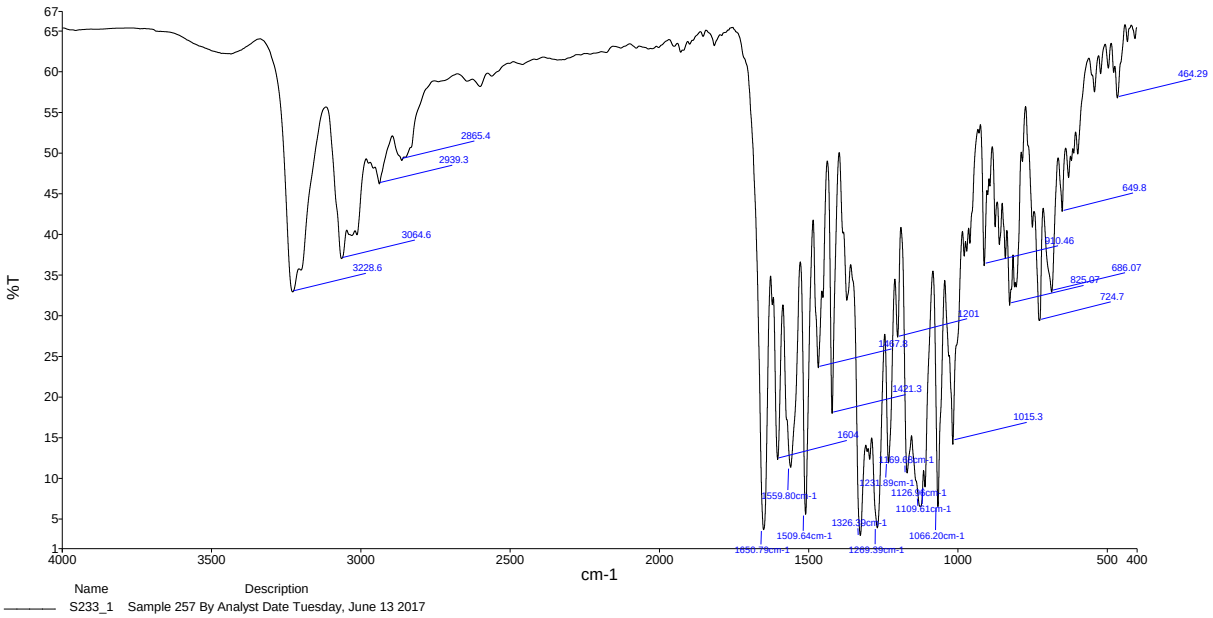

Figure S3. FT-IR spectra of (E)-3-bromo-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2c)

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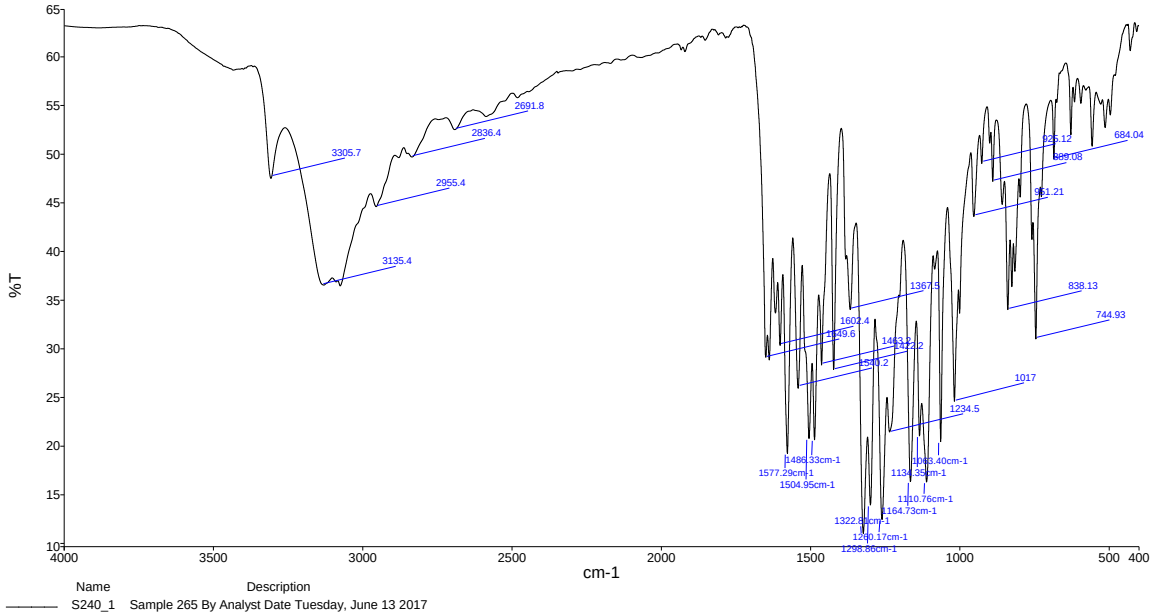

Figure S4. FT-IR spectra of (E)-3-hydroxy-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2d)

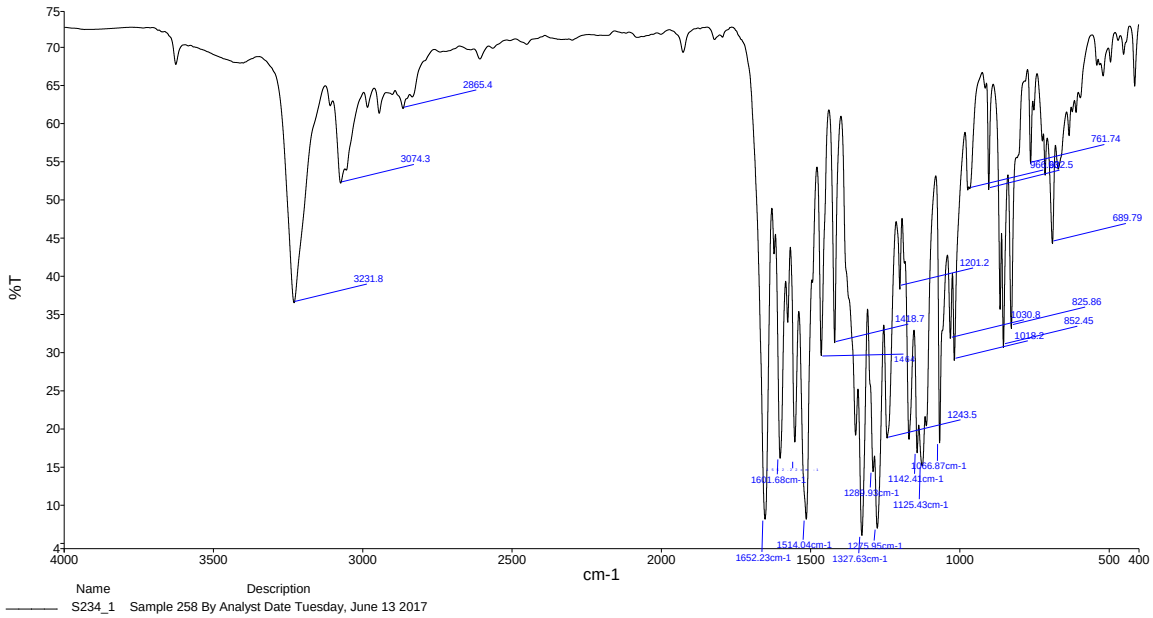

Figure S5. FT-IR spectra of (E)-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)-4-nitrobenzohydrazide (2e)

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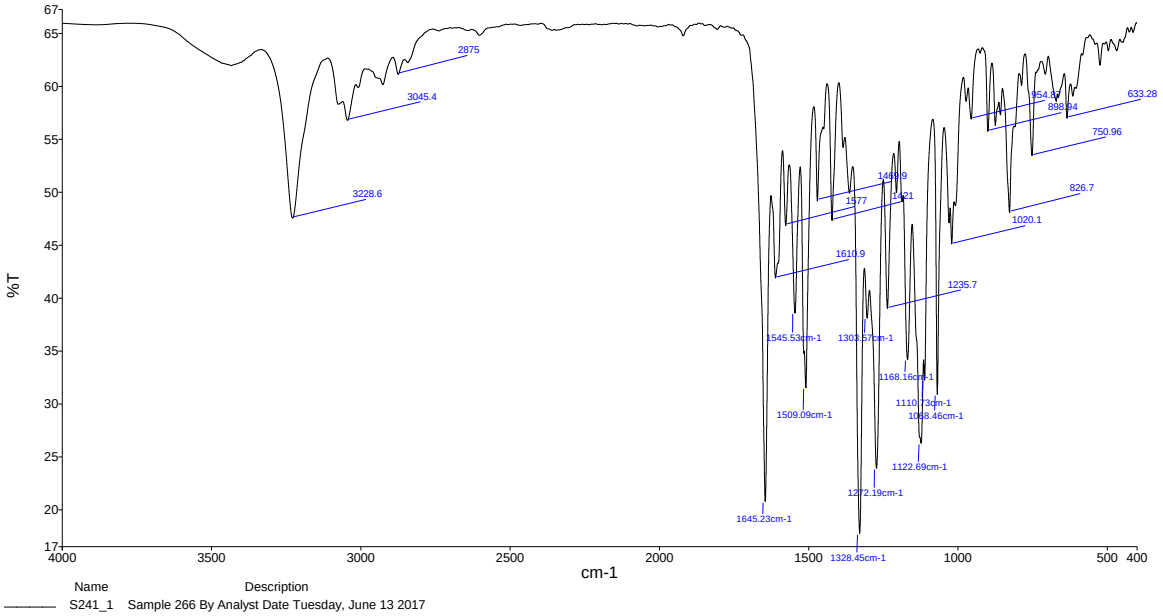

Figure S6. FT-IR spectra of (E)-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)-4-methylbenzohydrazide (2f)

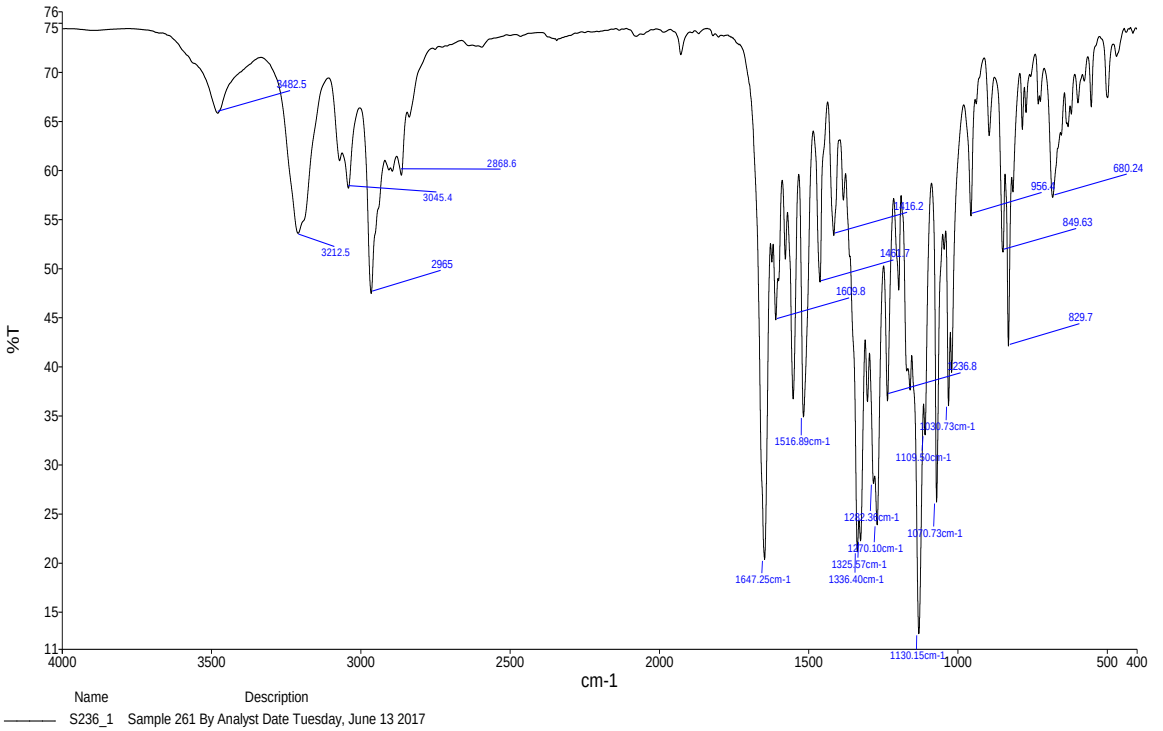

Figure S7. FT-IR spectra of (E)-4-(tert-butyl)-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2g)

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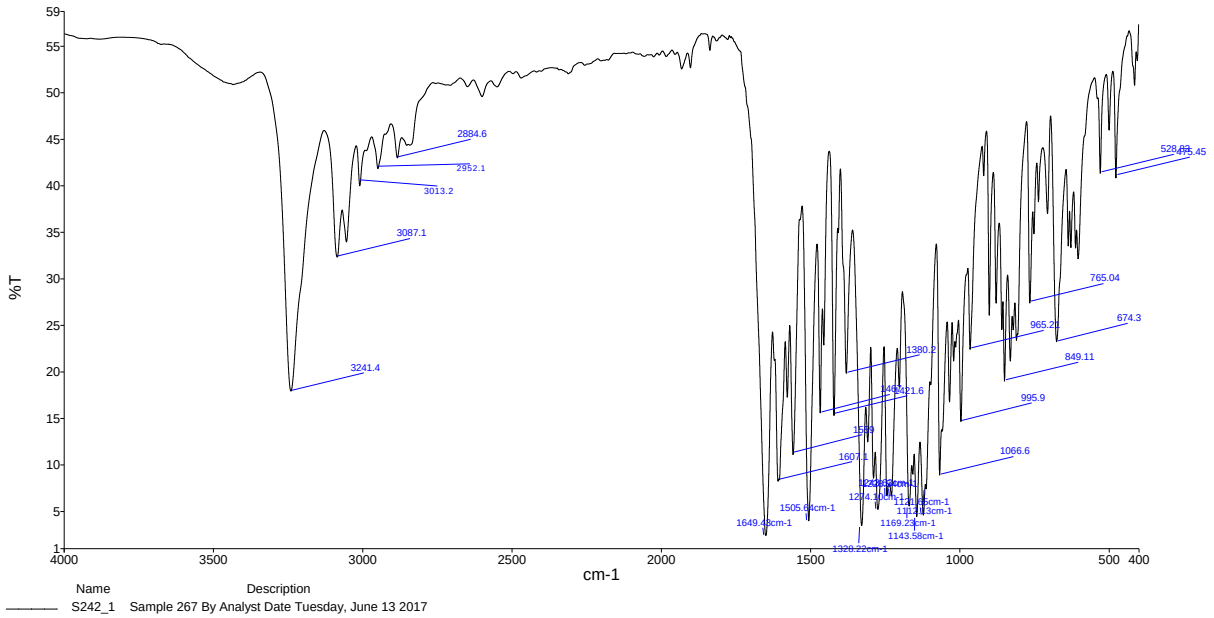

Figure S8. FT-IR spectra of (E)-4-fluoro-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2h)

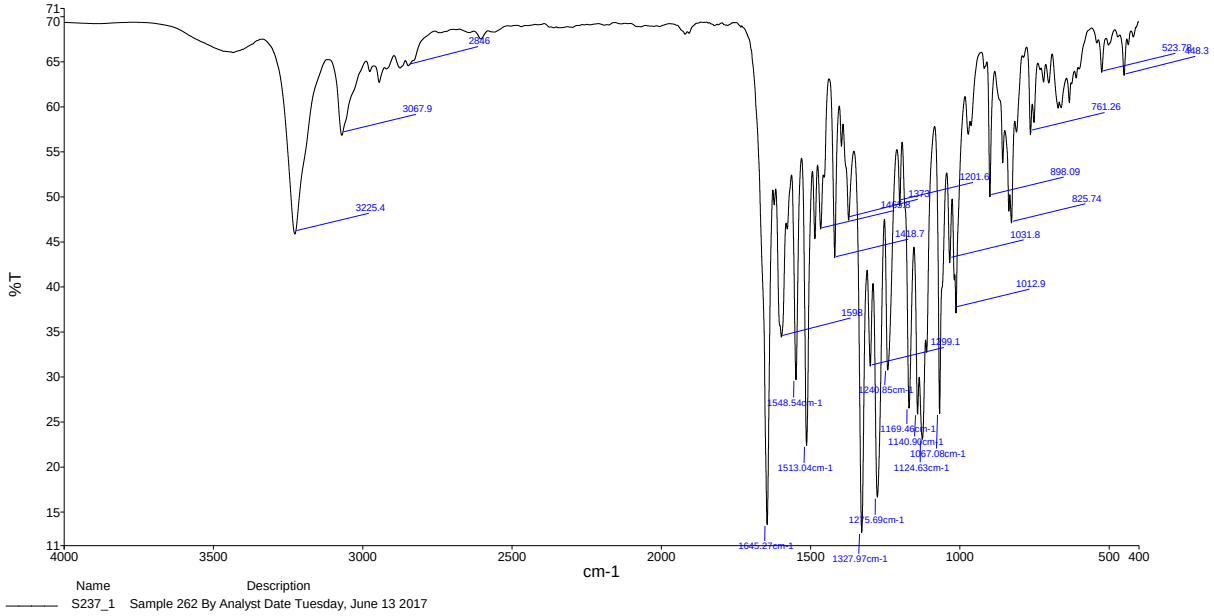

Figure S9. FT-IR spectra of (E)-4-bromo-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2i)

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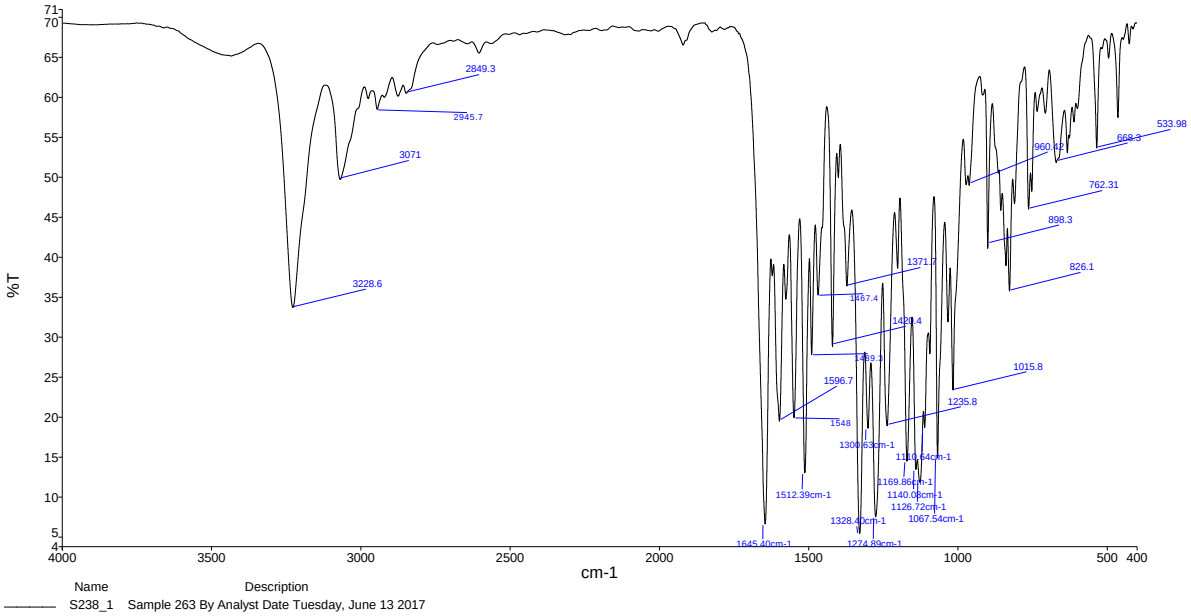

Figure S10. FT-IR spectra of (E)-4-chloro-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2j)

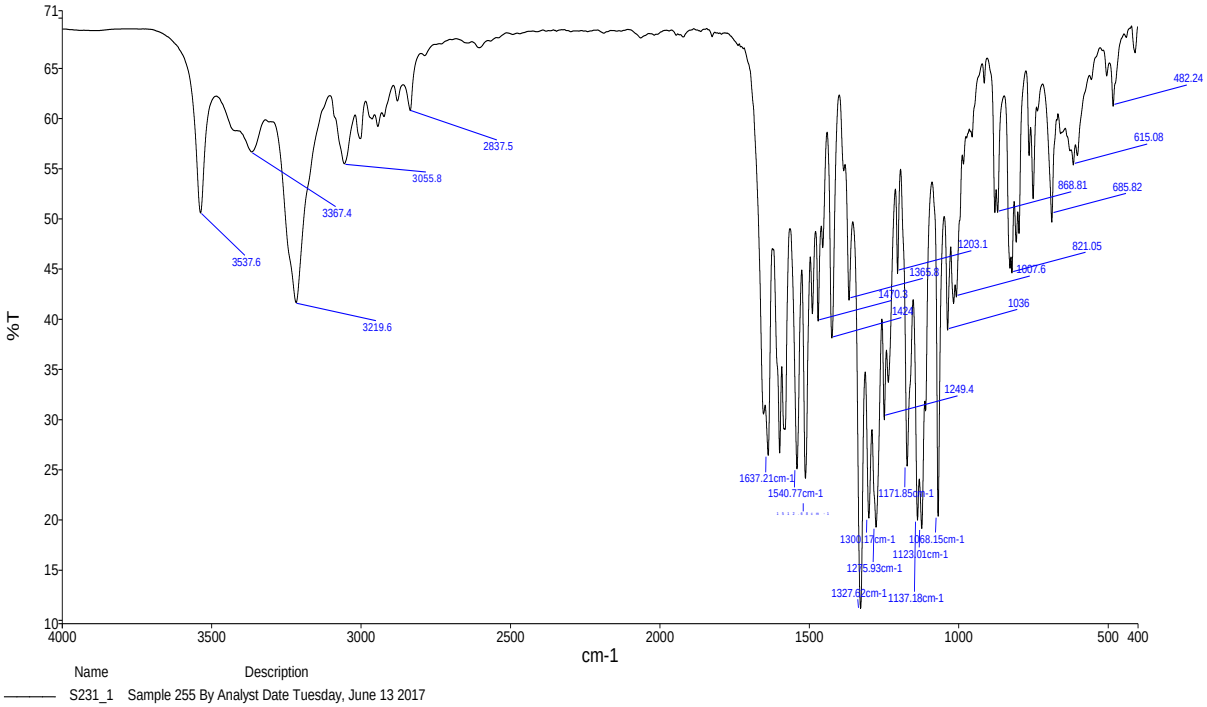

Figure S11. FT-IR spectra of (E)-3-methoxy-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2k)

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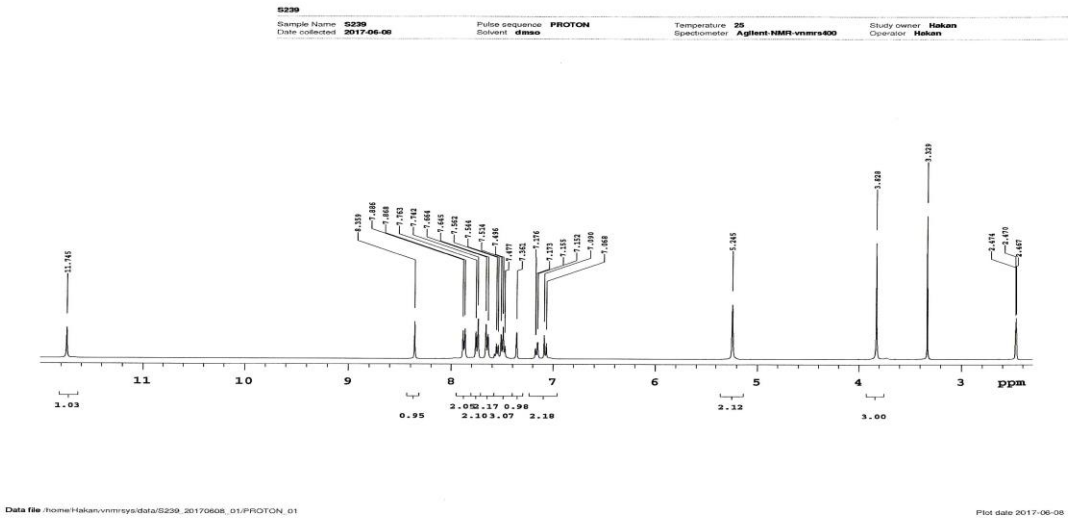

Figure S12. ^{1}H-NMR spectra of (E)-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2a)

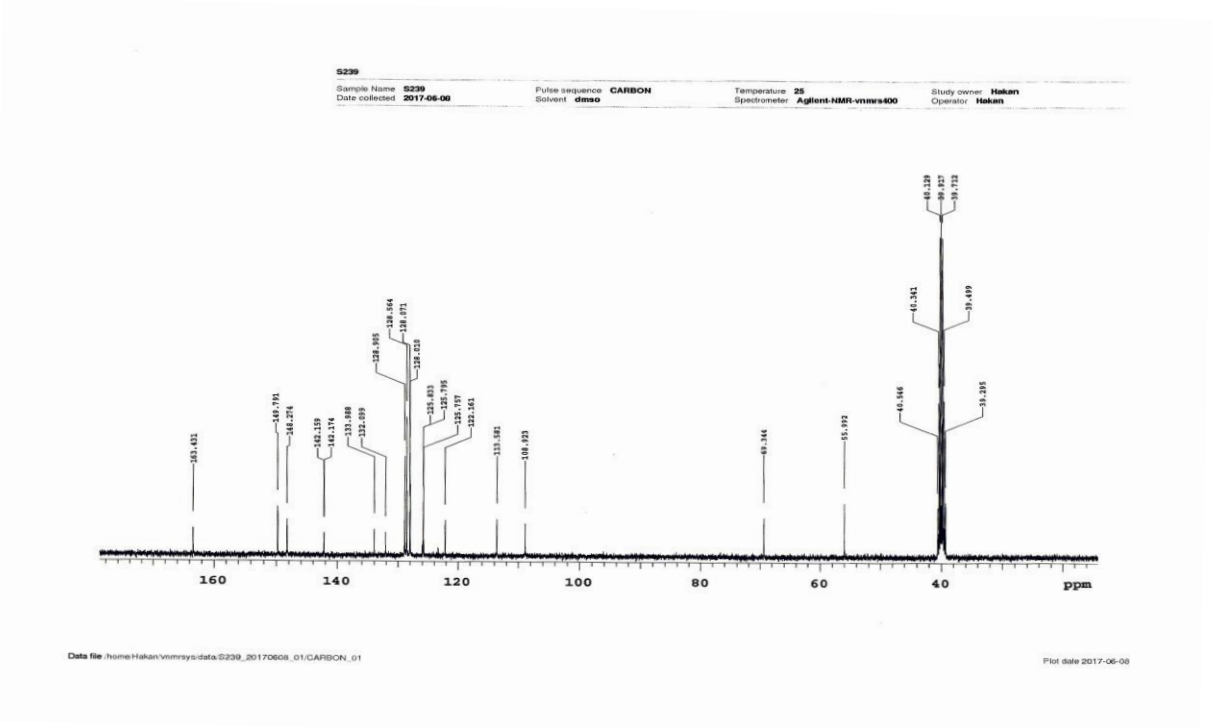

Figure S13. ^{13}C-NMR spectra of (E)-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2a)

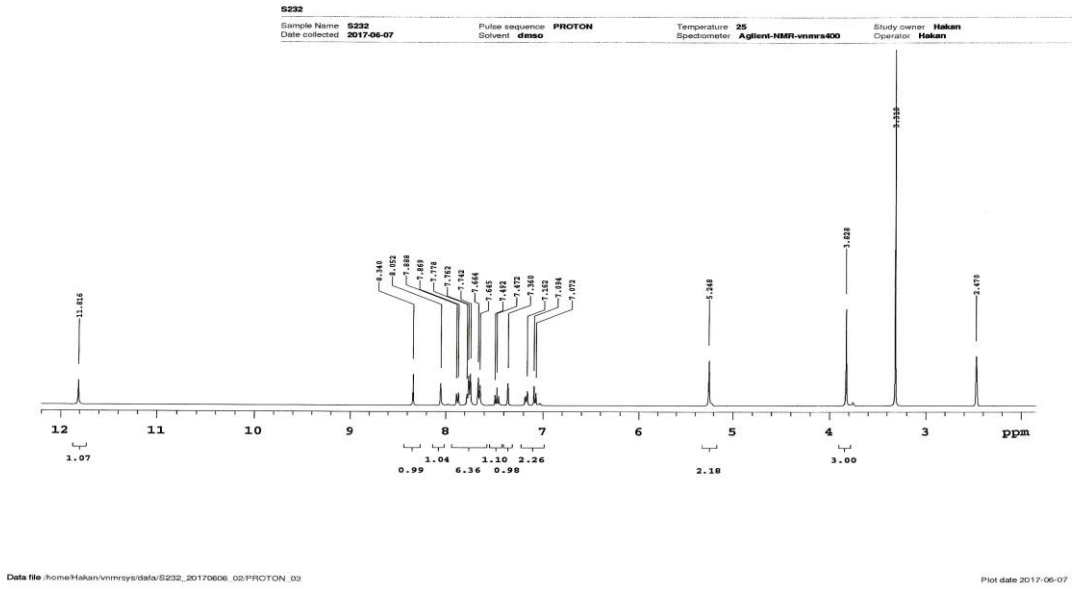

Figure S14. ^{1}H-NMR spectra of (E)-2-chloro-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2b)

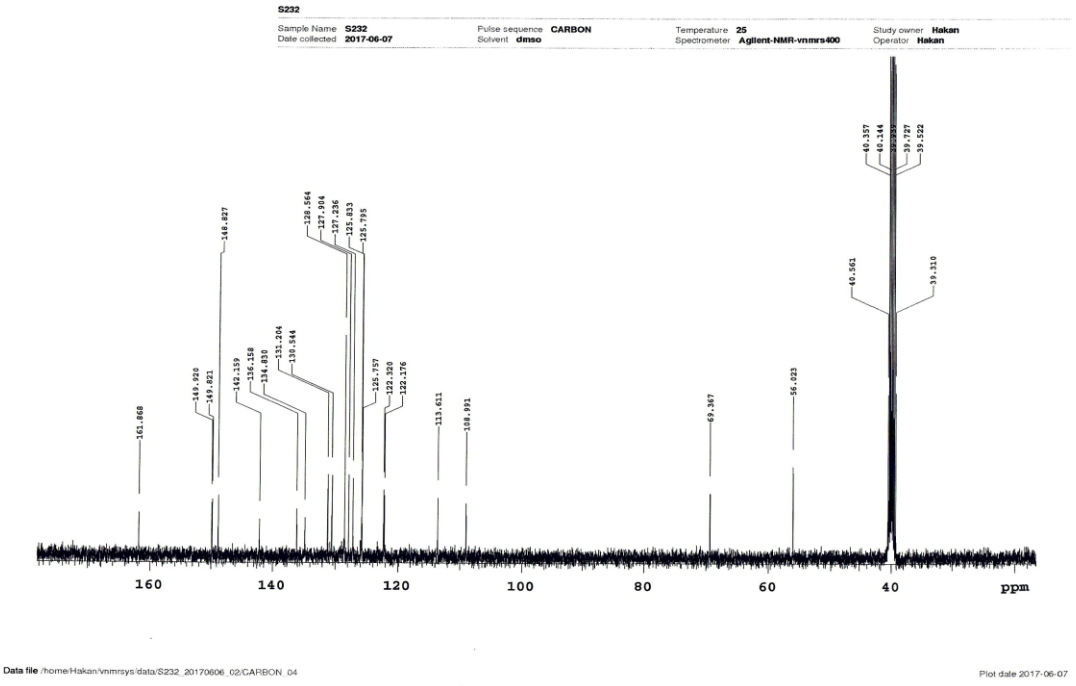

Figure S15. ^{13}C-NMR spectra of (E)-2-chloro-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2b)

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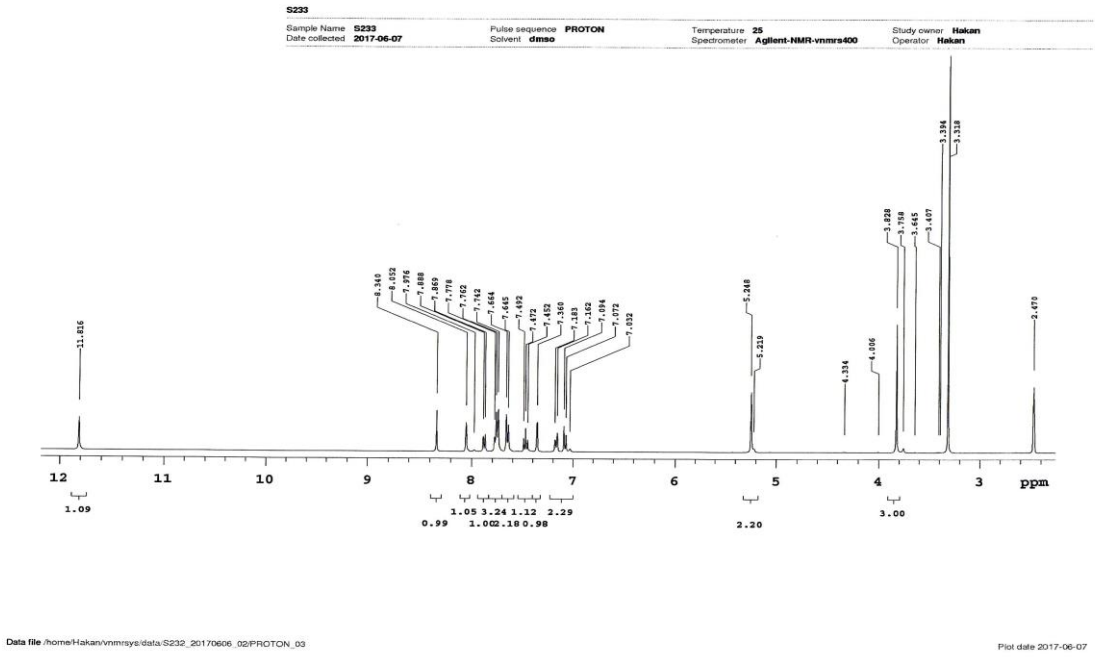

Figure S16. [1]H-NMR spectra of (E)-3-bromo-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2c)

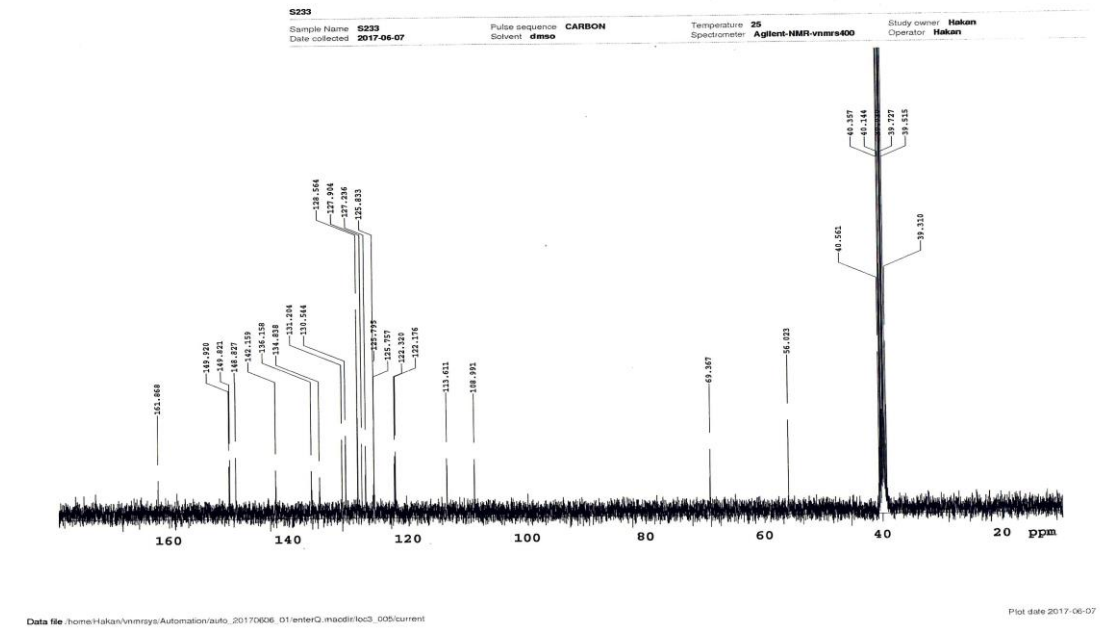

Figure S17. [13]C-NMR spectra of (E)-3-bromo-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2c)

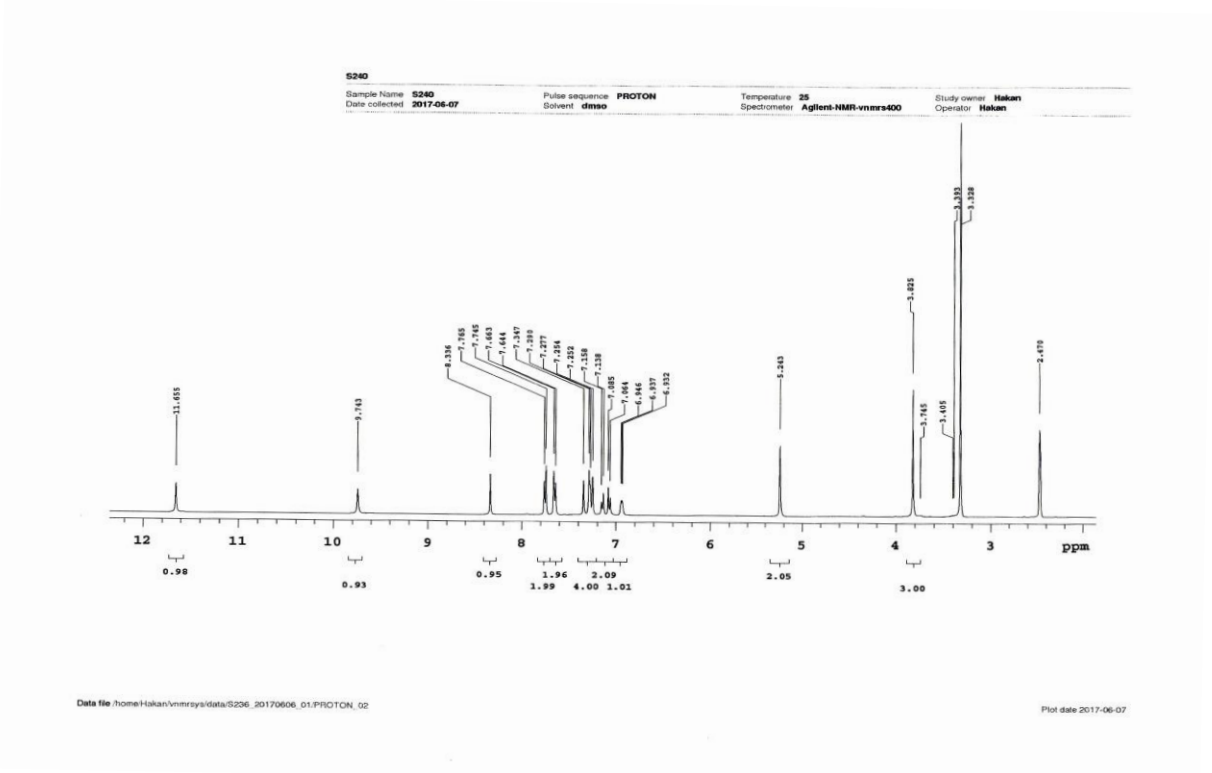

Figure S18. ^{1}H-NMR spectra of (E)-3-hydroxy-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2d)

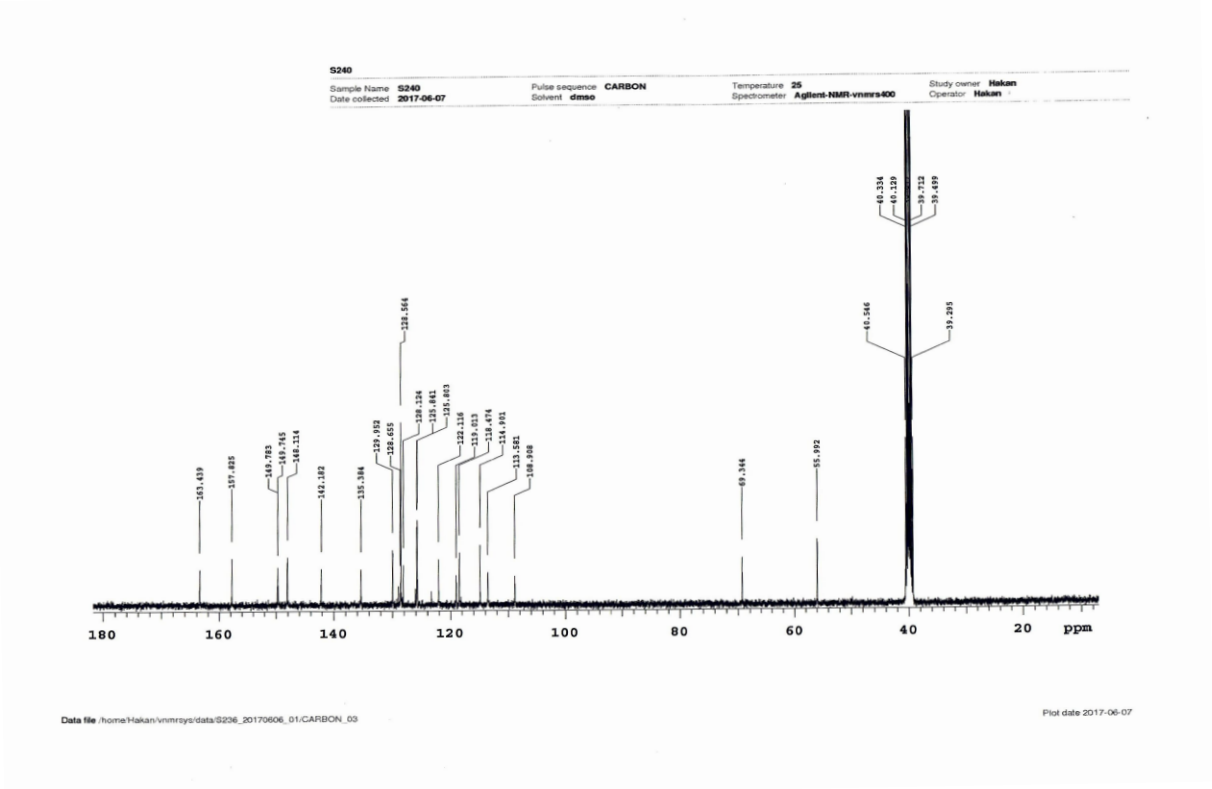

Figure S19. ^{13}C-NMR spectra of (E)-3-hydroxy-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2d)

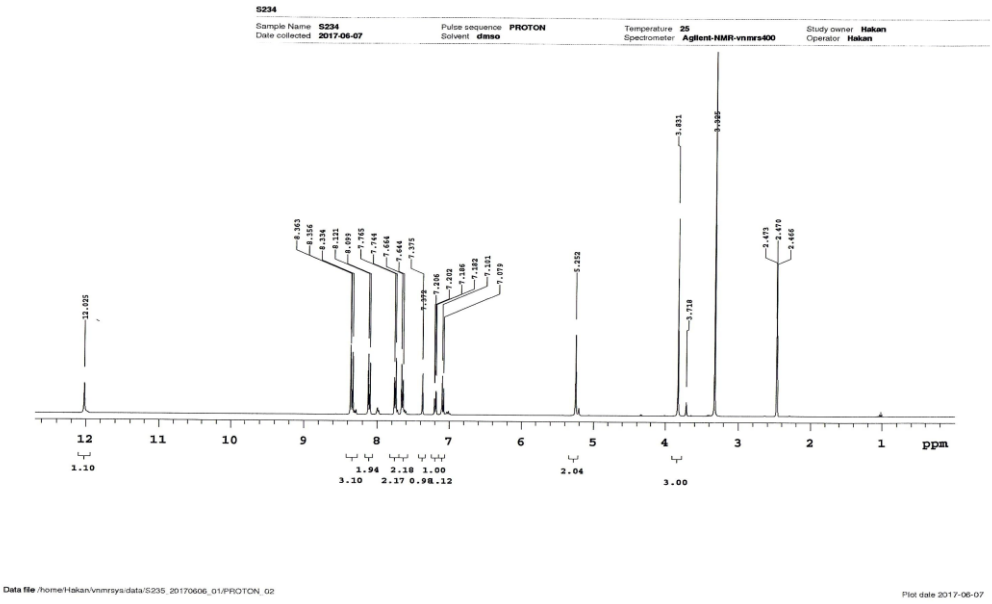

Figure S20. ^{1}H-NMR spectra of (E)-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)-4-nitrobenzohydrazide (2e)

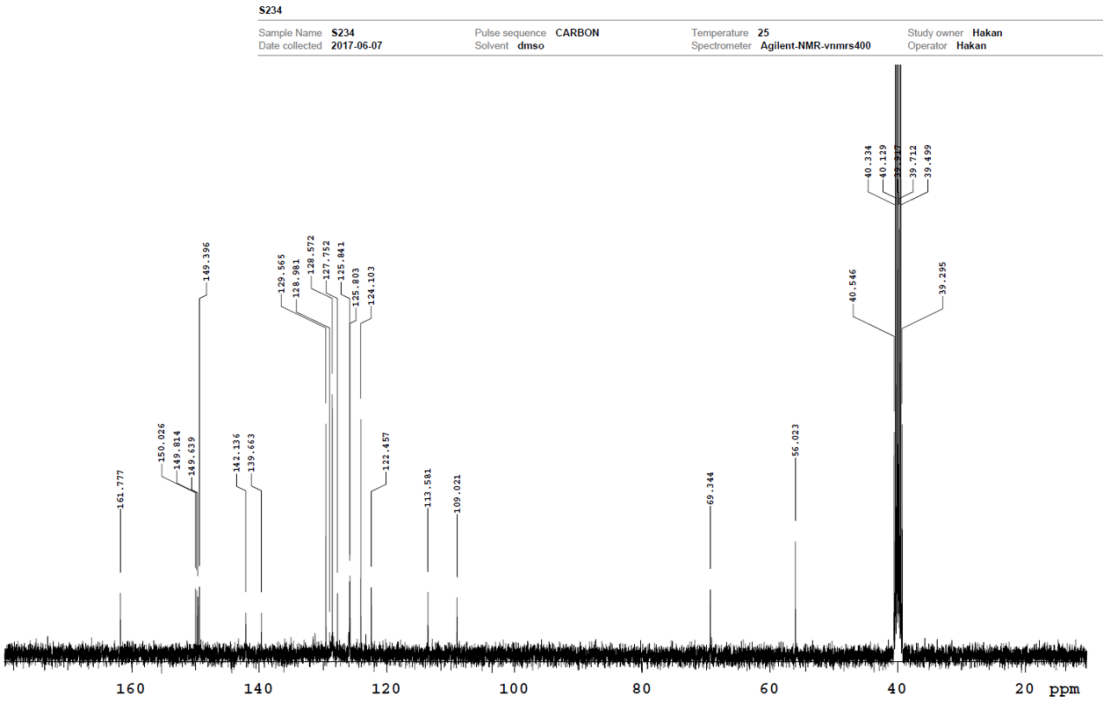

Figure S21. ^{13}C-NMR spectra of (E)-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)-4-nitrobenzohydrazide (2e)

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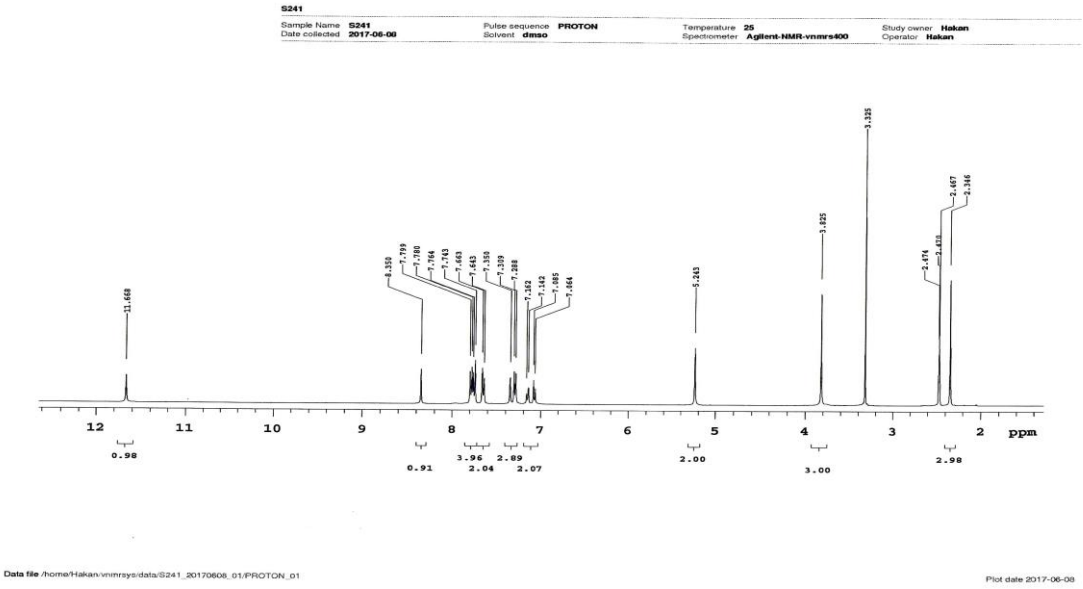

Figure S22. [1]H-NMR spectra of (E)-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)-4-methylbenzohydrazide (2f)

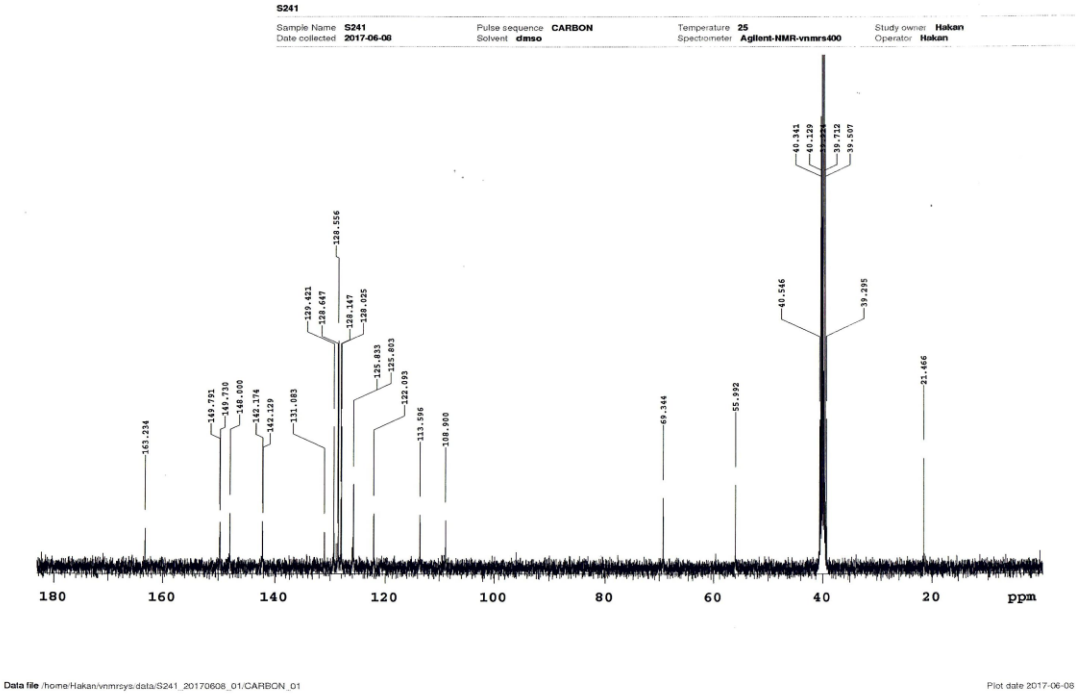

Figure S23. [13]C-NMR spectra of (E)-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)-4-methylbenzohydrazide (2f)

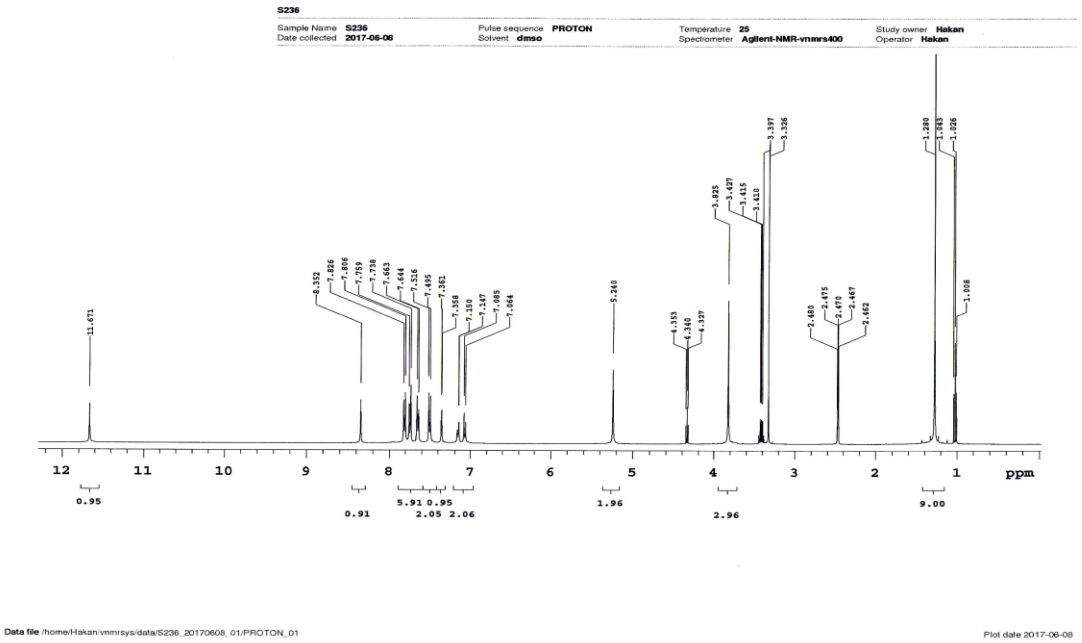

Figure S24. [1]H-NMR spectra of (E)-4-(tert-butyl)-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2g)

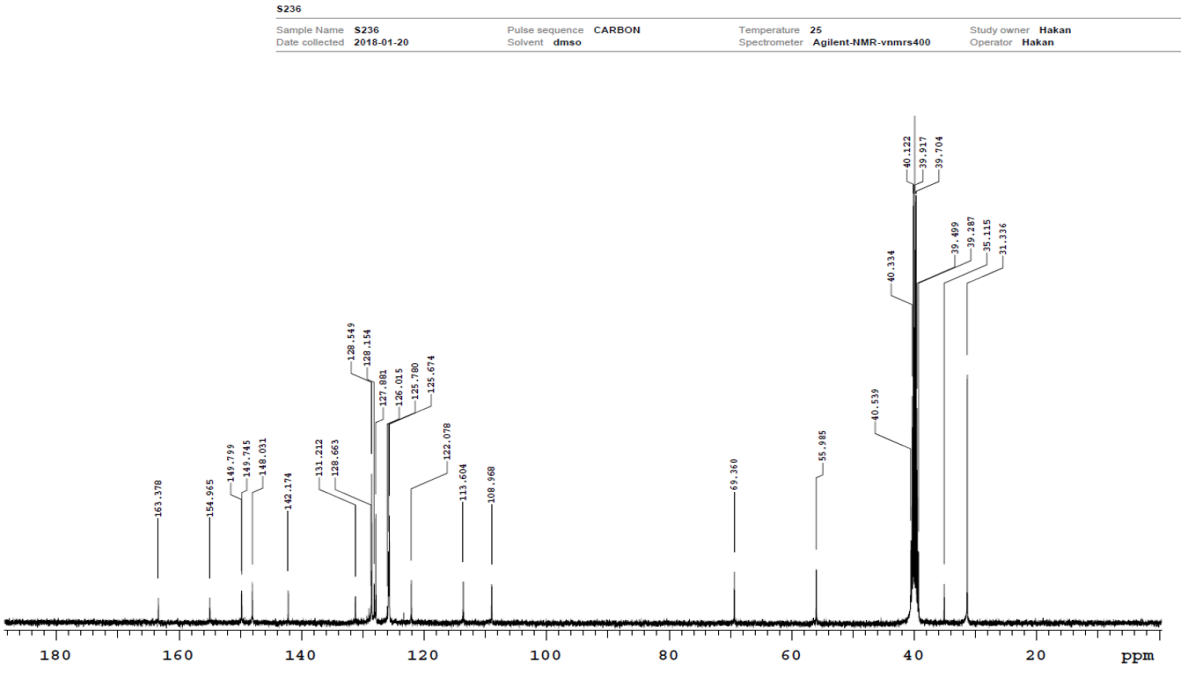

Figure S25. [13]C-NMR spectra of (E)-4-(tert-butyl)-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2g)

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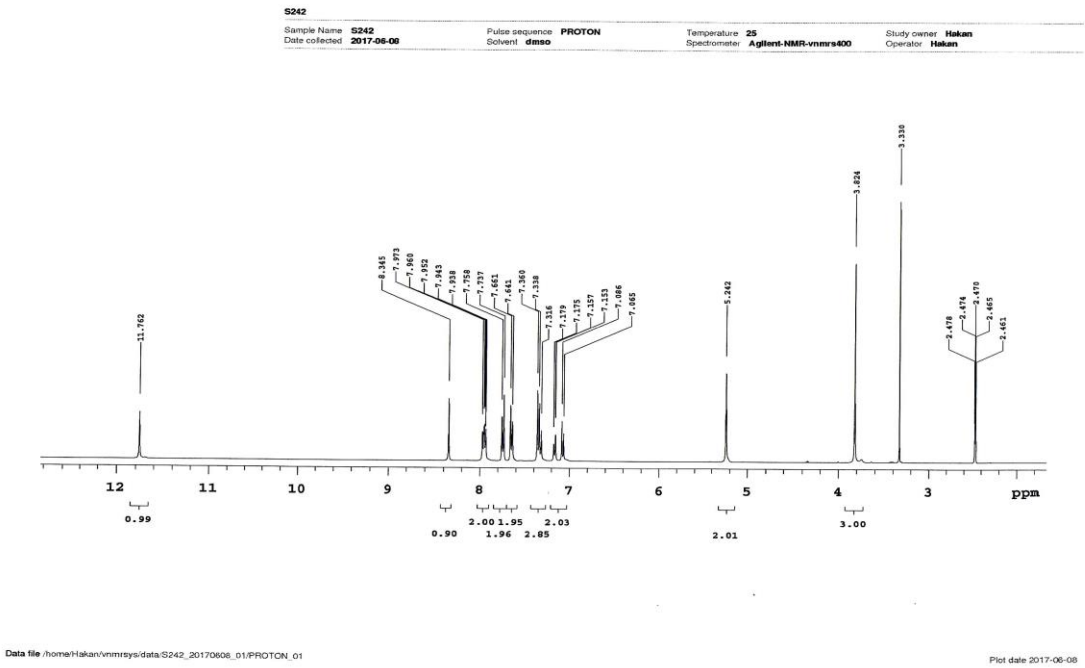

Figure S26. ^{1}H-NMR spectra of (E)-4-fluoro-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2h)

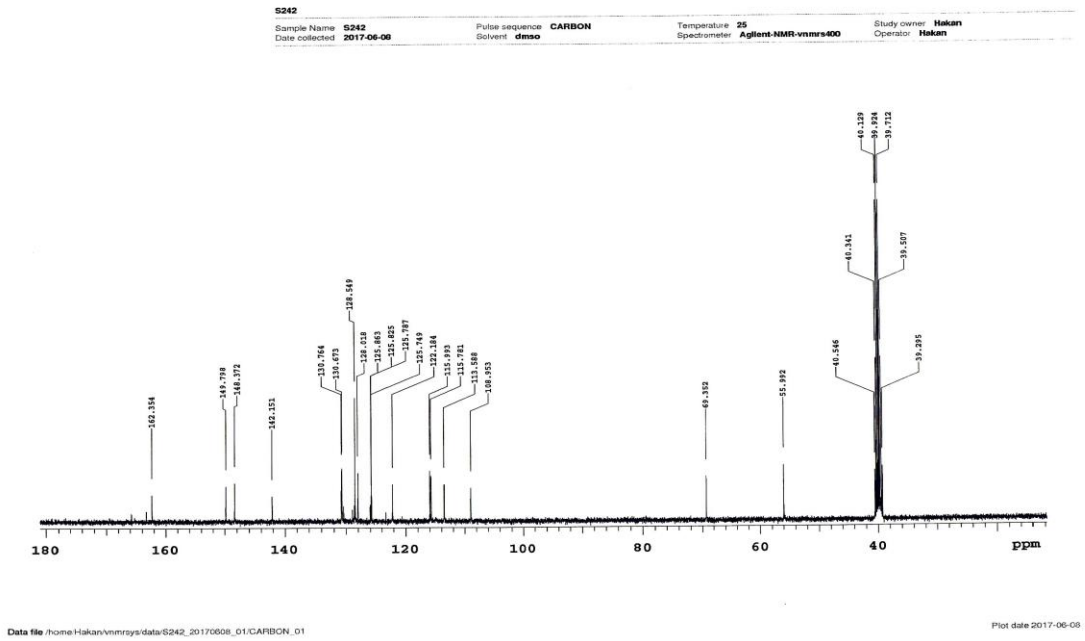

Figure S27. ^{13}C-NMR spectra of (E)-4-fluoro-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2h)

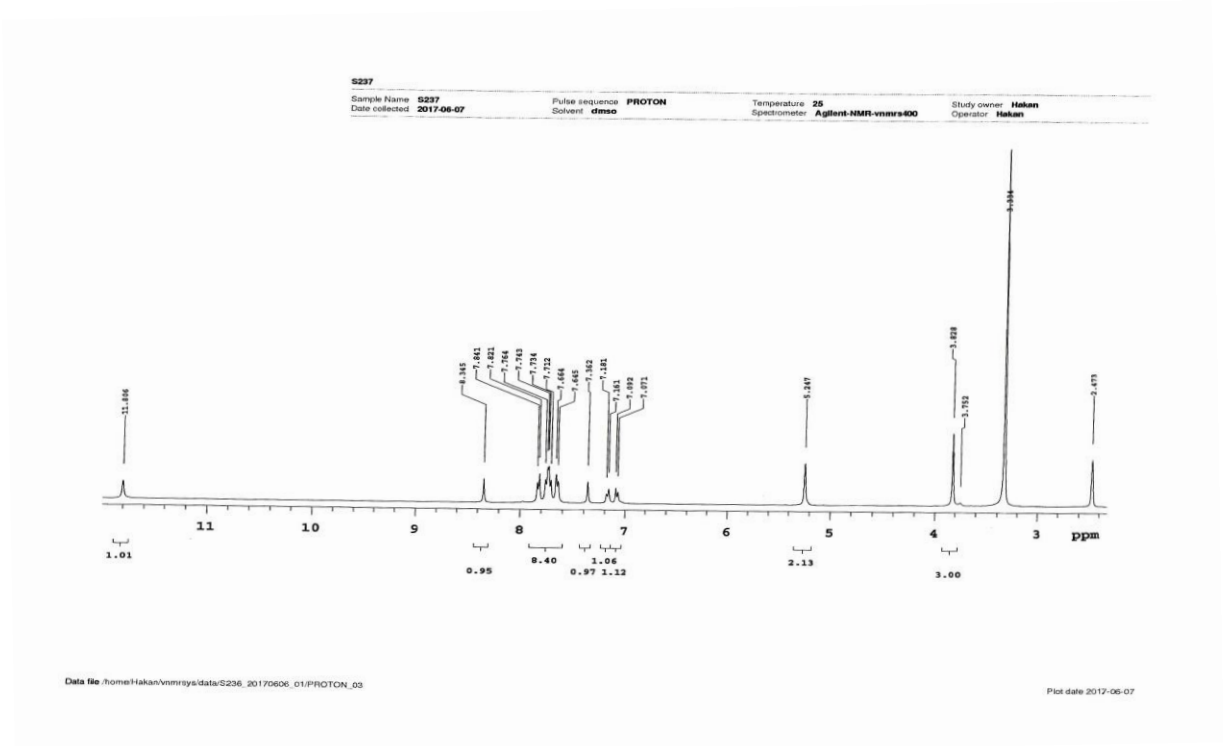

Figure S28. ^{1}H-NMR spectra of (E)-4-bromo-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2i)

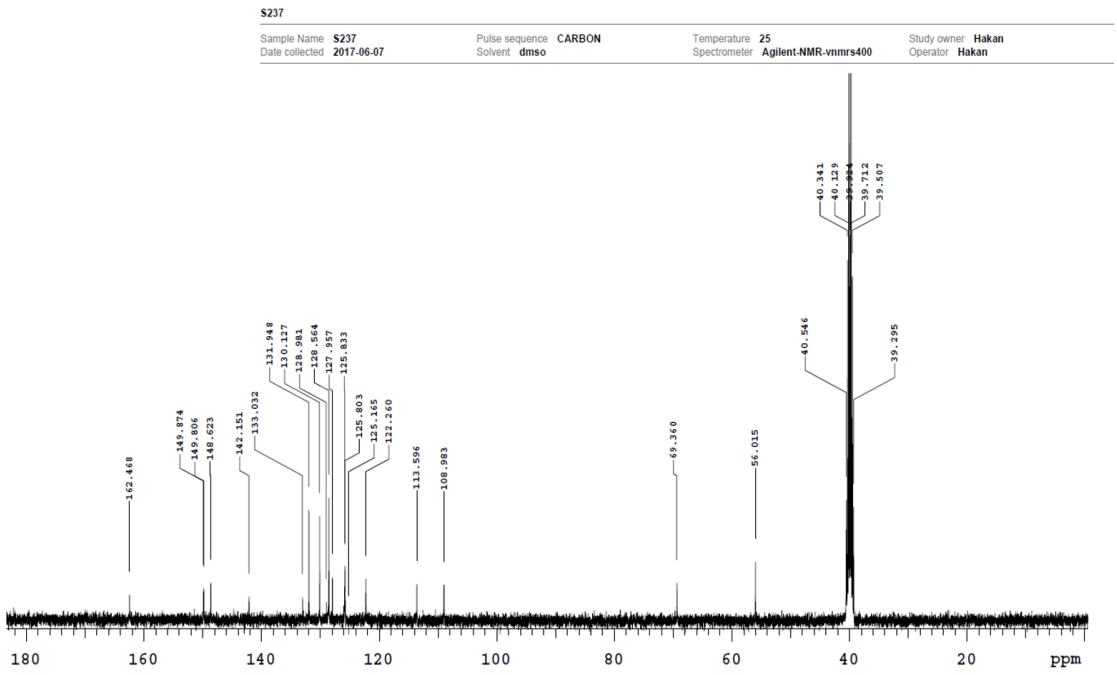

Figure S29. ^{13}C-NMR spectra of (E)-4-bromo-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2i)

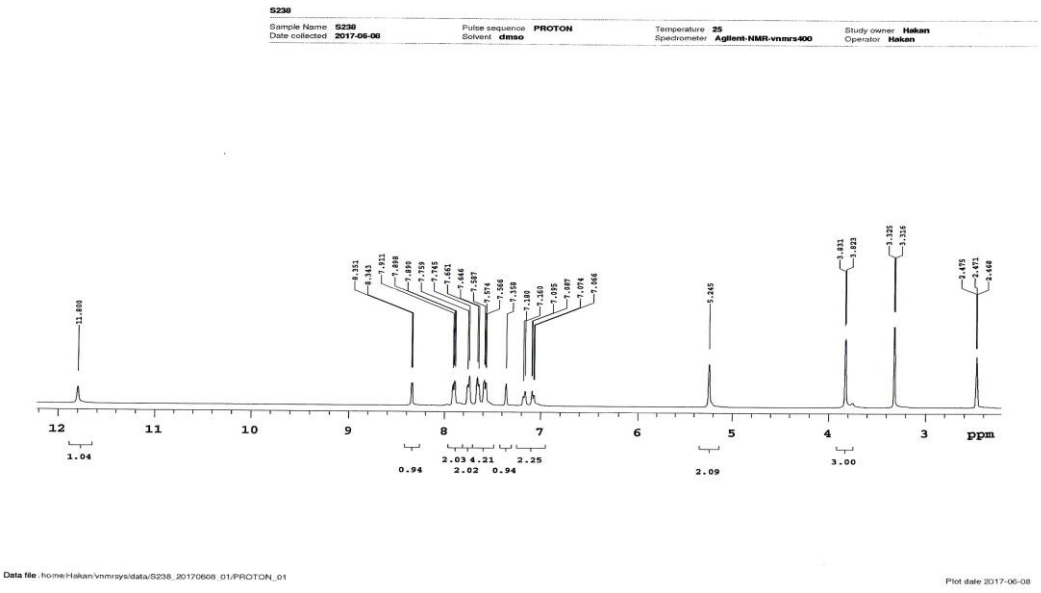

Figure S30. ^{1}H-NMR spectra of (E)-4-chloro-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2j)

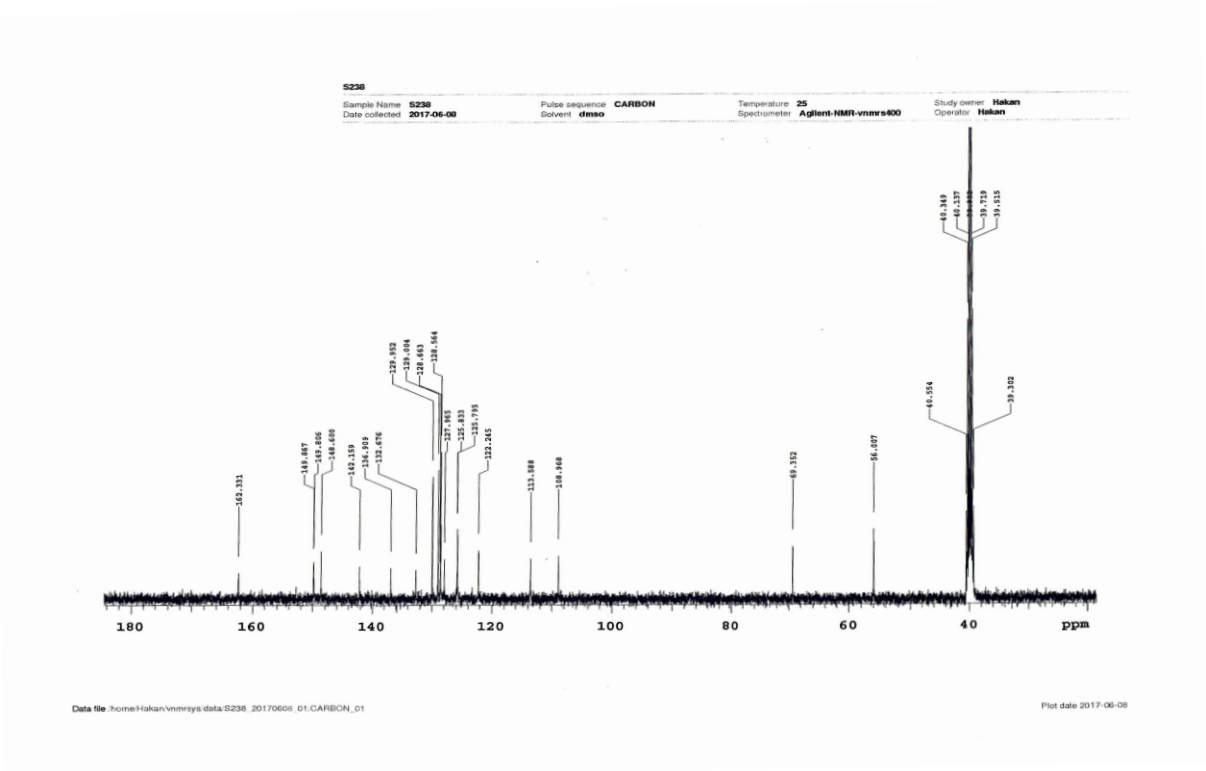

Figure S31. ^{13}C-NMR spectra of (E)-4-chloro-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2j)

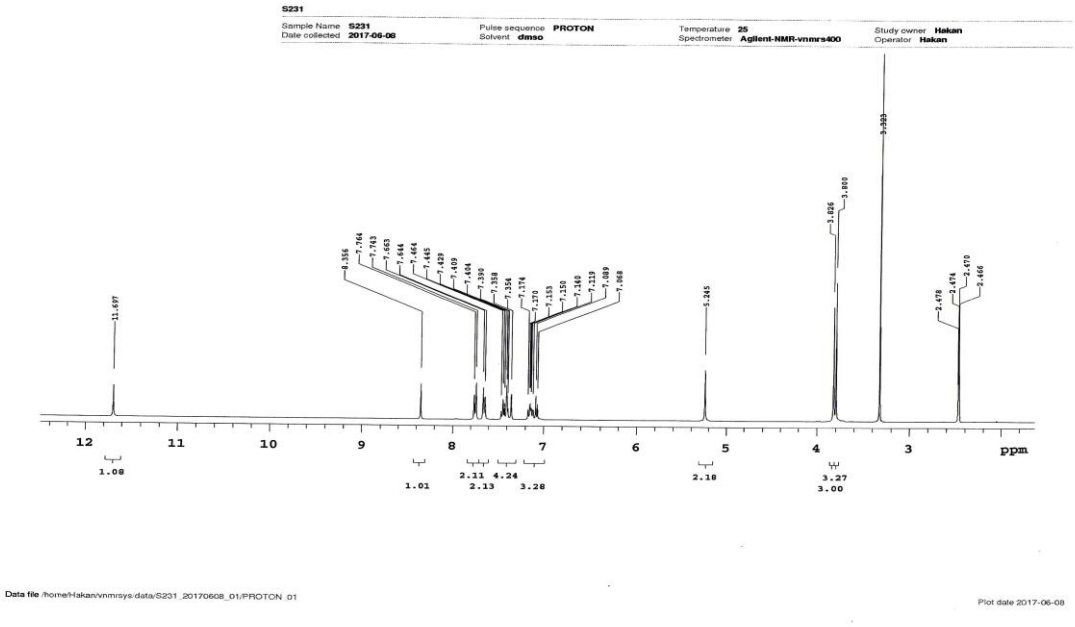

Figure S32. [1]H-NMR spectra of (E)-3-methoxy-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2k)

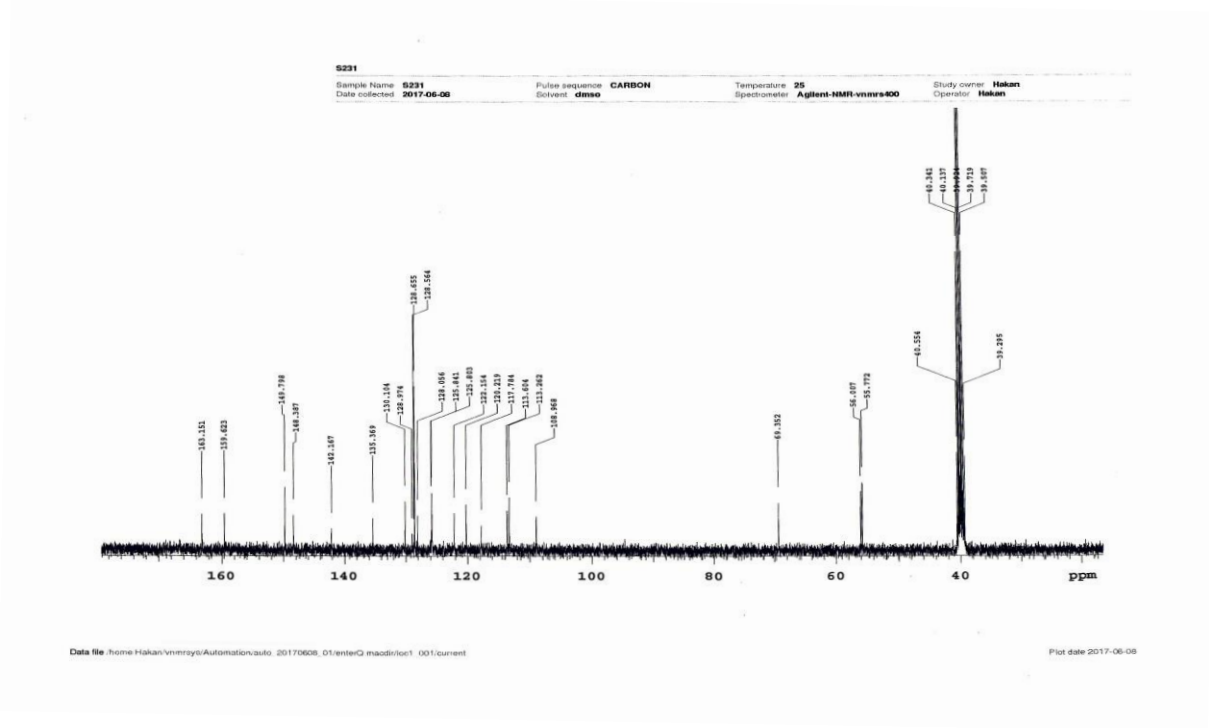

Figure S33. [13]C-NMR spectra of (E)-3-methoxy-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2k)

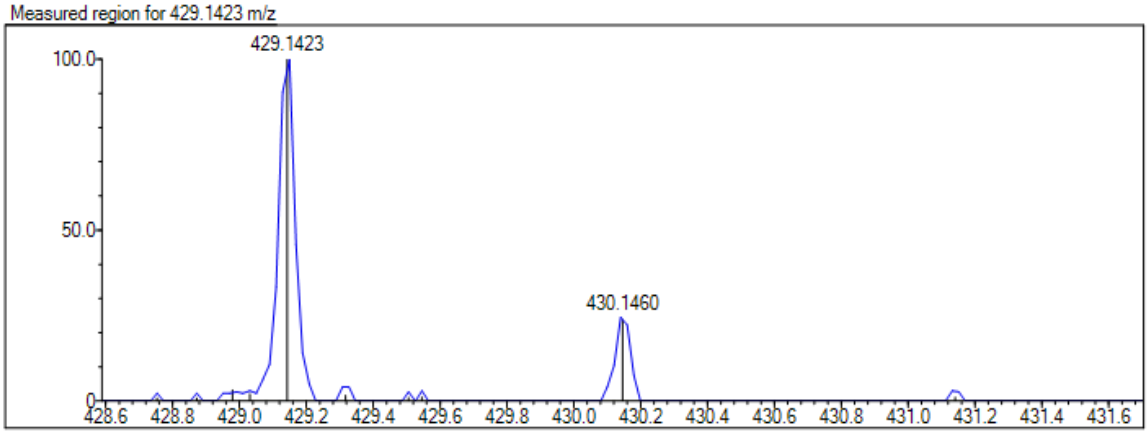

Figure S34. HR-Mass spectra of (E)-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2a)

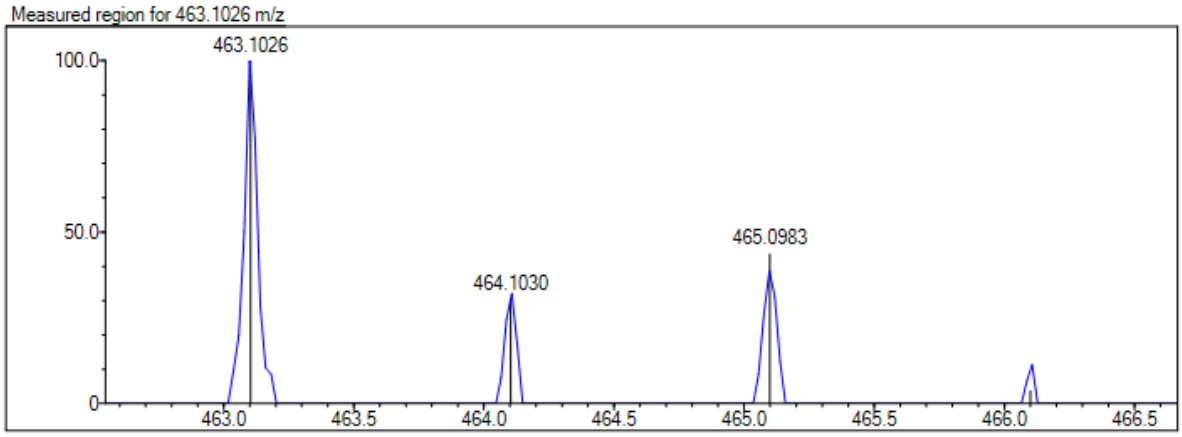

Figure S35. HR-Mass spectra of (E)-2-chloro-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2b)

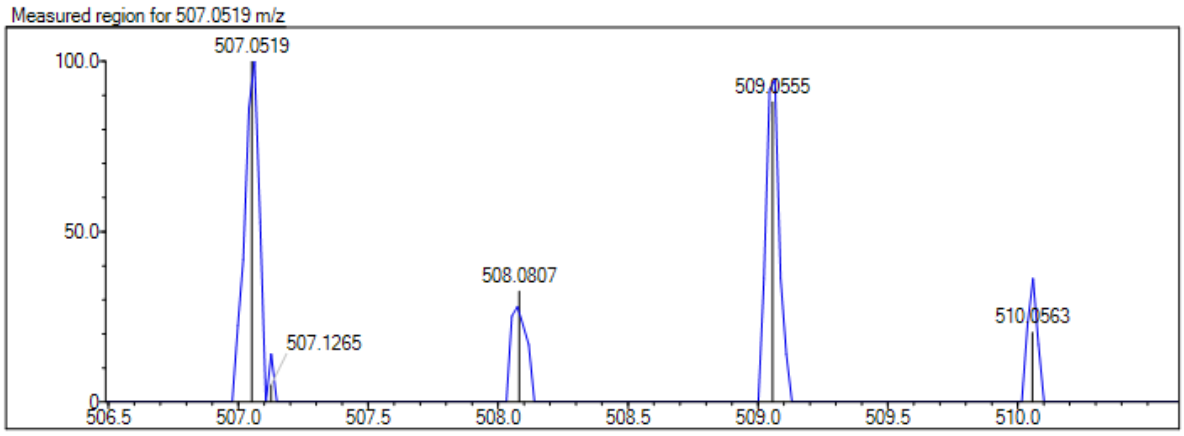

Figure S36. HR-Mass spectra of (E)-3-bromo-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2c)

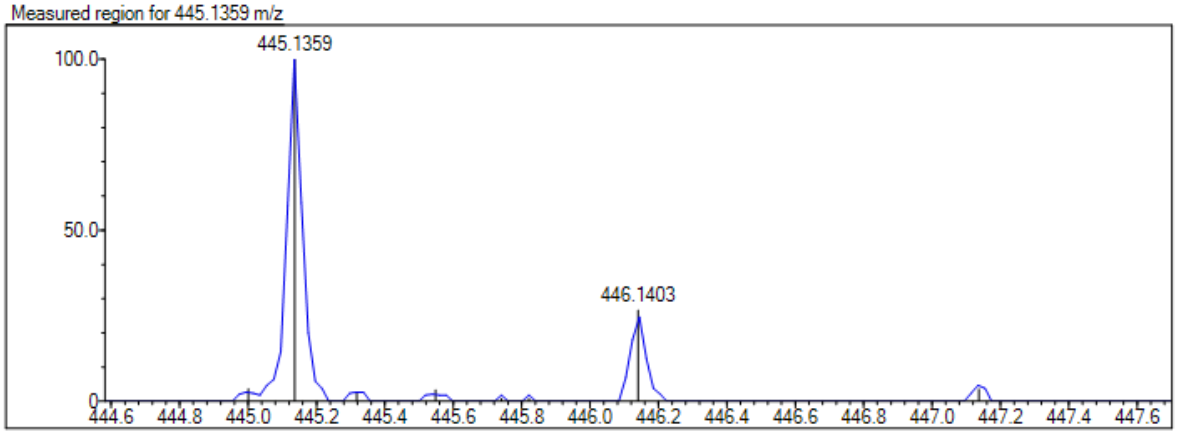

Figure S37. HR-Mass spectra of (E)-3-hydroxy-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2d)

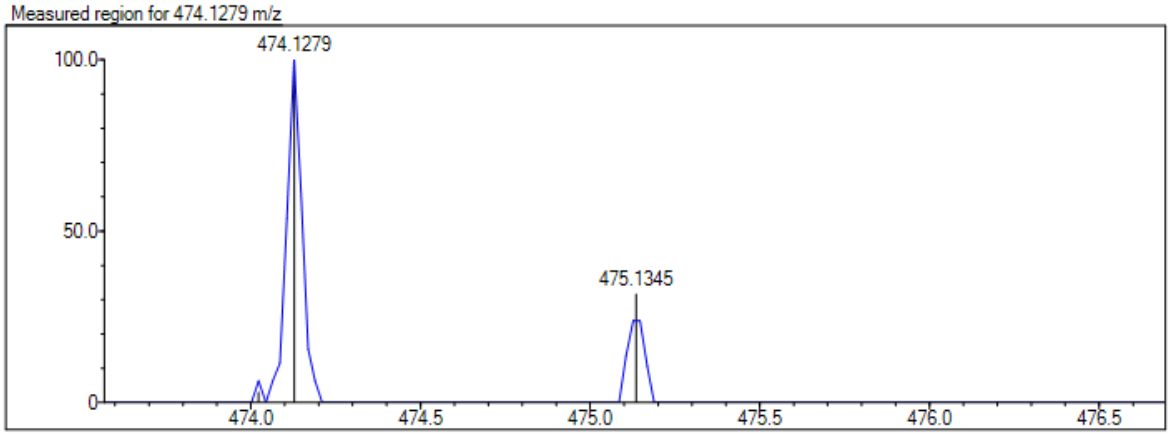

Figure S38. HR-Mass spectra of (E)-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)-4-nitrobenzohydrazide (2e)

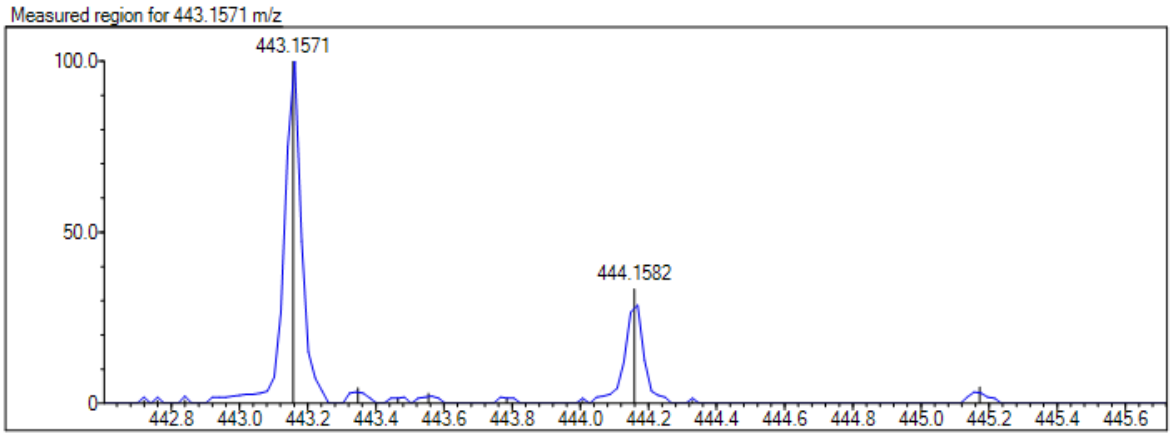

Figure S39. HR-Mass spectra of (E)-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)-4-methylbenzohydrazide (2f)

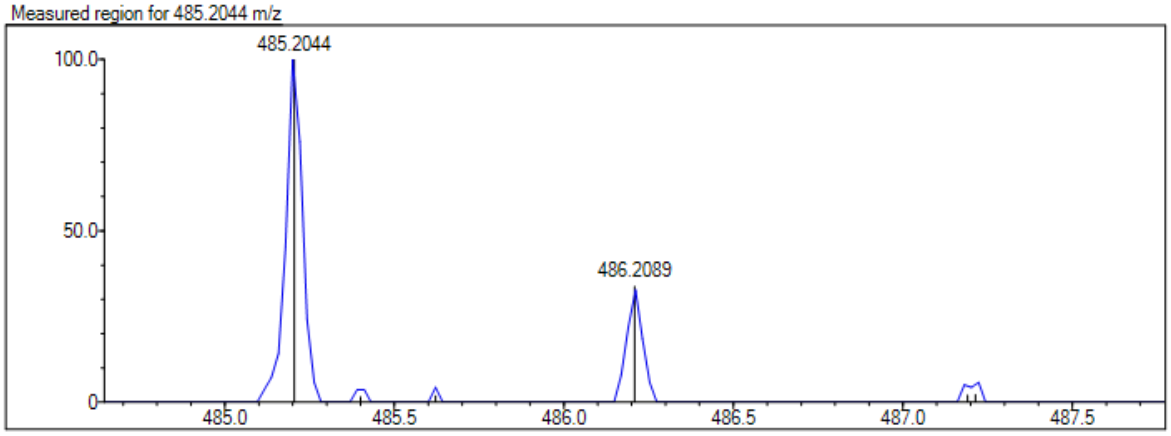

Figure S40. HR-Mass spectra of (E)-4-(tert-butyl)-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2g)

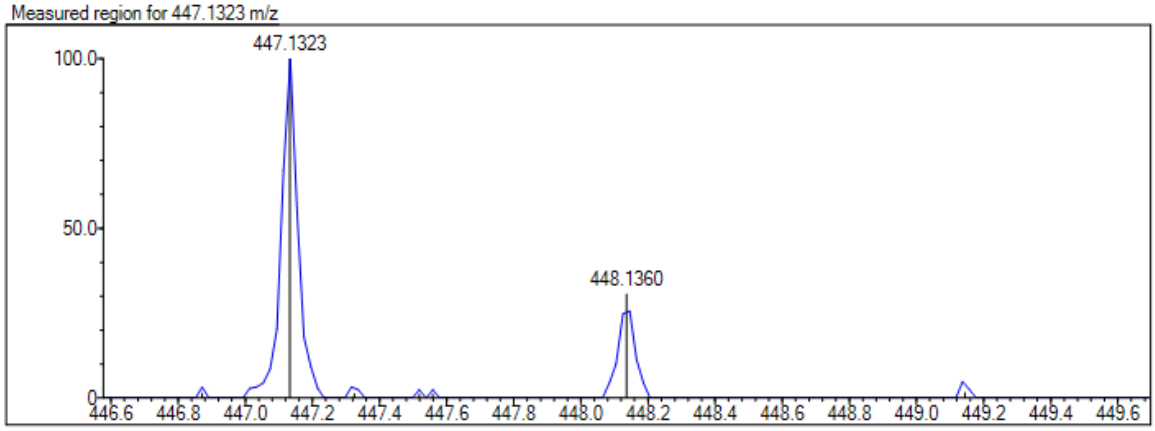

Figure S41. HR-Mass spectra of (E)-4-fluoro-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2h)

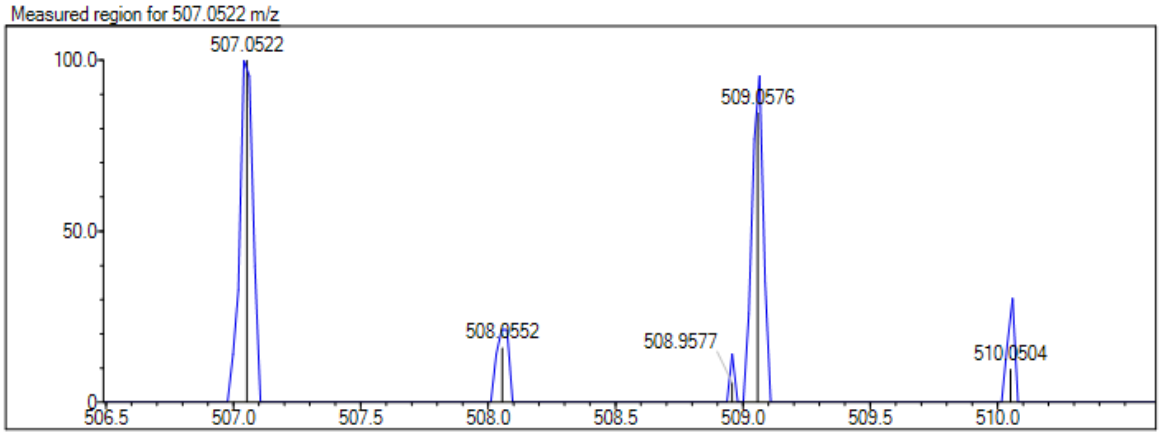

Figure S42. HR-Mass spectra of (E)-4-bromo-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2i)

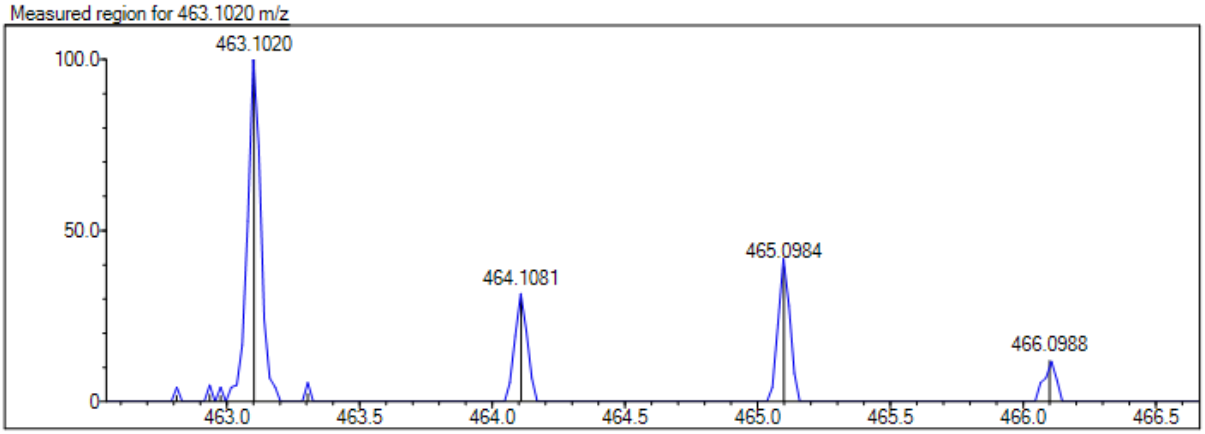

Figure S43. HR-Mass spectra of (E)-4-chloro-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2j)

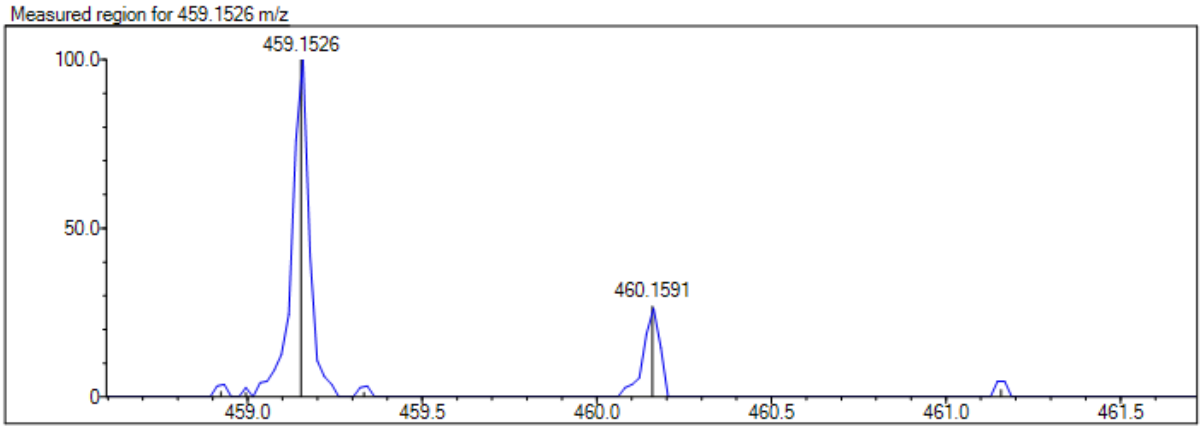

Figure S44. HR-Mass spectra of (E)-3-methoxy-N'-(3-methoxy-4-((4-(trifluoromethyl) benzyl)oxy)benzylidene)benzohydrazide (2k)