

The effect of 1,2,4-triazole-3-thiol derivatives bearing hydrazone moiety on cancer cell migration and growth of melanoma, breast and pancreatic cancer spheroids

Aida Šermukšnytė¹, Kristina Kantminienė^{2*}, Ilona Jonuškienė¹, Ingrida Tumosienė¹, and Vilma Petrikaitė^{3*}

¹ Department of Organic Chemistry, Kaunas University of Technology, Radvilėnų pl. 19, 50254 Kaunas, Lithuania

² Department of Physical and Inorganic Chemistry, Kaunas University of Technology, Radvilėnų pl. 19, 50254 Kaunas, Lithuania

³ Laboratory of Drug Targets Histopathology, Institute of Cardiology, Lithuanian University of Health Sciences, Sukilėlių pr. 13, 50162 Kaunas, Lithuania

* Correspondence: kristina.kantminiene@ktu.lt (K.K.); vilma.petrikaite@lsmuni.lt (V.P.)

Table of contents

Figure S1. ¹H NMR (400 MHz, DMSO-d₆) spectrum of **1**

Figure S2. ¹³C NMR (101 MHz, DMSO-d₆) spectrum of **1**

Figure S3. HRMS spectrum of **1**

Figure S4. ¹H NMR (400 MHz, DMSO-d₆) spectrum of **2**

Figure S5. ¹³C NMR (101 MHz, DMSO-d₆) spectrum of **2**

Figure S6. HRMS spectrum of **2**

Figure S7. ¹H NMR (400 MHz, DMSO-d₆) spectrum of **3**

Figure S8. ¹³C NMR (101 MHz, DMSO-d₆) spectrum of **3**

Figure S9. HRMS spectrum of **3**

Figure S10. ¹H NMR (400 MHz, DMSO-d₆) spectrum of **4**

Figure S11. ¹³C NMR (101 MHz, DMSO-d₆) spectrum of **4**

Figure S12. HRMS spectrum of **4**

Figure S13. ¹H NMR (400 MHz, DMSO-d₆) spectrum of **5**

Figure S14. ¹³C NMR (101 MHz, DMSO-d₆) spectrum of **5**

Figure S15. HRMS spectrum of **5**

Figure S16. ¹H NMR (400 MHz, DMSO-d₆) spectrum of **6**

Figure S17. ¹³C NMR (400 MHz, DMSO-d₆) spectrum of **6**

Figure S18. HRMS spectrum of **6**

Figure S19. ^1H NMR (400 MHz, DMSO-d_6) spectrum of **7**

Figure S20. ^{13}C NMR (101 MHz, DMSO-d_6) spectrum of **7**

Figure S21. HRMS spectrum of **7**

Figure S22. ^1H NMR (400 MHz, DMSO-d_6) spectrum of **8**

Figure S23. ^{13}C NMR (101 MHz, DMSO-d_6) spectrum of **8**

Figure S24. HRMS spectrum of **8**

Figure S25. ^1H NMR (400 MHz, DMSO-d_6) spectrum of **9**

Figure S26. ^{13}C NMR (101 MHz, DMSO-d_6) spectrum of **9**

Figure S27. HRMS spectrum of **9**

Figure S28. ^1H NMR (400 MHz, DMSO-d_6) spectrum of **10**

Figure S29. ^{13}C NMR (101 MHz, DMSO-d_6) spectrum of **10**

Figure S30. HRMS spectrum of **10**

Figure S31. ^1H NMR (400 MHz, DMSO-d_6) spectrum of **11**

Figure S32. ^{13}C NMR (101 MHz, DMSO-d_6) spectrum of **11**

Figure S33. HRMS spectrum of **11**

Figure S34. ^1H NMR (400 MHz, DMSO-d_6) spectrum of **12**

Figure S35. ^{13}C NMR (101 MHz, DMSO-d_6) spectrum of **12**

Figure S36. HRMS spectrum of **12**

Figure S37. ^1H NMR (400 MHz, DMSO-d_6) spectrum of **13**

Figure S38. ^{13}C NMR (101 MHz, DMSO-d_6) spectrum of **13**

Figure S39. HRMS spectrum of **13**

Figure S40. ^1H NMR (400 MHz, DMSO-d_6) spectrum of **14**

Figure S41. ^{13}C NMR (101 MHz, DMSO-d_6) spectrum of **14**

Figure S42. HRMS spectrum of **14**

Figure S43. ^1H NMR (400 MHz, DMSO-d_6) spectrum of **15**

Figure S44. ^{13}C NMR (101 MHz, DMSO-d_6) spectrum of **15**

Figure S45. HRMS spectrum of **15**

Figure S46. ^1H NMR (400 MHz, DMSO-d_6) spectrum of **16**

Figure S47. ^{13}C NMR (101 MHz, DMSO-d_6) spectrum of **16**

Figure S48. HRMS spectrum of **16**

Figure S49. ^1H NMR (400 MHz, DMSO-d_6) spectrum of **17**

Figure S50. ^{13}C NMR (101 MHz, DMSO-d_6) spectrum of **17**

Figure S51. HRMS spectrum of **17**

Figure S52. ^1H NMR (400 MHz, DMSO-d_6) spectrum of **18**

Figure S53. ^{13}C NMR (101 MHz, DMSO- d_6) spectrum of **18**

Figure S54. HRMS spectrum of **18**

Figure S55. ^1H NMR (400 MHz, DMSO- d_6) spectrum of **19**

Figure S56. ^{13}C NMR (101 MHz, DMSO- d_6) spectrum of **19**

Figure S57. HRMS spectrum of **19**

Figure S58. The effect of 10 μM compound concentration on MDA-MB-231 cell line viability after 24 and 48 h.

Figure S59. The effect of 10 μM compound concentration on IGR39 cell viability after 24 h and 48 h.

Figure S60. The effect of 10 μM compound concentration on Panc-1 cell line viability after 24, 48, and 72 h.

Procedure S1. Evaluation of 10 μM compound concentration effect on cell line viability.

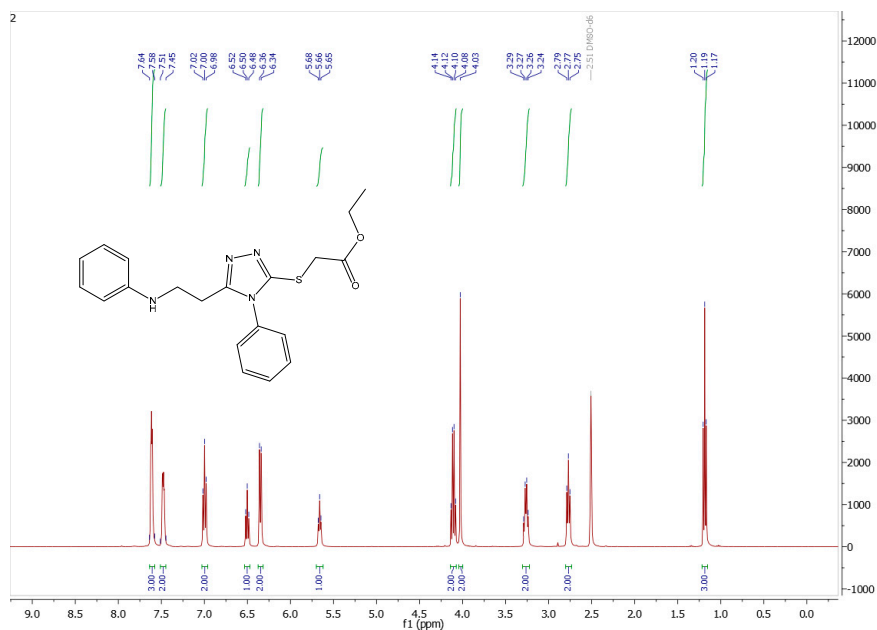


Figure S1. ¹H NMR (400 MHz, DMSO-d₆) spectrum of 2

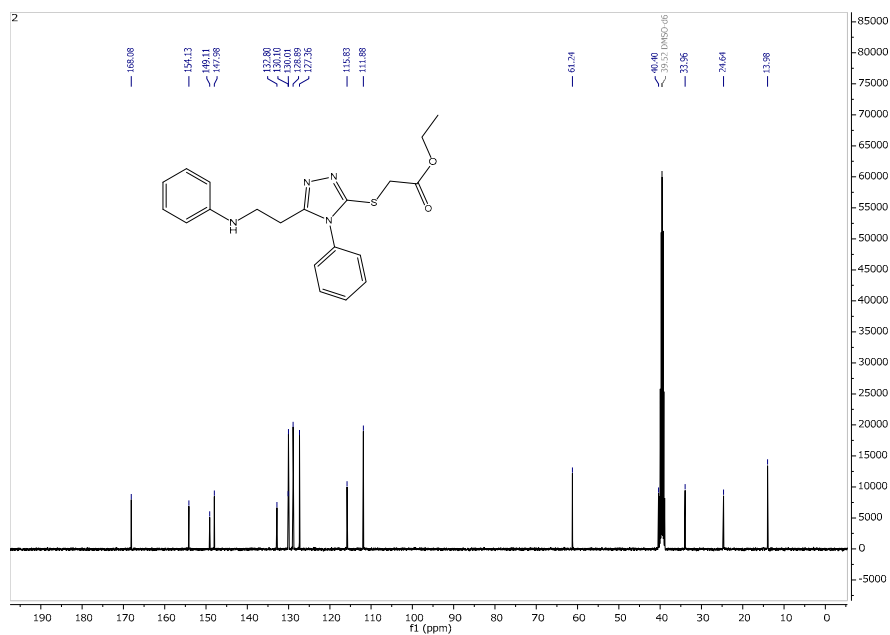


Figure S2. ¹³C NMR (101 MHz, DMSO-d₆) spectrum of 2

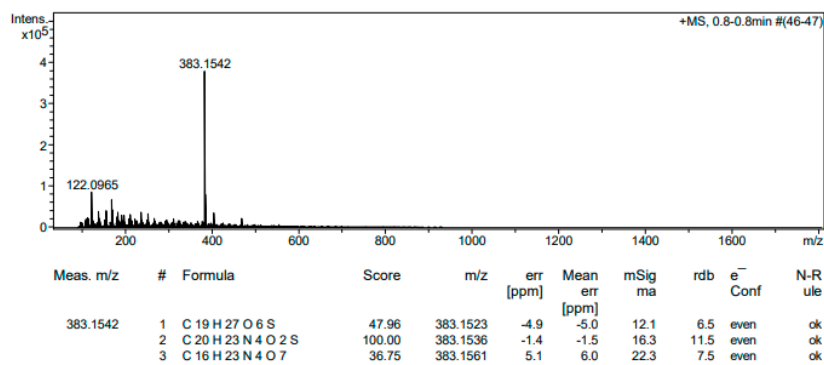


Figure S3. HRMS spectrum of 2



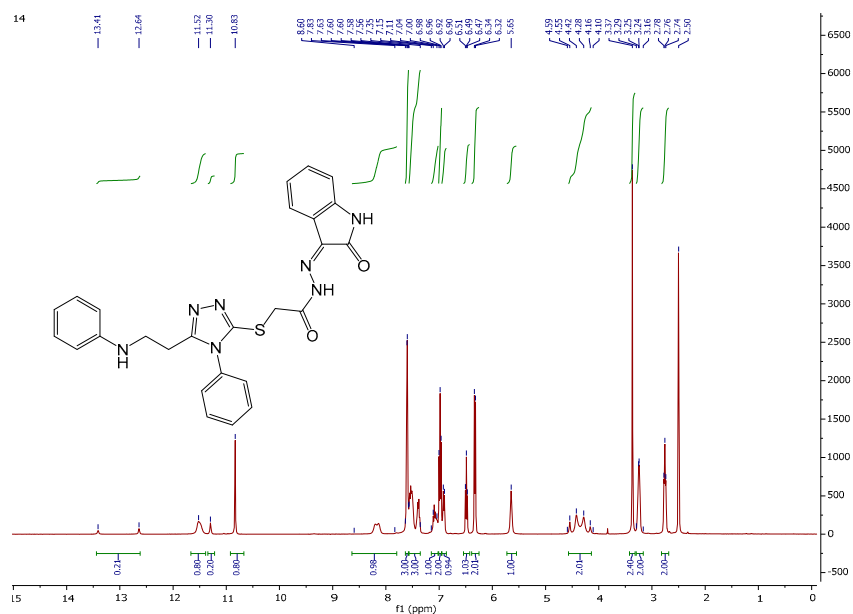


Figure S10. ¹H NMR (400 MHz, DMSO-d₆) spectrum of 4

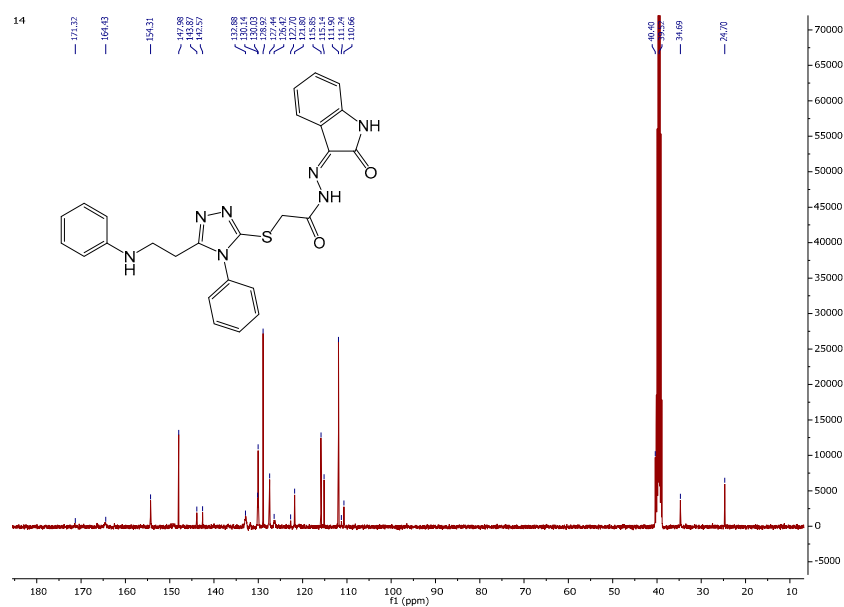


Figure S11. ¹³C NMR (101 MHz, DMSO-d₆) spectrum of 4

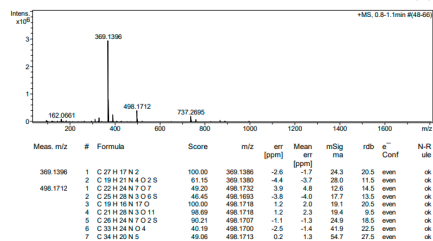
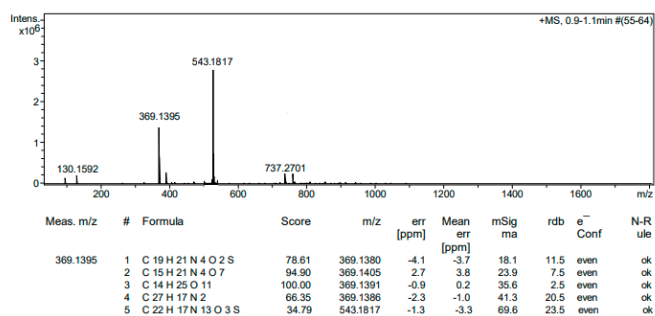
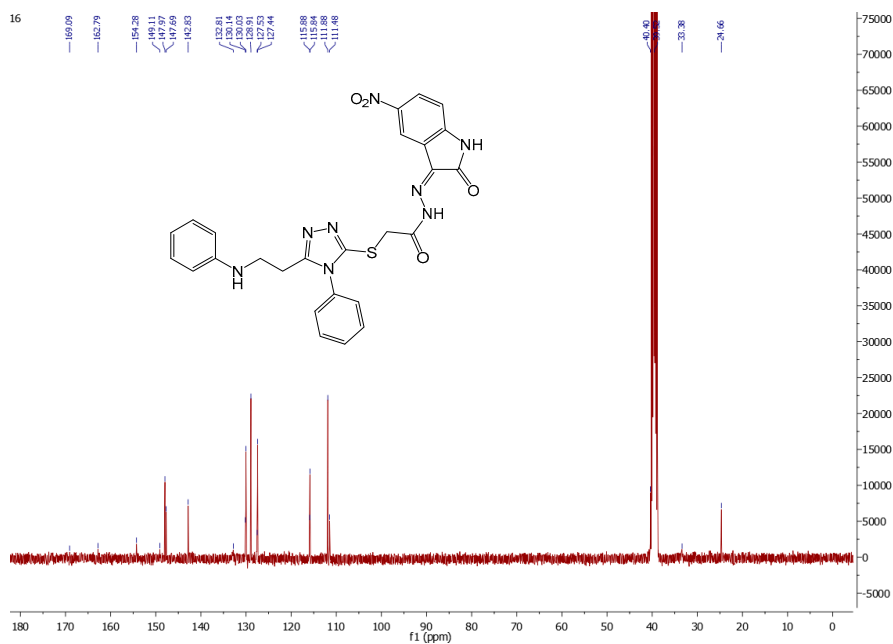
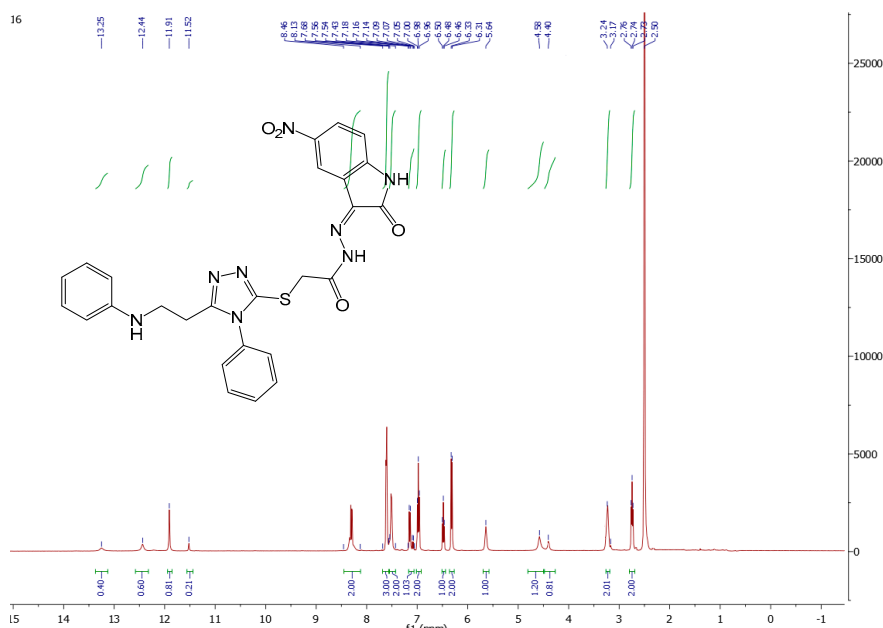


Figure S12. HRMS spectrum of 4







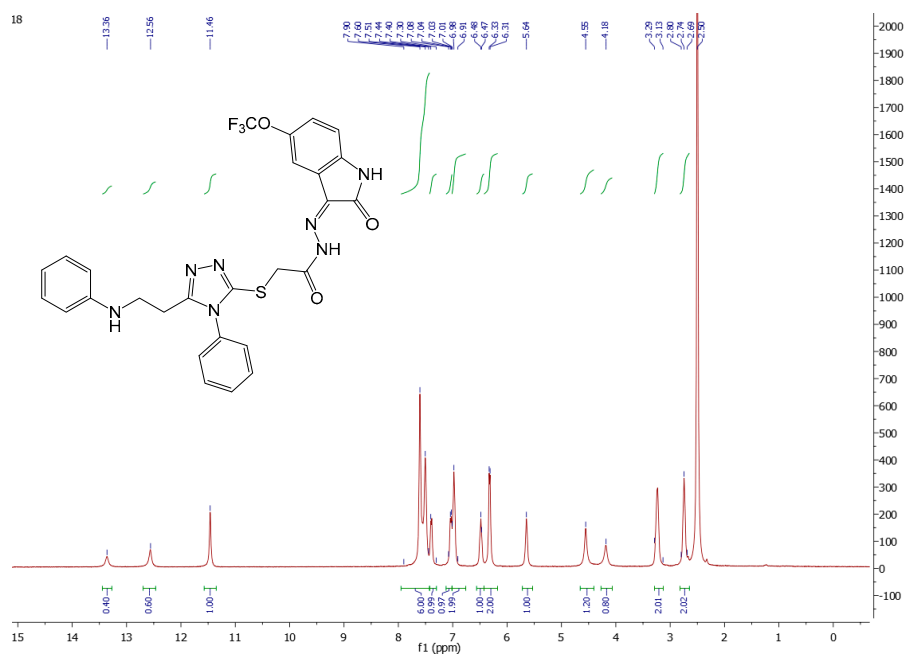


Figure S22. ¹H NMR (400 MHz, DMSO-d₆) spectrum of 8

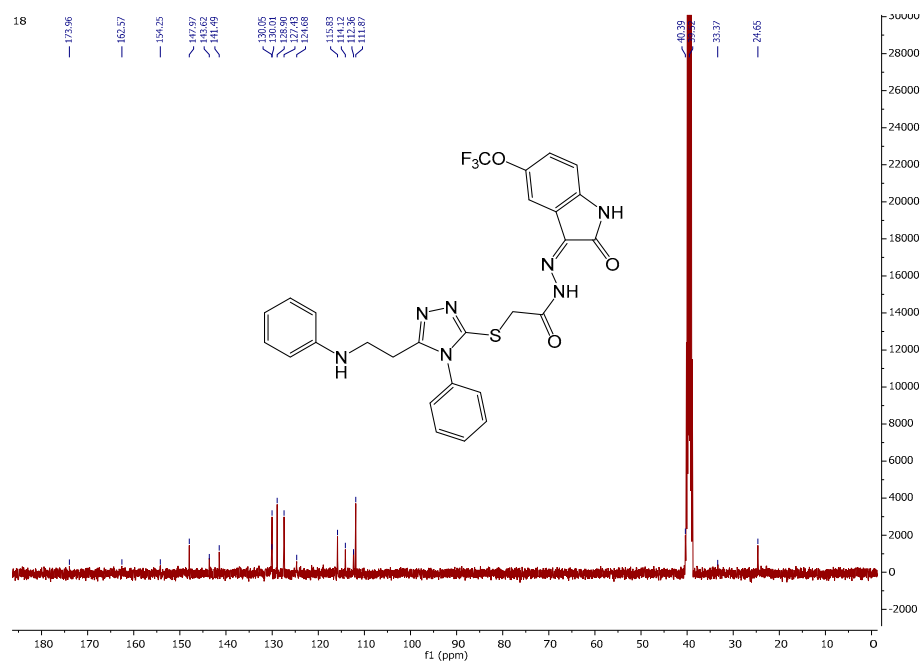


Figure S23. ¹³C NMR (101 MHz, DMSO-d₆) spectrum of 8

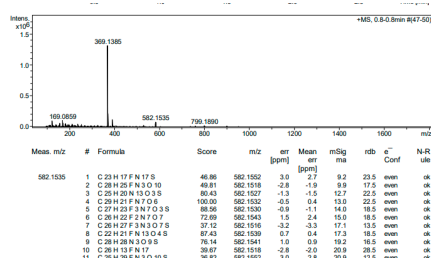


Figure S24. HRMS spectrum of 8





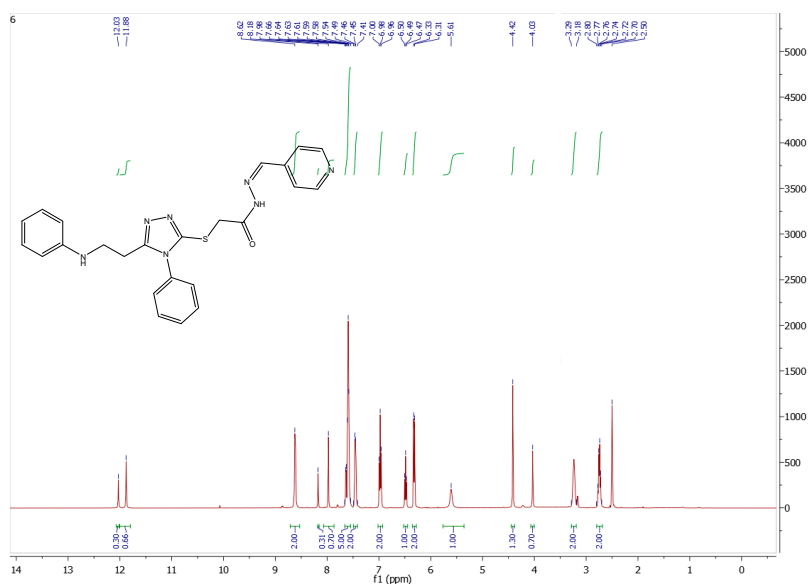


Figure S31. ¹H NMR (400 MHz, DMSO-d₆) spectrum of 11

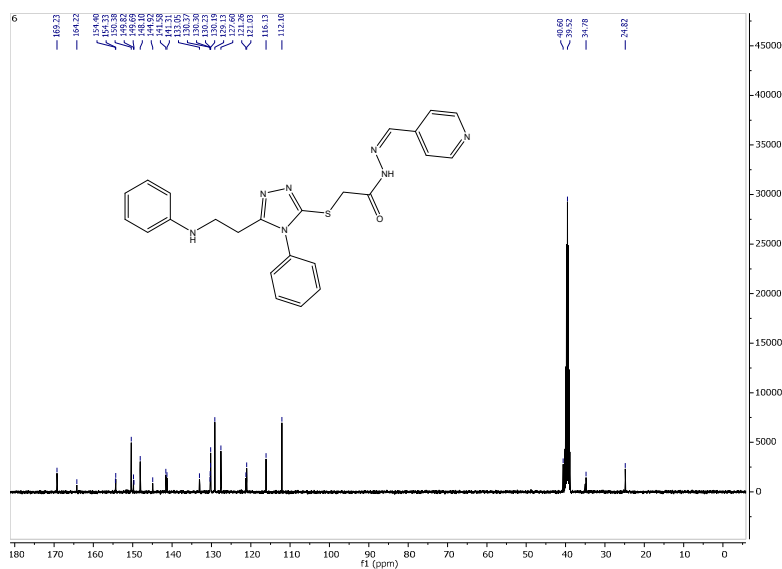


Figure S32. ¹³C NMR (400 MHz, DMSO-d₆) spectrum of 11

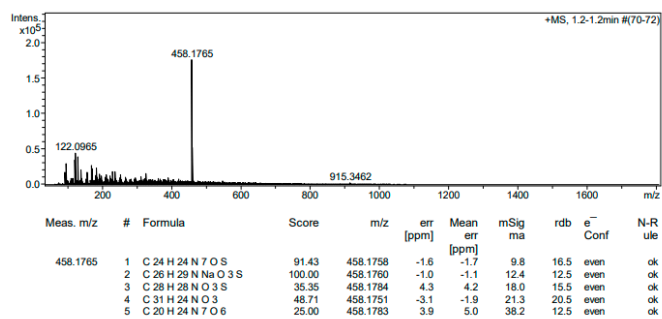


Figure S33. HRMS spectrum of 11

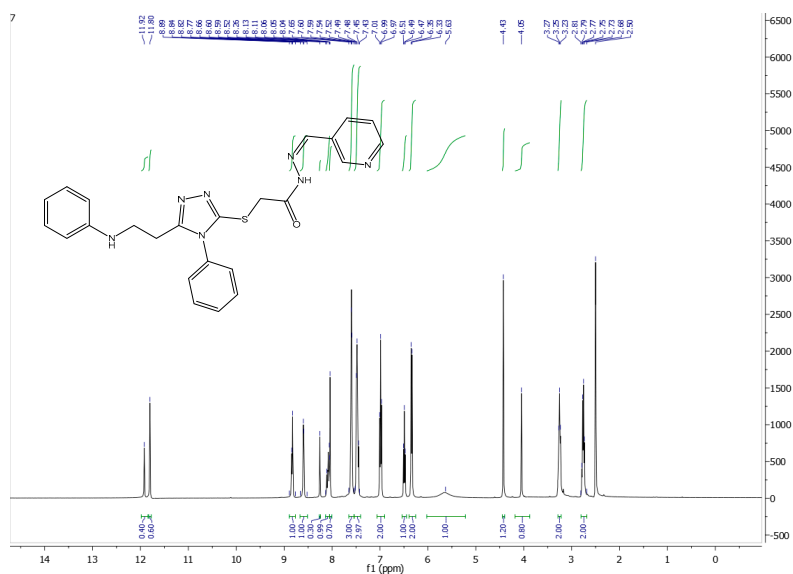


Figure S34. ^1H NMR (400 MHz, DMSO-d_6) spectrum of 12

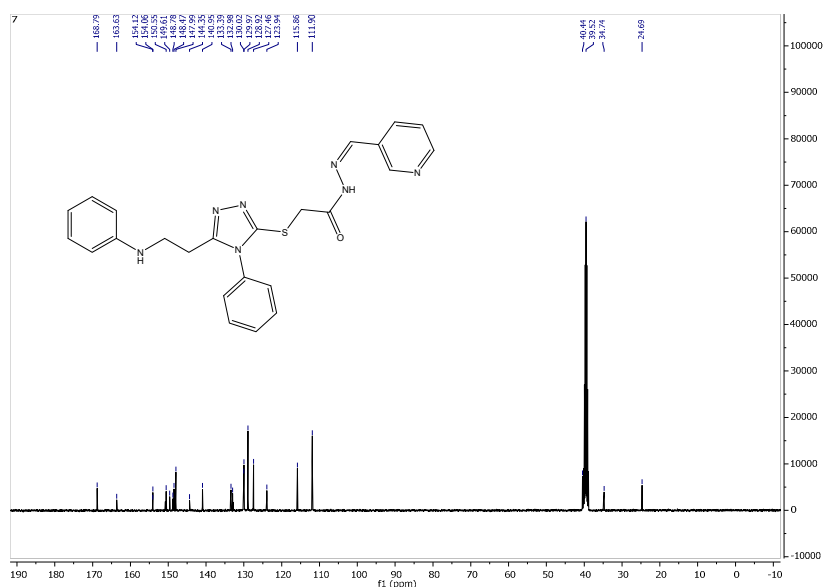


Figure S35. ^{13}C NMR (101 MHz, DMSO-d_6) spectrum of 12

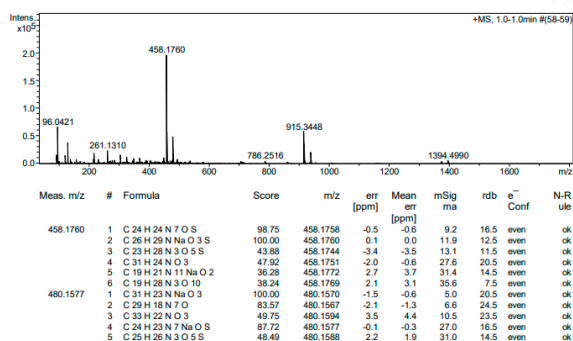
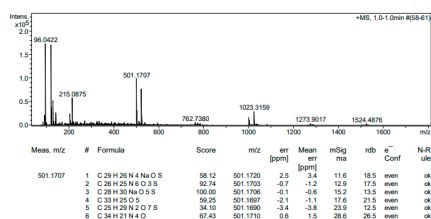
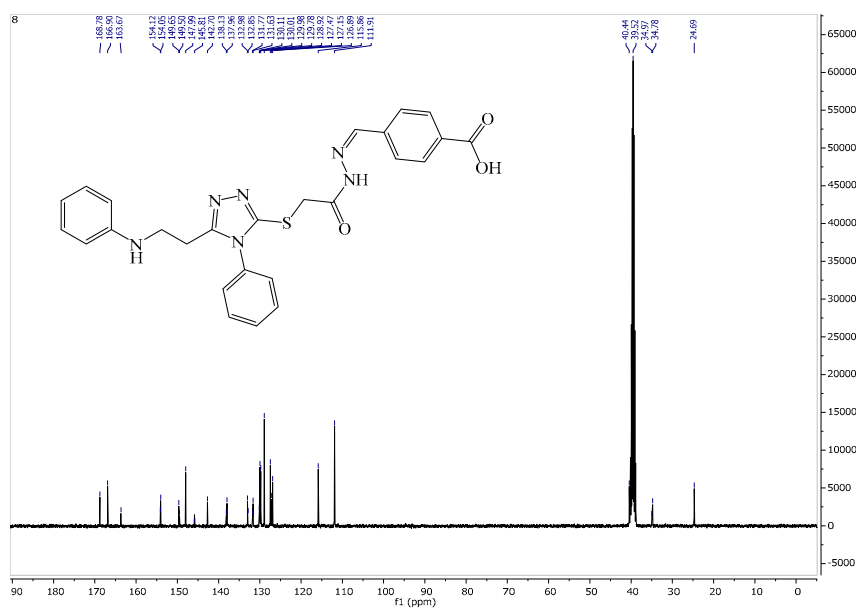
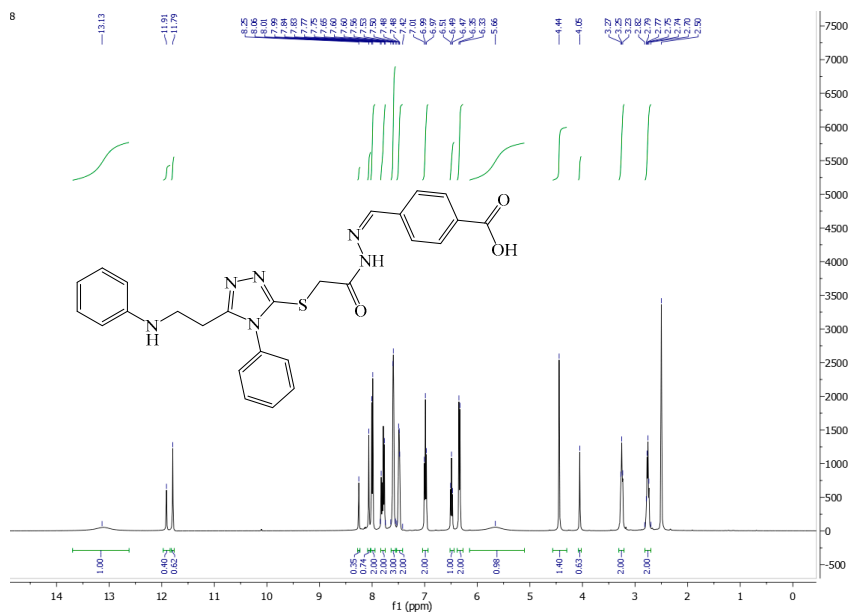


Figure S36. HRMS spectrum of 12



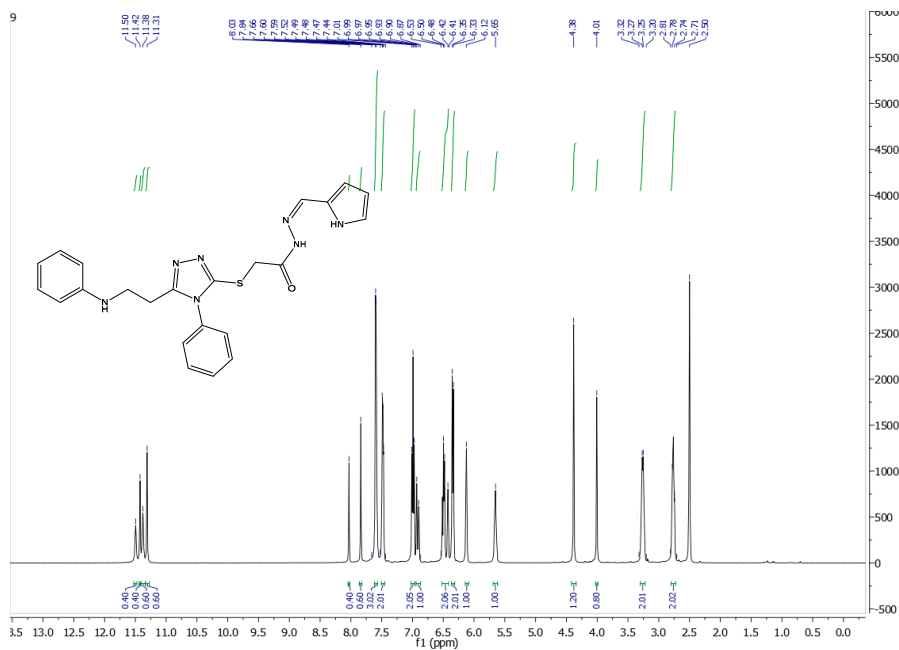


Figure S40. ^1H NMR (400 MHz, DMSO-d_6) spectrum of 14

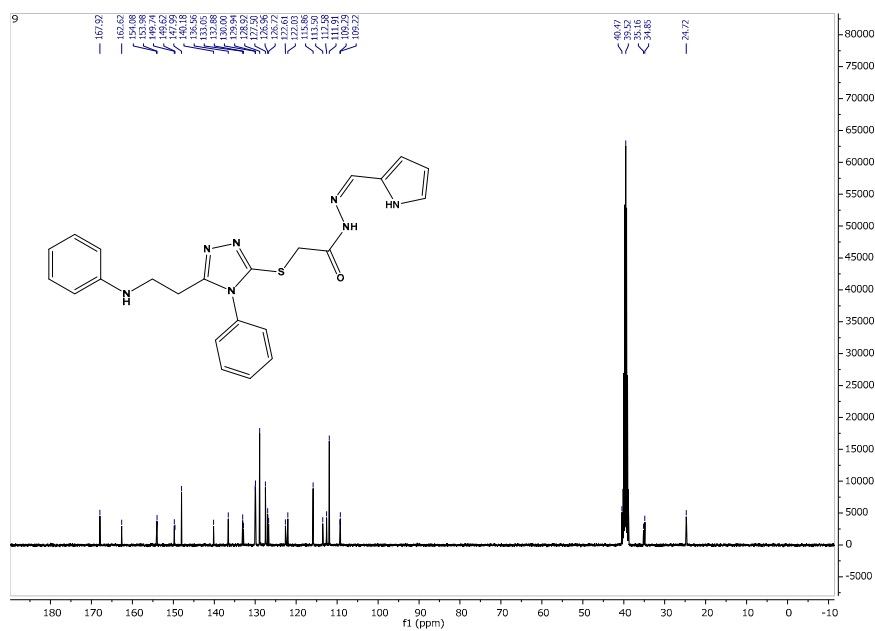


Figure S41. ^{13}C NMR (101 MHz, DMSO-d_6) spectrum of 14

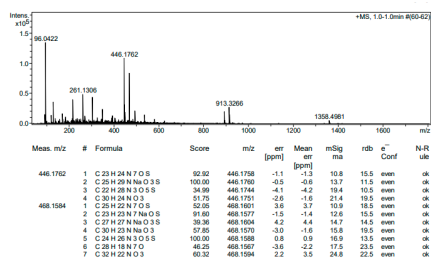


Figure S42. HRMS spectrum of 14

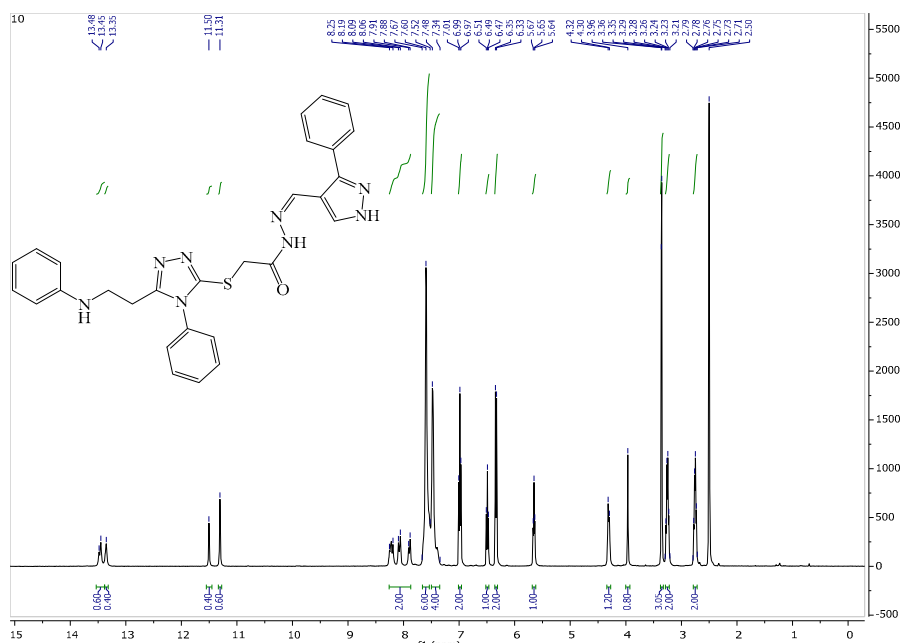


Figure S43. ¹H NMR (400 MHz, DMSO-d₆) spectrum of 15

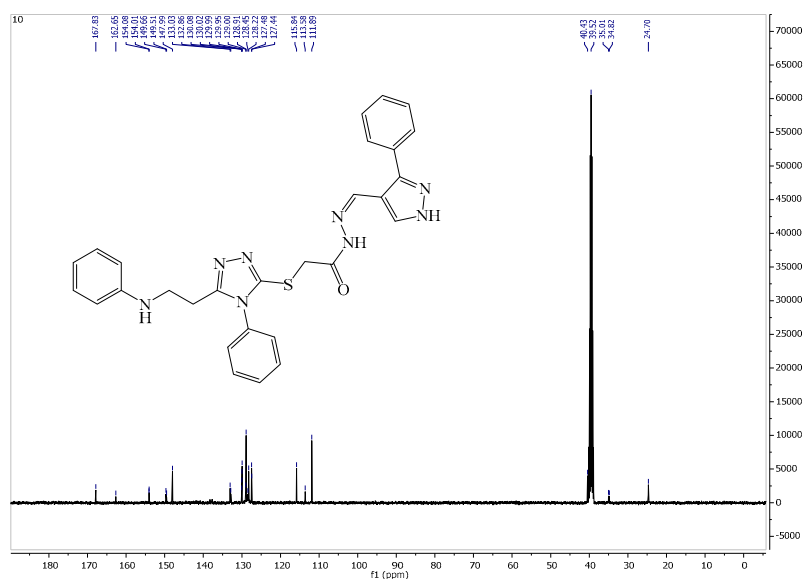


Figure S44. ¹³C NMR (101 MHz, DMSO-d₆) spectrum of 15

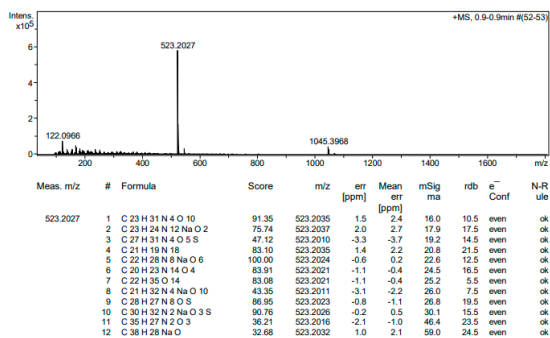


Figure S45. HRMS spectrum of 15

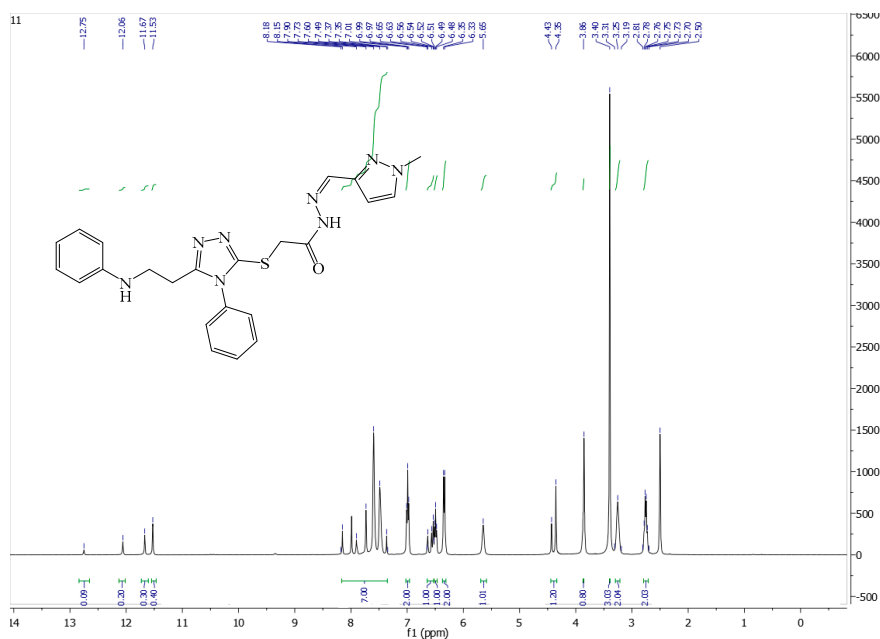


Figure S46. ¹H NMR (400 MHz, DMSO-d₆) spectrum of 16

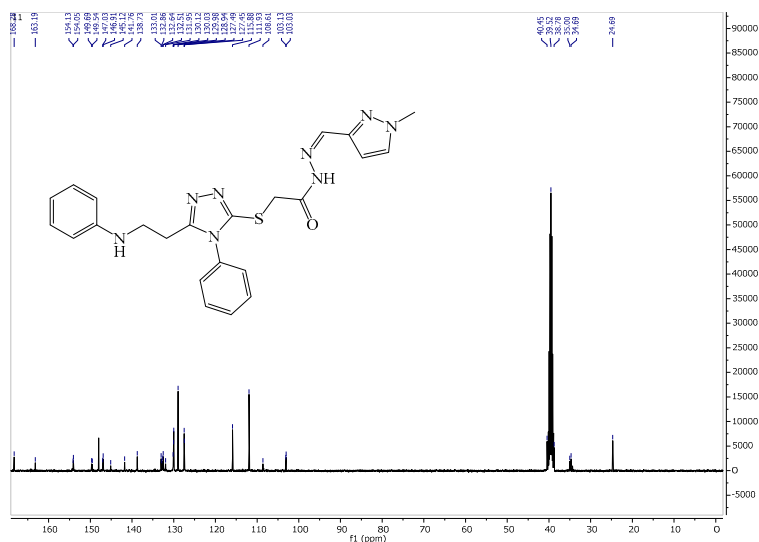


Figure S47. ¹³C NMR (101 MHz, DMSO-d₆) spectrum of 16

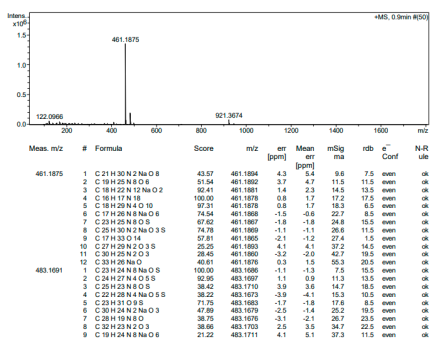


Figure S48. HRMS spectrum of 16

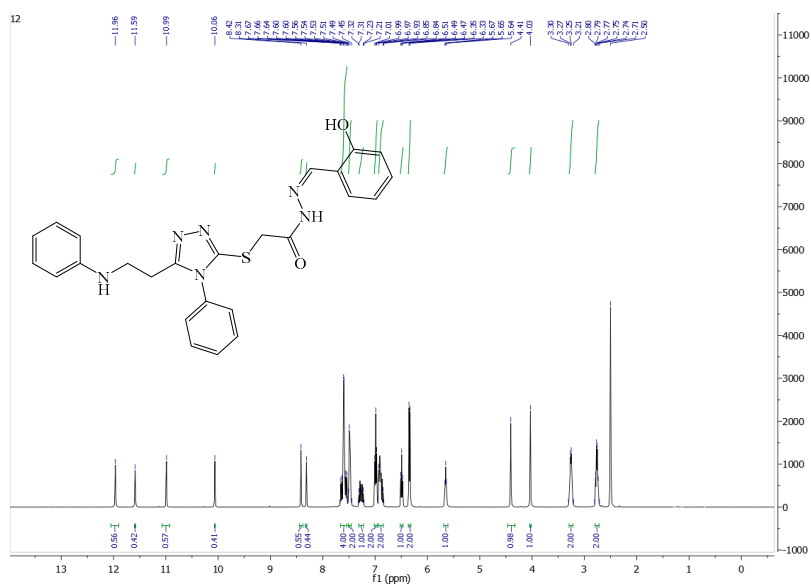


Figure S49. ¹H NMR (400 MHz, DMSO-d₆) spectrum of 17

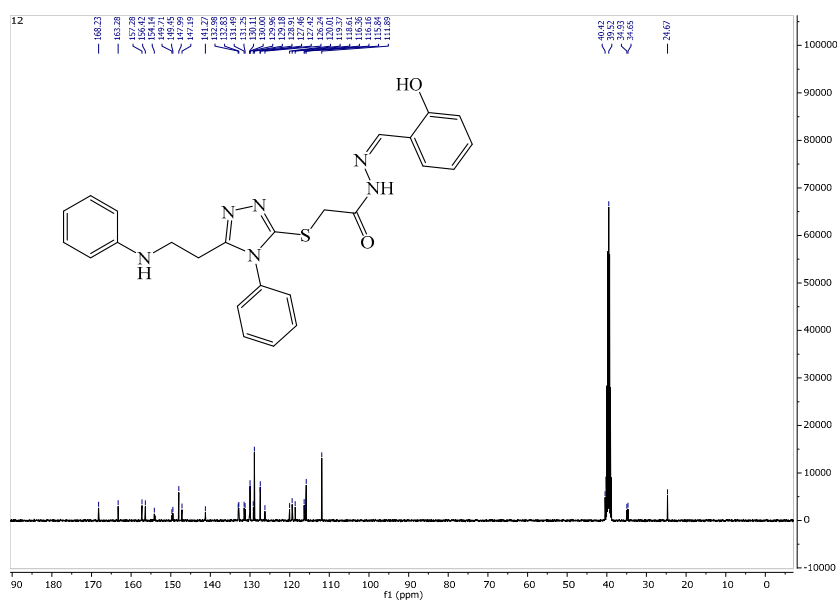


Figure S50. ¹³C NMR (101 MHz, DMSO-d₆) spectrum of 17

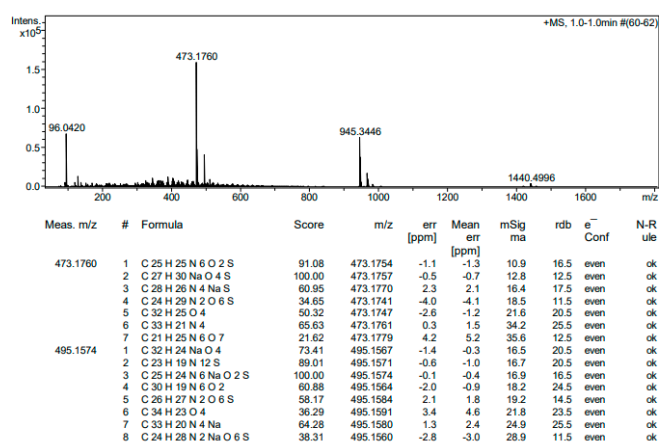


Figure S51. HRMS spectrum of 17



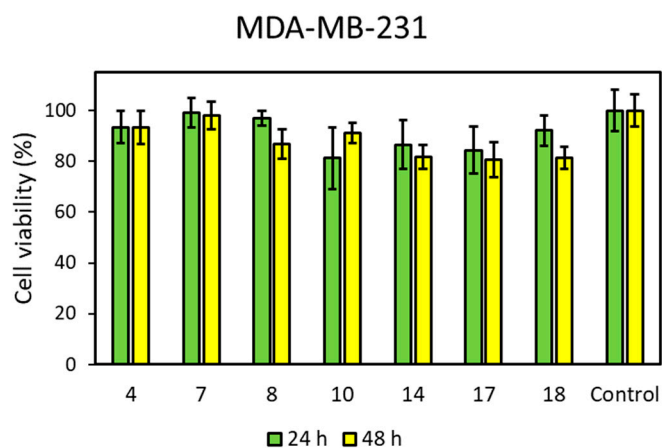


Figure S58. The effect of 10 μ M compound concentration on MDA-MB-231 cell line viability after 24 and 48 h.

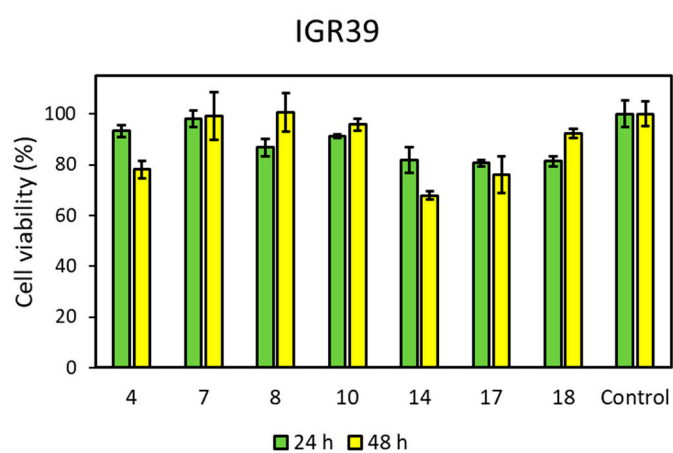


Figure S59. The effect of 10 μ M compound concentration on IGR39 cell line viability after 24 and 48 h.

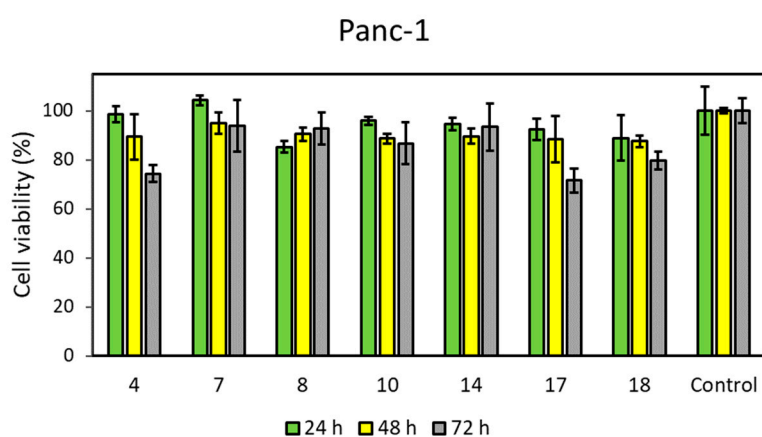


Figure S60. The effect of 10 μ M compound concentration on Panc-1 cell line viability after 24, 48, and 72 h.

Procedure S1. Evaluation of 10 μ M compound concentration effect on cell line viability.

The effect of 10 μ M compounds on cell viability was studied using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich Co., St Louis, MO, USA) assay. IGR39, MDA-MB-231, and Panc-1 cells were seeded in 96-well plates (Corning) in triplicates at a volume of 100 μ L at 4×10^3 cells/well). After 24 h, the cells were treated with 10 μ M of compounds **4**, **7**, **8**, **10**, **14**, **17**, and **18**. After 24 h, 48 h and 72 h (only Panc-1 cells), the MTT reagent has been added and cells were incubated for 4 h. Then the medium was aspirated, and the formed formazan crystals were dissolved in 100 μ L DMSO (Sigma-Aldrich Co., St. Louis, MO, USA). The absorbance was measured at 570 and 630 nm using a multi-detection microplate reader. Compound effect on cell viability was calculated according to a formula:

$$\text{Relative cell viability (\%)} = \frac{A - A_0}{A_{NC} - A_0}$$

Where:

A – mean of absorbance of tested compound,

A₀ – mean of absorbance of blank (no cells, positive control),

A_{NC} – mean of absorbance of negative control (only cells, no treatment).