Supporting Information

New multitarget antidiabetic potential agents based on sulfaguanidine: Design, synthesis, and biological evaluation

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Contents

1. ^{1}H, ^{13}C NMR spectra, IR and HRMS of the synthesized compounds (Figures S1-S44) **S2-S23**
2. Equipment and analytical technique **S24**
3. Biological procedures **S24-S27**
4. 2D diagram and 3D representation of molecular docking of all compounds in the binding pocket (PDB: 2QMJ). **S27-S34**
5. References **S34**

1. NMR, IR and HRMS Spectra of products 3-13.

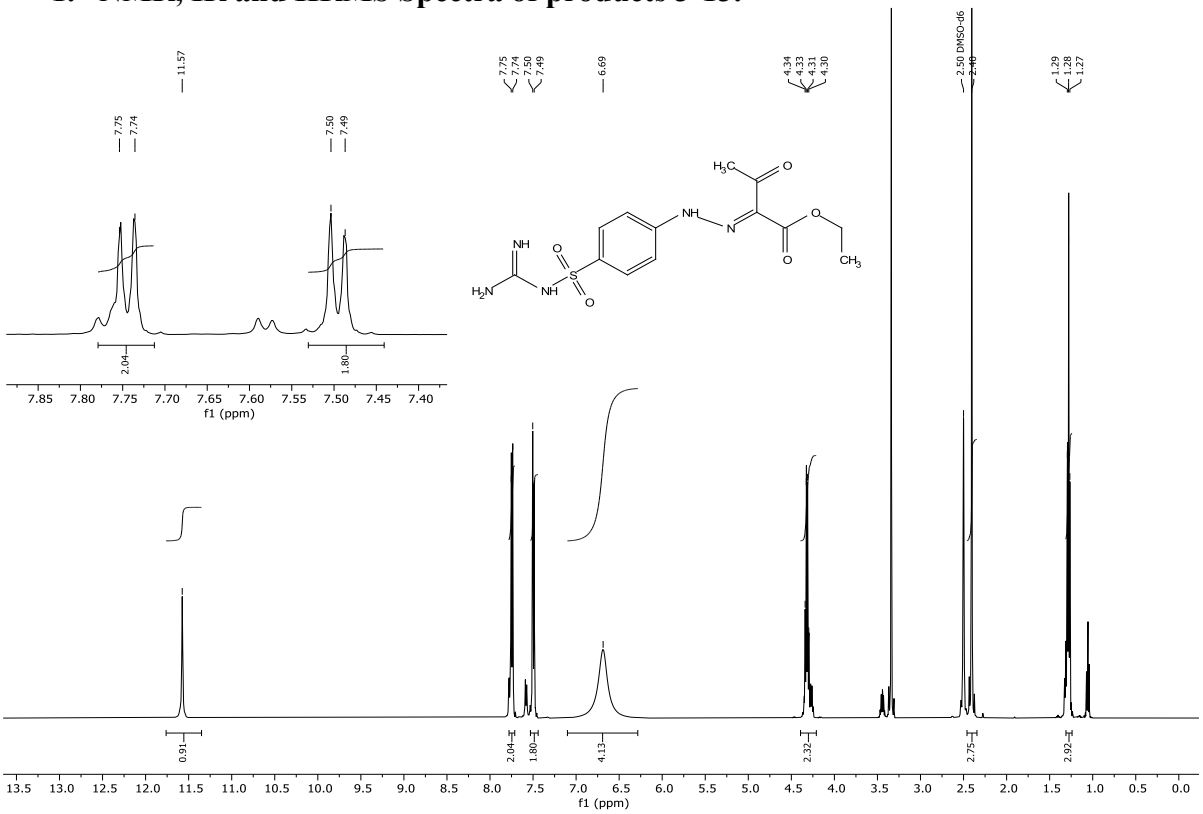

Figure S1. ^{1}H NMR of **3** (500 MHz, DMSO-d_6).

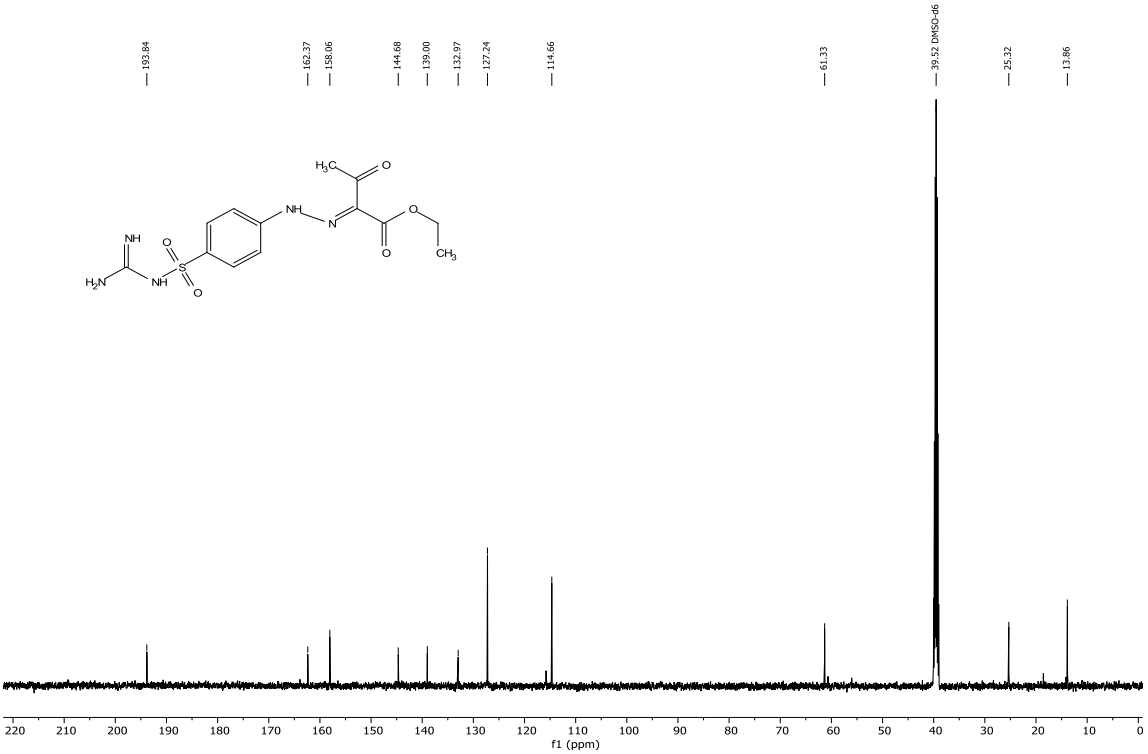

Figure S2. ^{13}C NMR of **3** (500 MHz, DMSO-d_6).

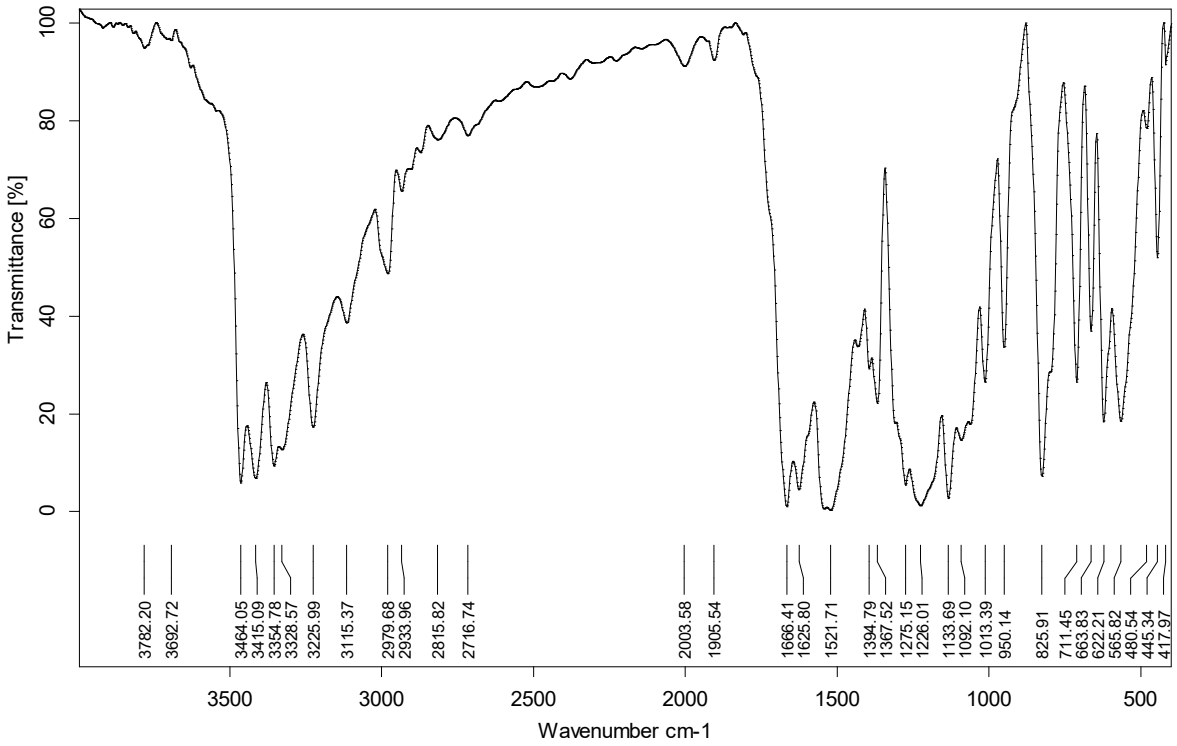

Figure S3. IR (KBr) of **3**.

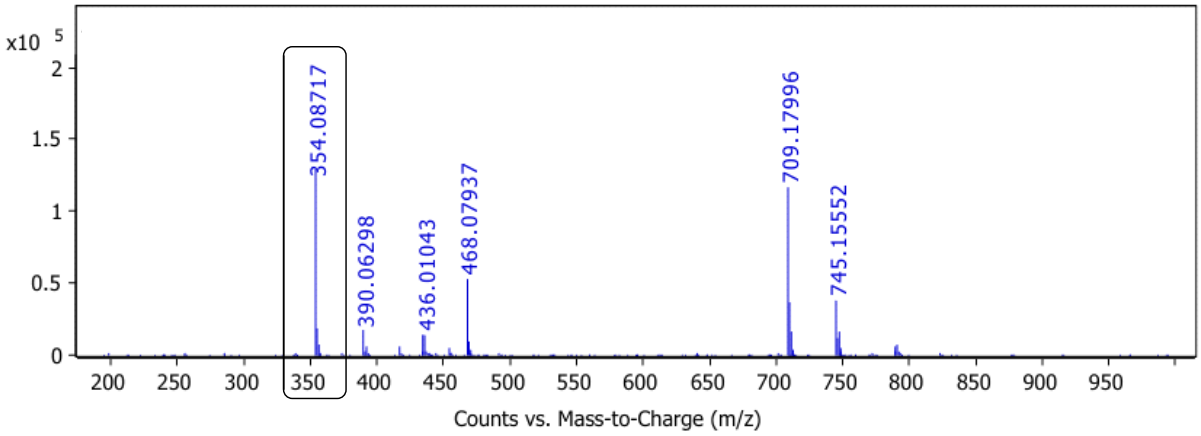

Figure S4. HRMS (ESI$^-$) of compound **3**.

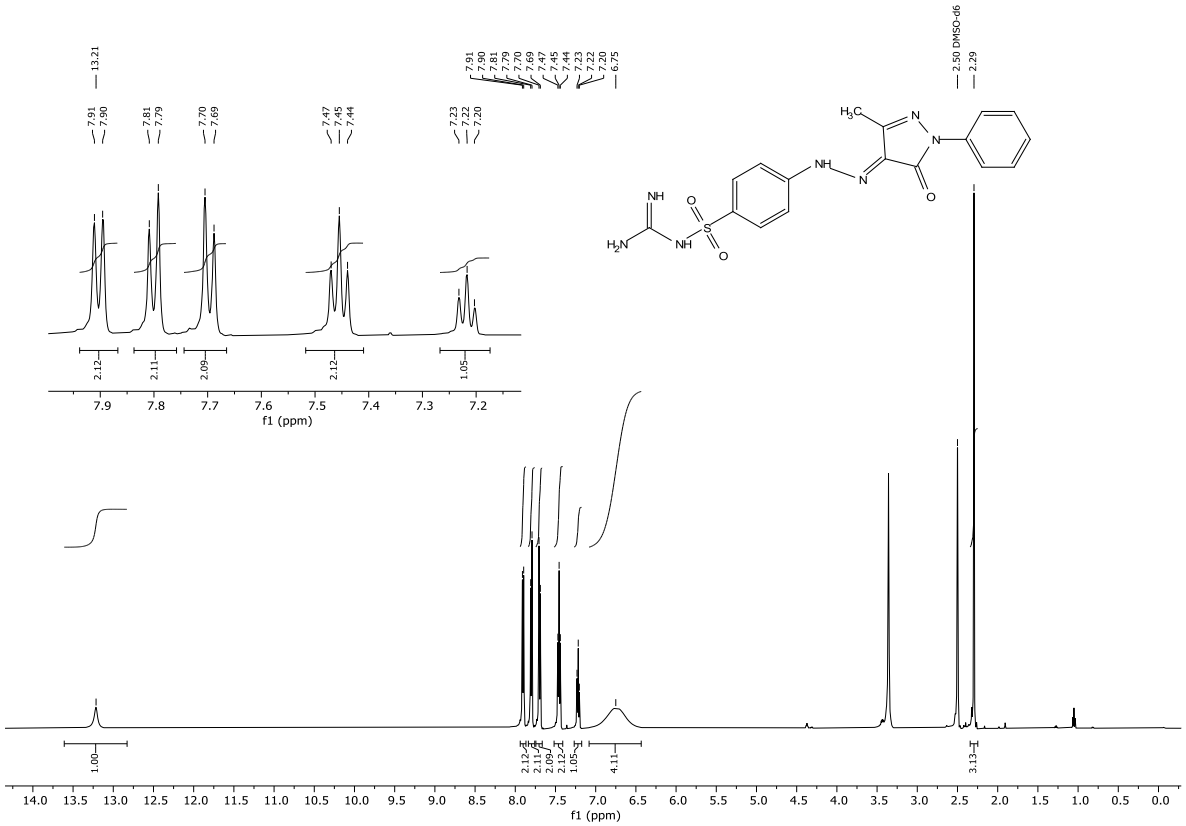

Figure S5. ^{1}H NMR of **4** (500 MHz, DMSO-d_6).

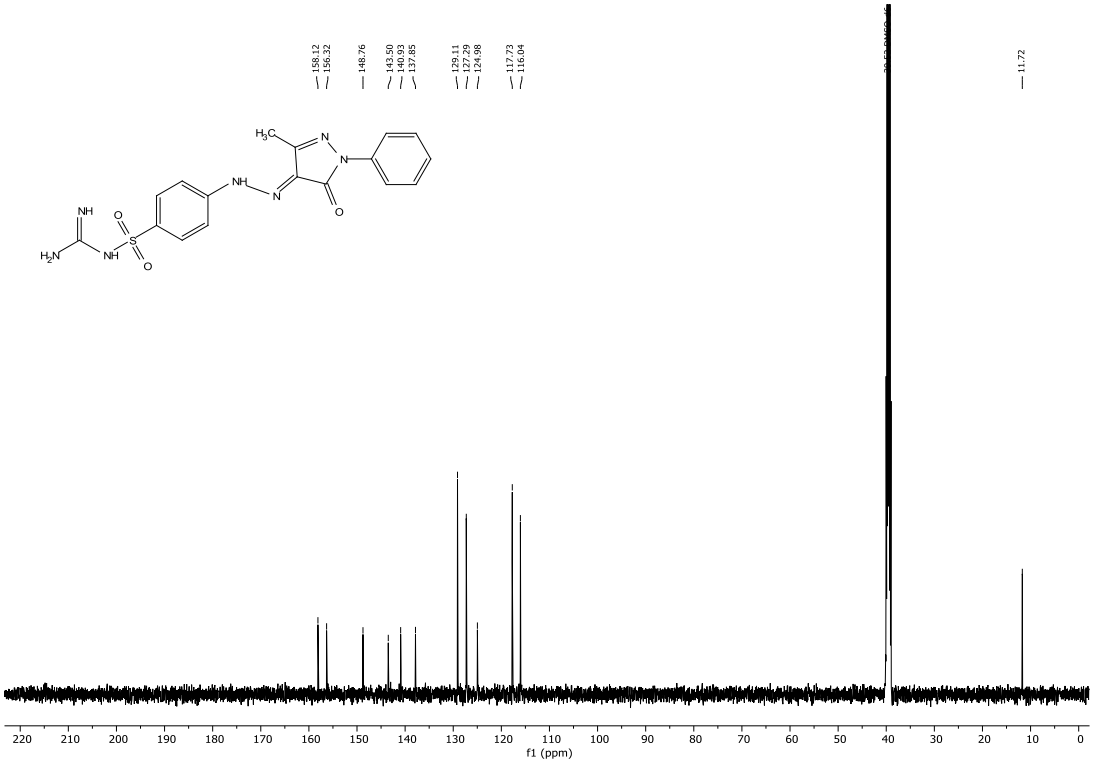

Figure S6. ^{13}C NMR of **4** (500 MHz, DMSO-d_6).

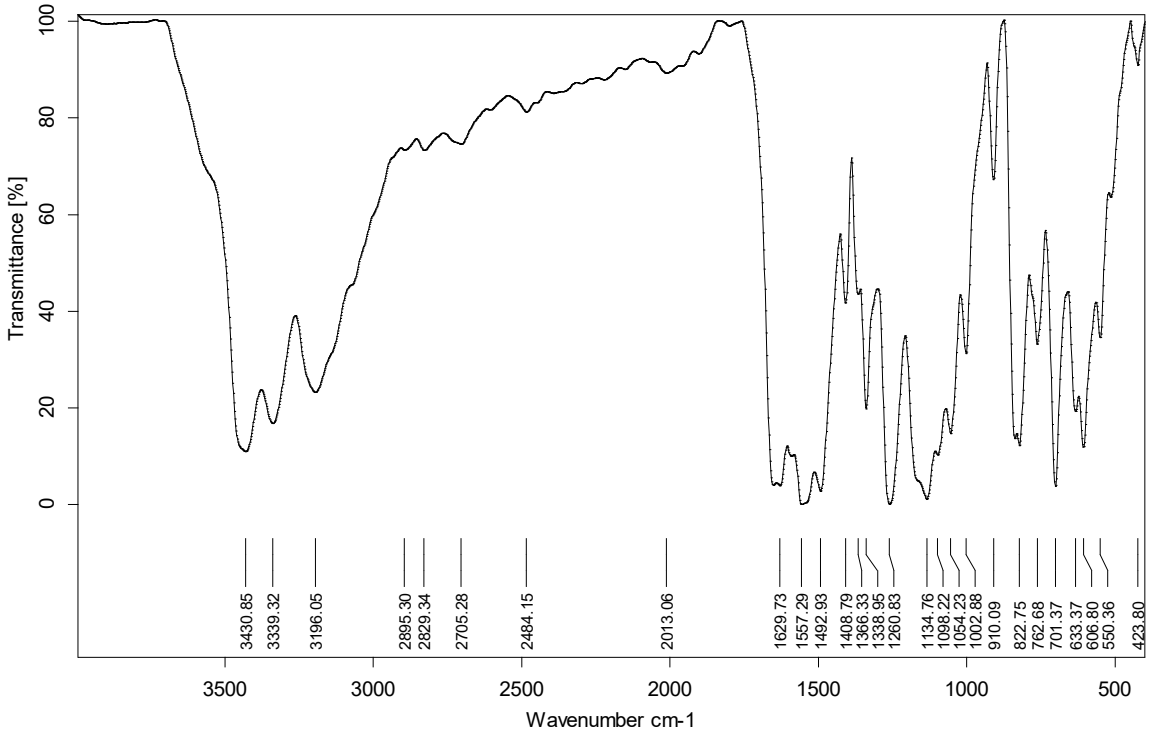

Figure S7. IR (KBr) of **4**.

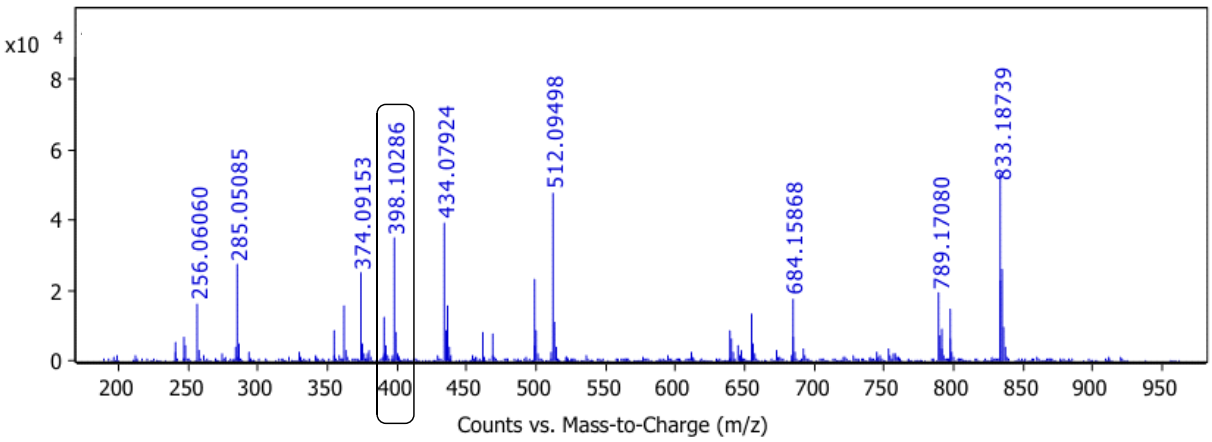

Figure S8. HRMS (ESI$^-$) of compound **4**.

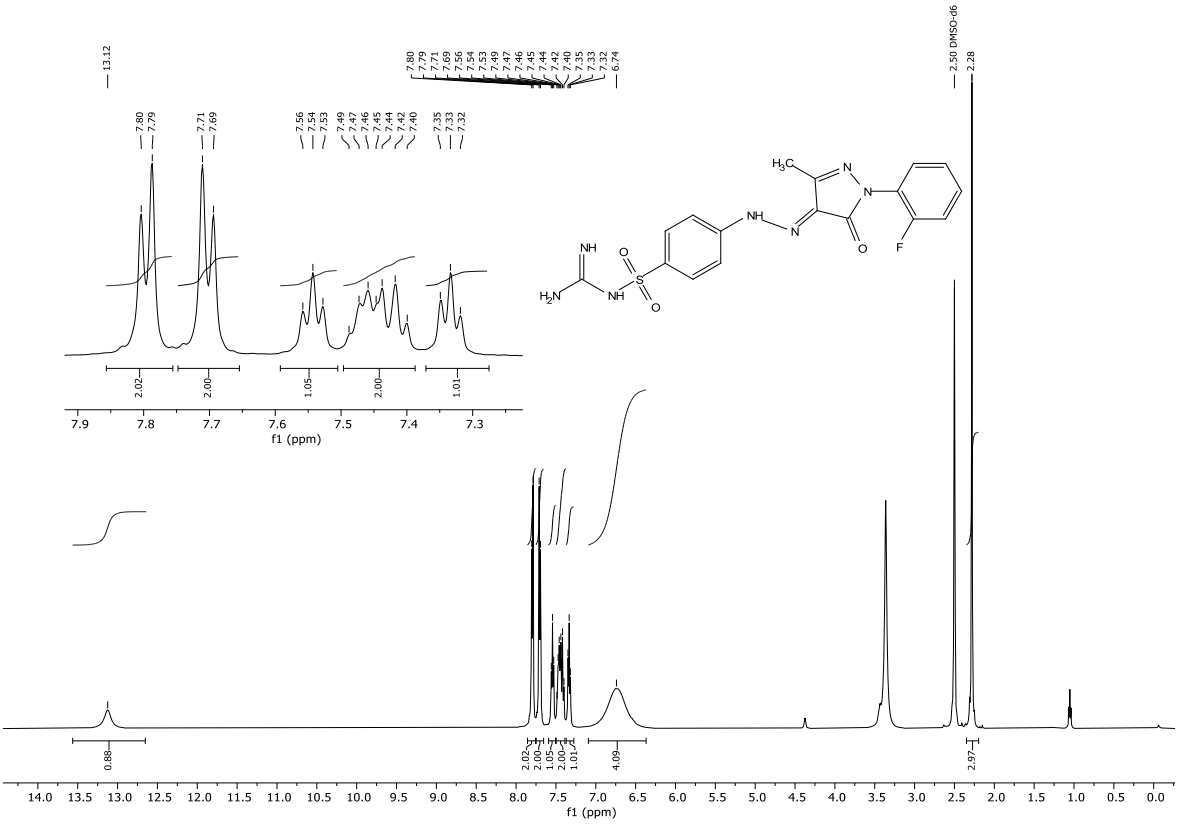

Figure S9. ^{1}H NMR of **5** (500 MHz, DMSO-d_6).

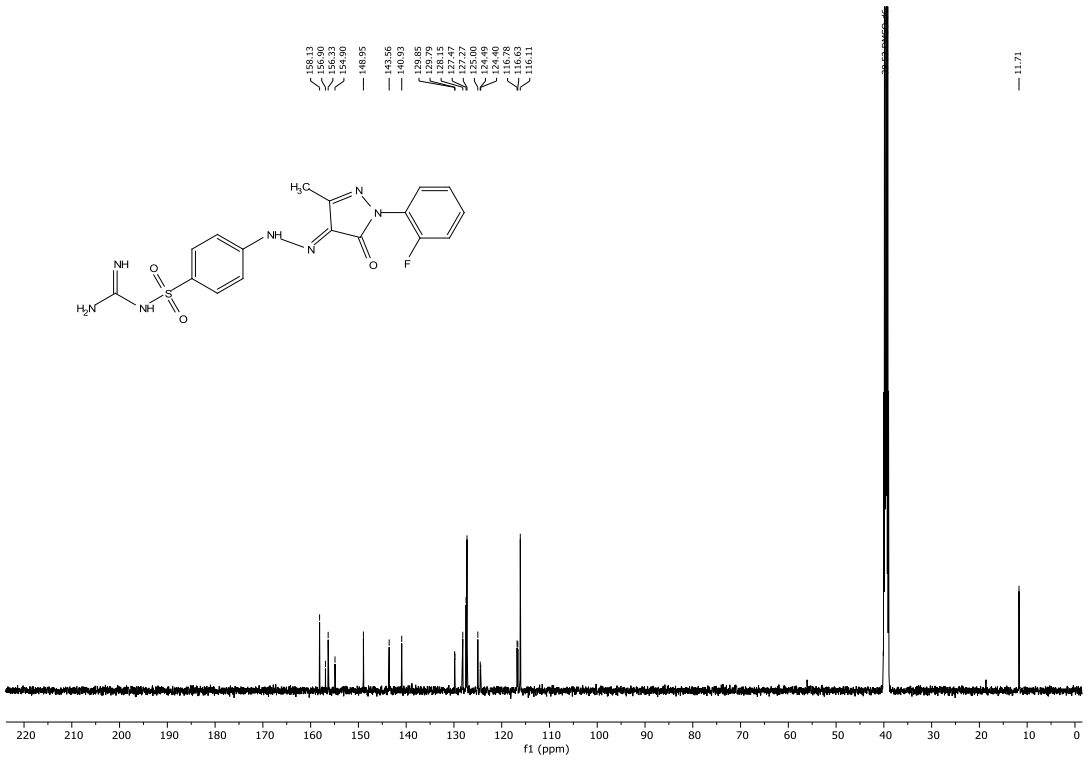

Figure S10. ^{13}C NMR of **5** (500 MHz, DMSO-d_6).

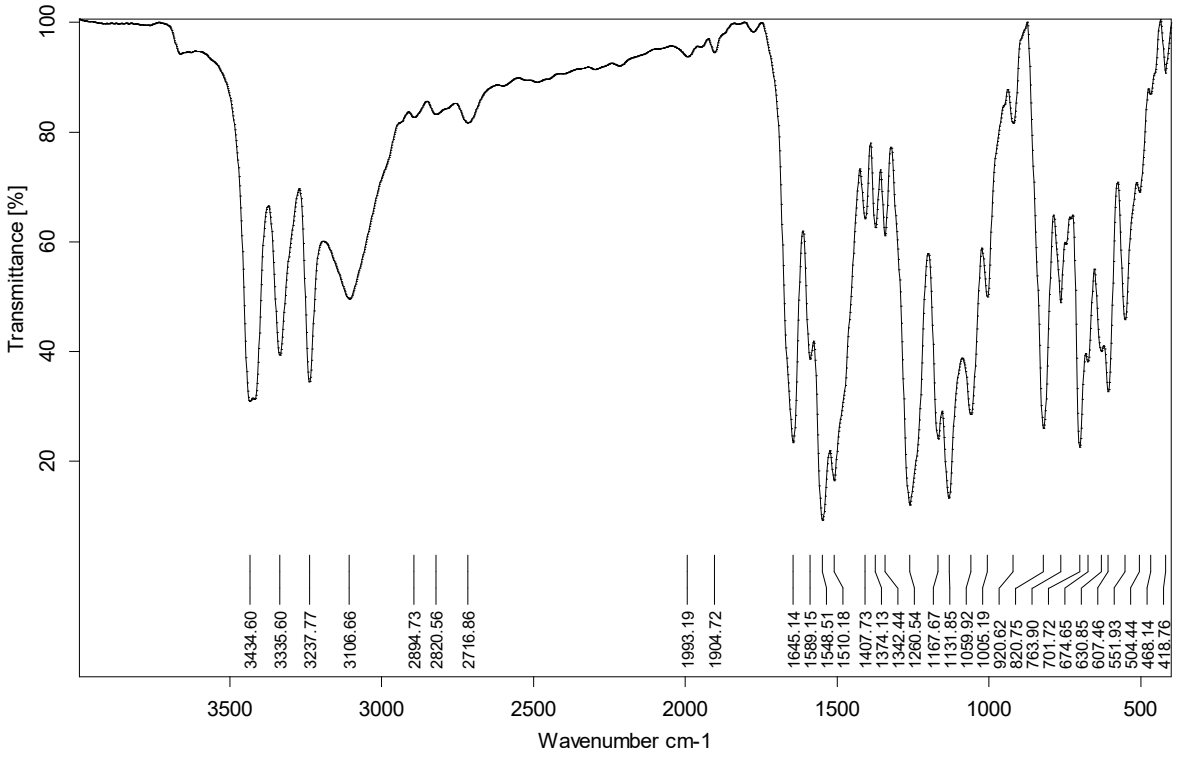

Figure S11. IR (KBr) of **5**.

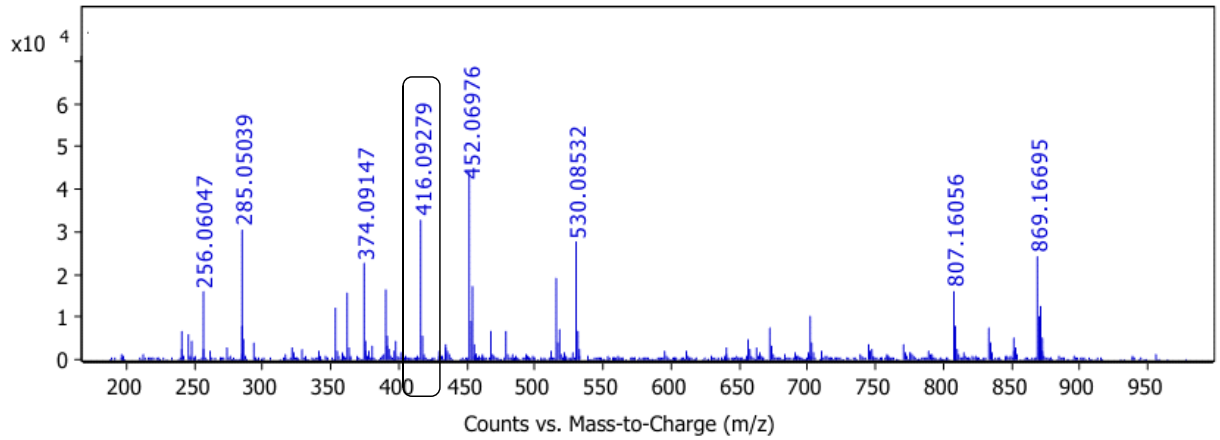

Figure S12. HRMS (ESI$^-$) of compound **5**.

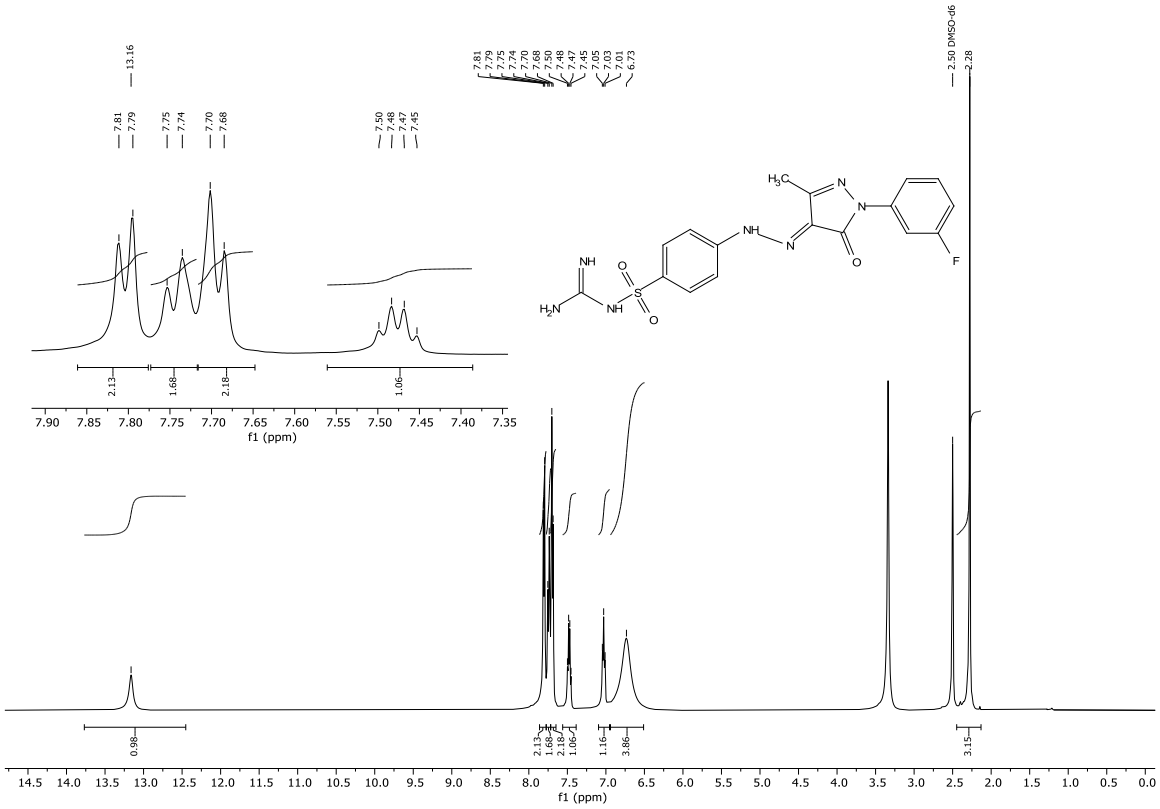

Figure S13. ^{1}H NMR of **6** (500 MHz, DMSO-d_6).

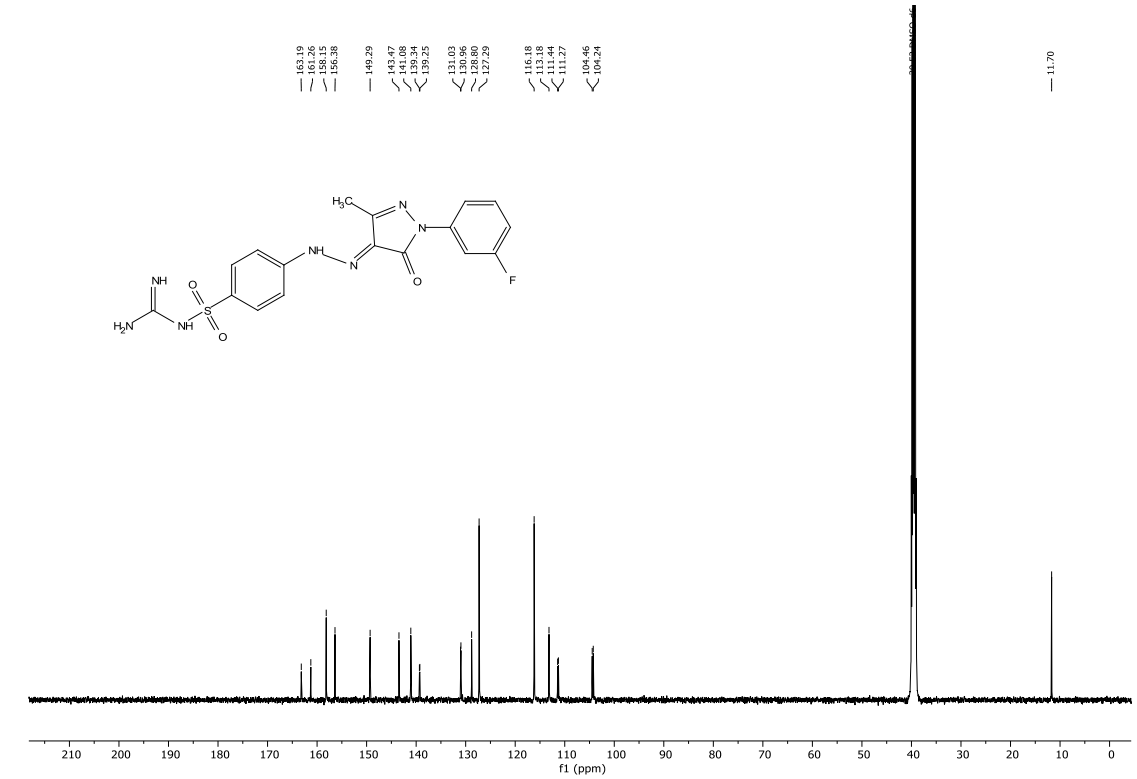

Figure S14. ^{13}C NMR of **6** (500 MHz, DMSO-d_6).

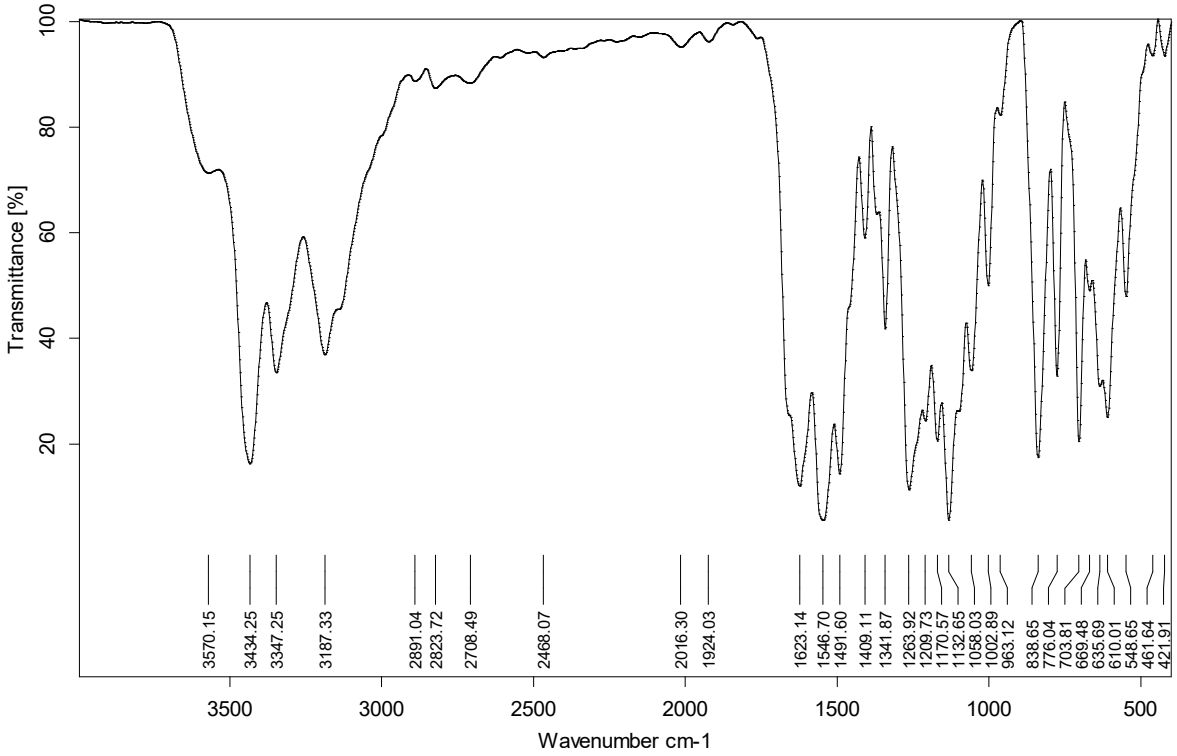

Figure S15. IR (KBr) of **6**.

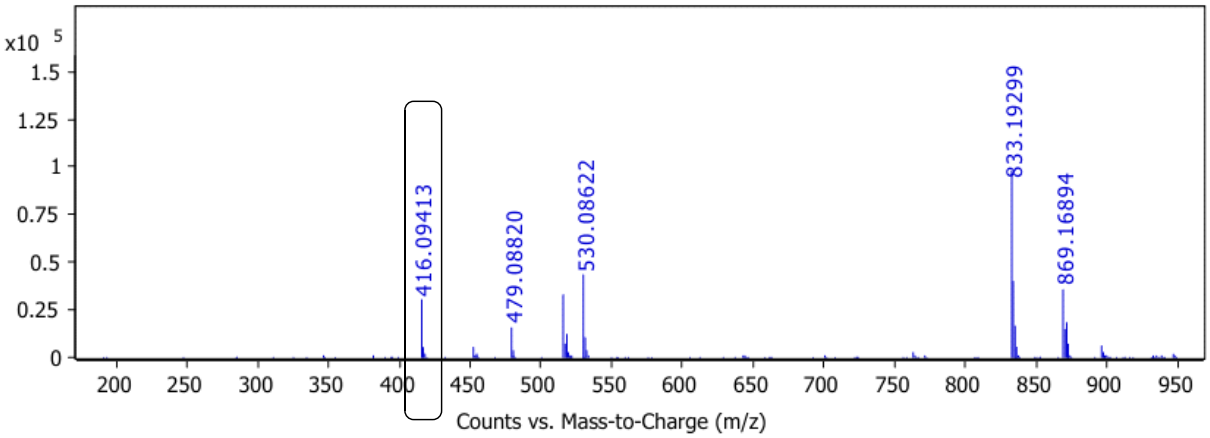

Figure S16. HRMS (ESI$^-$) of compound **6**.

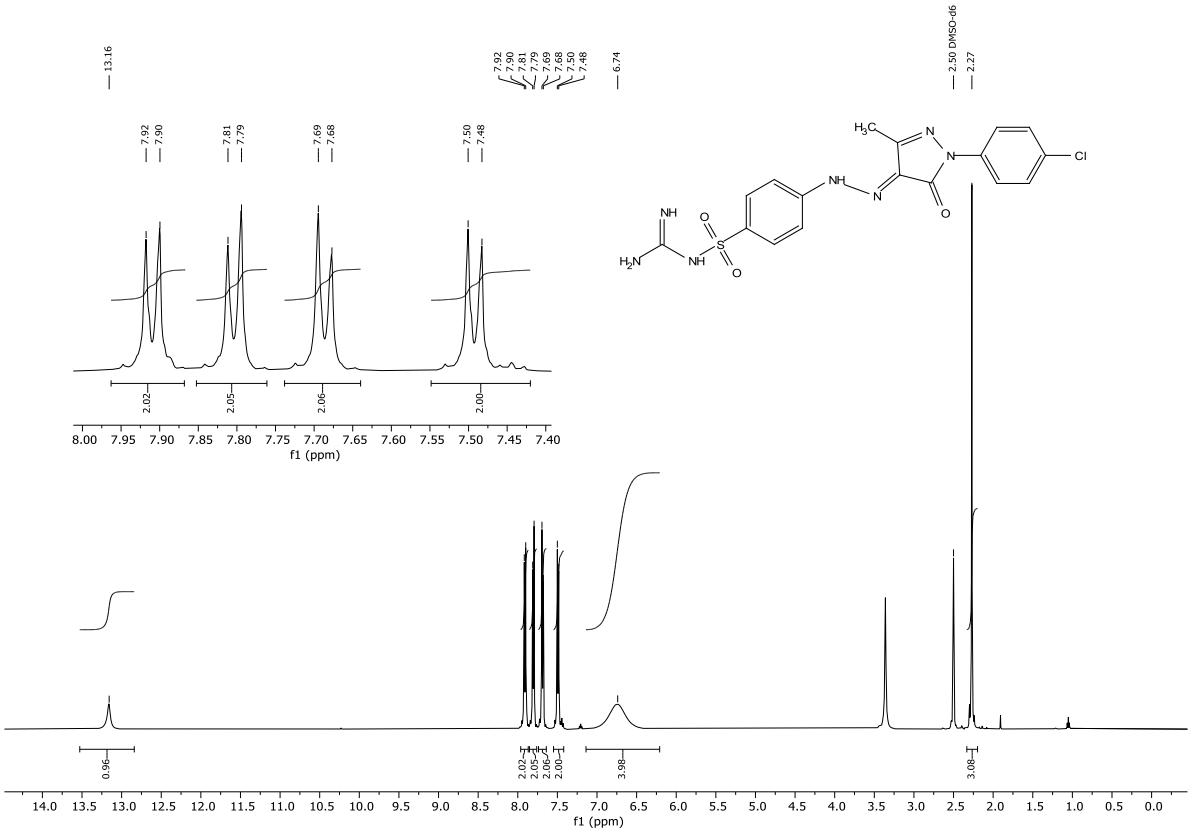

Figure S17. ^{1}H NMR of **7** (500 MHz, DMSO-d_6).

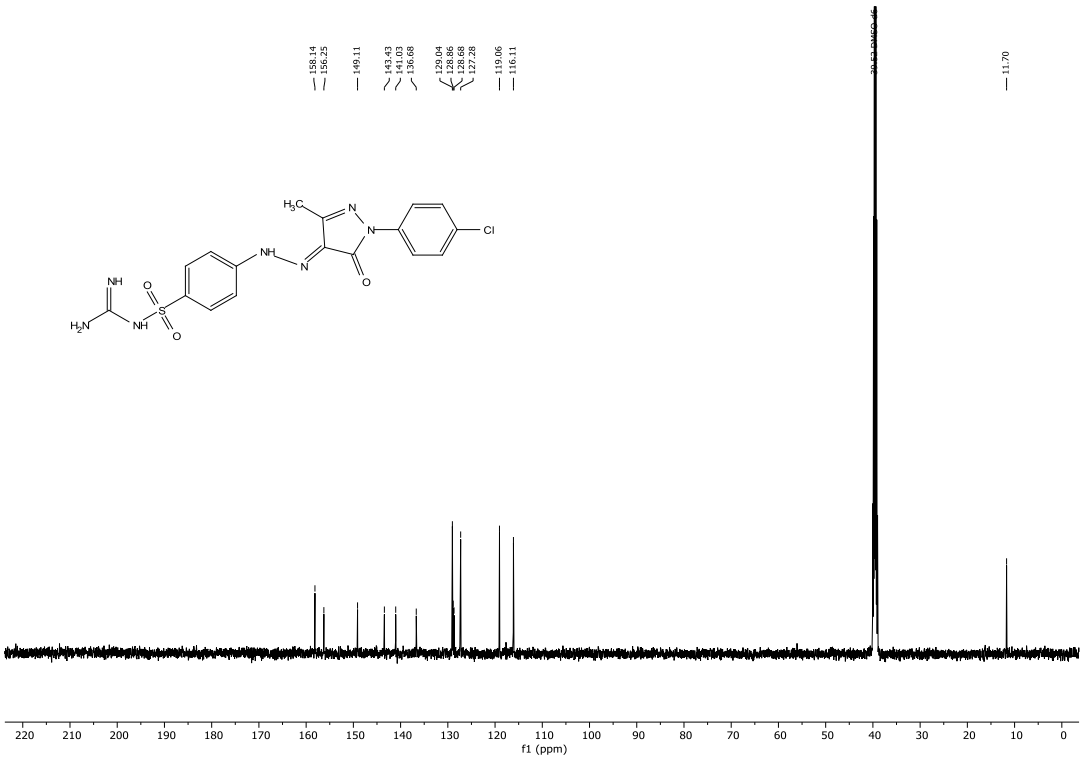

Figure S18. ^{13}C NMR of **7** (500 MHz, DMSO-d_6).

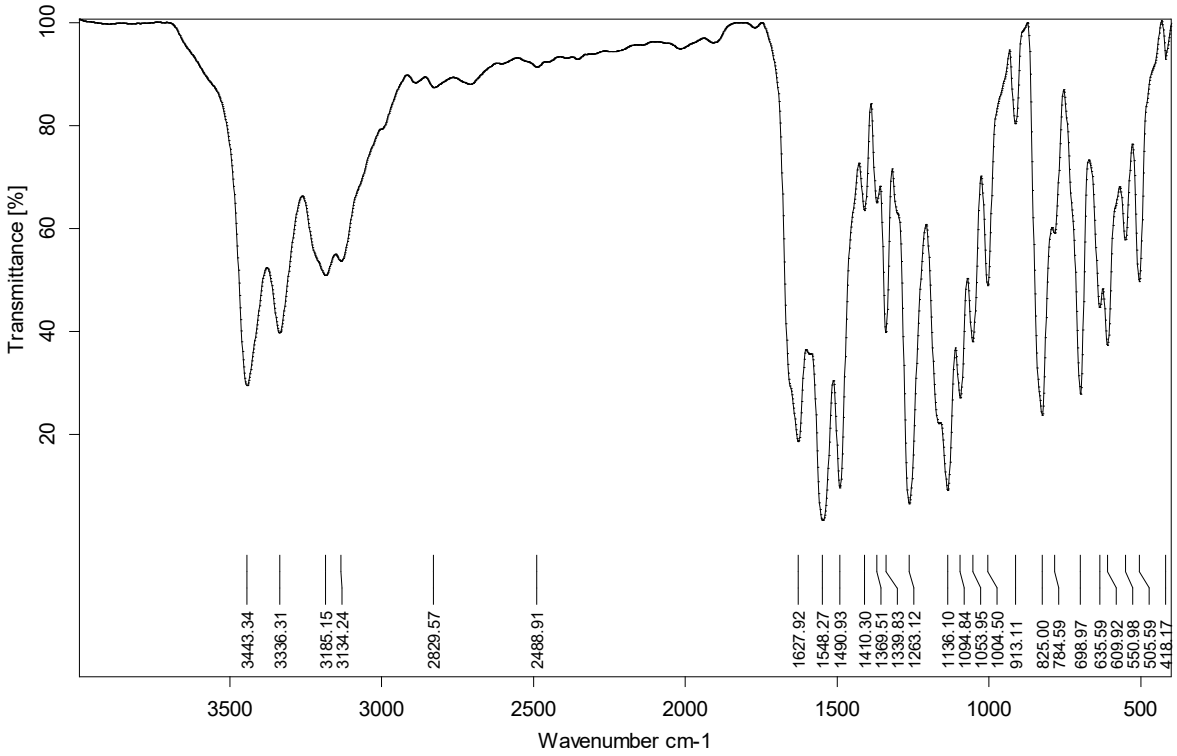

Figure S19. IR (KBr) of **7**.

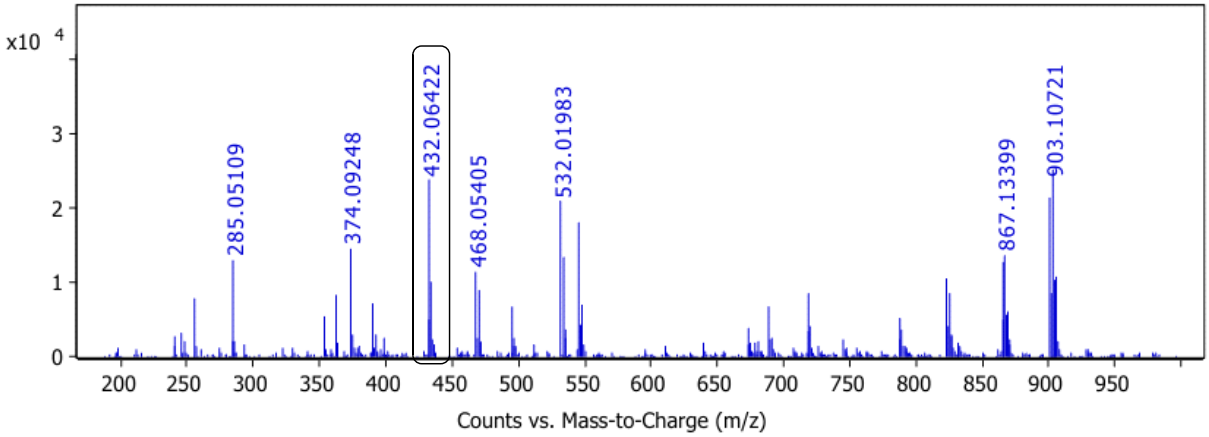

Figure S20. HRMS (ESI^-) of compound **7**.

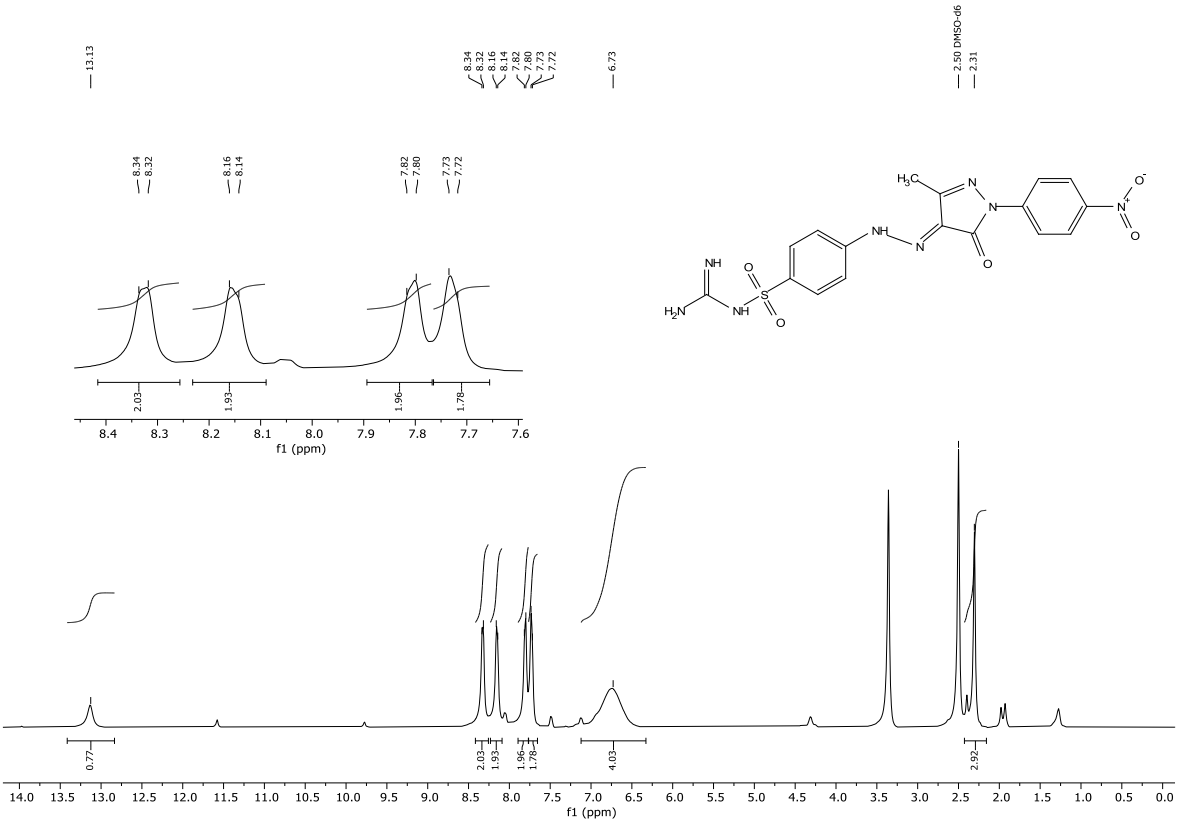

Figure S21. ^{1}H NMR of **8** (500 MHz, DMSO-d_6).

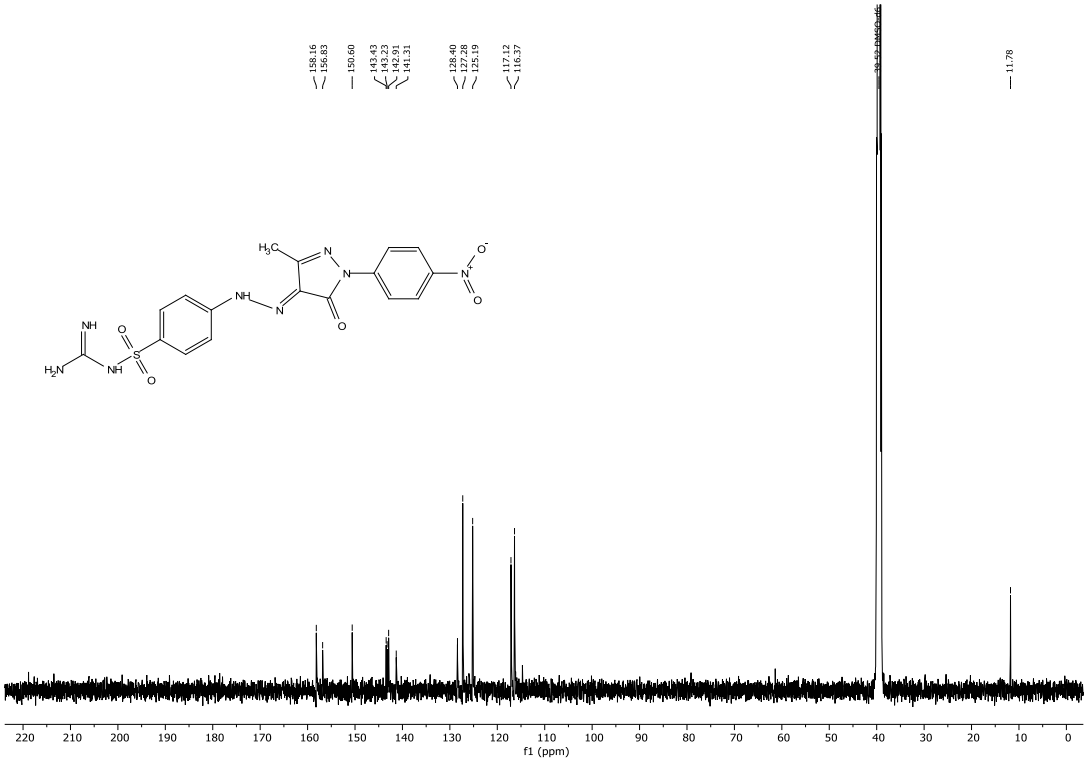

Figure S22. ^{13}C NMR of **8** (500 MHz, DMSO-d_6).

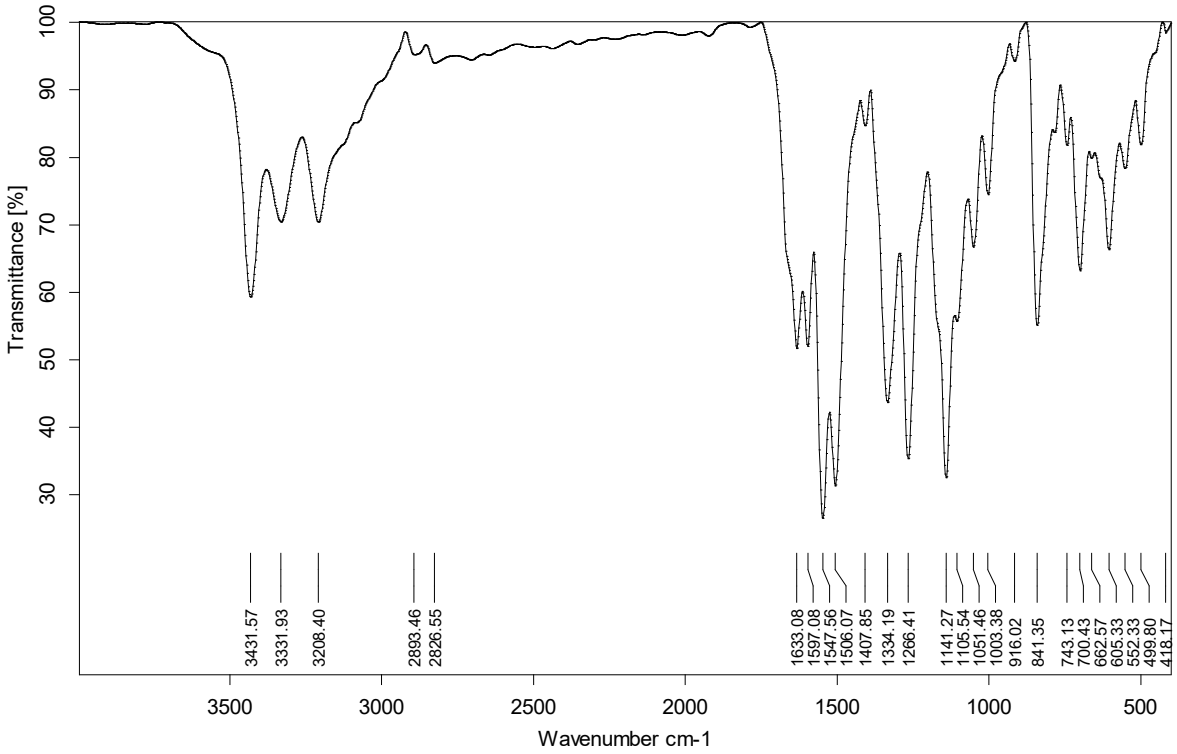

Figure S23. IR (KBr) of **8**.

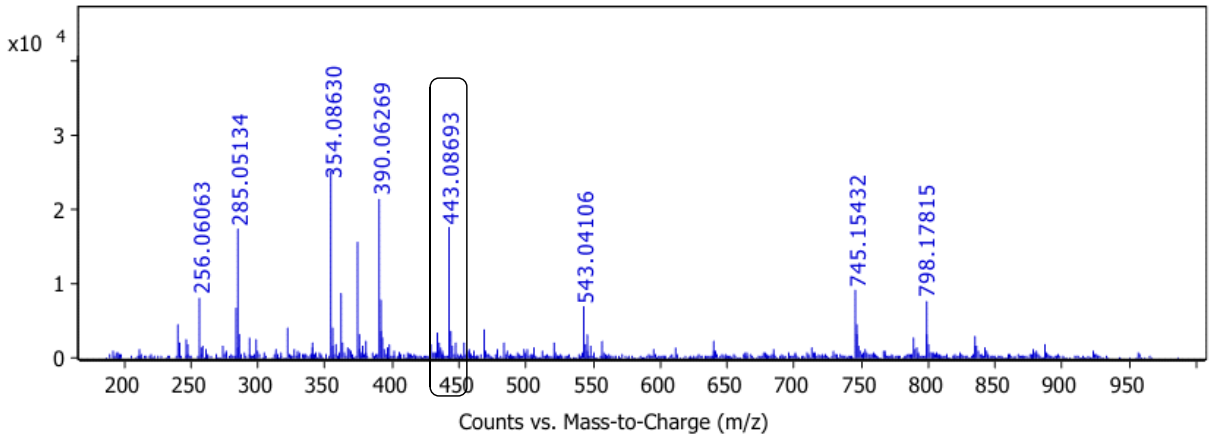

Figure S24. HRMS (ESI$^-$) of compound **8**.

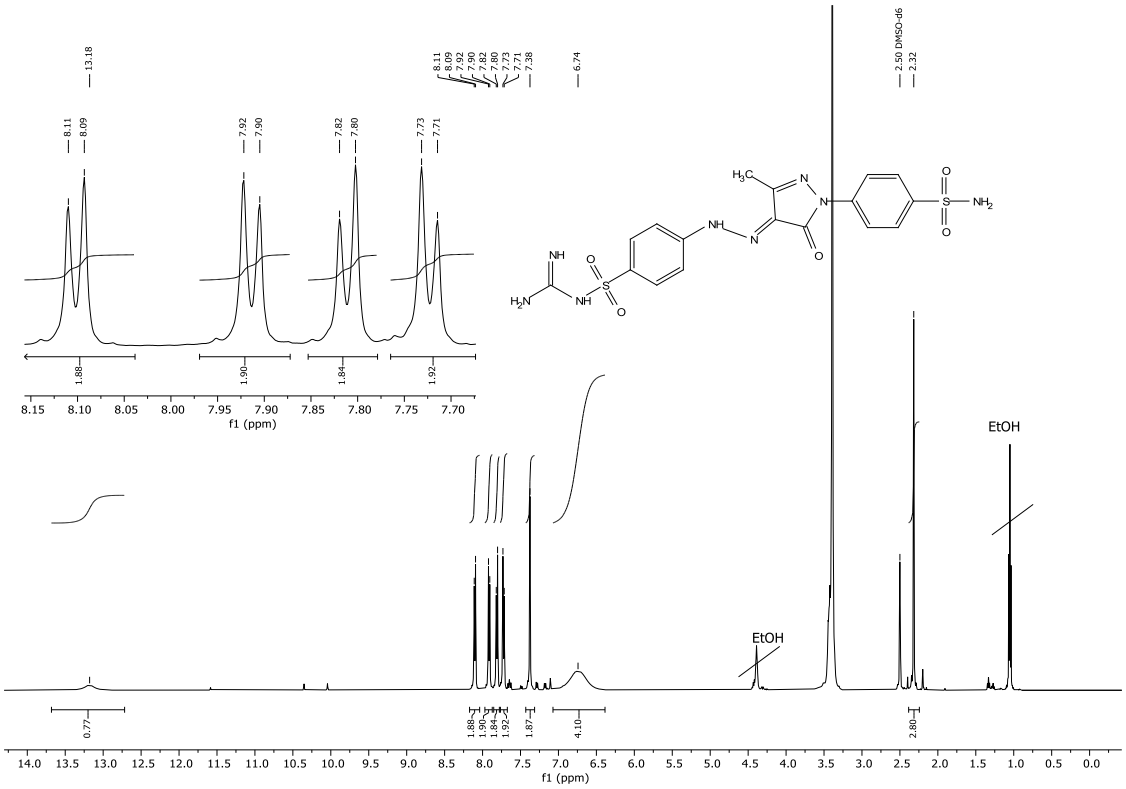

Figure S25. ^{1}H NMR of **9** (500 MHz, DMSO-d_6).

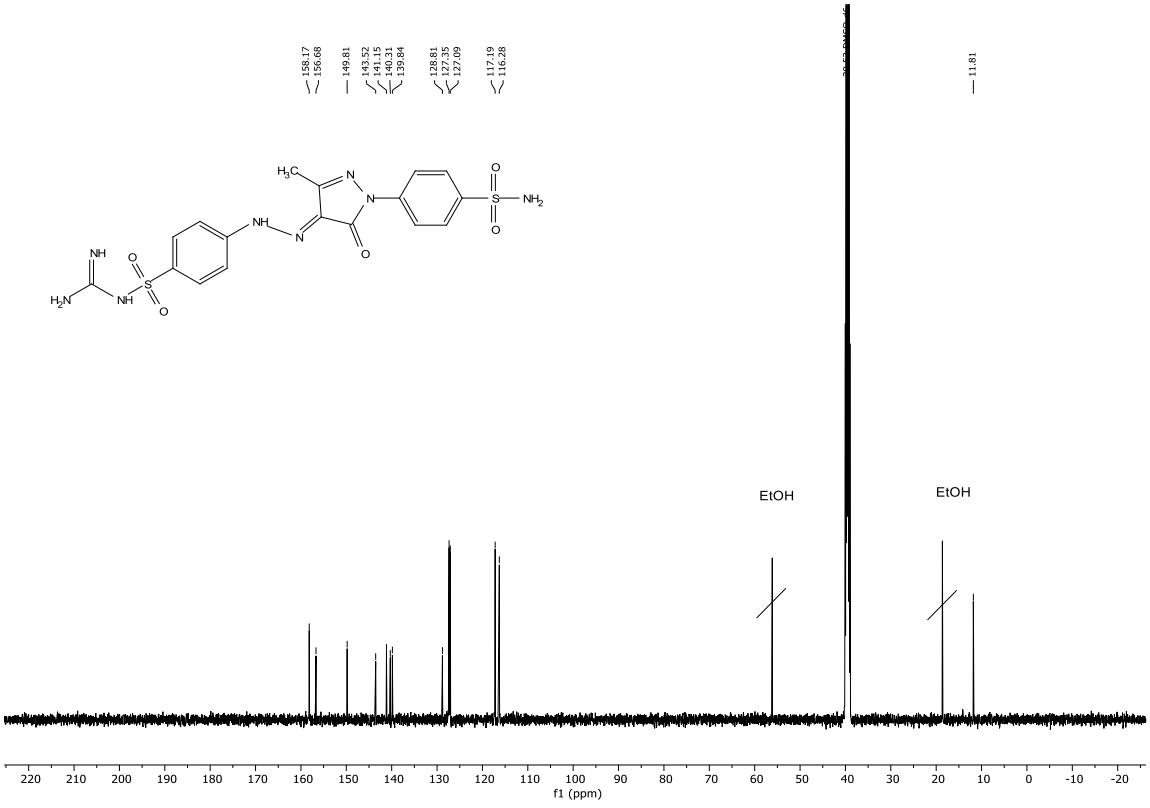

Figure S26. ^{13}C NMR of **9** (500 MHz, DMSO-d_6).

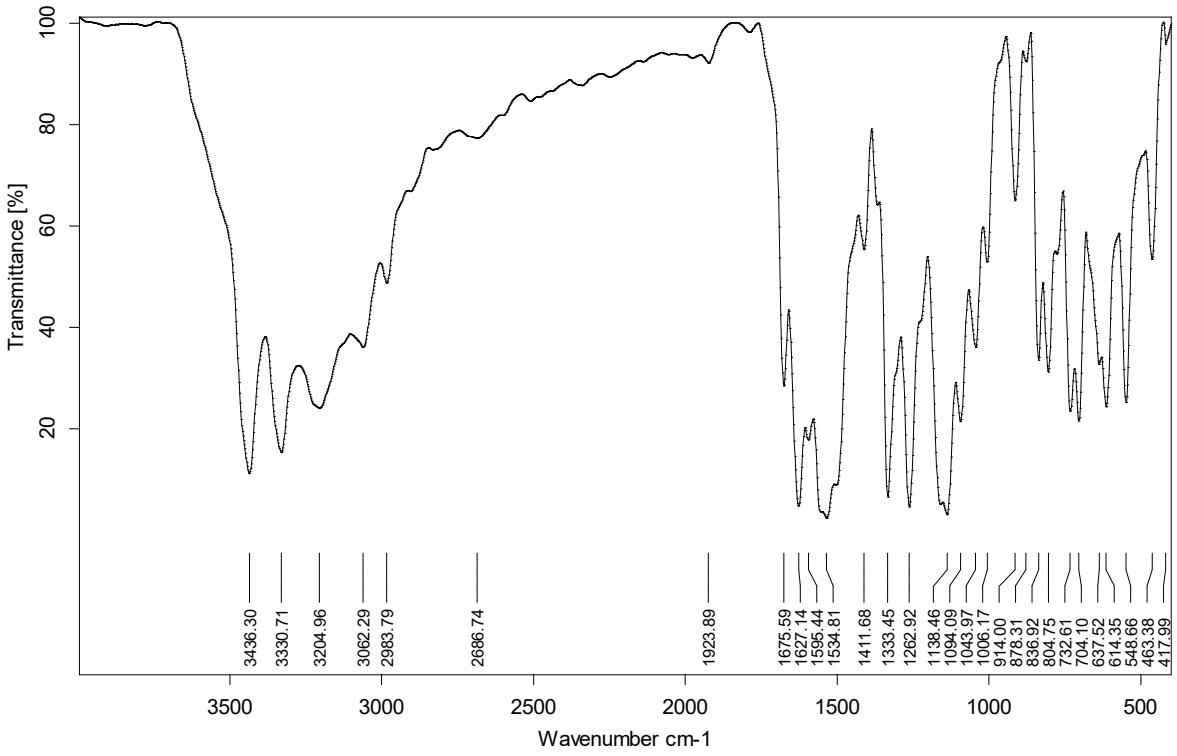

Figure S27. IR (KBr) of **9**.

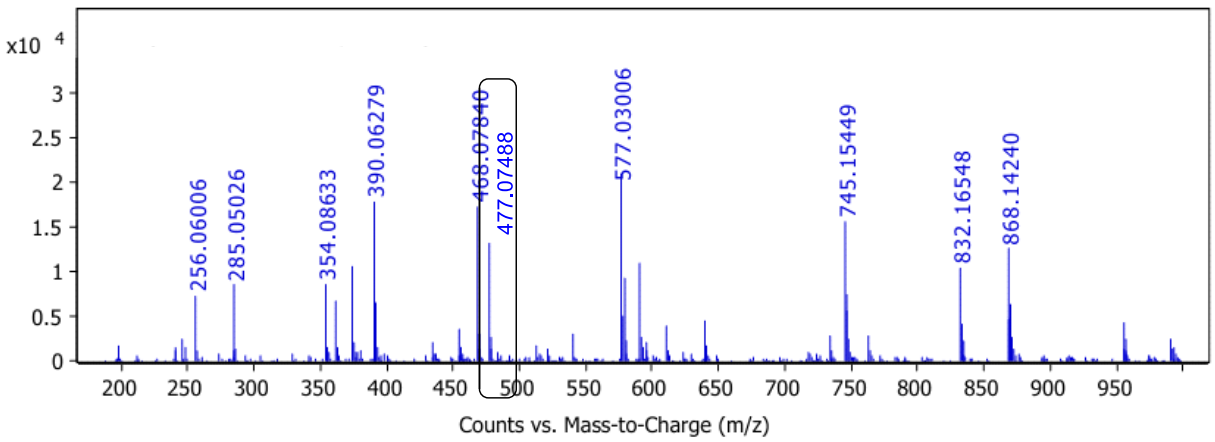

Figure S28. HRMS (ESI$^-$) of compound **9**.

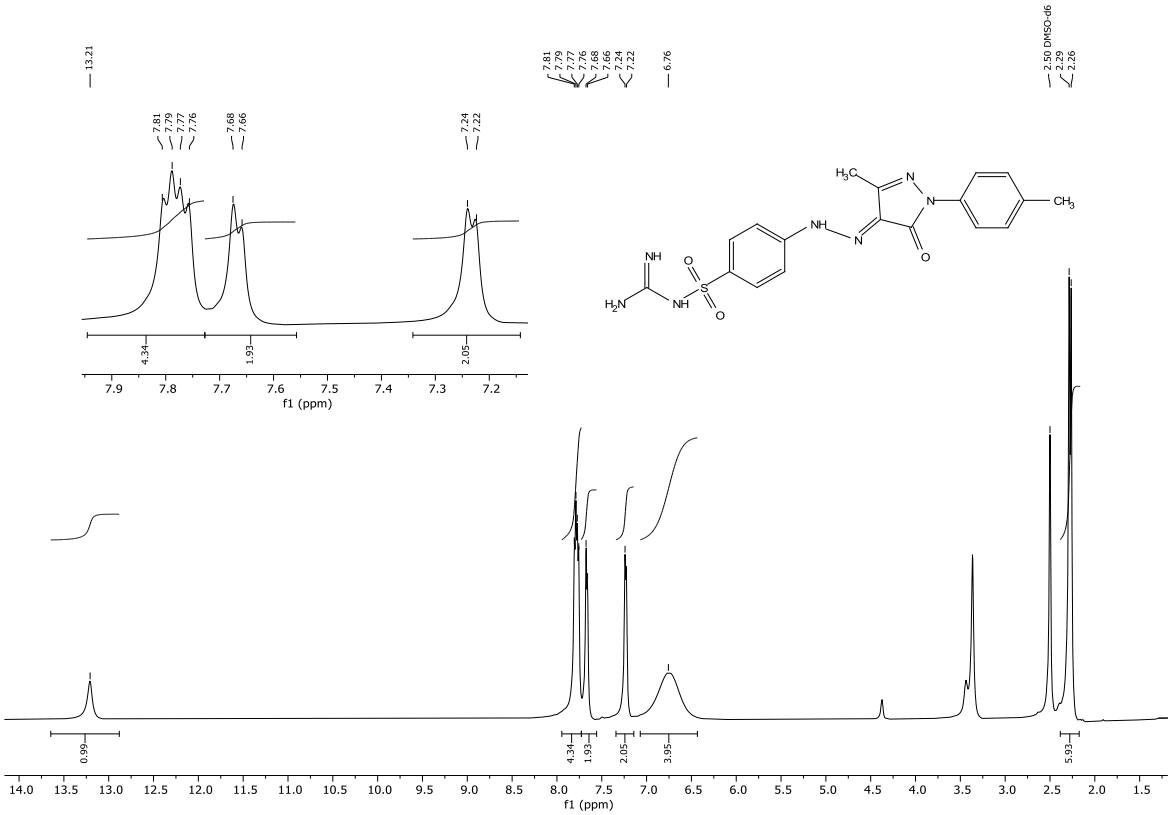

Figure S29. ^{1}H NMR of **10** (500 MHz, DMSO-d_6).

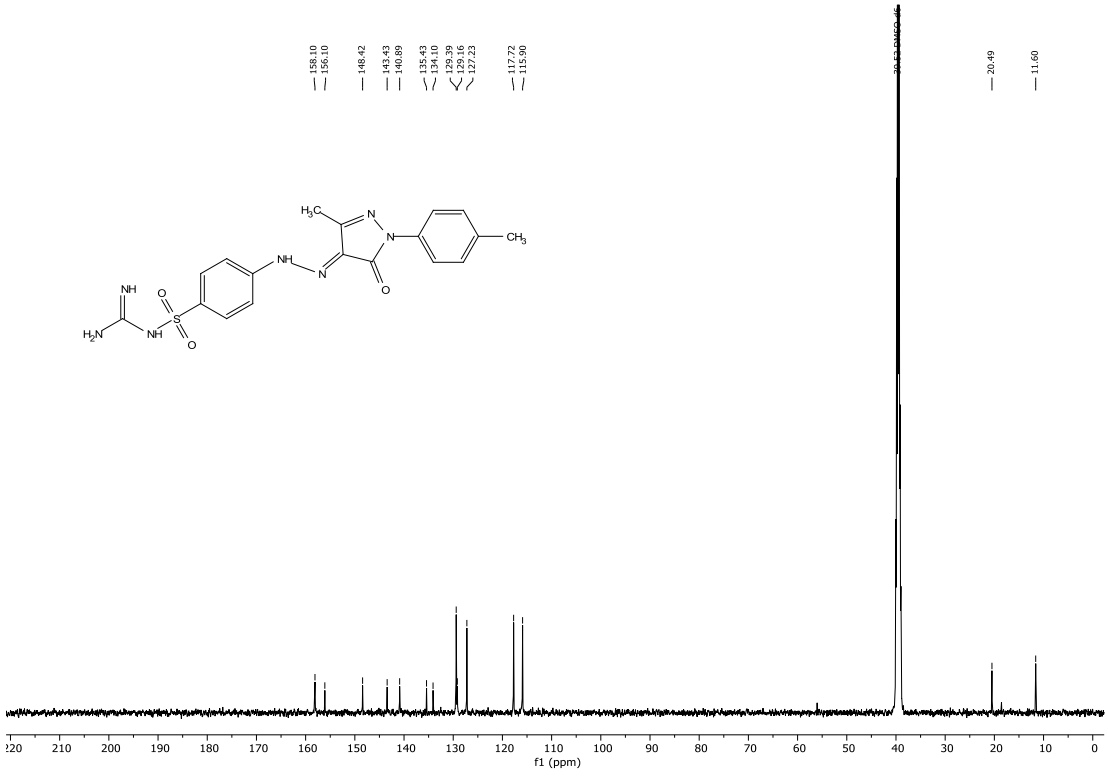

Figure S30. ^{13}C NMR of **10** (500 MHz, DMSO-d_6).

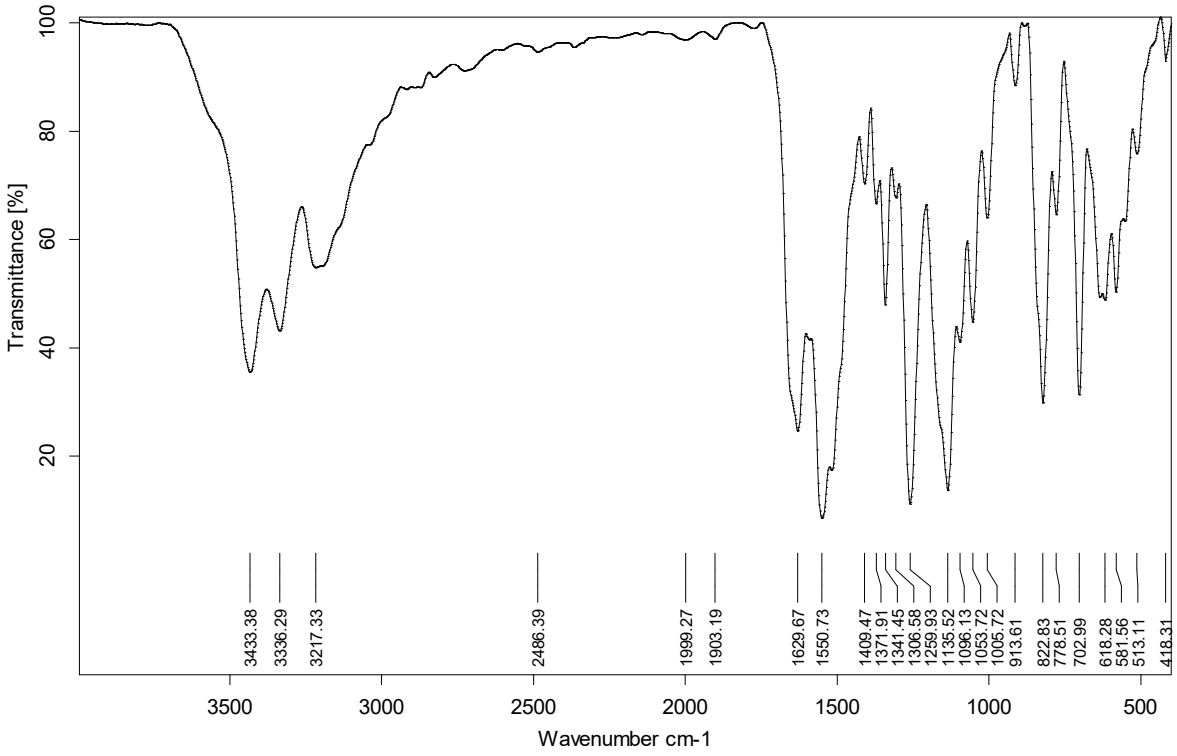

Figure S31. IR (KBr) of **10**.

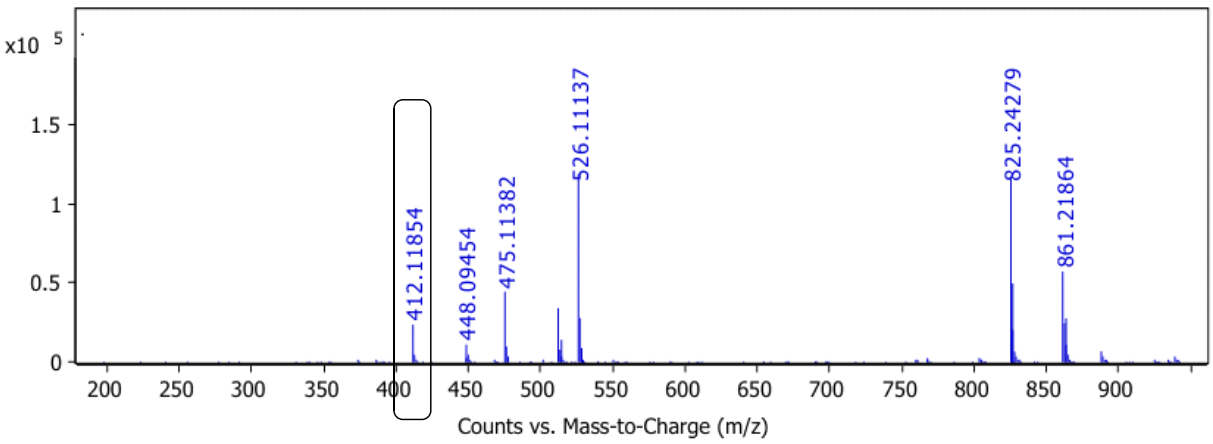

Figure S32. HRMS (ESI$^-$) of compound **10**.

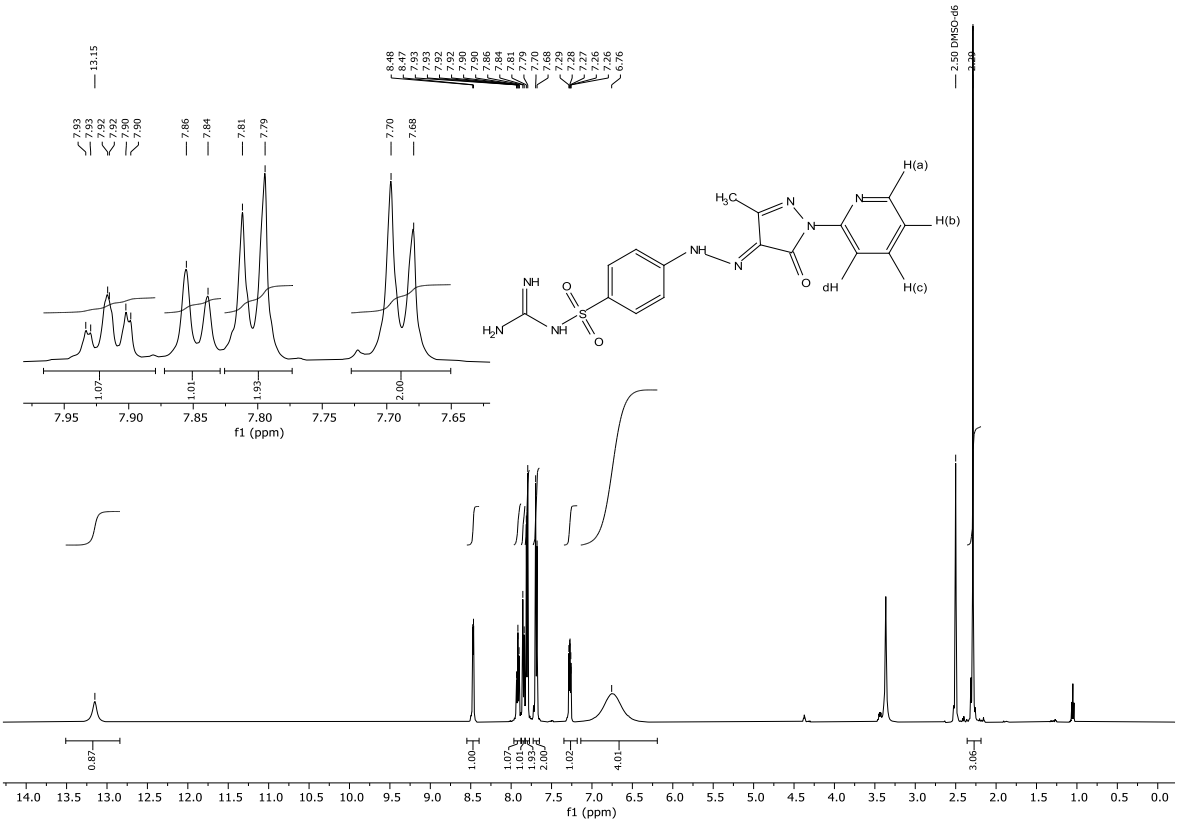

Figure S33. ^{1}H NMR of **11** (500 MHz, DMSO-d_6).

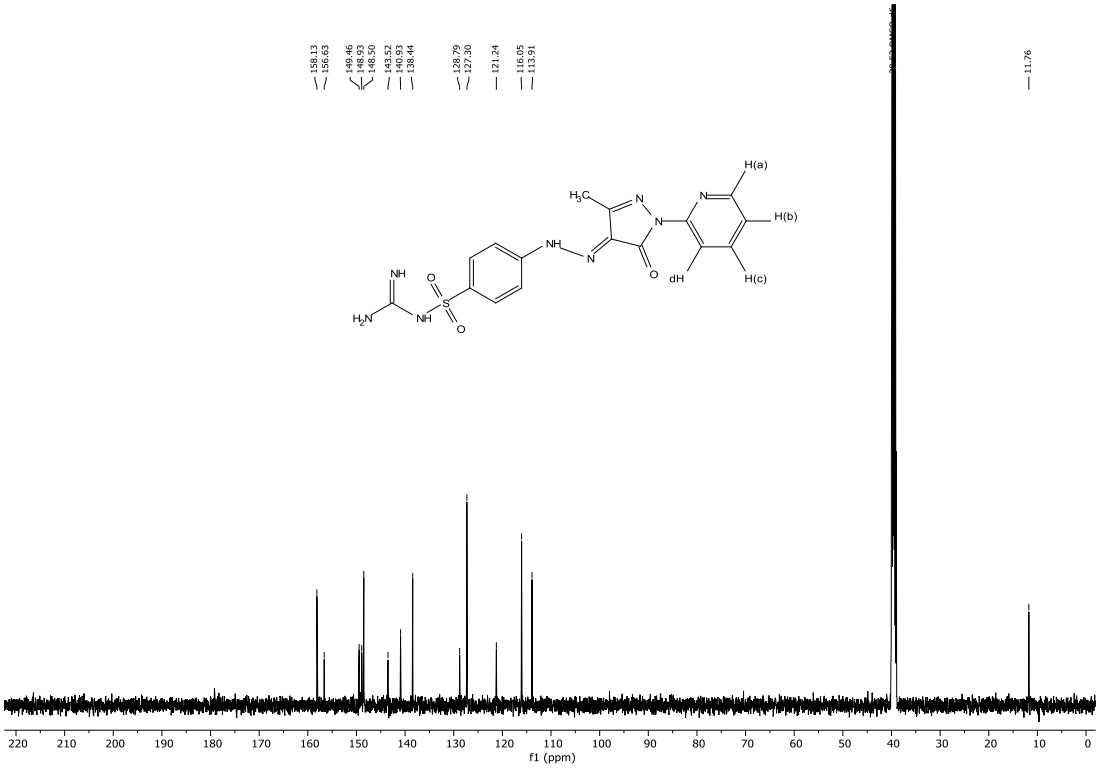

Figure S34. ^{13}C NMR of **11** (500 MHz, DMSO-d_6).

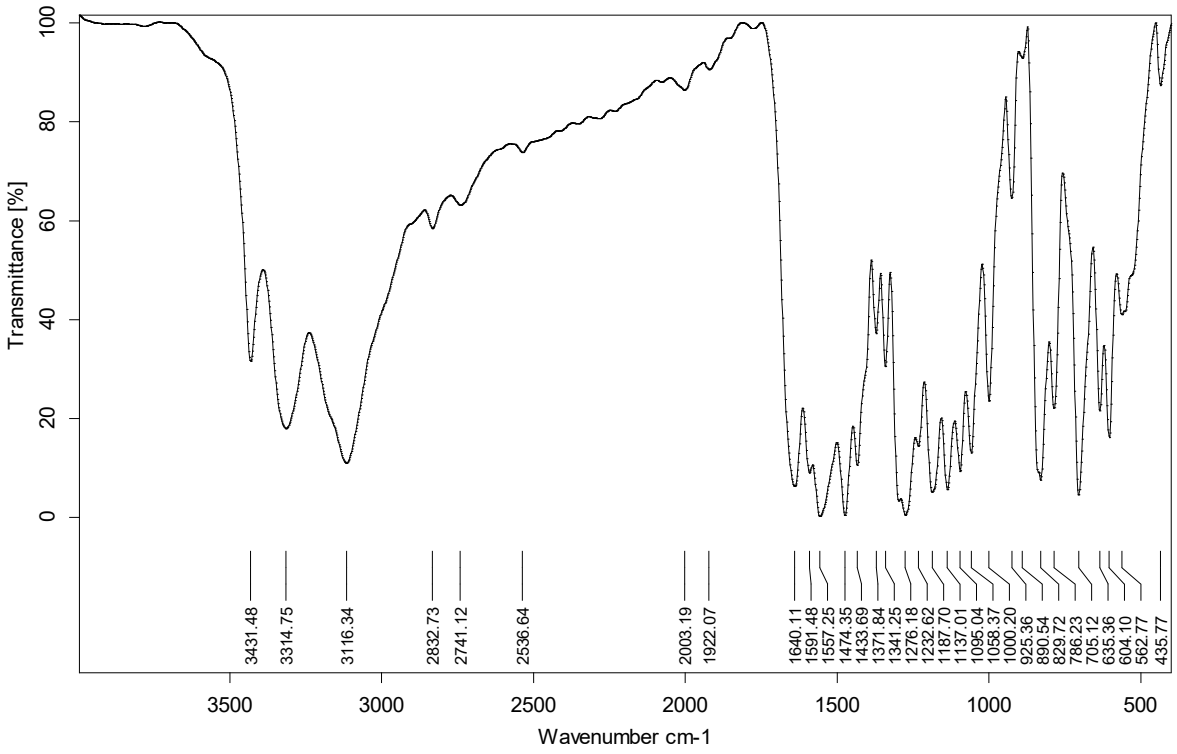

Figure S35. IR (KBr) of **11**.

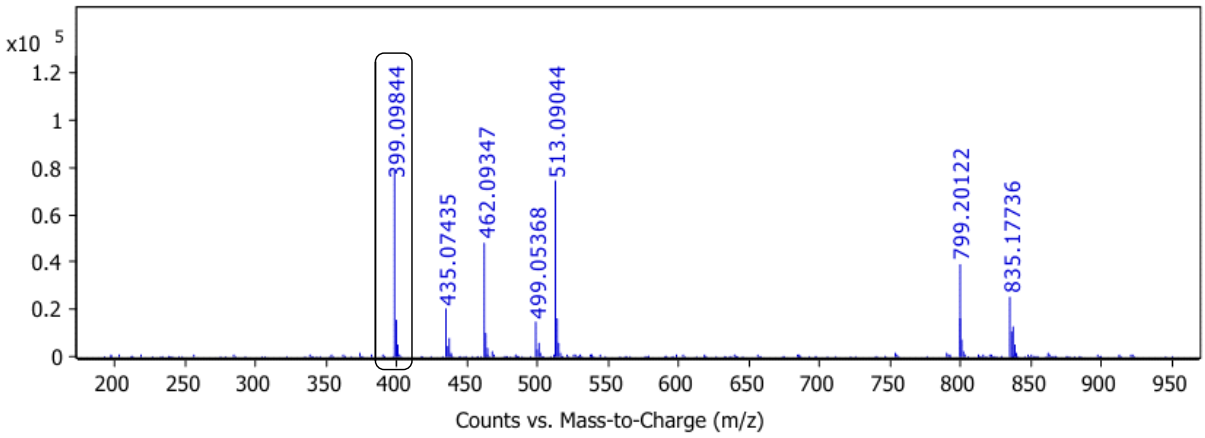

Figure S36. HRMS (ESI$^-$) of compound **11**.

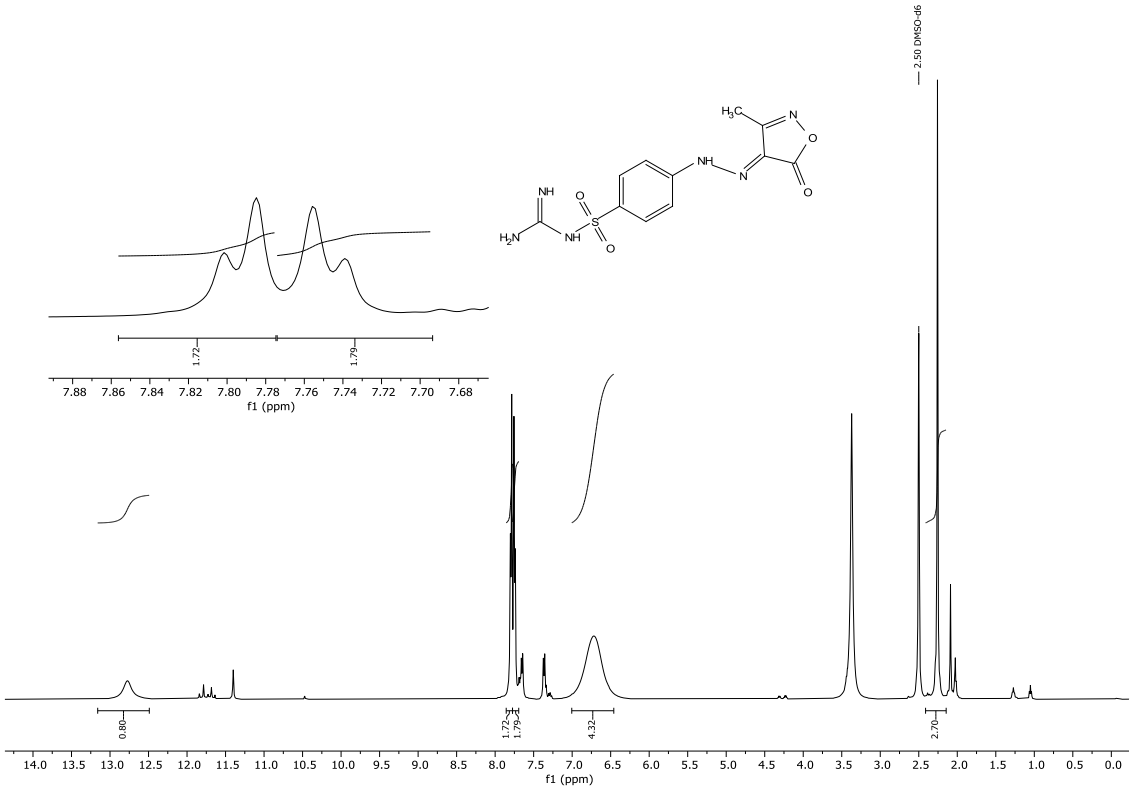

Figure S37. ^{1}H NMR of **12** (500 MHz, DMSO-d_6).

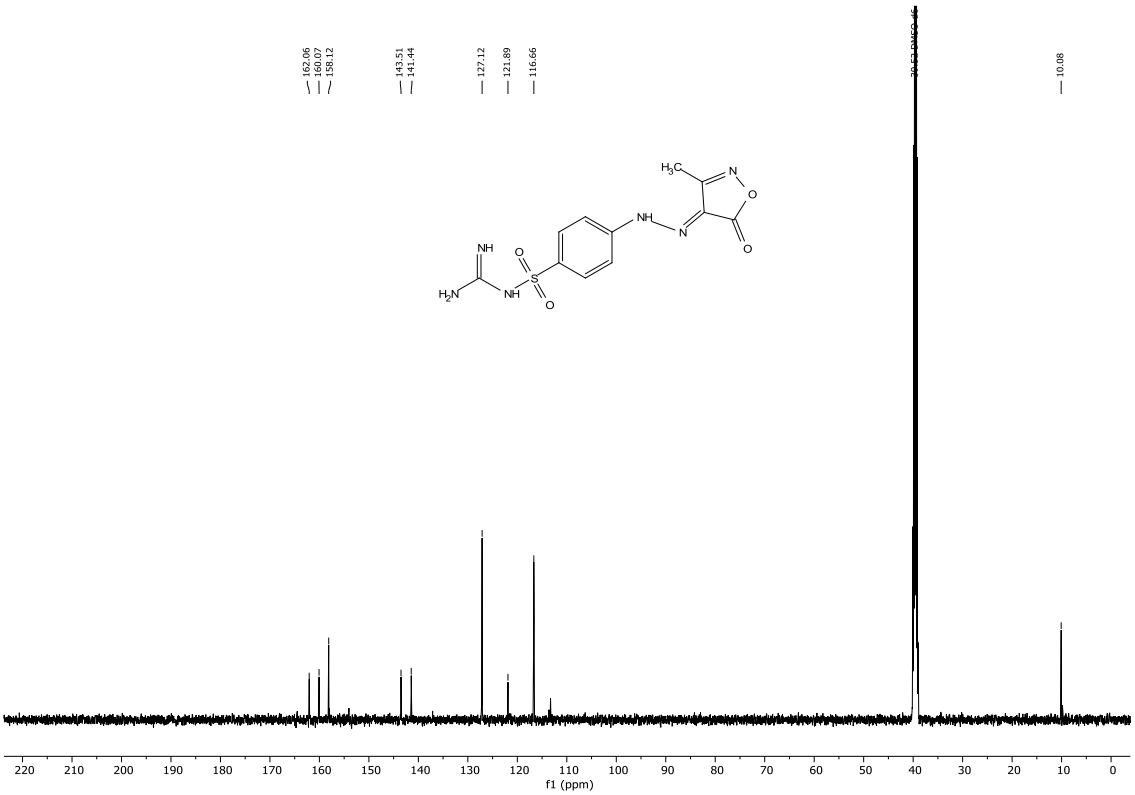

Figure S38. ^{13}C NMR of **12** (500 MHz, DMSO-d_6).

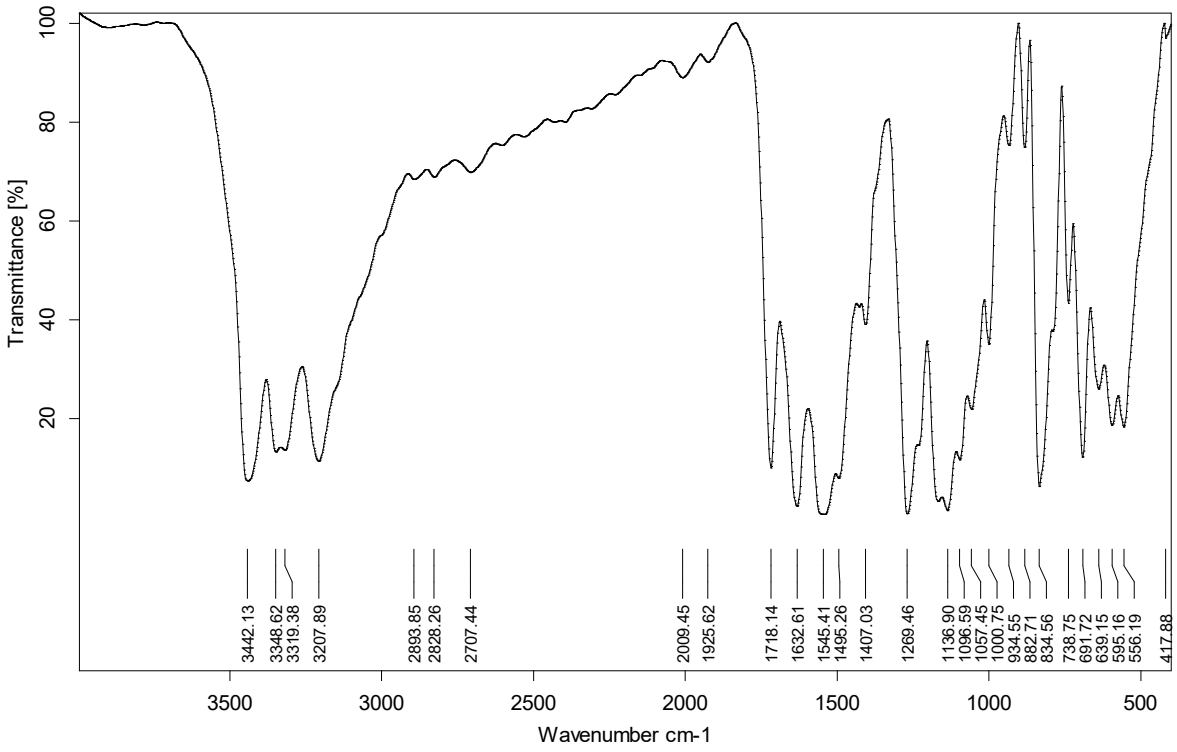

Figure S39. IR (KBr) of **12**.

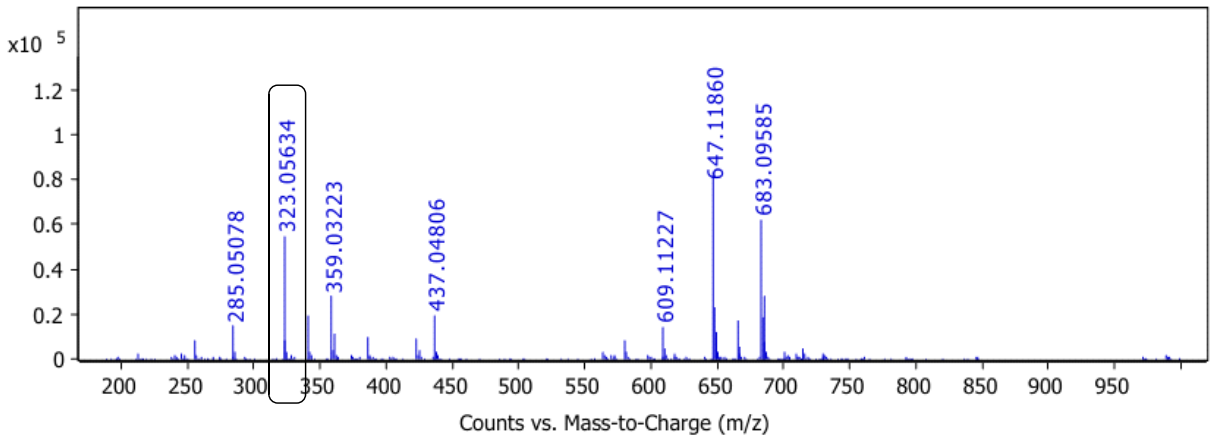

Figure S40. HRMS (ESI$^-$) of compound **12**.

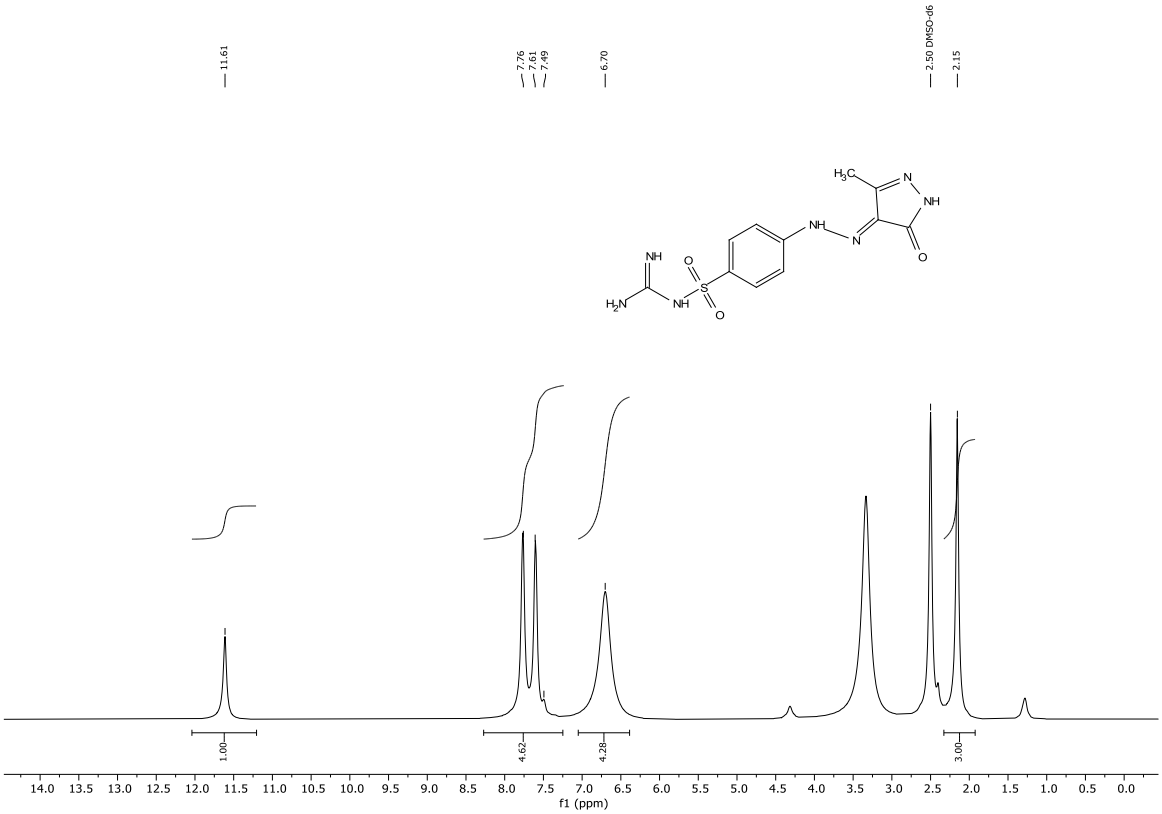

Figure S41. ^{1}H NMR of **13** (500 MHz, DMSO-d_6).

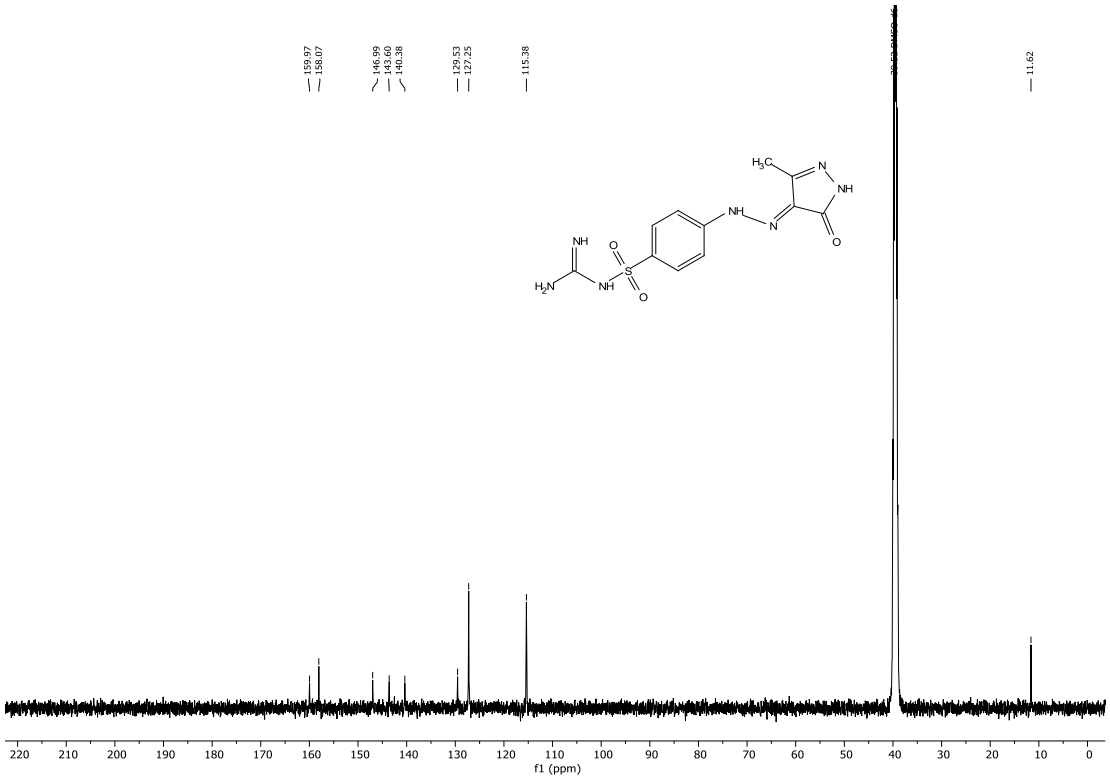

Figure S42. ^{13}C NMR of **13** (500 MHz, DMSO-d_6).

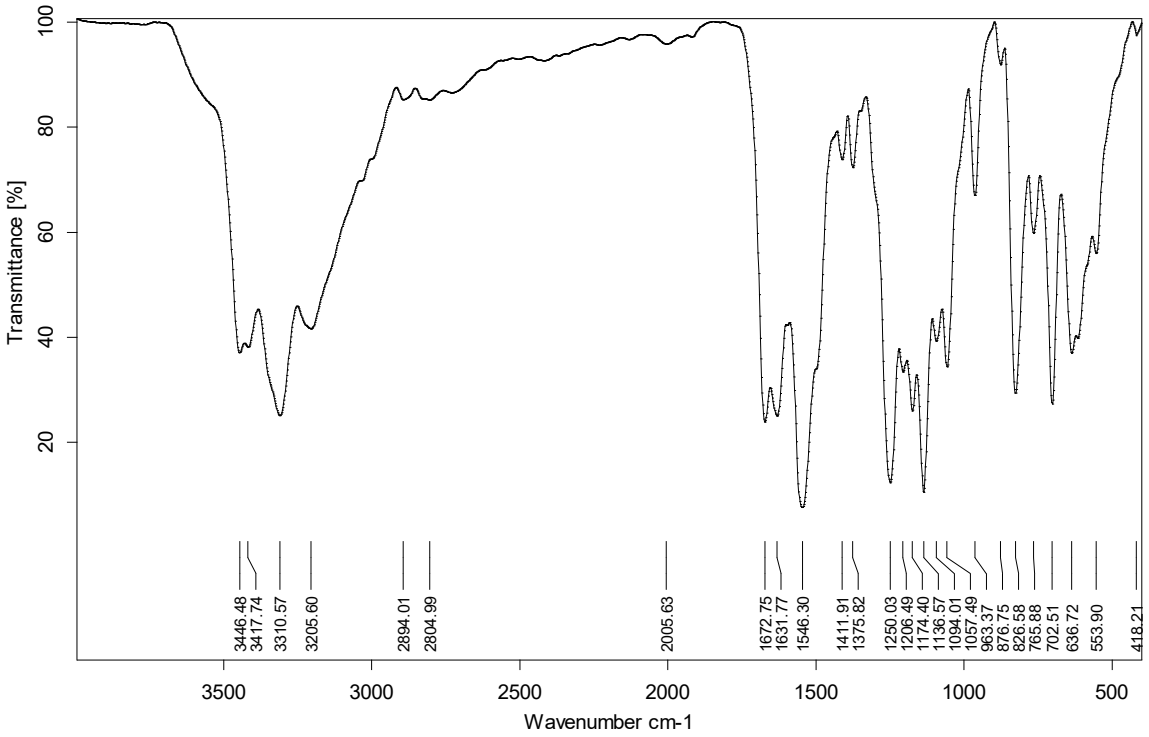

Figure S43. IR (KBr) of **13**.

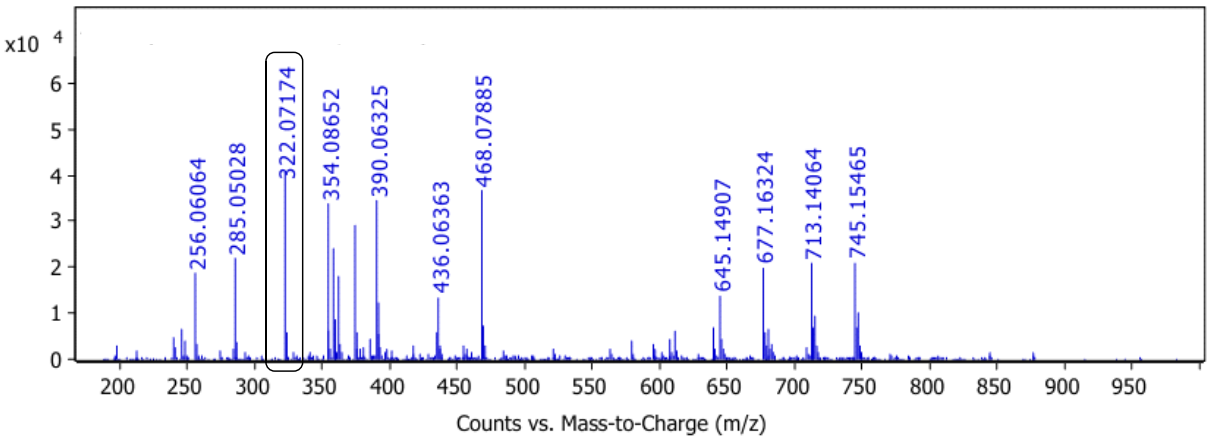

Figure S44. HRMS (ESI$^-$) of compound **13**.

2. Equipment and analytical technique

All reactions were carried out in dried glassware. NMR spectra were measured using a JEOL JNM-ECX 500 spectrometer. The deuterated solvent was used as an internal deuterium lock. ^{13}C NMR spectra were recorded using the UDEFT pulse sequence and broad-band proton decoupling at 125 MHz. All chemical shifts (δ) are stated in units of parts per million (ppm) and presented using TMS as the standard reference point. Melting points were recorded using Thermo Scientific, Model No. 1002D, 220-240V; 200 W; 50/60 Hz and are uncorrected. The reaction progress was monitored by TLC on Merck silica gel aluminum cards (0.2 mm thickness) with a fluorescent indicator at 254 nm. Visualization of the TLC during reaction monitoring was performed using a UV VILBER LOURMAT 4w-365 nm or 254 nm tube.

3. Biological procedures

Antidiabetic activity

4.3.1. Alpha-glucosidase inhibitory assay [1]

Principle:

α-glucosidase (EC.3.2.1.20) enzyme catalyzes the hydrolysis of maltose into glucose and water. In presence of glucose oxidase (GOD) enzyme, glucose and water undergo oxidation and reduction reaction yielding gluconic acid and hydrogen peroxide. Peroxidase (POD) enzyme catalyzes hydrogen peroxide to oxidize phenol and 4-aminoantipyrine (4-AAP) to form a red violet quinonimine dye.

Reagents:

- Phosphate buffer: (0.1 M at pH=7.4)
- Bovine pancreatin enzyme solution: (5 mg/0.5 ml in 0.1 M phosphate buffer at pH= 7.4 then dilute 0.5 ml in 5 ml phosphate buffer)
- Maltose substrate solution: (1% in distilled H_2O)
- Glucose kit reagent:
    1. Phosphate buffer (PB, 100 mM/l).
    2. Phenol (4 mM/l).
    3. 4-amino-antipyrine (AAP, 1 mM/l).
    4. Glucose oxidase (GOD, > 20 KU/l).
    5. Peroxidase (POD, >2 KU/l).
    6. Sodium azide (NaN_3, 8 mmol/l).

Procedure:

10 µl of the compound (test and test blank)/ DMSO (negative control) in microtiter plate wells were mixed with 110 µl of diluted pancreatin enzyme. Microtiter plate wells were incubated at 37°C for 30 min. After incubation, 60 µl of maltose then incubated at 37°C for 20 min and 100 µl of glucose kit

reagent were added to all wells (add 100 µl of phosphate buffer in test blank only). The absorbance was measured at 490 nm using spectrophotometer.

Calculation:

The α-glucosidase inhibition activity of the test compound was estimated from the following formula

Alpha-glucosidase inhibition activity of the test compound (%) = $(\frac{AC-AS}{AC})$ *100

Where;

A_C: The mean absorbance of negative control.

A_S: The mean absorbance of the test compound - the mean absorbance of the test blank.

100: Percentage of inhibition.

The α-glucosidase inhibition activity of the test compound was expressed as IC_{50}. IC_{50} value (mg/ml) is the inhibitory concentration at which 50% of α-glucosidase are repressed. It was calculated by interpolation from the graph of inhibition percentage against sample concentration using linear regression equations (for test compound, y= ax+b and R^2)

Where;

Y= 50.

a: Slope of linear regression equation.

x: IC_{50} value of the test compound (ug/ml) at which 50% of α-glucosidase are repressed.

b: Intercept of linear regression equation.

R^2: Regression squared.

4.3.2. Alpha-amylase inhibitory assay [2]

Principle:

α-Amylase (EC 3.2. 1.1) enzyme catalyzes the hydrolysis of dextrin into glucose and water. In presence of glucose oxidase (GOD) enzyme, glucose and water undergo oxidation and reduction reaction yielding gluconic acid and hydrogen peroxide. Peroxidase (POD) enzyme catalyzes hydrogen peroxide to oxidize phenol and 4-aminoantipyrine (4-AAP) to form a red violet quinonimine dye.

Preparations:

- Phosphate buffer: (0.1 M at pH=6.9)
- α-Amylase enzyme solution: (5 mg/0.5 ml in 0.1 M phosphate buffer at pH= 6.9 then dilute 0.5 ml in 5 ml phosphate buffer)
- Dextrin substrate solution: (1% in distilled H_2O)
- Glucose kit reagent:
  1. Phosphate buffer (PB, 100 mM/l).
  2. Phenol (4 mM/l).
  3. 4-amino-antipyrine (AAP, 1 mM/l).
  4. Glucose oxidase (GOD, > 20 KU/l).
  5. Peroxidase (POD, >2 KU/l).
  6. Sodium azide (NaN_3, 8 mmol/l).

Procedure:

10 µl of the compound (test and test blank)/ DMSO (negative control) in microtiter plate wells were mixed with 110 µl of diluted amylase enzyme. Microtiter plate wells were incubated at 37°C for 30 min. After incubation, 60 µl of dextrin then incubated at 37°C for 20 min and 100 µl of glucose kit reagent were added to all wells (add 100 µl of phosphate buffer in test blank only). The absorbance was measured at 490 nm using spectrophotometer.

Calculation:

The α-amylase inhibition activity of the test compound was estimated from the following formula:

Alpha-amylase inhibition activity of the test compound (%) = $(\frac{AC-AS}{AC})$ *100

Where;

A_C: The mean absorbance of negative control.

A_S: The mean absorbance of the test compound - the mean absorbance of the test blank.

100: Percentage of inhibition.

The α-amylase inhibition activity of the test compound was expressed as IC_{50}. IC_{50} value (mg/ml) is the inhibitory concentration at which 50% of α-amylase are repressed. It was calculated by interpolation from the graph of inhibition percentage against sample concentration using linear regression equations (for test compound, y= ax+b and R^2)

Where;

Y= 50.

a: Slope of linear regression equation.

x: IC_{50} value of the test compound (ug/ml) at which 50% of α-amylase are repressed.

b: Intercept of linear regression equation.

R^2: Regression squared.

.4.3.3. Glucose uptake assay [3]

Principle:

Yeast uptakes glucose through glucose transporters with the help of the test compound then glucose was metabolized into glucose-6-phopshate. The remaining glucose was detected by glucose oxidase and Peroxidase kit reagent.

Preparations:

- Baker yeast
- Glucose solution (25 mM in distilled water)
- Glucose kit reagent:

1. Phosphate buffer (PB, 100 mM/l)
2. Phenol (4 mM/l).
3. 4-amino-antipyrine (AAP, 1 mM/l).
4. Glucose oxidase (GOD, > 20 KU/l).
5. Peroxidase (POD, >2 KU/l).
6. Sodium azide (NaN_3, 8 mmol/l).

Procedure:

Baker yeast was washed by centrifugation (3000 xg; 5min) using distilled water until the supernatant was clear and 10 % (v/v) suspension was prepared in distilled water. 1ml of the test compound added to 1 ml of glucose solution and incubated for 10 min at 37°C. 100 µl of yeast suspension was added and further incubated at 37°C for 60 min. All tubes were centrifuged (2500 xg, 5min). The remaining glucose was estimated by glucose oxidase Peroxidase kit reagent in sample/control tubes (phosphate buffer/DMSO were added in tubes instead of samples).

Calculation:

The glucose uptake of the test compound was estimated from the following formula:

The glucose uptake of the test compound (%) = $(\frac{AC-AS}{AC})$ *100

Where;

A_C: The mean absorbance of negative control.

A_S: The mean absorbance of the test compound - the mean absorbance of the test blank.

100: Percentage of inhibition.

The glucose uptake of the test compound was expressed as IC_{50}. IC_{50} value (mg/ml) is the inhibitory concentration at which 50% of glucose uptake are repressed. It was calculated by interpolation from the graph of inhibition percentage against sample concentration using linear regression equations (for the test compound, y= ax+b and R^2)

Where;

Y= 50.

a: Slope of linear regression equation.

x: IC_{50} value of the test compound (mg/ml) at which 50% of glucose uptake are repressed.

b: Intercept of linear regression equation.

R^2: Regression squared.

4. 2D diagram and 3D representation of molecular docking of all compounds in the binding pocket (PDB: 2QMJ)

Table 1: 2D diagram and 3D representation of molecular docking of all compounds in the binding pocket (PDB: 2QMJ).

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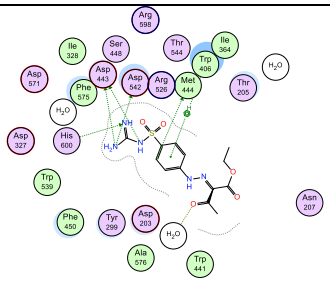
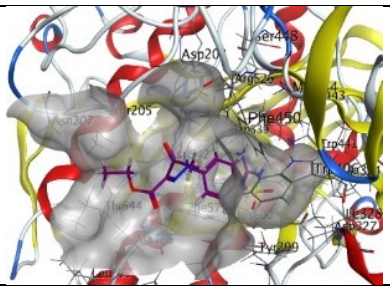
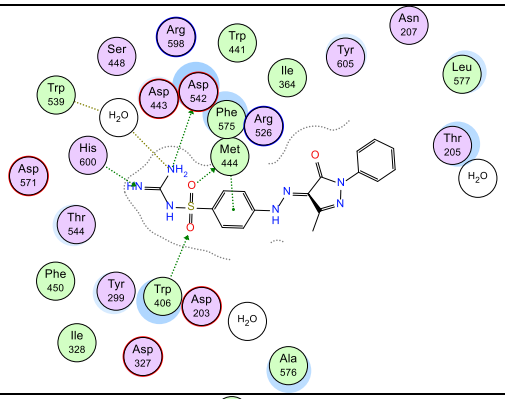
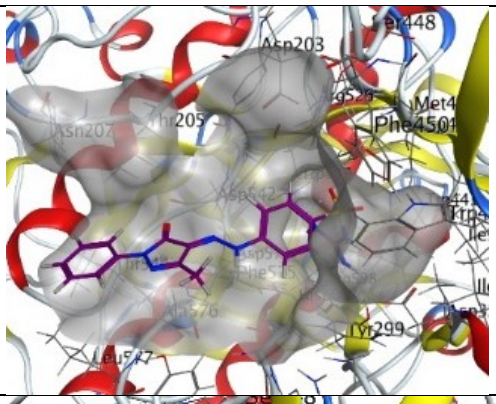
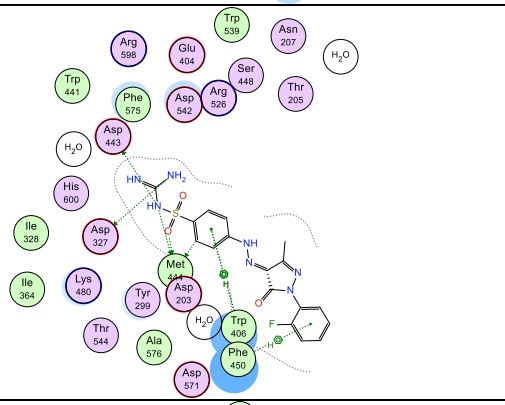
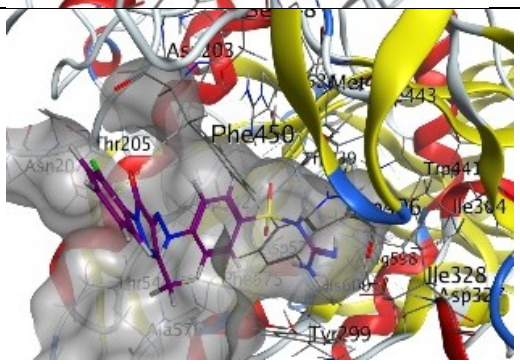
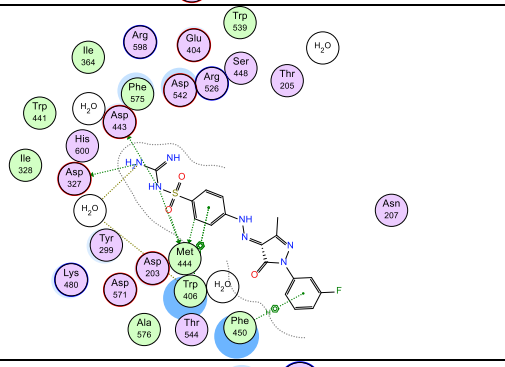
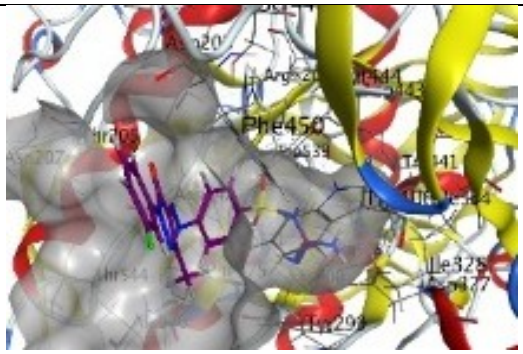
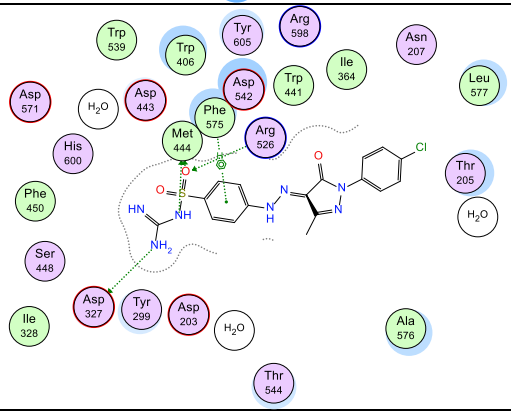
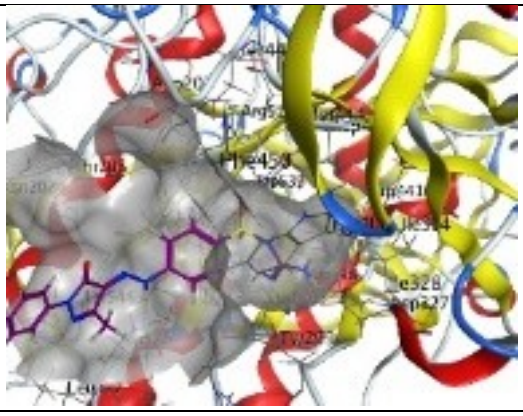
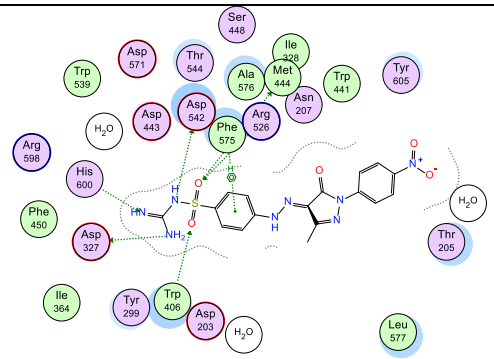
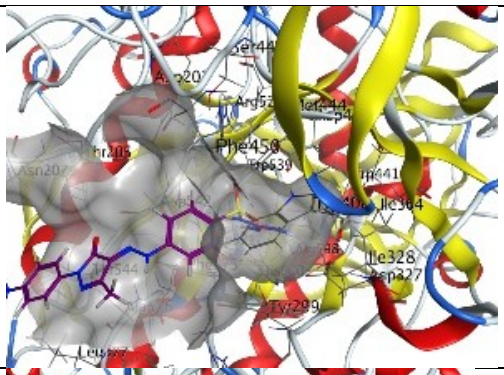
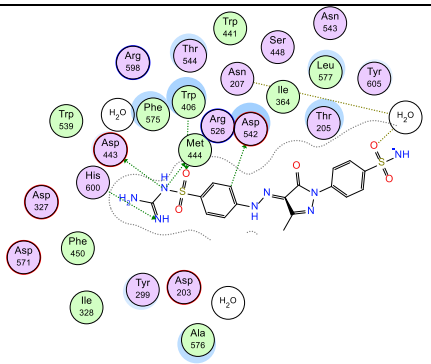
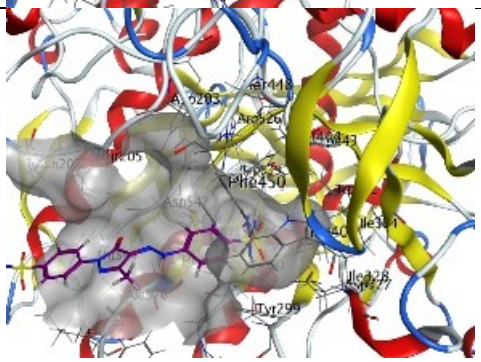
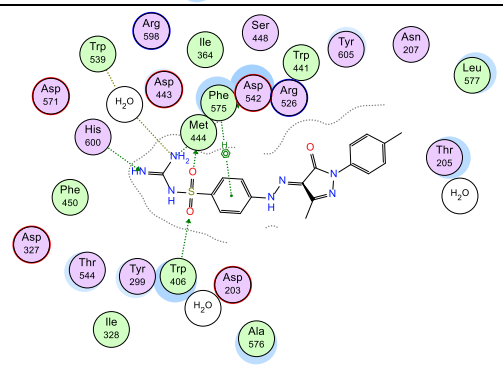
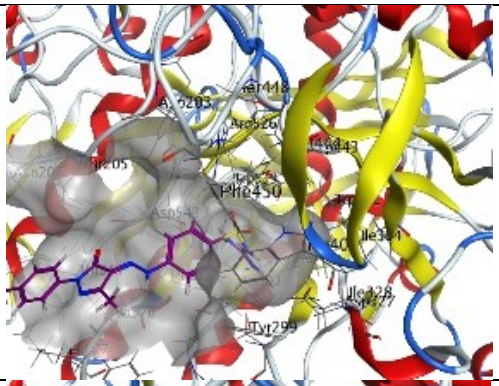
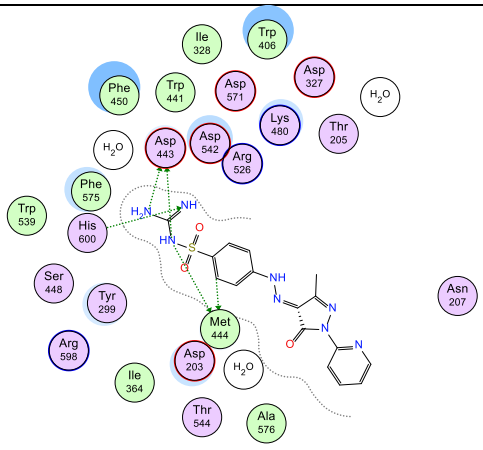
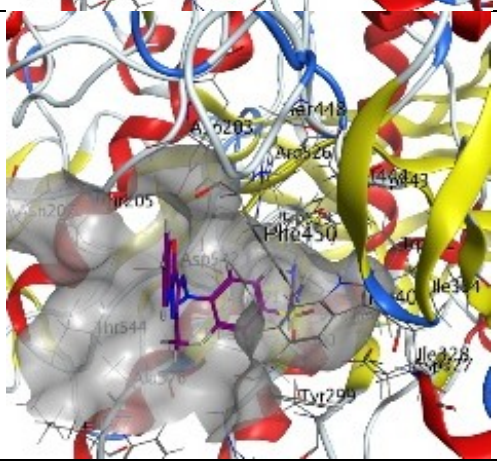
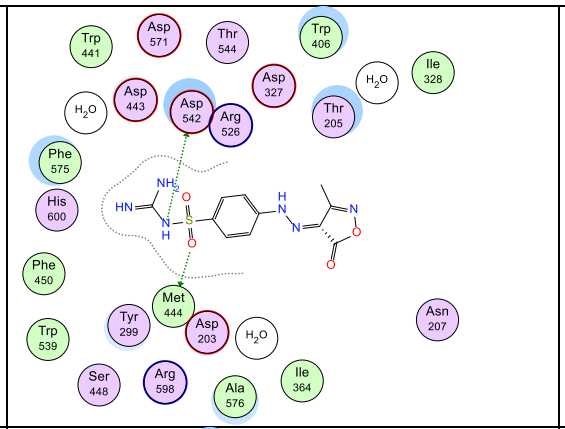
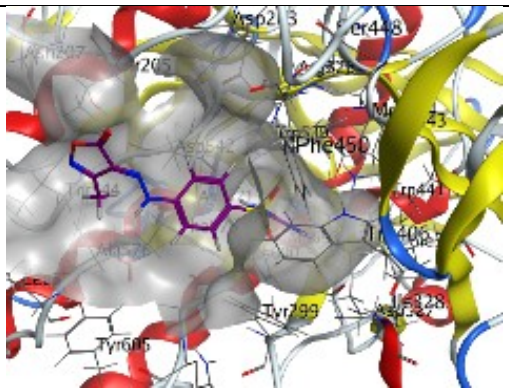
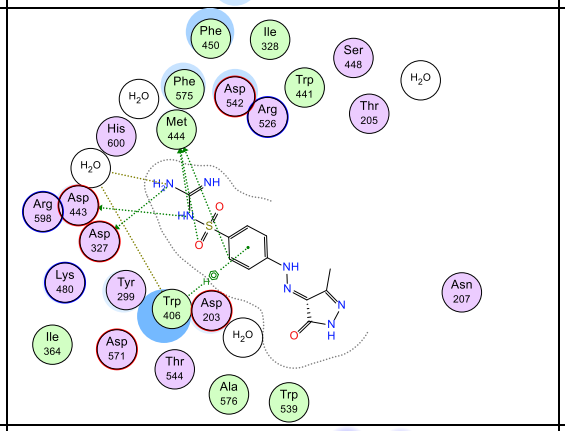
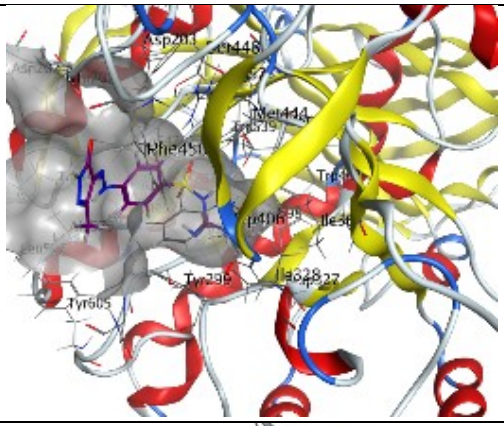
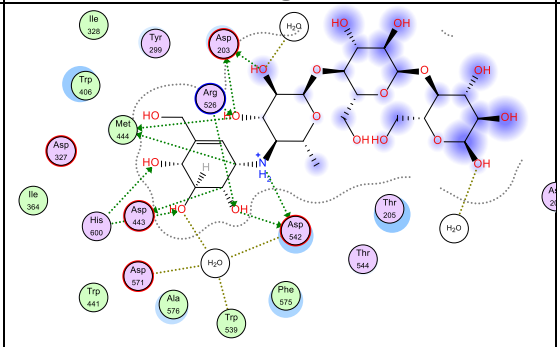
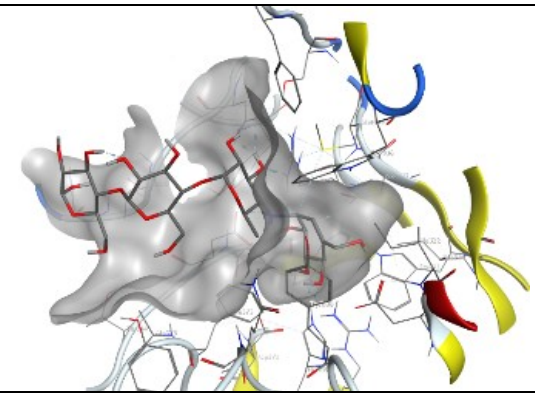
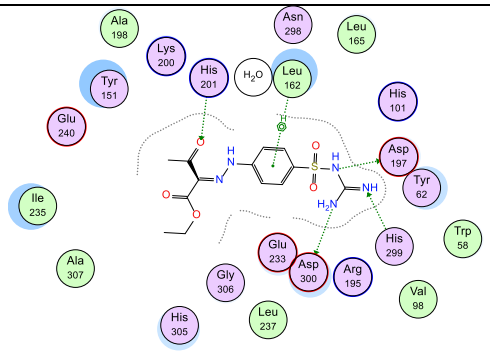
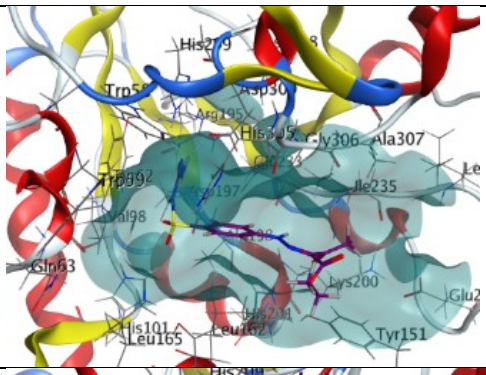
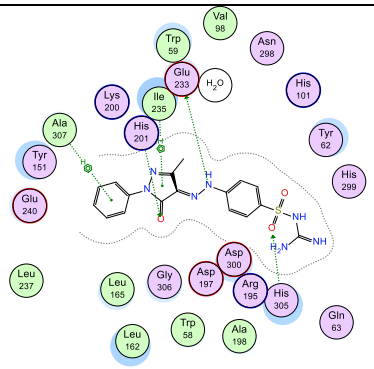
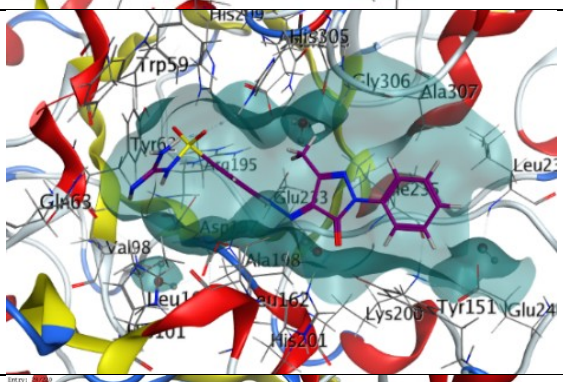
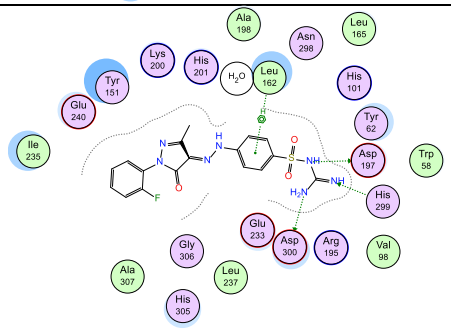
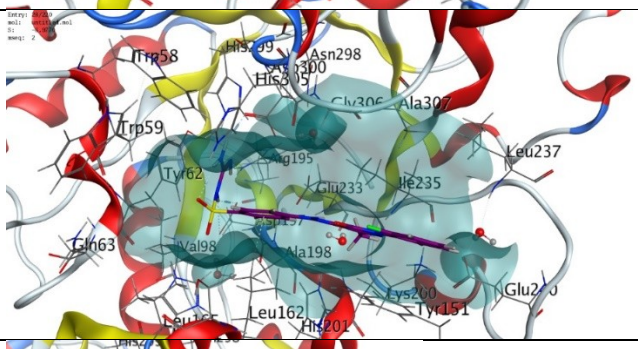
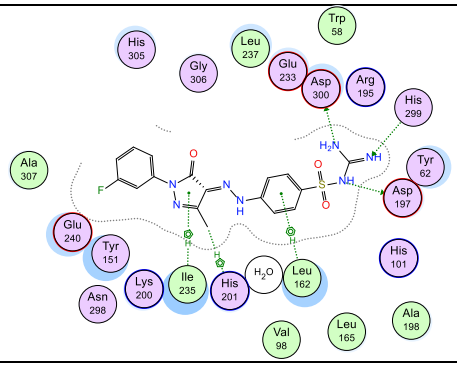
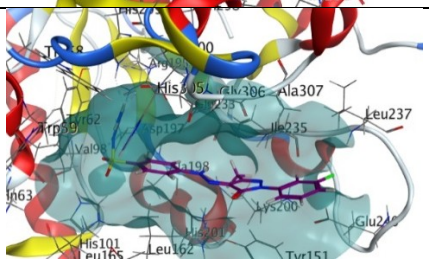
3		

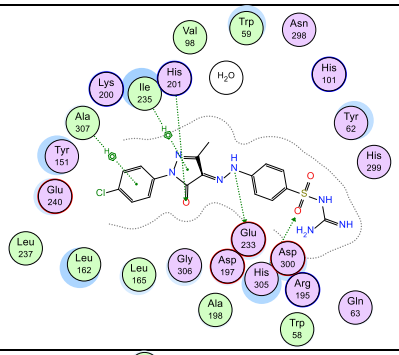
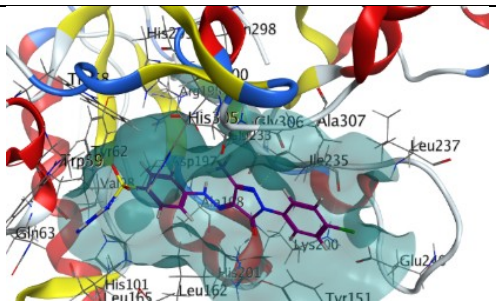
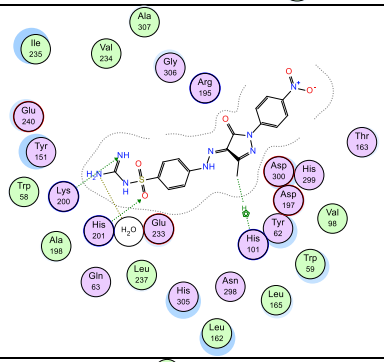
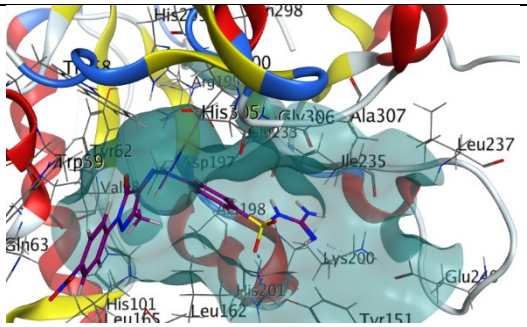
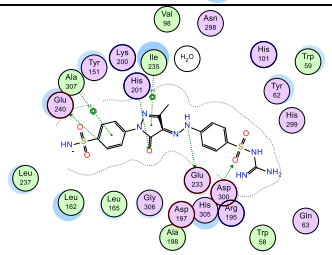
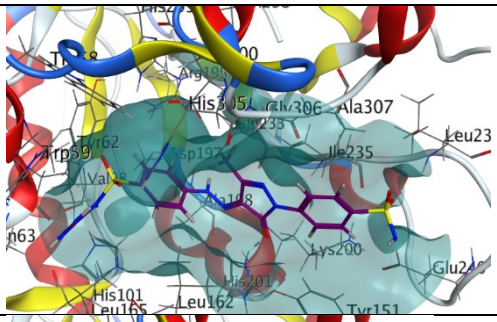
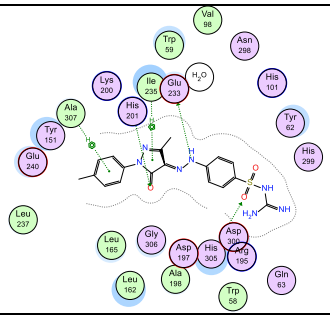
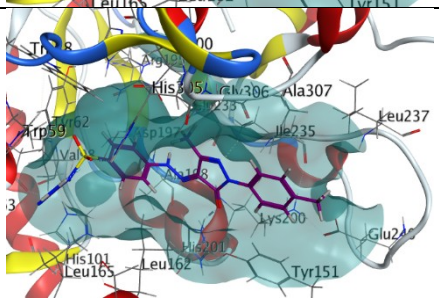
4		
5		
6		
7		

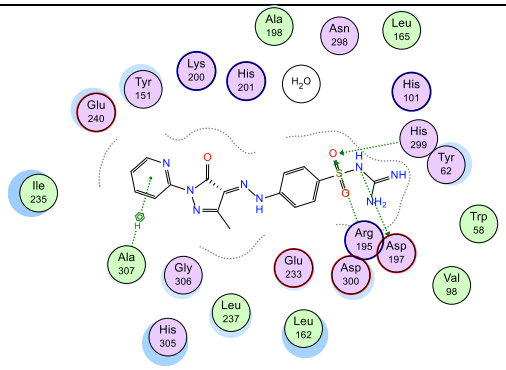
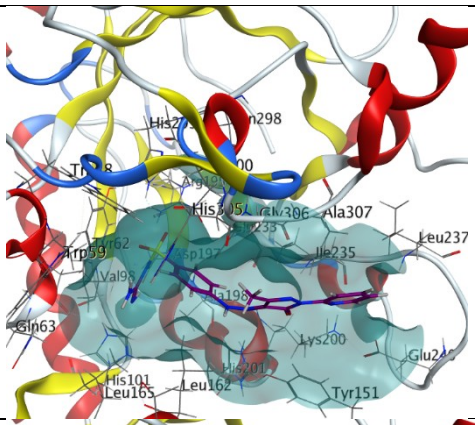
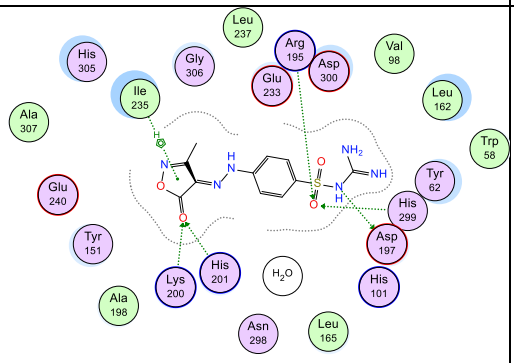
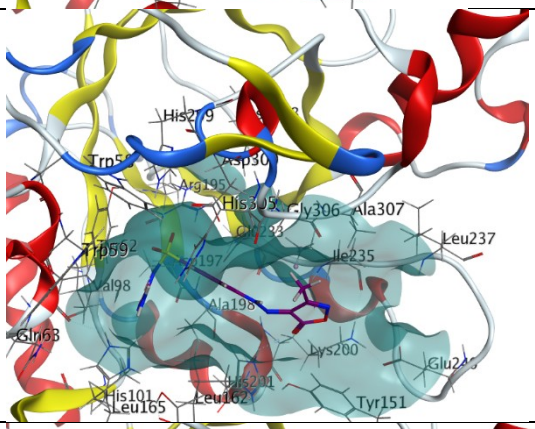
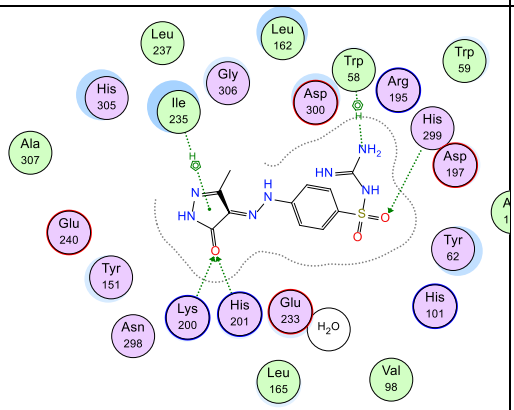
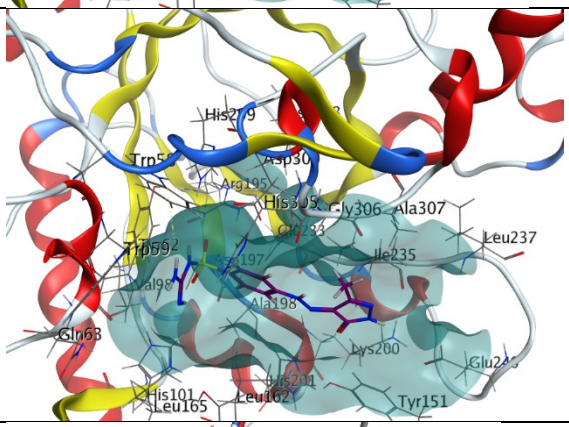
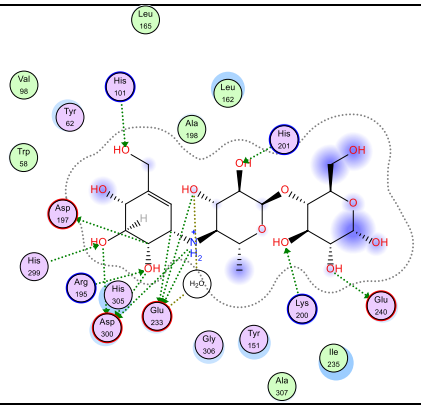
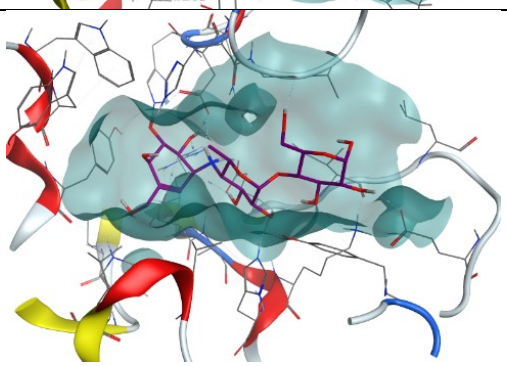
8		
9		
10		
11		

12		
13		
Acarbose		

Compound	**2D (PDB: 1XCW) (kcal/mol)**	**3D (PDB: 1XCW) (kcal/mol)**

3		
4		
5		
6		

7		
8		
9		
10		

11		
12		
13		
Acarbose		

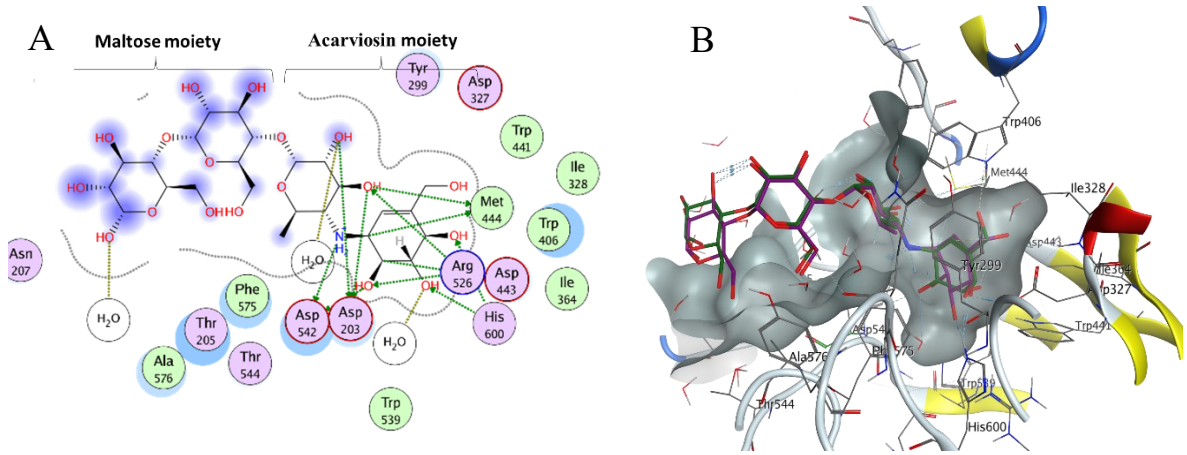


Figure S23. A) Two-dimensional diagram of acarbose binding with alpha-glucosidase binding pocket; B) Three-dimensional diagram of both the co-crystallized acarbose inhibitor (green) and its re-docked pose (magenta) in the active site of alpha-glucosidase showing RMSD (0.76 Å) within the acceptable range.

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