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J. Serb. Chem. Soc. 87 (10) S360–S378 (2022) *Supplementary material*

SUPPLEMENTARY MATERIAL TO

Synthesis of some benzylidene thiosemicarbazide derivatives and evaluation of their cytotoxicity on U87, MCF-7, A549, 3T3 and HUVEC cell lines

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EXPERIMENTAL DETAILS

The crude products obtained during the synthesis reactions were first recrystallized from ethanol and then their purity was checked by a TLC-Grade silica gel-G/UV 254 nm plate using EtOAc/n-hexane (1:2) as eluent. Melting points were determined on an electrothermal IA9100 melting point apparatus and were not corrected. In the case of known compounds, their melting points were compared with the reference and their IR spectra was analysed for identification purpose. The IR spectra were recorded on an FT-IR Tensor 27 infrared spectrophotometer (Bruker) using KBr as a matrix. For the novel compounds, in addition to IR spectra, ^{1}H & ^{1}3C NMR spectra were also recorded. ^{1}H NMR and ^{13}C NMR spectra were taken by an FT-NMR Bruker Avance Ultra Shield Spectrometer (300 and 75 MHz in frequencies for ^{1}H and ^{13}C, respectively) using DMSO-d_6 as solvent and as the internal standard. Chemical shifts are expressed in ppm (δ / ppm) values and coupling constants in Hz (J / Hz).

General procedures for the synthesis of compounds 1-13

To a solution of 0.1 g NaOH in 5 mL water, 1 mmol corresponding aldehyde was added under stirring. Then 1 mmol thiosemicarbazide was gradually added to the solution and under room temperature the mixture was stirred overnight. Then 5 mL ethyl alcohol was poured into the mixture, and it was refluxed for 1 h. After completion of the reaction, monitored by TLC using EtOAc/n-hexane (1:2) as eluent, the reaction mixture was filtered off to separate precipitate. Next, the precipitate was recrystallized from boiling ethanol to afford pure crystalline. The structure of novel compounds was confirmed by application of spectroscopic methods.

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*(E)-1-benzylidenethiosemicarbazide (**1**):*

Yield 75 %; m.p. 160-161 °C (literature data: 162-165 °C[1,2]); IR (KBr) ν_{max} (cm^{-1}): 3423 (NH), 3253, 3156 (NH_2), 1590 (C=N), 1299 (C=S).

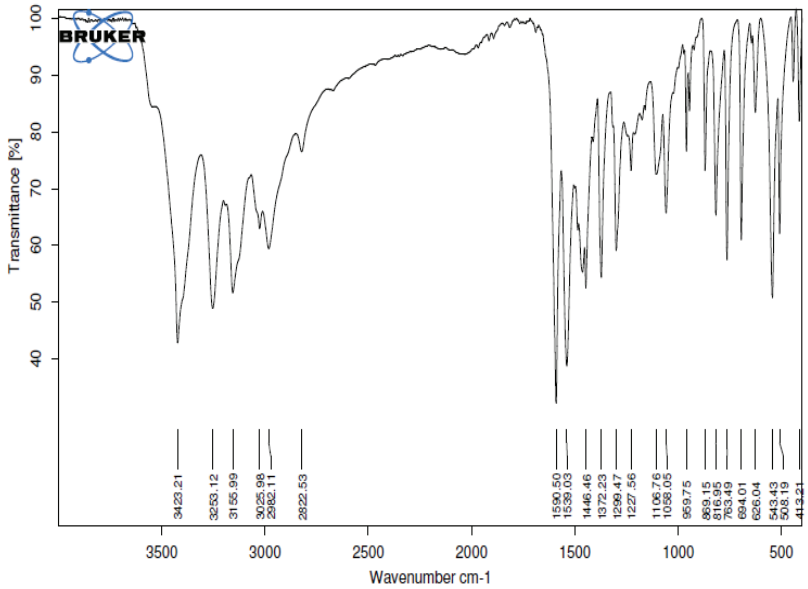

Fig S-1. IR spectrum of compound **1**

*(E)-1-(4-chlorobenzylidene)thiosemicarbazide (compound **2**)*

Yield 90 %; m.p. 222-225 °C (literature data: 217-220 °C[3]); IR (KBr) ν_{max} (cm^{-1}): 3436 (NH), 3280, 3164 (NH_2), 1600 (C=N), 1281 (C=S), 815 (C-Cl).

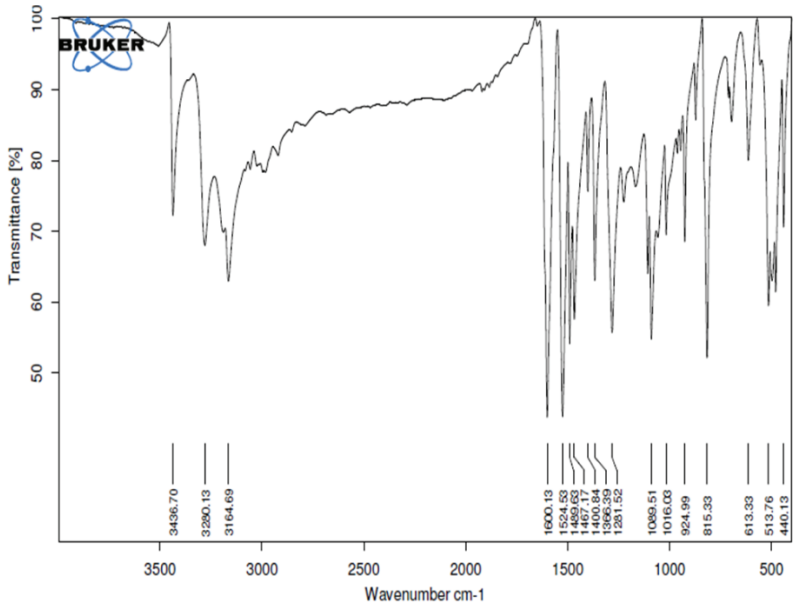

Fig. S-2. IR spectrum of compound **2**

*(E)-1-(4-(4-chlorobenzyloxy)benzylidene)thiosemicarbazide (compound **3**)*

Yield 85 %; m.p. 194-195 °C (literature data: 194 °C[4]); IR (KBr) ν_{max} (cm^{-1}): 3419 (NH), 3288, 3165 (NH_2), 1594 (C=N), 1231 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.36 (1H, s, NH), 8.15 (1H, s, NH), 8.02 (1H, s, NH), 7.95 (1H, s, CH), 7.77 (2H, d, J = 9 Hz, 2CHAr), 7.52-7.45 (4H, m, 4CHAr), 7.04 (2H, d, J = 9 Hz, 2CHAr), 5.16 (2H, s, OCH_2). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 178.1, 160.0, 142.5, 136.3, 132.9, 130.0, 129.3, 128.9, 127.5, 115.4, 68.9 (CH_2).

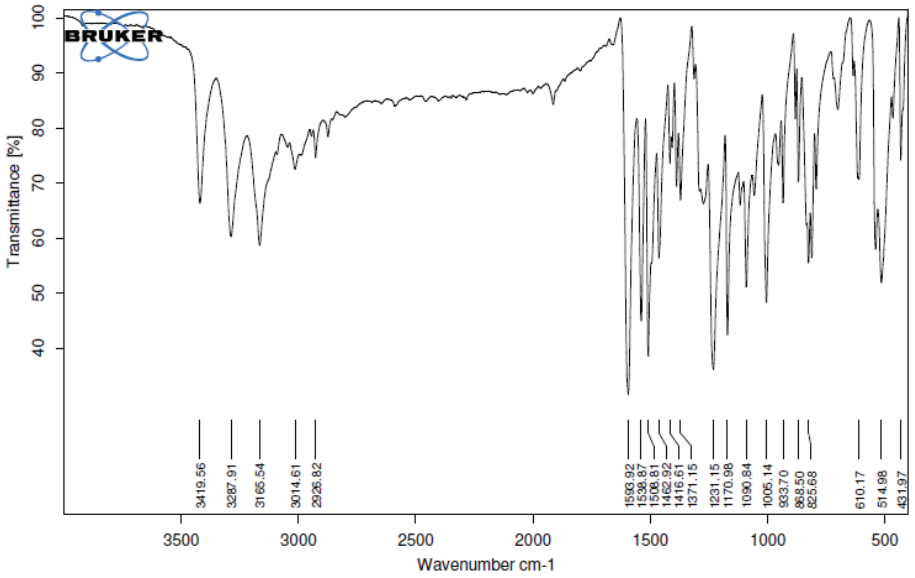

Fig. S-3. IR spectrum of compound **3**

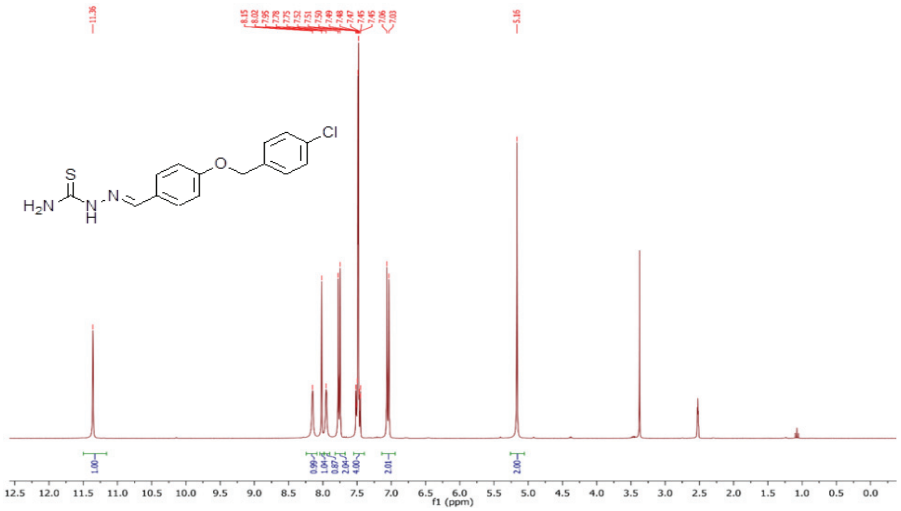

Fig S-4. ^{1}H NMR spectrum of compound **3**

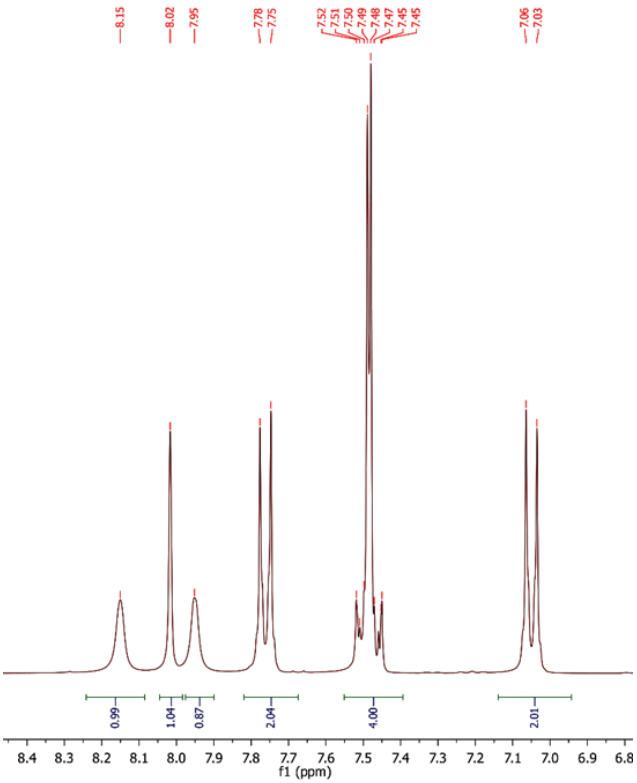

Fig S-5. ^{1}H NMR expanded spectrum of compound **3**

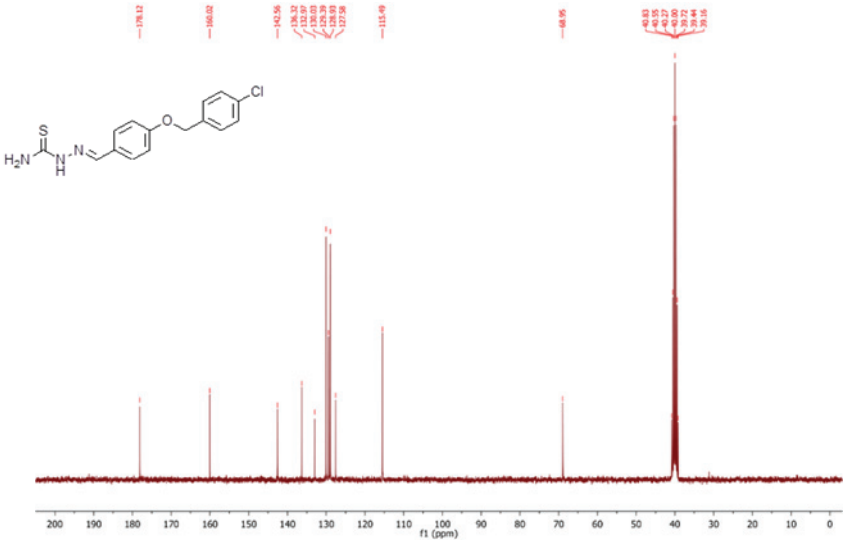

Fig S-6. ^{13}C NMR spectrum of compound **3**

*(E)-1-(3-(4-chlorobenzyloxy)benzylidene)thiosemicarbazide (compound **4**)*

Yield 85%; m.p. 165-167 °C (literature data: 160 °C[5]); IR (KBr) ν_{max} (cm^{-1}): 3429 (NH), 3252, 3155 (NH_2), 1610(C=N), 1269 (C=S). ^1H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.47 (1H, s, NH), 8.26 (1H, s, NH), 8.1 (1H, s, NH), 8.04 (1H,s, CHAr), 7.6 (1H, s, CHAr), 7.54-7.46 (4H, m, 4CHAr), 7.36-7.27 (2H, m, 2CHAr), 7.06-7.03(1H, m, CHAr), 5.17(2H, s, OCH_2). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 178.4, 158.9, 142.4, 136.5, 136.1, 132.9, 130.2, 130.1, 128.9, 121.5, 117.4, 112.2, 68.9 (CH_2).

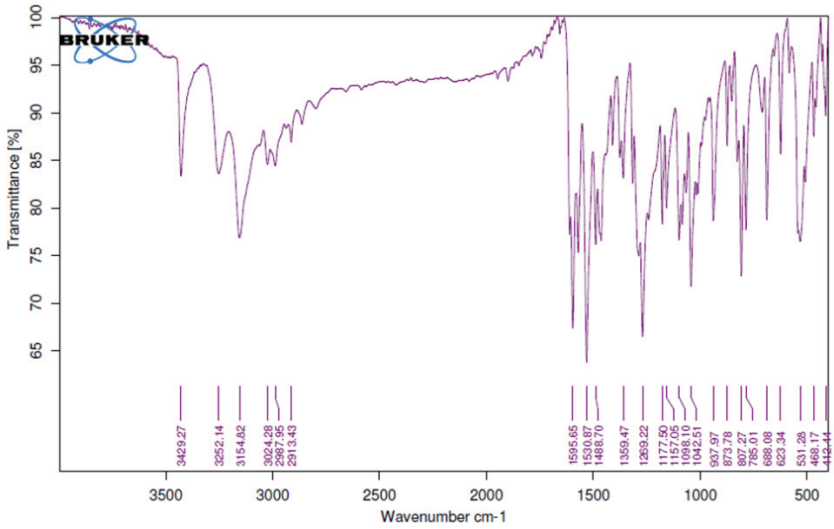

Fig S-7. IR spectrum of compound **4**

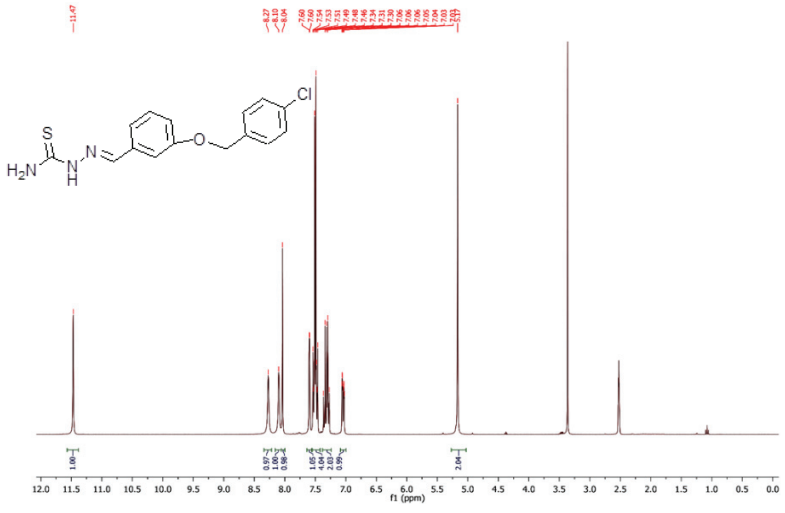

Fig S-8. ^1H NMR spectrum of compound **4**

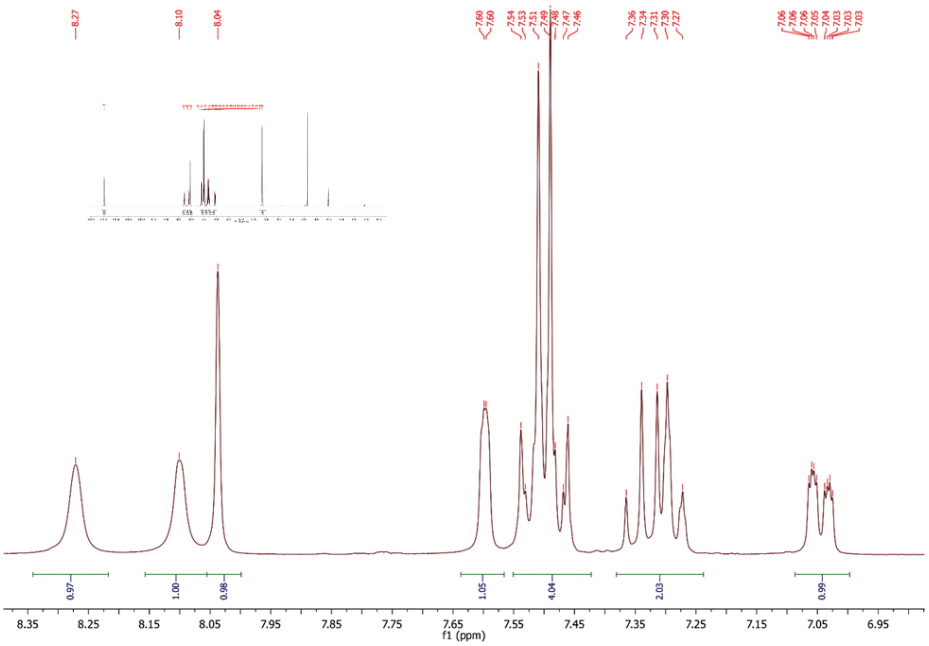

Fig S-9. ^{1}H NMR expanded spectrum of compound **4**

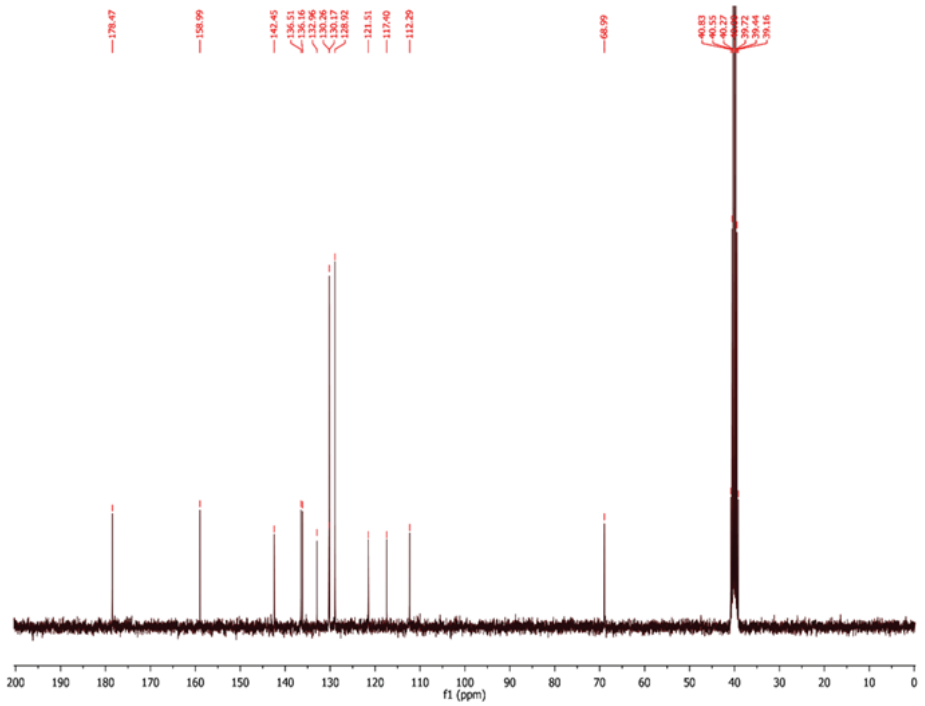

Fig S-10. ^{13}C NMR spectrum of compound **4**

*(E)-1-(4-(dimethylamino)benzylidene)thiosemicarbazide (compound **5**)*

Yield 80 %; m.p. 209-212 °C (literature data: 210-211 °C[6,7]); IR (KBr) ν_{max} (cm^{-1}): 3378 (NH), 3257, 3157 (NH_2), 1598 (C=N), 1229 (C=S).

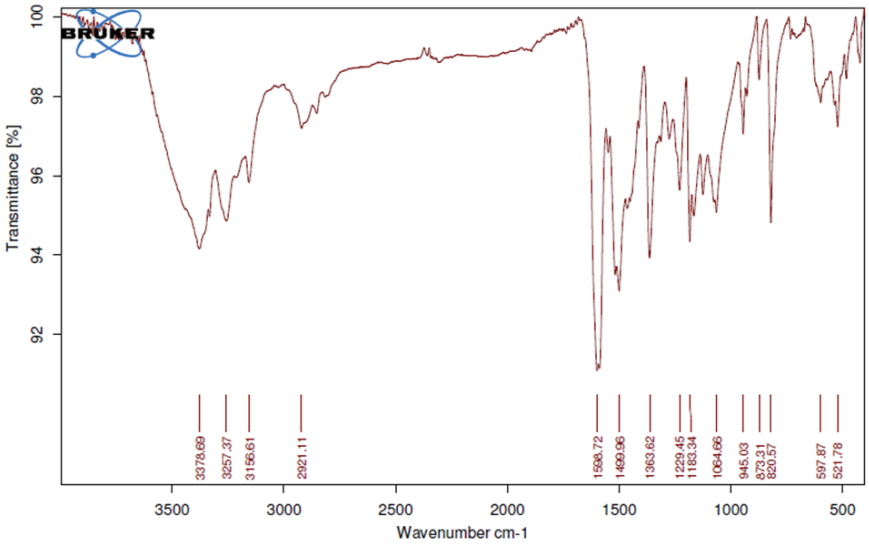

Fig S-11. IR spectrum of compound **5**

*(E)-1-(4-morpholinobenzylidene)thiosemicarbazide (compound **6**)*

Yield 80 %; m.p 210-213 °C (decomp.) (literature data: 208.5 °C with decomposition[8]); IR (KBr) ν_{max} (cm^{-1}): 3375 (NH), 3266, 3157 (NH_2), 1605 (C=N), 1230 (C=S).

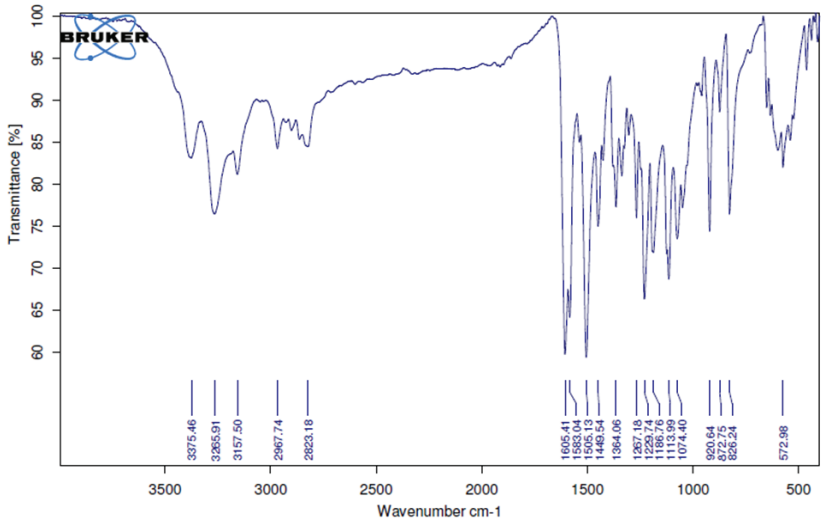

Fig S-12. IR spectrum of compound **6**

(E)-1-(4-(4-bromobenzyloxy)benzylidene)thiosemicarbazide (compound 7)

Yield 85 %; m.p. 199-201 °C (literature data: 187 ºC[4]); IR (KBr) ν_{max} (cm^{-1}): 3419 (NH), 3289, 3161 (NH_2), 1601 (C=N), 1583, 1244 (C=S). ^{1}H NMR (300 MHz, DMSO-d6) δ (ppm): 11.36 (1H, s, NH), 8.15 (H, s, NH), 8.02 (1H, s, CH), 9.95 (1H, s, NH), 7.77 (2H, d, J = 9 Hz, 2CHAr), 7.61 (2H, d, J = 9 Hz, 2CHAr), 7.44 (2H, d, J = 9 Hz, 2CHAr), 7.05 (2H, d, J = 9 Hz, 2CHAr), 5.15 (2H, s, OCH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 178.5, 158.9, 142.5, 136.9, 136.2, 131.8, 130.5, 130.3, 121.9, 117.4, 112.3, 69.0 (CH_2).

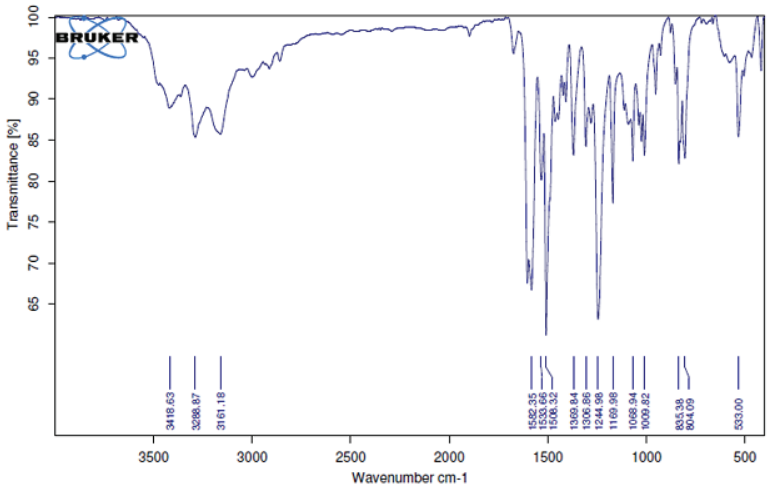

Fig S-13. IR spectrum of compound **7**

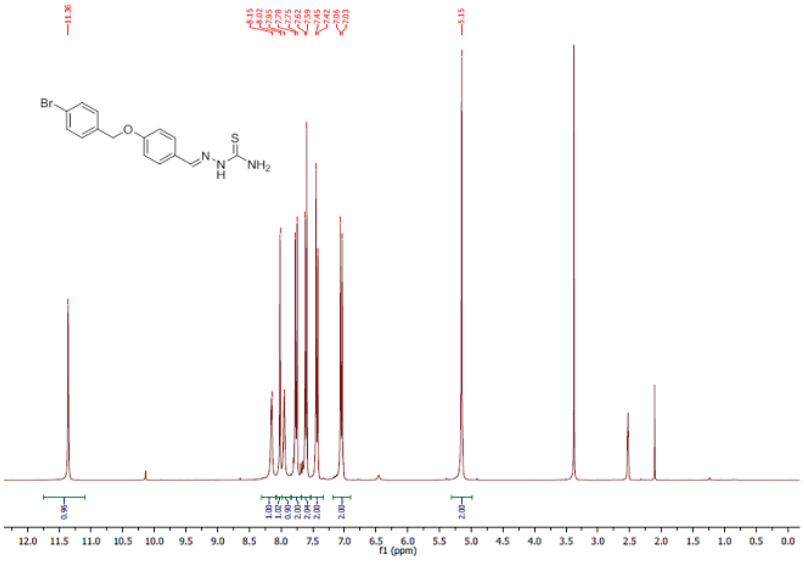

Fig S-14. ^{1}H NMR spectrum of compound **7**

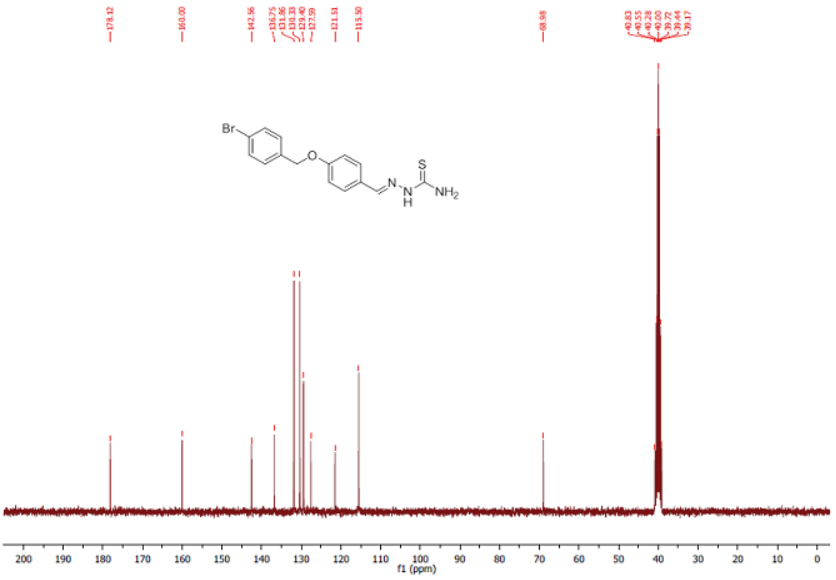

Fig S-15. ^{13}C NMR spectrum of compound **7**

*(E)-1-(3-(4-bromobenzyloxy)benzylidene)thiosemicarbazide (compound **8**)*

Yield 85%; m.p. 171-173.5 °C (literature data: 165 °C[5]); IR (KBr) ν_{max} (cm^{-1}): 3393 (NH), 3242, 3160 (NH_2), 1600 (C=N), 1262 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.48 (1H, s, NH), 8.27 (H, s, NH), 8.10 (1H, s, NH), 8.04 (1H, s, CH), 7.61 (3H, d, J = 6 Hz, 3CHAr), 7.46 (2H, d, J = 9 Hz, 2CHAr), 7.35 (2H, d, J = 6 Hz, 2CHAr), 7.32 (2H, m, 2CHAr), 7.04 (1H, m, CHAr), 5.15 (2H, s, OCH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 178.1, 160.0, 142.6, 136.8, 131.9, 130.3, 129.4, 127.9, 121.5, 115.9, 68.9 (CH_2).

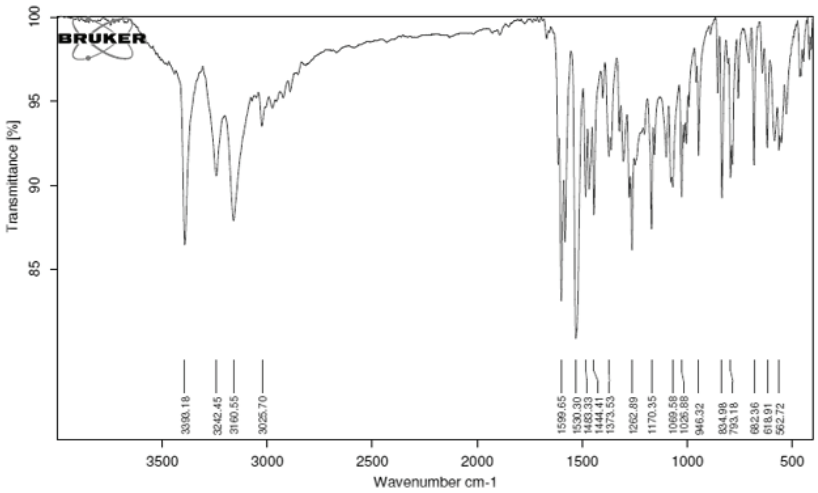

Fig S-16. IR spectrum of compound **8**

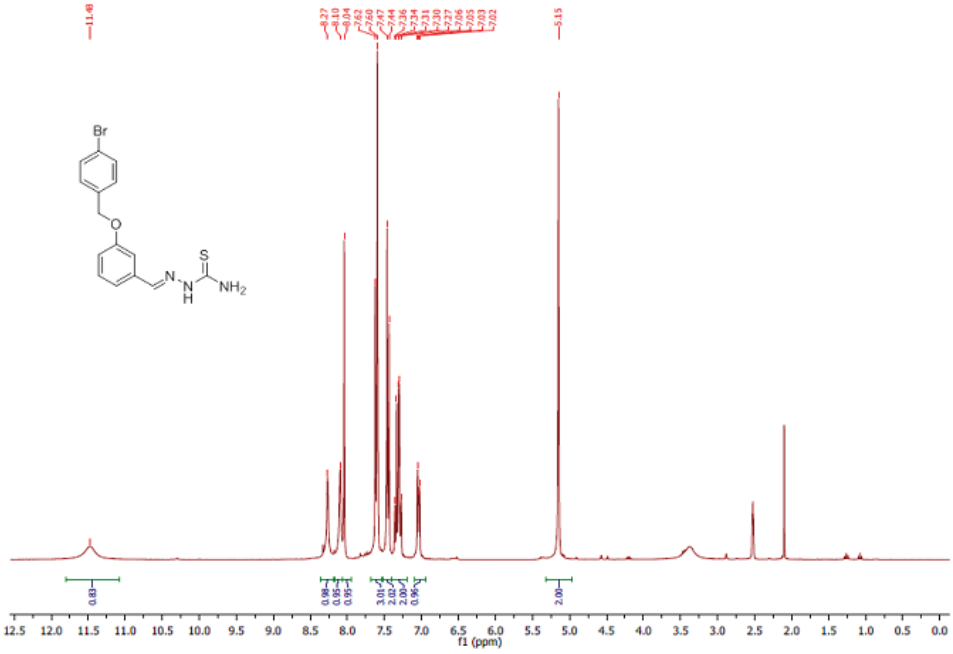

Fig S-17. ^{1}H NMR spectrum of compound **8**

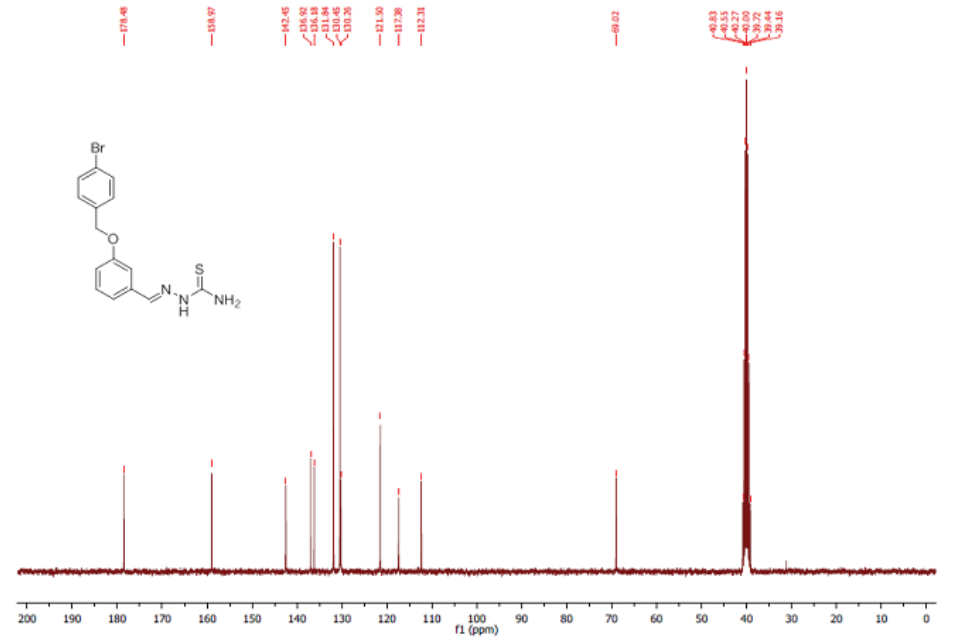

Fig S-18. ^{13}C NMR spectrum of compound **8**

*(E)-1-(4-(2-morpholinoethoxy)benzylidene)thiosemicarbazide (compound **9**)*

Yield 80 %; m.p. 216-217.4 °C (literature data: 216-217 °C[9]); IR (KBr) ν_{max} (cm^{-1}): 3438 (NH), 3204, 3113 (NH_2), 1599 (C=N), 1243 (C=S).

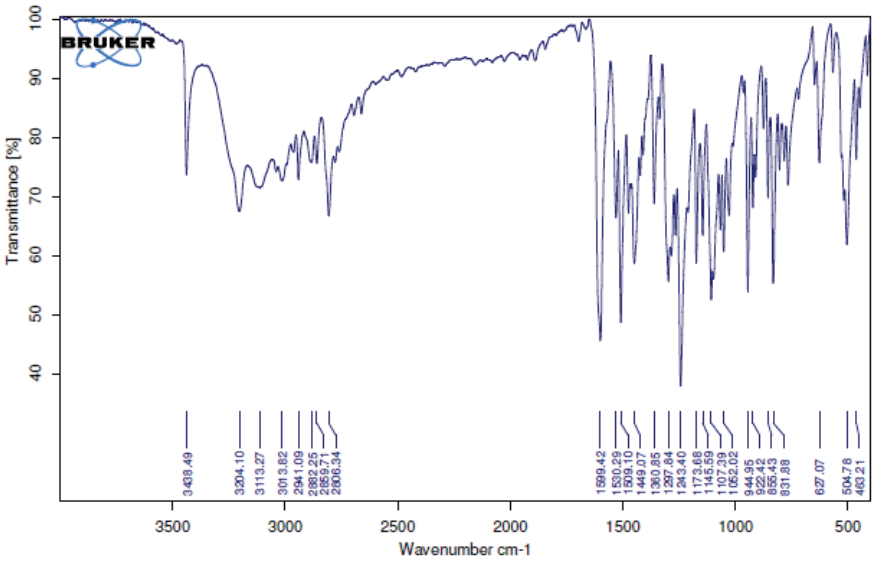

Fig S-19. IR spectrum of compound **9**

*(E)-1-(4-(3-chlorobenzyloxy)-3-methoxybenzylidene)thiosemicarbazide (compound **10**)*

Yield 85 %; m.p. 174-176 °C; IR (KBr) ν_{max} (cm^{-1}): 3421 (NH), 3308, 3144 (NH_2), 1599 (C=N), 1268 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.35 (1H, s, NH), 8.19 (H, s, NH), 8.05 (1H, s, NH), 7.96 (1H, s, CH), 7.55 (1H, d, J =1.8 Hz, 1CHAr), 7.51 (1H, t, J = 2.1 Hz, 1CHAr), 7.40-7.44 (3H, m, 3CHAr), 7.13 (1H, dd, J = 1.8, 8.3 Hz, 1CHAr), 7.03 (1H, d, J = 8.4 Hz, 1CHAr), 5.14 (2H, s, OCH_2), 3.84 (3H, s, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 177.6, 149.4, 149.2, 142,4, 139.5, 133.1, 130.4, 127.9, 127.5, 126.4, 122.0, 68.9 (CH_2), 55.8 (CH_3).

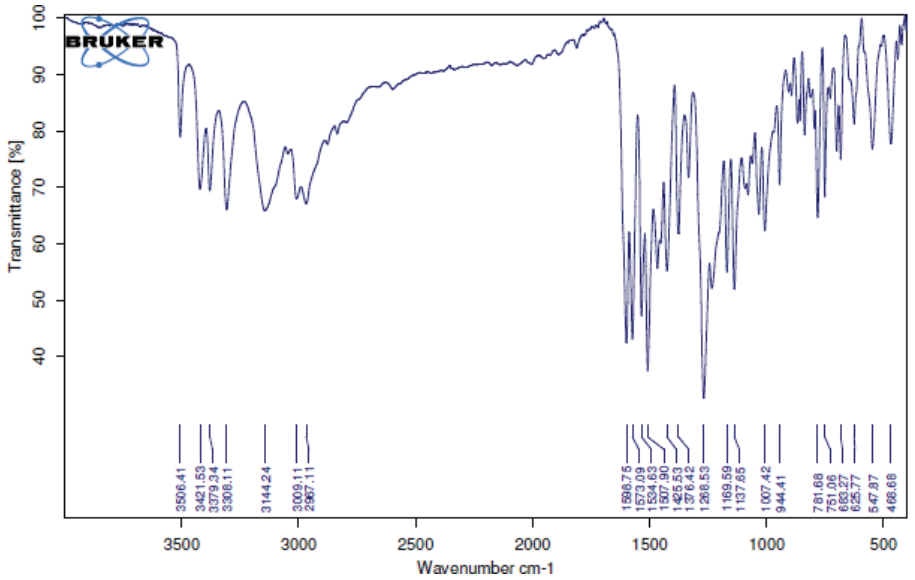

Fig S-20. IR spectrum of compound **10**

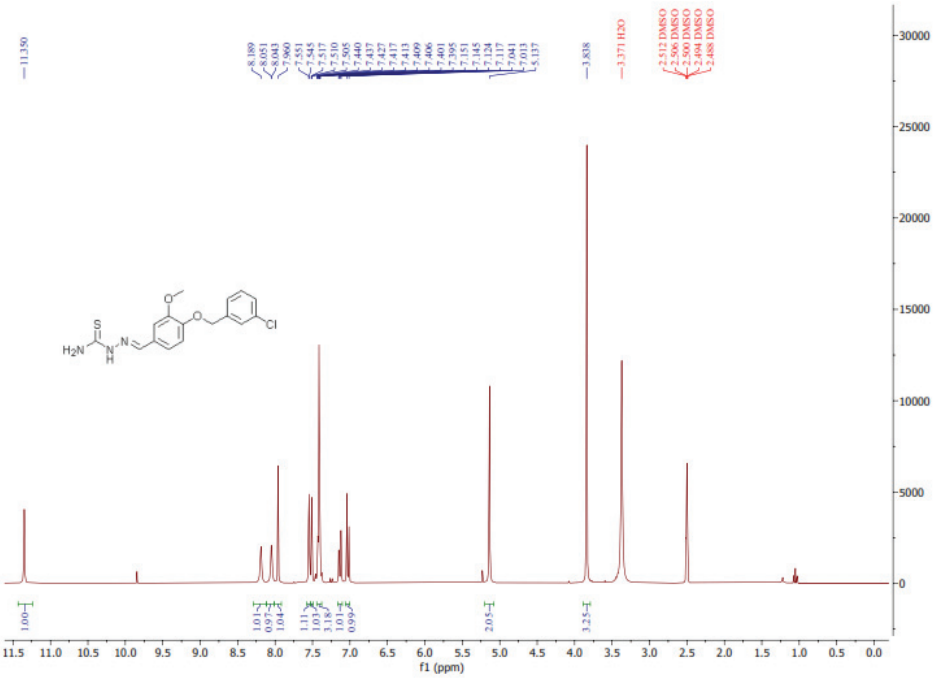

Fig S-21. ^{1}H NMR spectrum of compound **10**

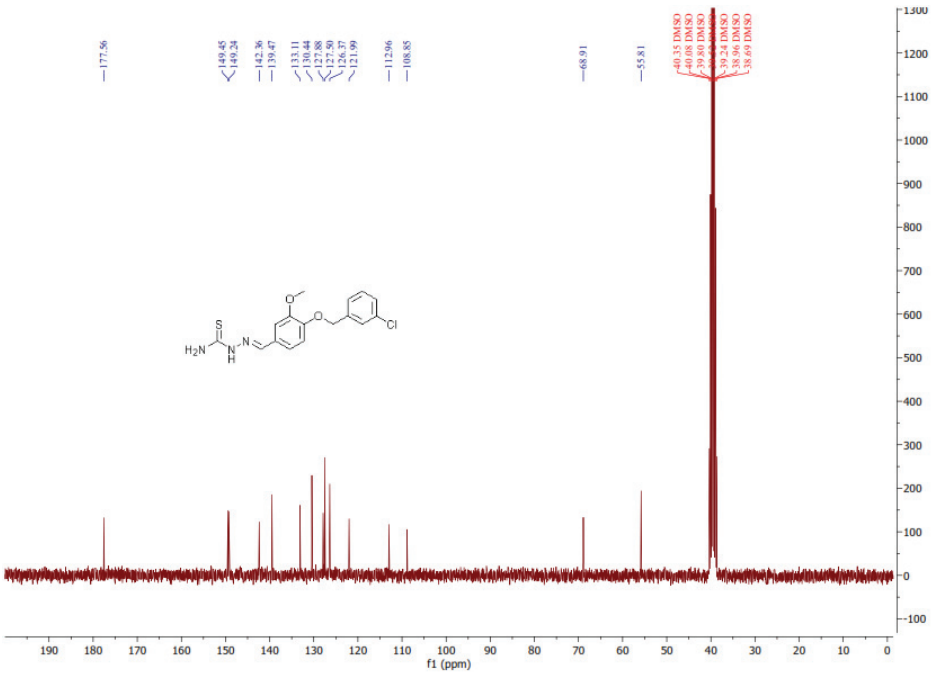

Fig S-22. ^{13}C NMR spectrum of compound **10**

*(E)-1-(4-(piperidin-1-yl)benzylidene)thiosemicarbazide (compound **11**)*

Yield 95 %; m.p. 99-101 °C (literature data: 105-106 °C[10]); IR (KBr) ν_{max} (cm^{-1}): 3371 (NH), 3266, 3160 (NH_2), 1604 (C=N), 1530, 1227 (C=S).

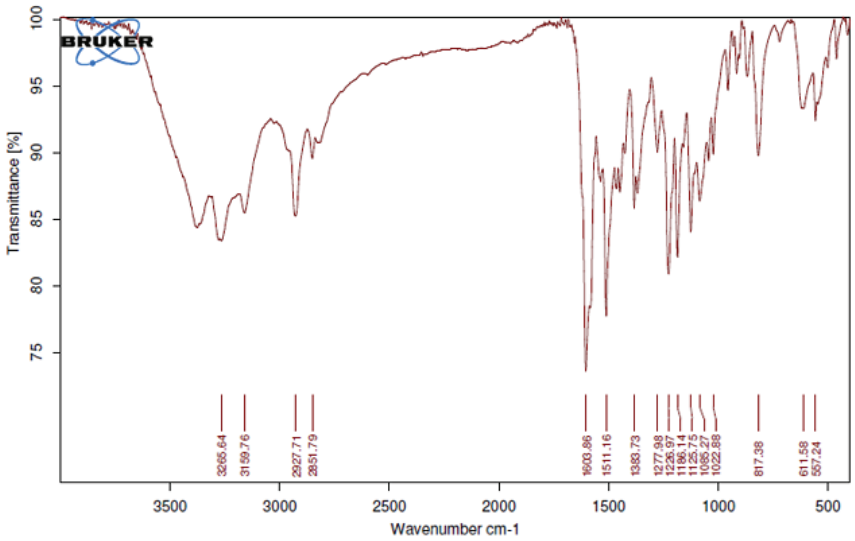

Fig S-23. IR spectrum of compound **11**

*(E)-1-(4-(2-(piperidin-1-yl)ethoxy)benzylidene)thiosemicarbazide (compound **12**)*

Yield 90 %; m.p. 166.166.6 °C; IR (KBr) ν_{max} (cm^{-1}): 3442 (NH), 3320, 3159 (NH_2), 1607 (C=N), 1247 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.33 (1H, s, NH), 8.12 (1H, s, NH), 8.01 (H, s, CH), 7.93 (H, s, NH), 7.74 (2H, d, J = 9 Hz, 2CHAr), 6.98 (2H, d, J = 9 Hz, 2CHAr), 4.11 (2H, t, J = 6 Hz, OCH_2), 2.66 (2H, t, J = 6 Hz, NCH_2), 2.43 (4H, t, J = 6 Hz, CH_2NCH_2), 1.5 (4H, quint, J = 6 Hz, CH_2), 1.40 (2H, t, J = 6 Hz, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 178.1, 160.4, 142.9, 129.4, 127.2, 115.2, 66.2, 57.8, 54.9, 26.1, 24.4.

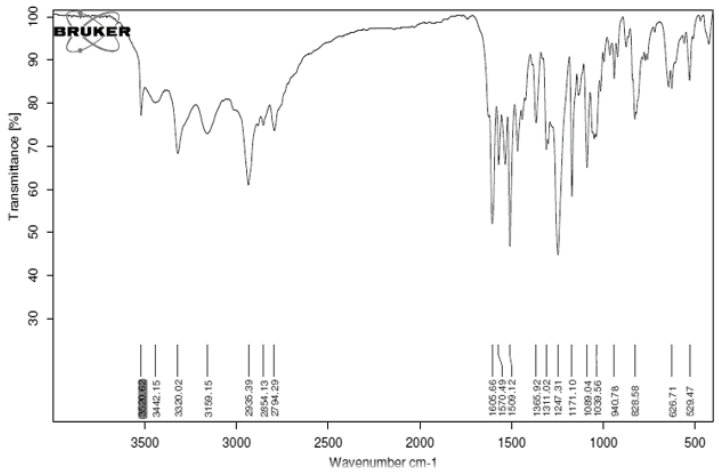

Fig S-24. IR spectrum of compound **12**

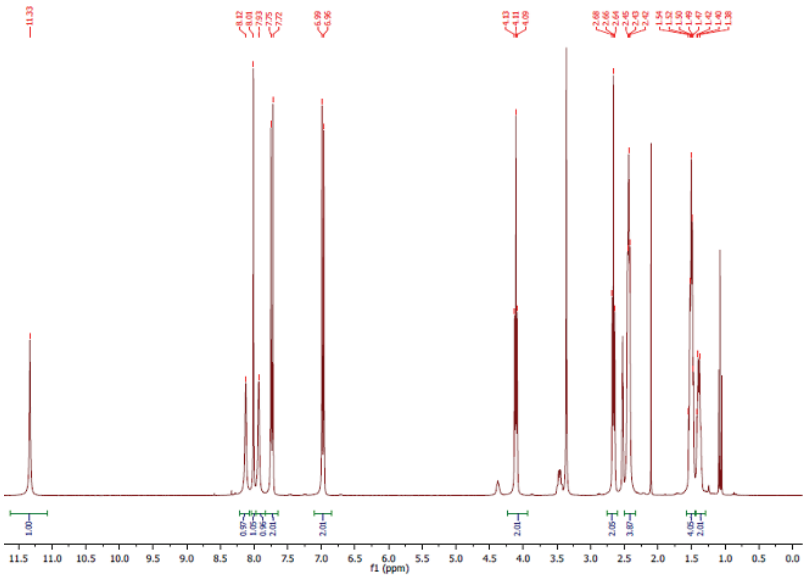

Fig S-25. ^{1}H NMR spectrum of compound **12**

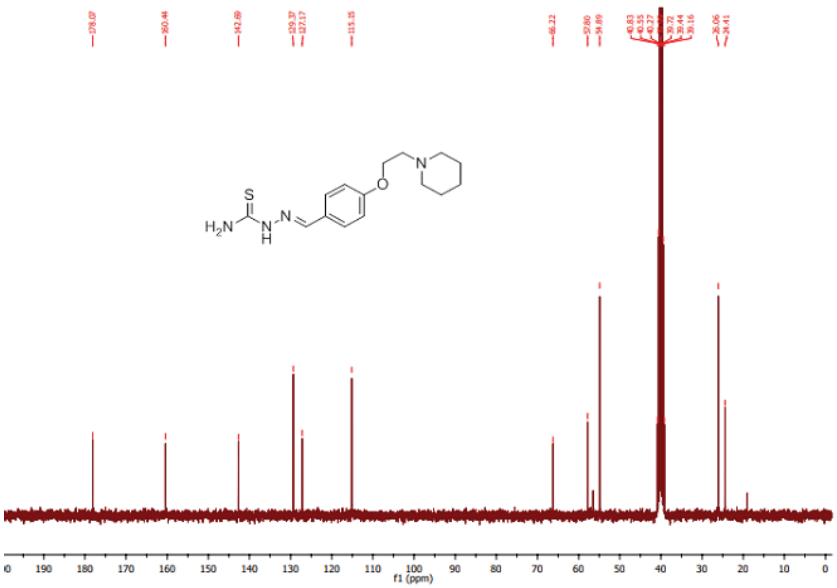

Fig S-26. ^{13}C NMR spectrum of compound **12**

*(E)-1-(4-(3-(piperidin-1-yl)propoxy)benzylidene)thiosemicarbazide (compound **13**)*

Yield 90%; m.p. 137-139 °C (decomp.); IR (KBr) ν_{max} (cm^{-1}): 3476 (NH), 3302, 3160 (NH_2), 1602 (C=N), 1253 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.33 (1H, s, NH), 8.13 (H, s, NH), 8.02 (1H, s, NH), 7.92 (1H, s, CH), 7.73 (2H, d, J = 9 Hz, 2CHAr), 6.97 (2H, d, J = 9 Hz, 2CHAr), 4.04 (2H, t, J = 6 Hz, OCH_2), 2.39 (2H, t, J = 6 Hz, CH_2), 2.34 (2H, t, CH_2), 1.87 (2H, quint, J = 6 Hz, CH_2), 1.52-1.39 (6H, m, $3CH_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 178.0, 160.5, 142.7, 129.3, 127.1, 115.0, 66.5, 55.5, 54.5, 26.6, 26.0, 24.6.

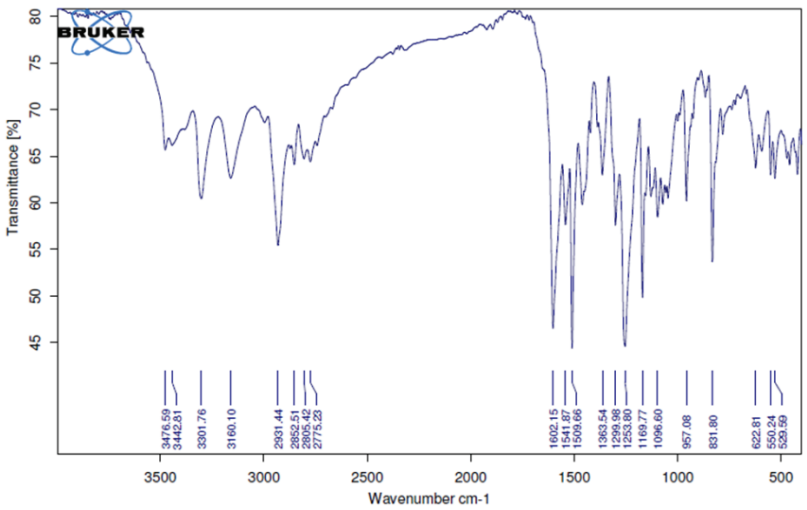

Fig S-27. IR spectrum of compound **13**

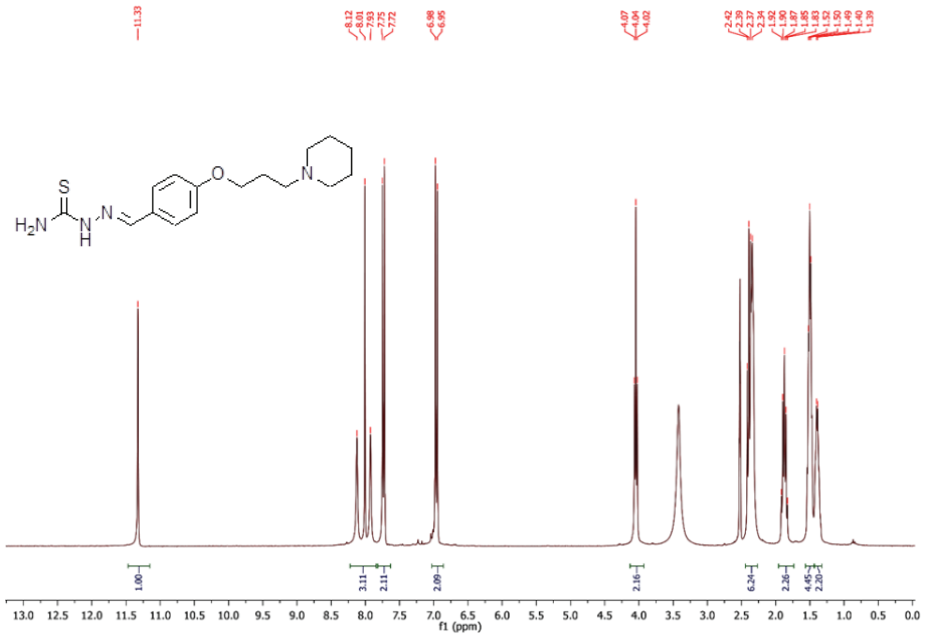

Fig S-28. ^{1}H NMR spectrum of compound **13**

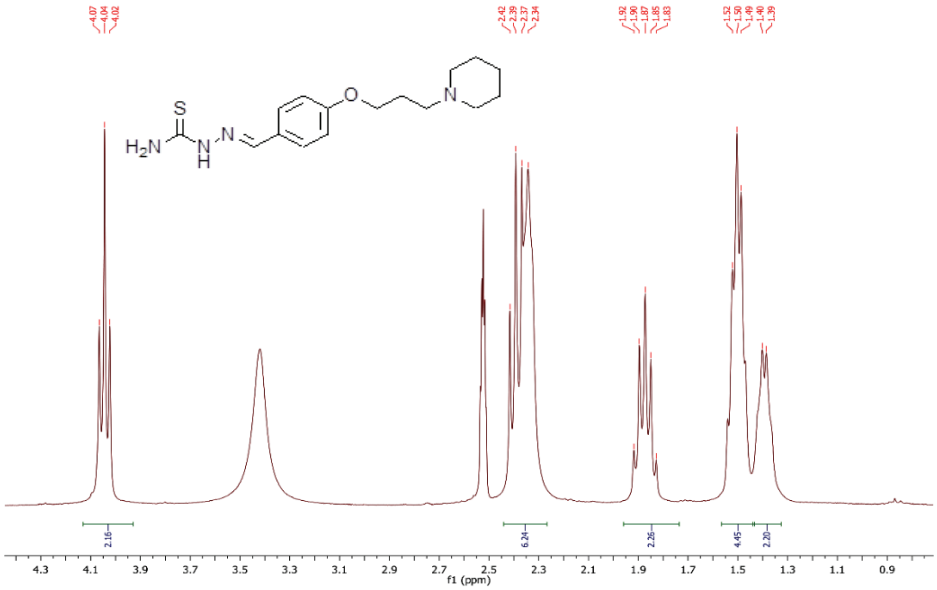

Fig S-29. ^{1}H NMR expanded spectrum of compound **13**

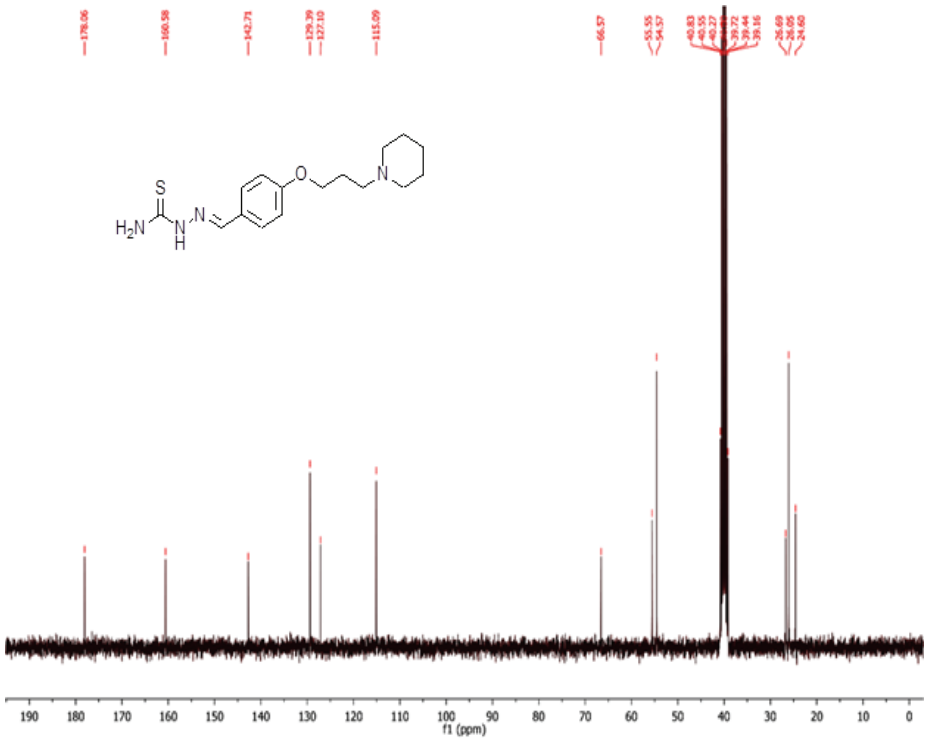

Fig S-30. ^{13}C NMR spectrum of compound **13**

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