Design and Synthesis of 2-(4-bromophenyl)quinoline-4-carbohydrazide Derivatives *via* Molecular Hybridization as Novel Microbial DNA-gyrase Inhibitors

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Supplementary data

The supplementary data are divided into three parts;

1. **The molecular docking studies (pages S2-S10)**
2. **Spectral data (^{1}H-NMR, ^{13}C-NMR and MS) of all synthesized compounds (pages S11-S55)**
3. **Biological Studies (pages S56-S62)**

Part 1: Molecular docking studies:

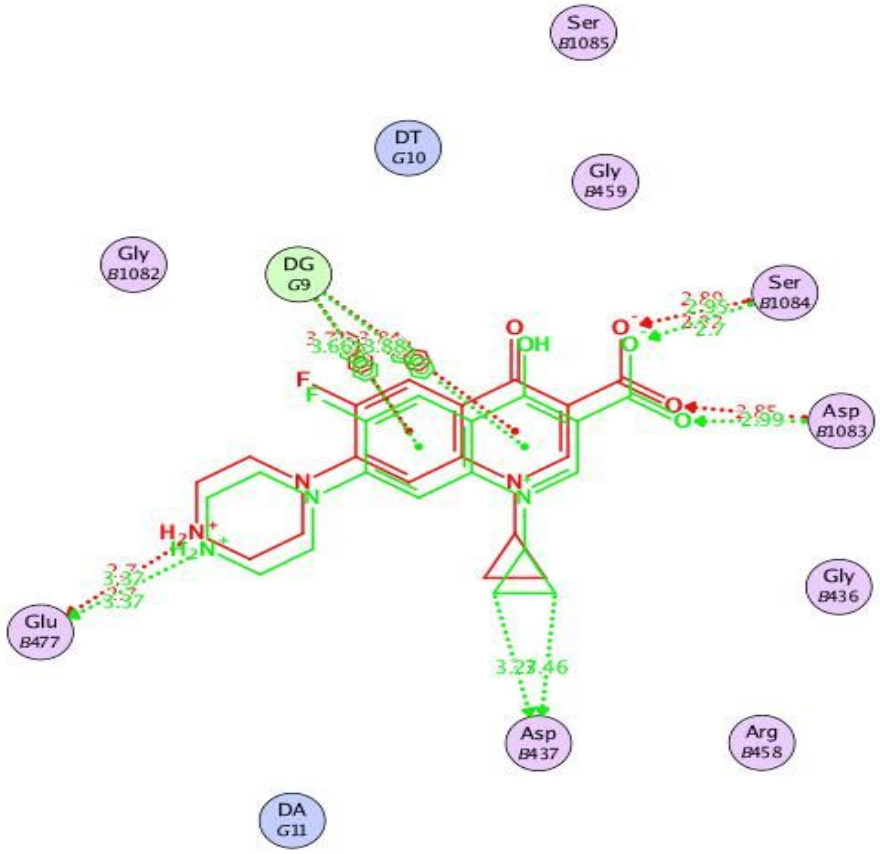
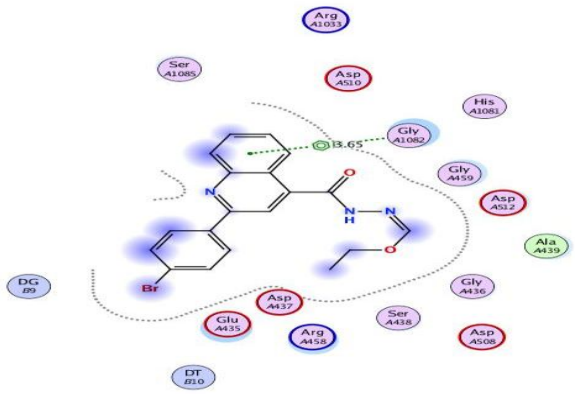
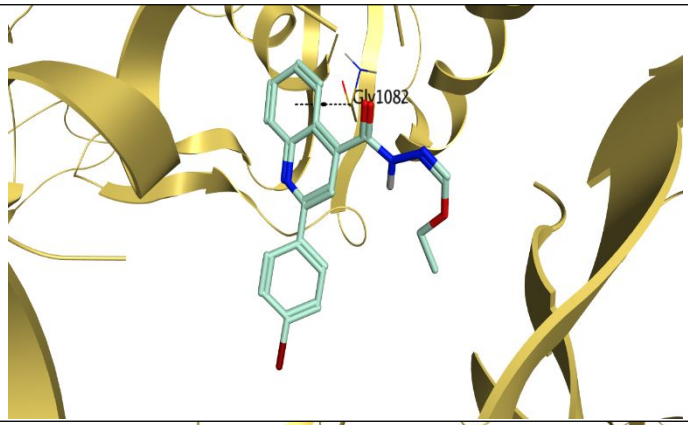
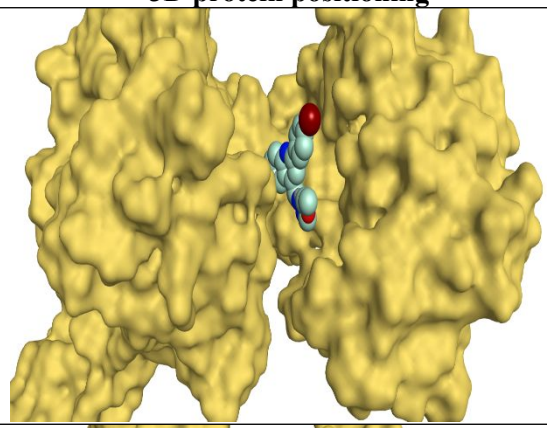
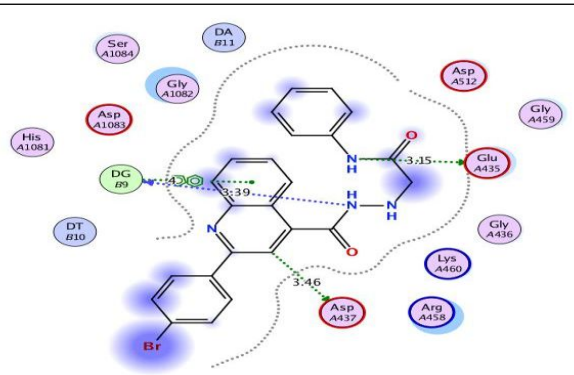
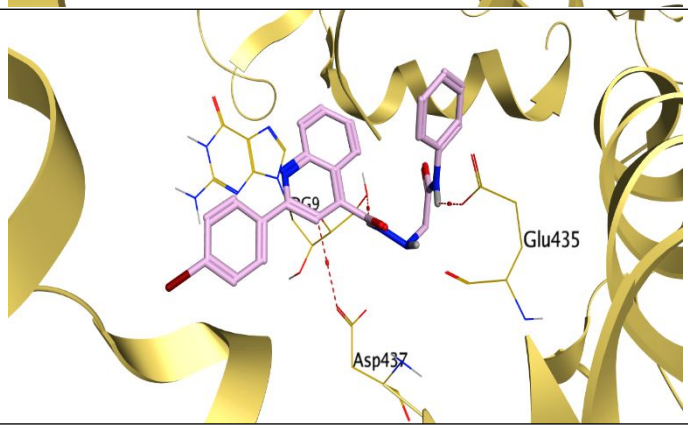
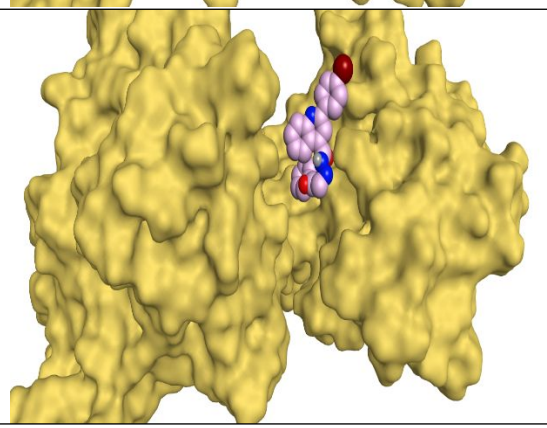
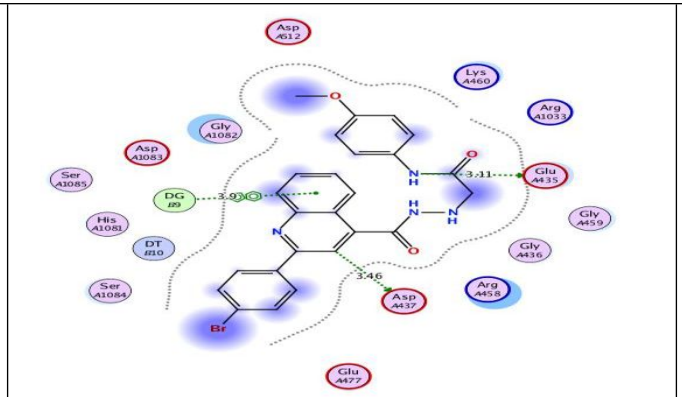
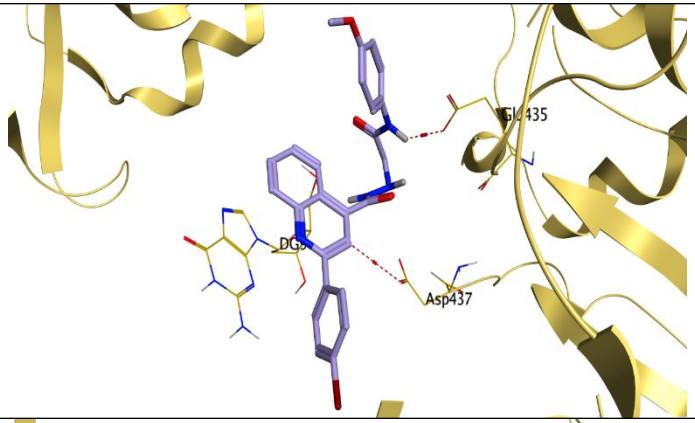
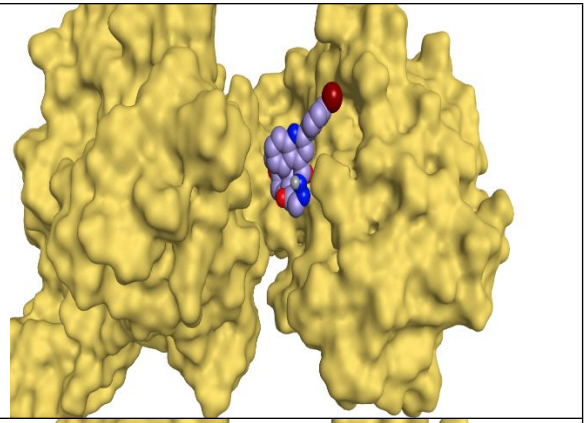
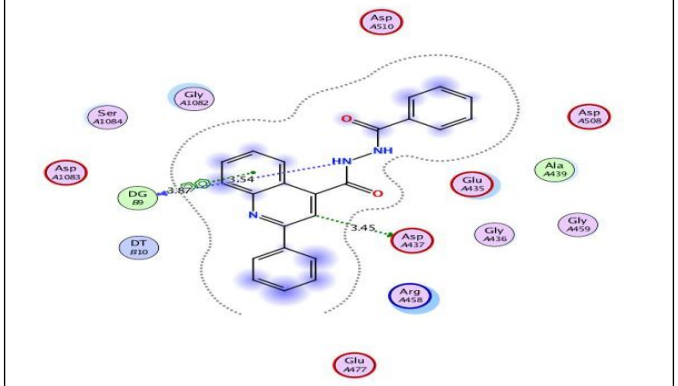
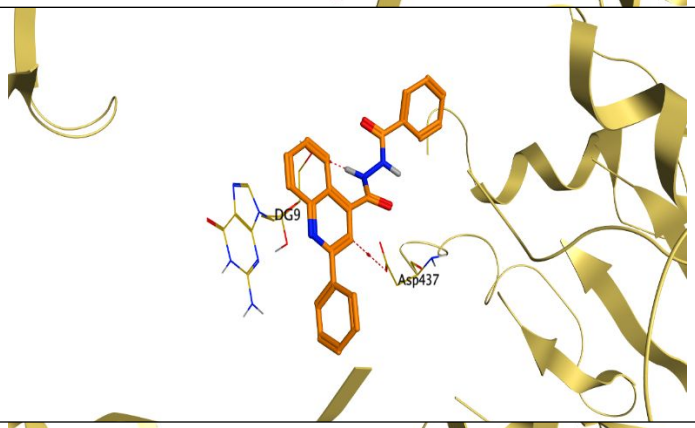
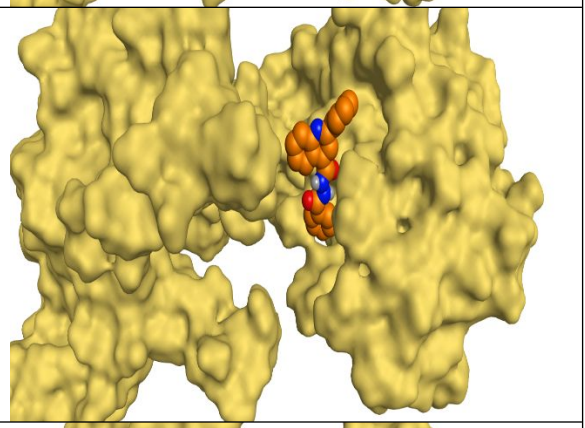
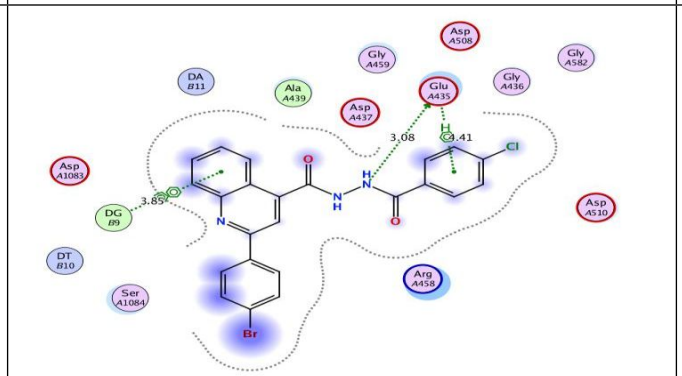
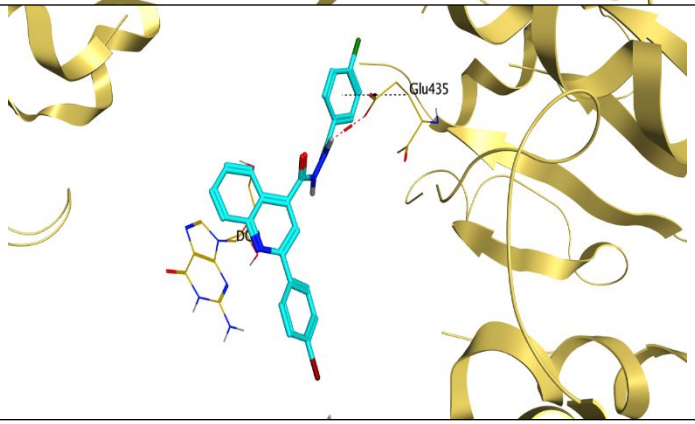
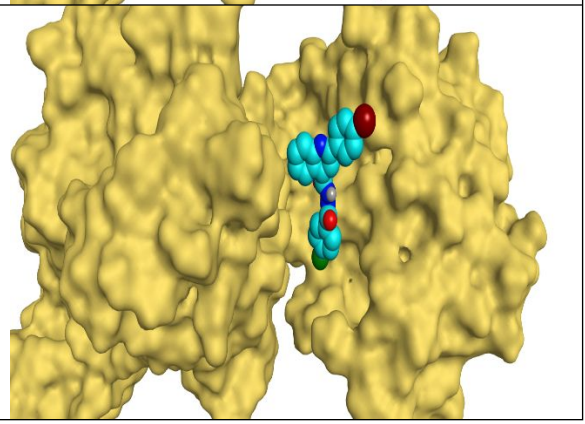
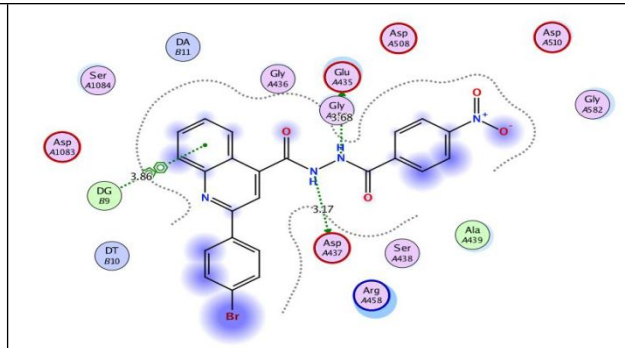
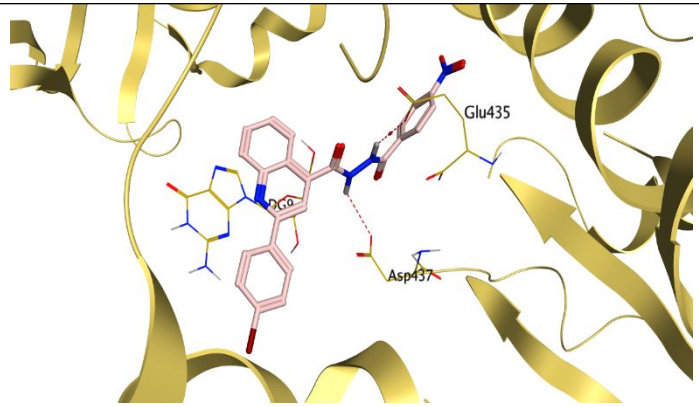
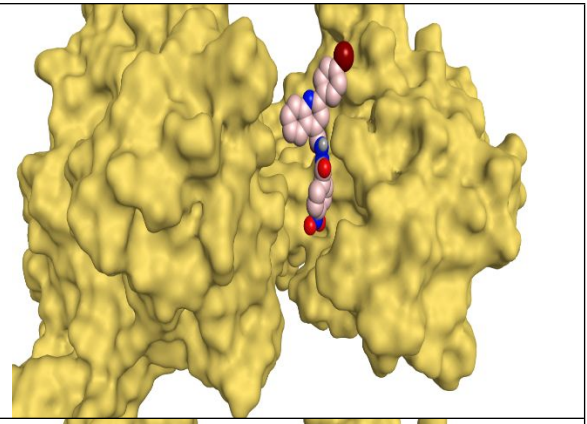
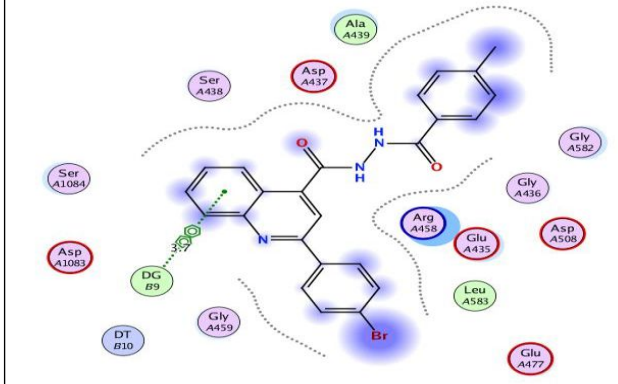
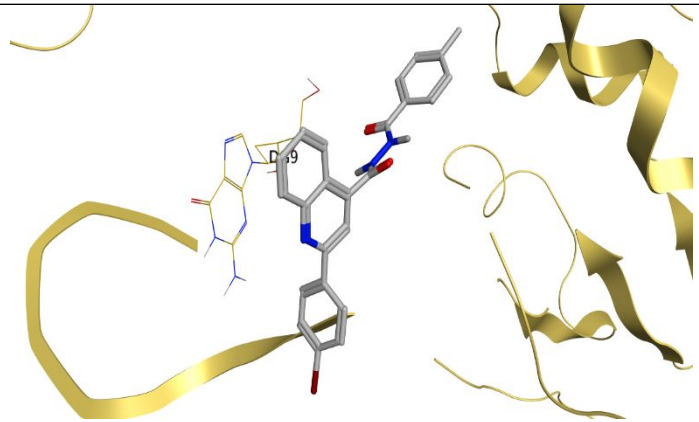
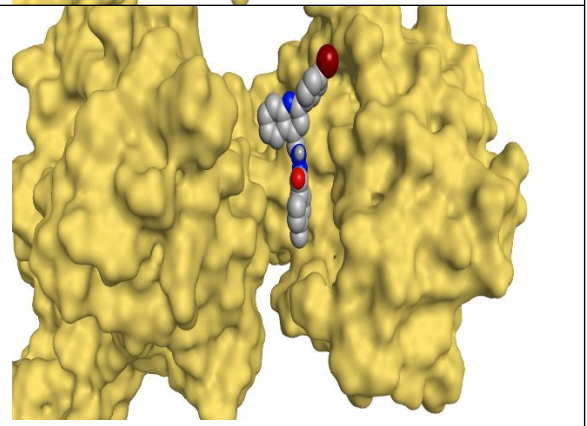
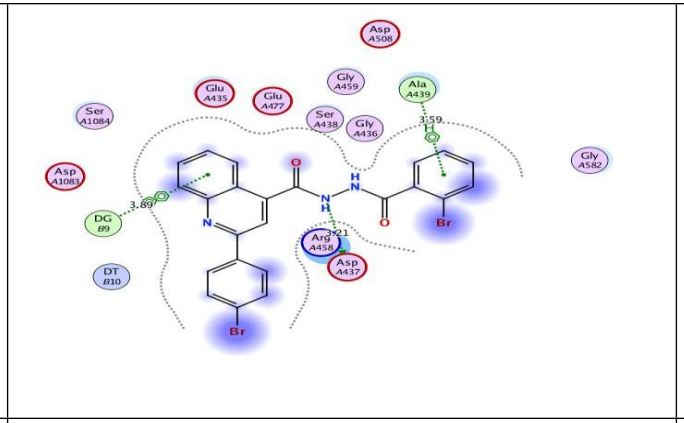
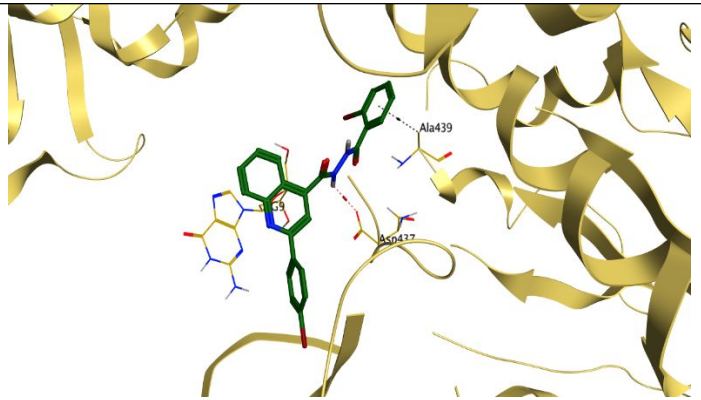
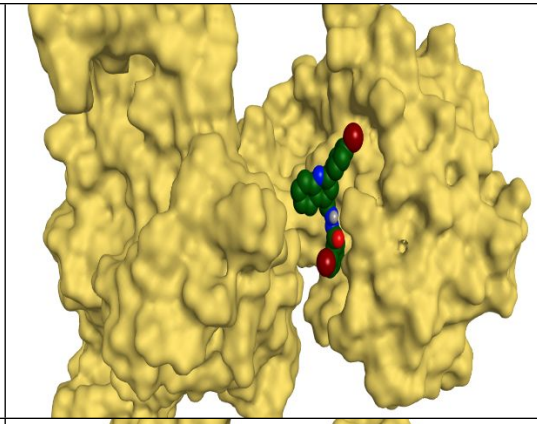
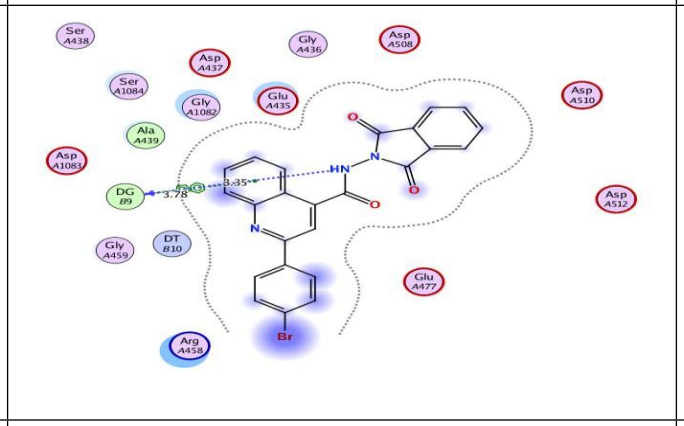
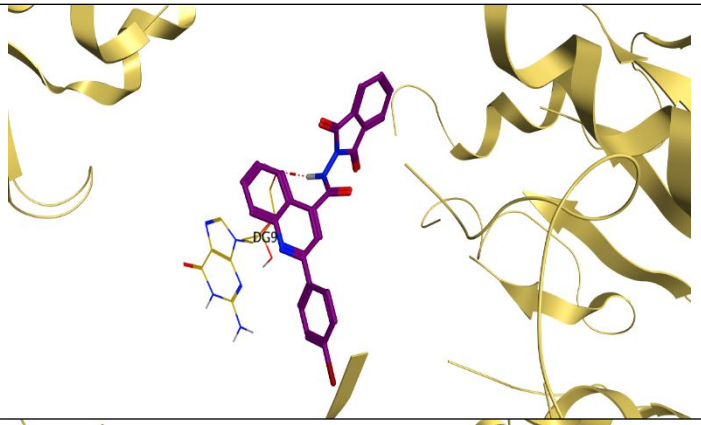
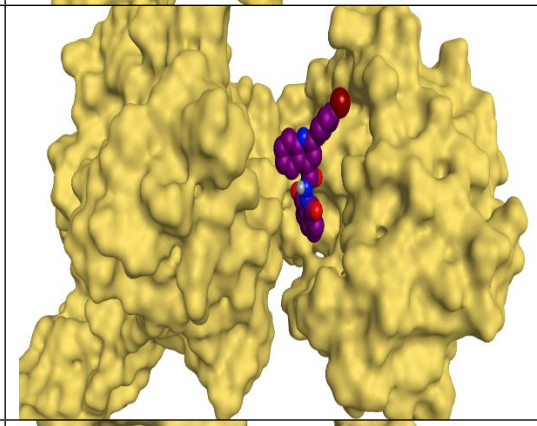
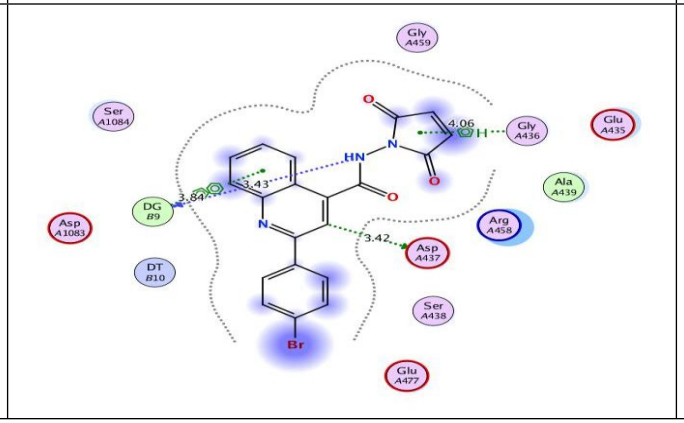
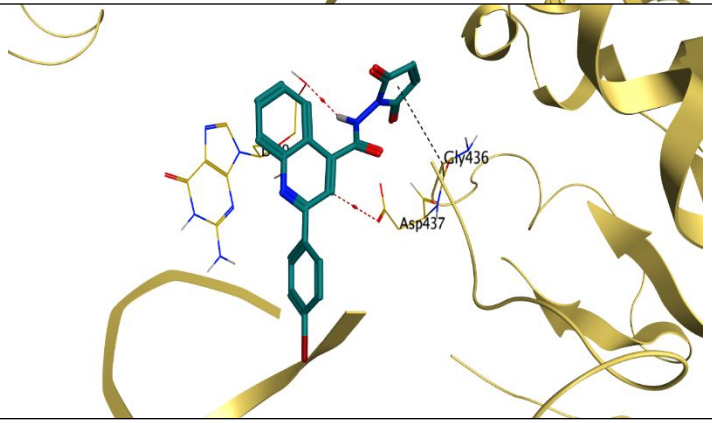
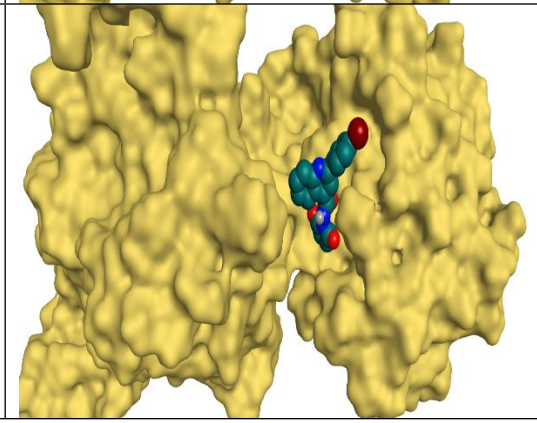


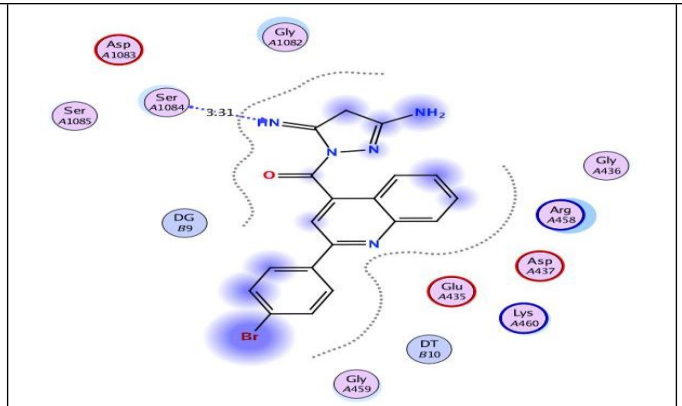
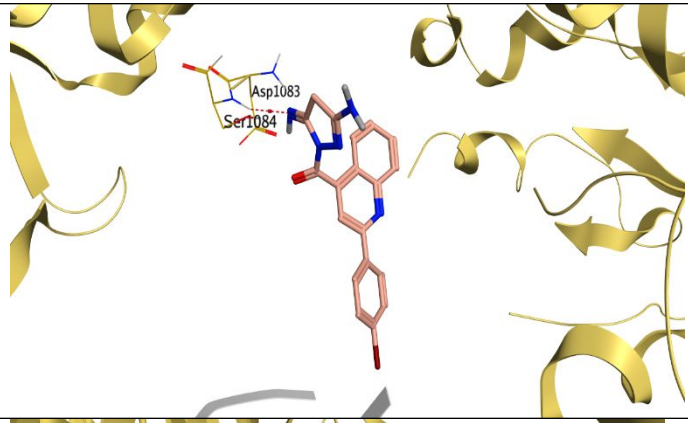
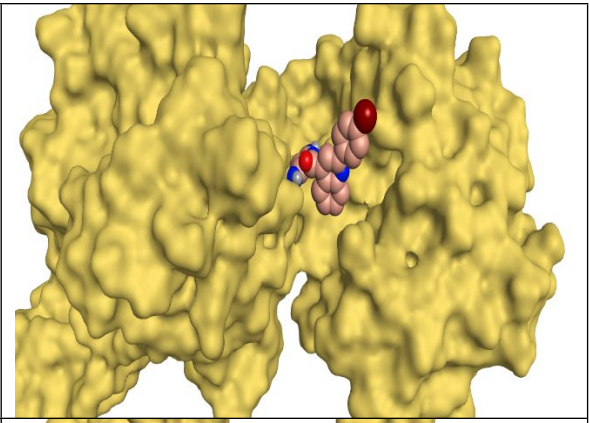
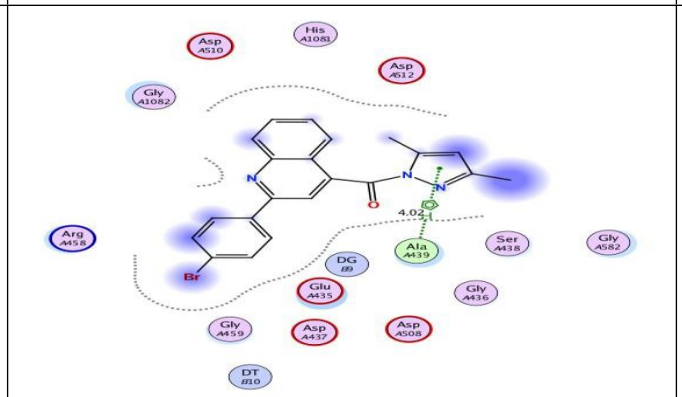
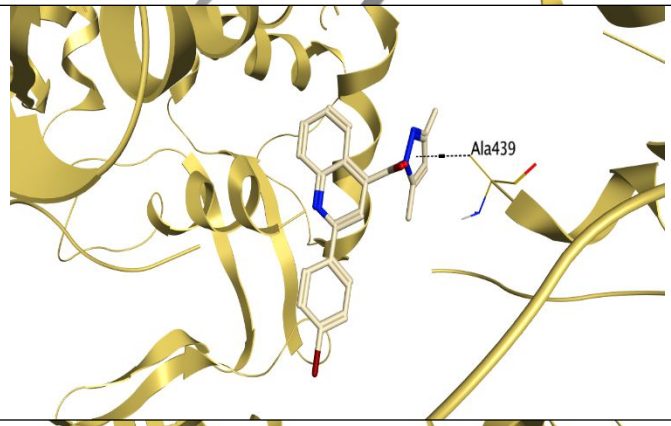
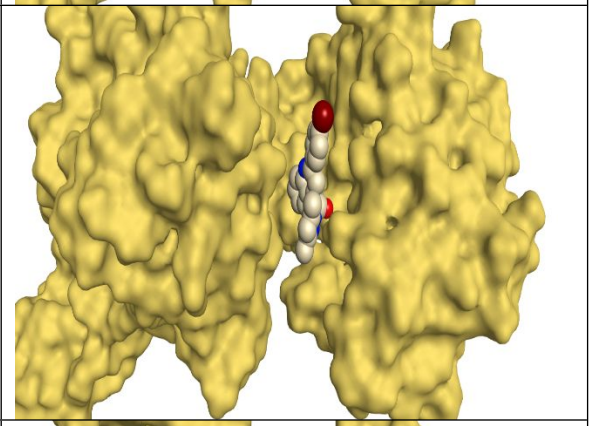
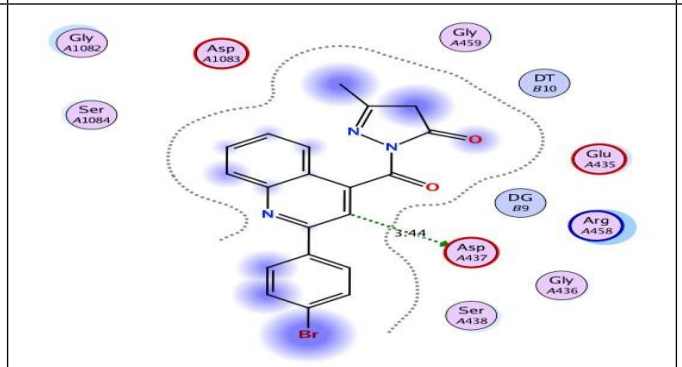
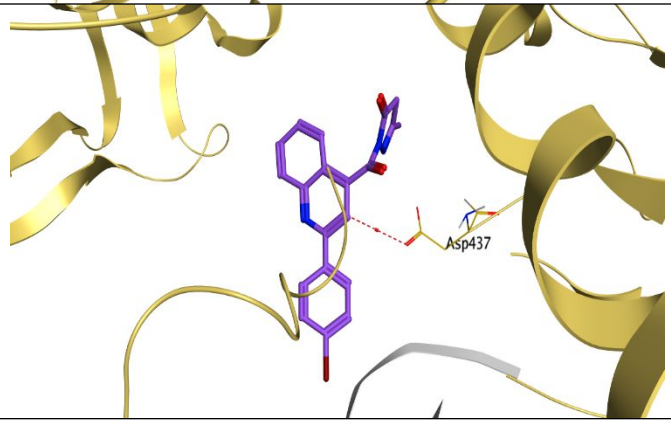
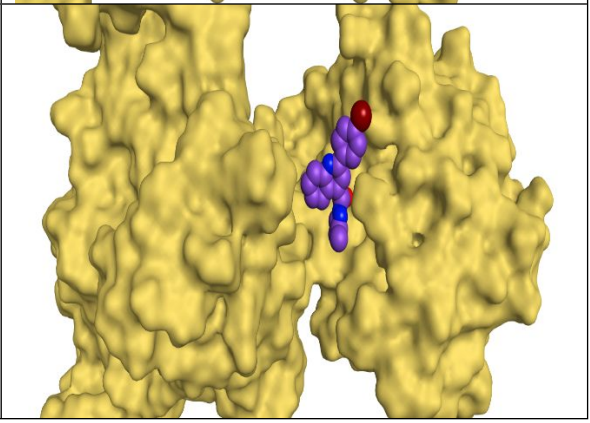
Figure S1: The 2D superimposition of the native co-crystallized ciprofloxacin, and the re-docked co-crystallized Ciprofloxacin at *S. aureus* DNA gyrase target protein

No.	2D interactions	3D interactions	3D protein positioning
5			
6a			

6b			
7a			
7b			

7c	 DA B11 Asp A508 Asp A510 Ser A1084 Gly A436 Glu A435 Gly A368 Gly A582 Asp A1083 DG B9 3.86 3.17 DT B10 Asp A437 Ser A438 Ala A439 Arg A458 Br	 Glu435 DG9 Asp437	
7d	 Ala A439 Asp A437 Ser A438 Gly A582 Ser A1084 Gly A436 Arg A458 Asp A508 Glu A435 Asp A1083 DG B9 Leu A583 Gly A459 DT B10 Br Glu A477	 DG9	

7e			
8			
9			

10	 Asp A1083 Gly A1082 Ser A1085 Ser A1084 3.31 HN NH2 N N O Gly A436 DG B9 Arg A458 Asp A437 Glu A435 Lys A460 Br DT B10 Gly A459	 Asp1083 Ser1084	
11	 Asp A510 His A1081 Asp A512 Gly A1082 N N O 4.02 Arg A458 Br Ala A439 Ser A438 Gly A582 DG B9 Glu A435 Gly A436 Gly A459 Asp A437 Asp A508 DT B10	 Ala439	
12	 Gly A1082 Asp A1083 Gly A459 DT B10 Ser A1084 N N O O Glu A435 DG B9 Arg A458 3.44 Asp A437 Gly A436 Ser A438 Br	 Asp437	

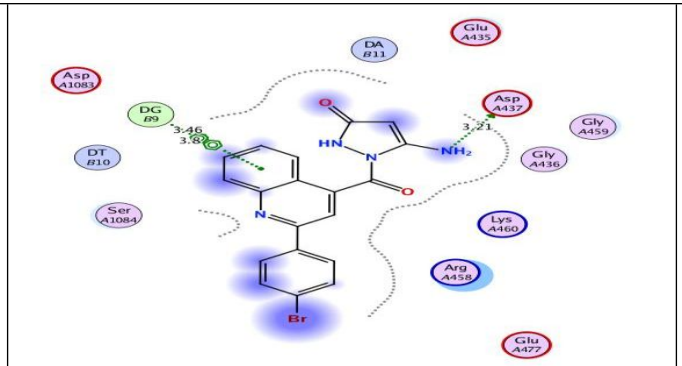
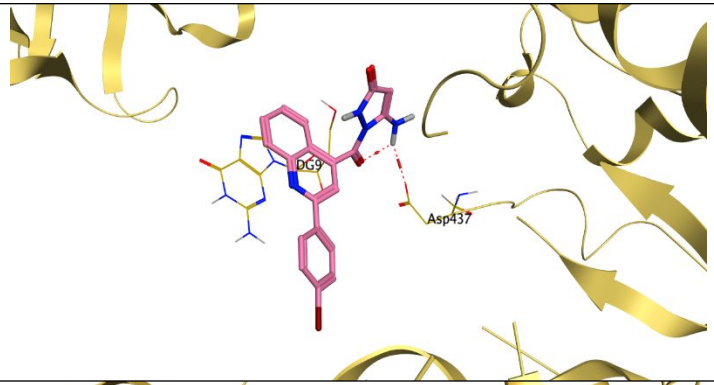
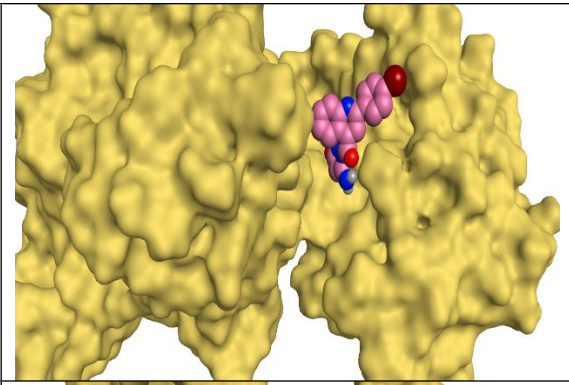
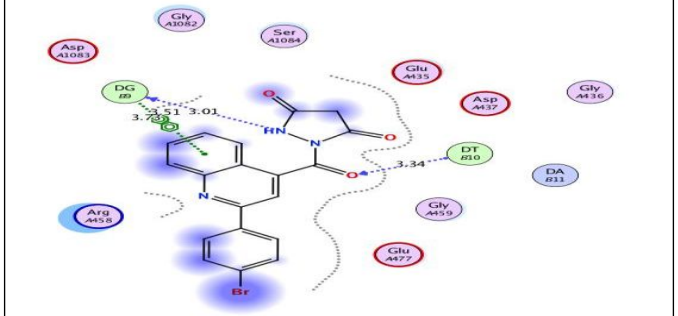
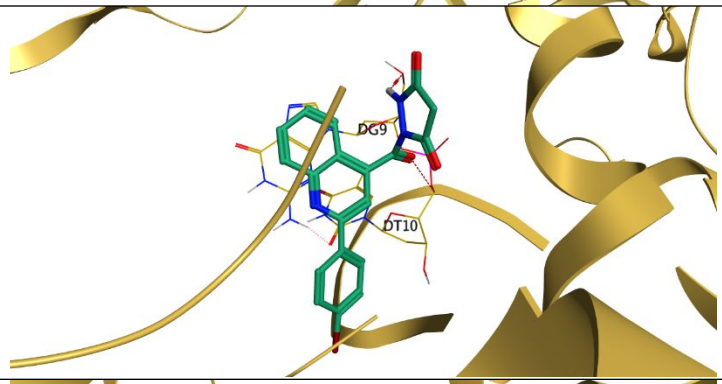
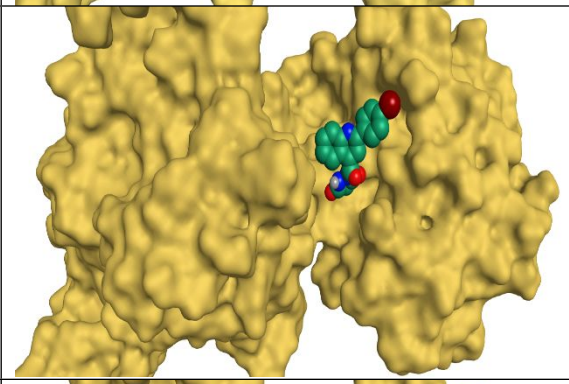
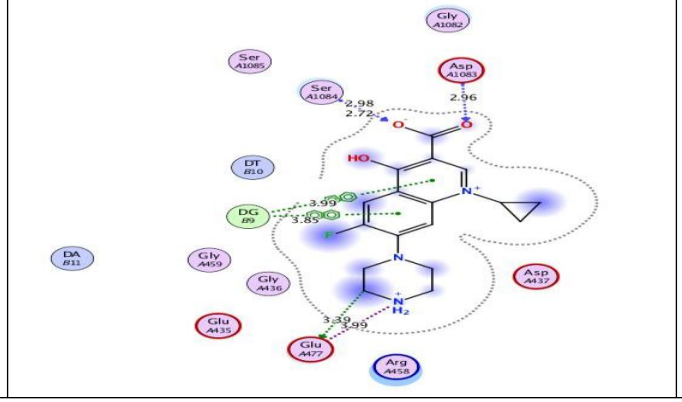
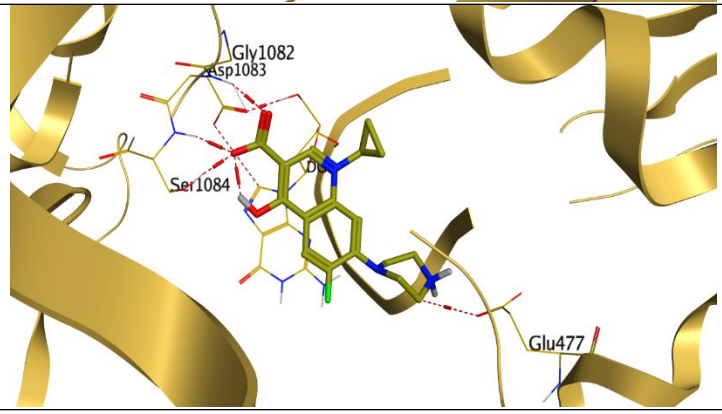
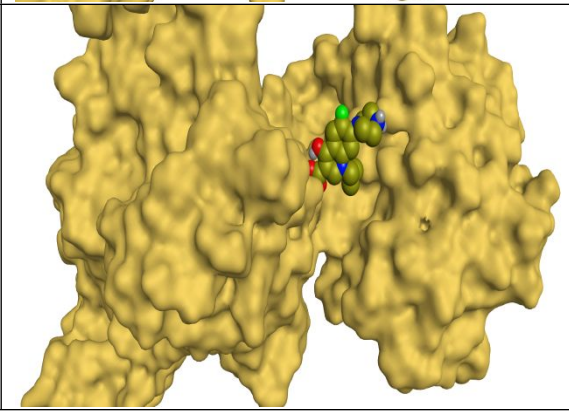
13	 Glu A435 DA B11 Asp A1083 DG B9 Asp A437 Gly A459 DT B10 Gly A436 Ser A1084 Lys A460 Arg A458 Br Glu A477	 DG9 Asp437	
14	 Gly A1082 Asp A1083 Ser A1084 Glu A435 DG B9 Asp A437 Gly A436 DT B10 DA B11 Arg A458 Gly A459 Glu A477 Br	 DG9 DT10	
Cipro	 Gly A1082 Ser A1085 Asp A1083 Ser A1084 DT B10 DG B9 DA B11 Gly A459 Gly A436 Asp A437 Glu A435 Glu A477 Arg A458	 Gly1082 Asp1083 Ser1084 Glu477	

Figure S2: The 2D, and 3D binding interactions for the all afforded compounds (5-14) along with their corresponding 3D protein positioning

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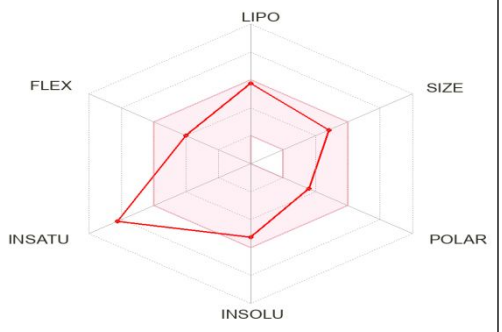
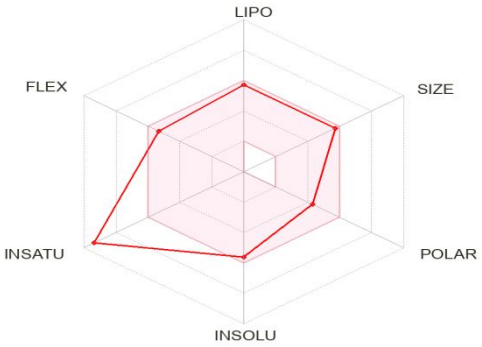
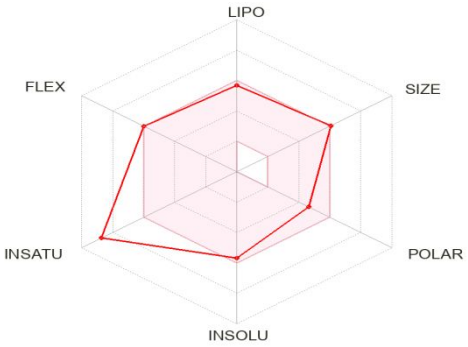
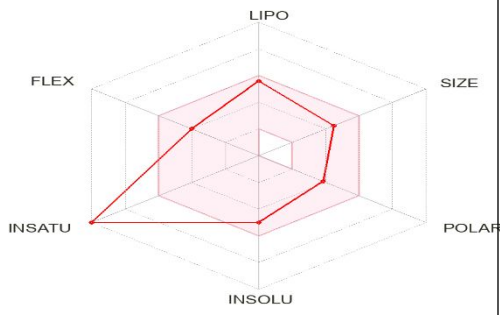
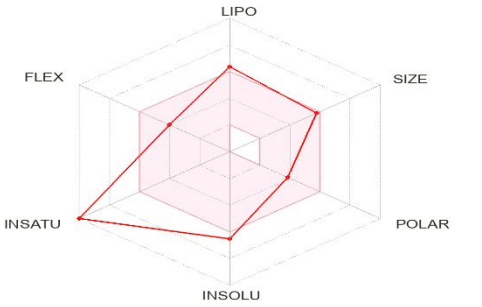
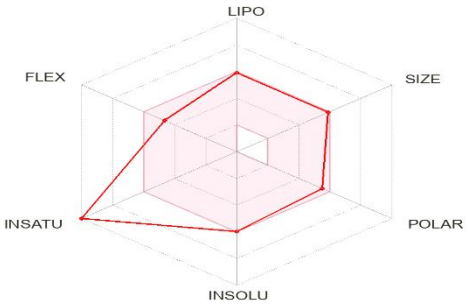
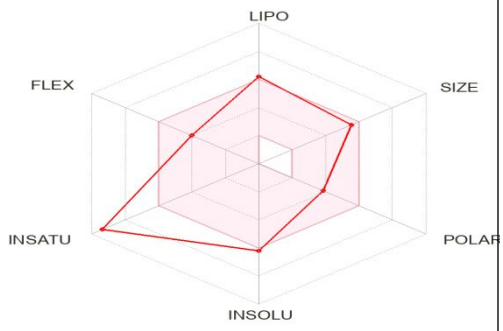
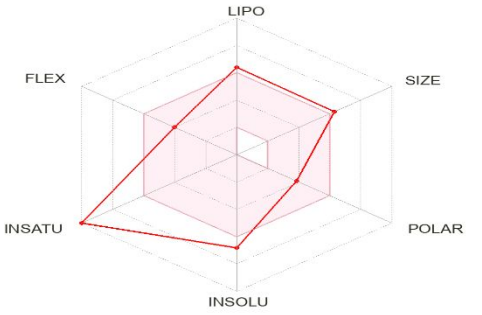
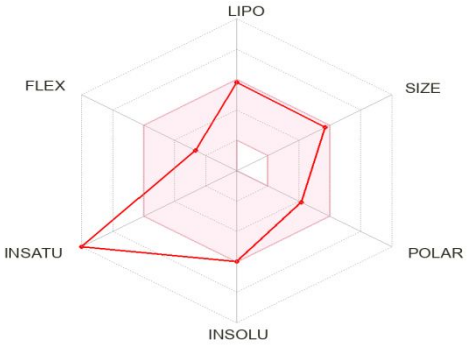
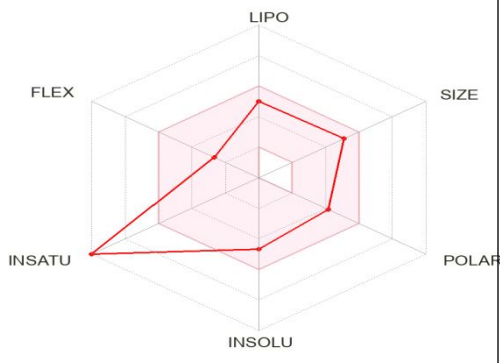
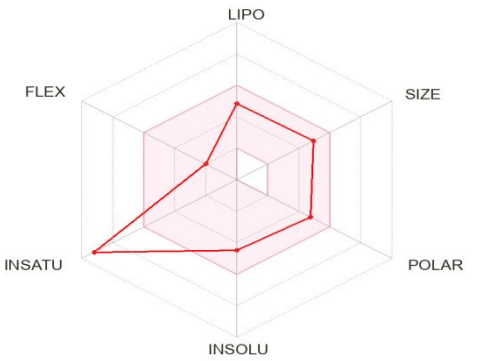
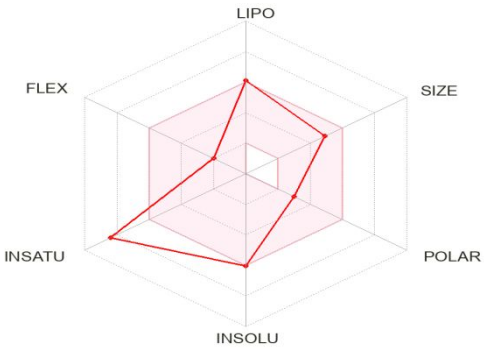
		
7a	**7b**	**7c**
		
7d	**7e**	**8**
		
9	**10**	**11**
		

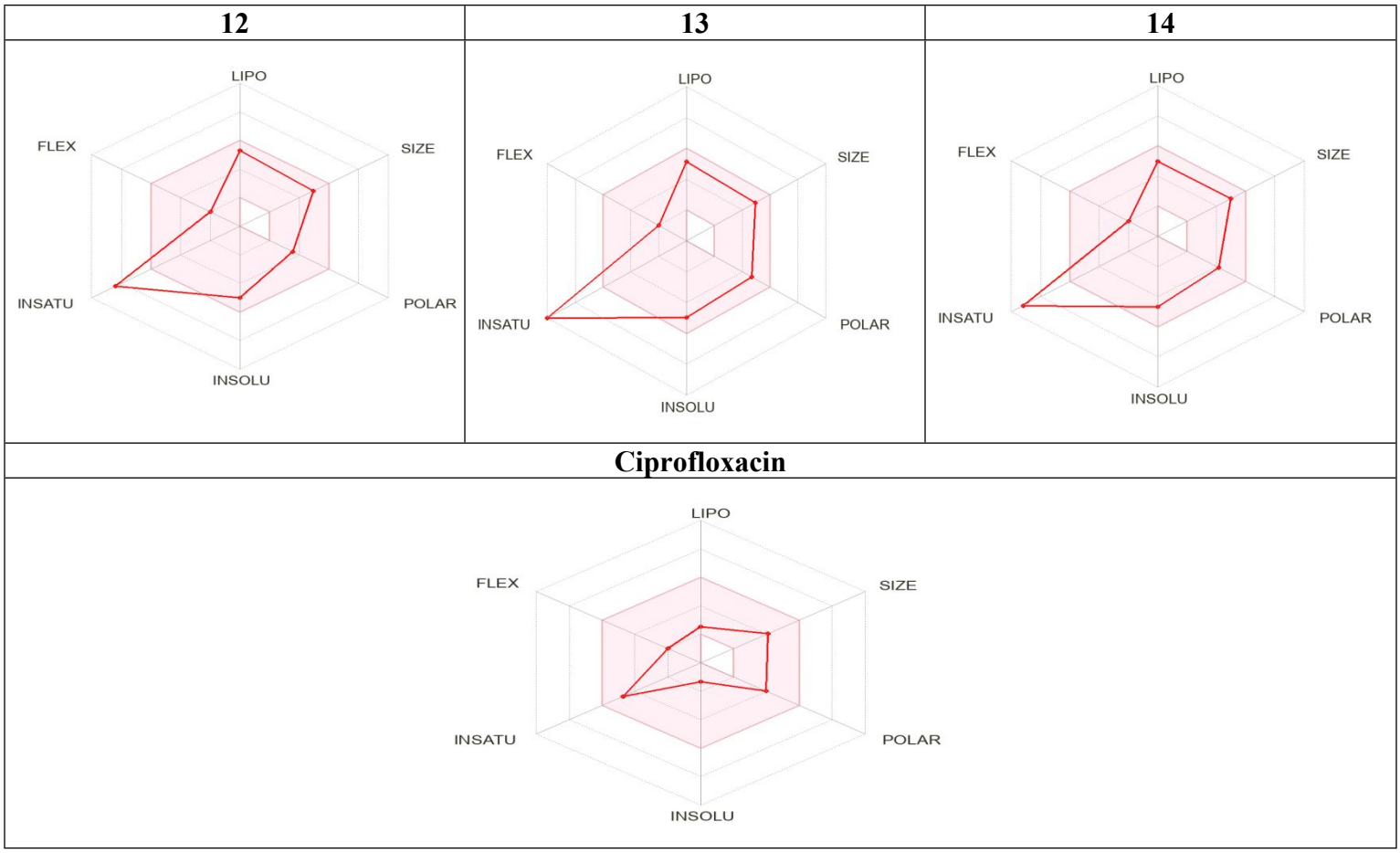


Figure S3: Radar bioavailability for studied compounds in which the area in pink displays specific property optimal range. (LIPO = lipophilicity expressed as XLOGP3 (range from -0.7 to 5.0). SIZE= size expressed as molecular weight (range from 150 g/mol to 500 g/mol). POLAR = polarity expressed as TPSA (topological polar surface area) (range from 20 $Å^2$ to 130 $Å^2$). INSOLU = water insolubility by log S (ESOL) (range from -6 to 0). INSATU = insaturation expressed as for each carbons fraction in sp3 hybridization (range from 0.25 to 1). FLEX = flexibility, expressed as number of rotatable bonds (range from 0 to 9).

Part 2: Spectral data:

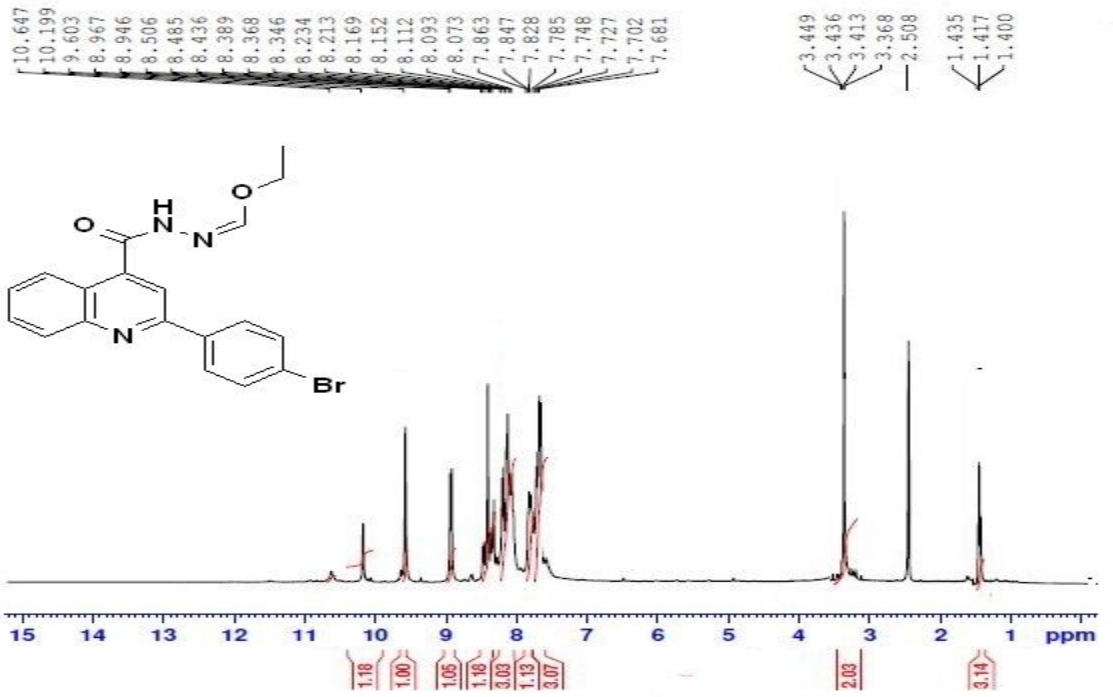


Figure S4: ^{1}H NMR spectrum of the compound **5**.

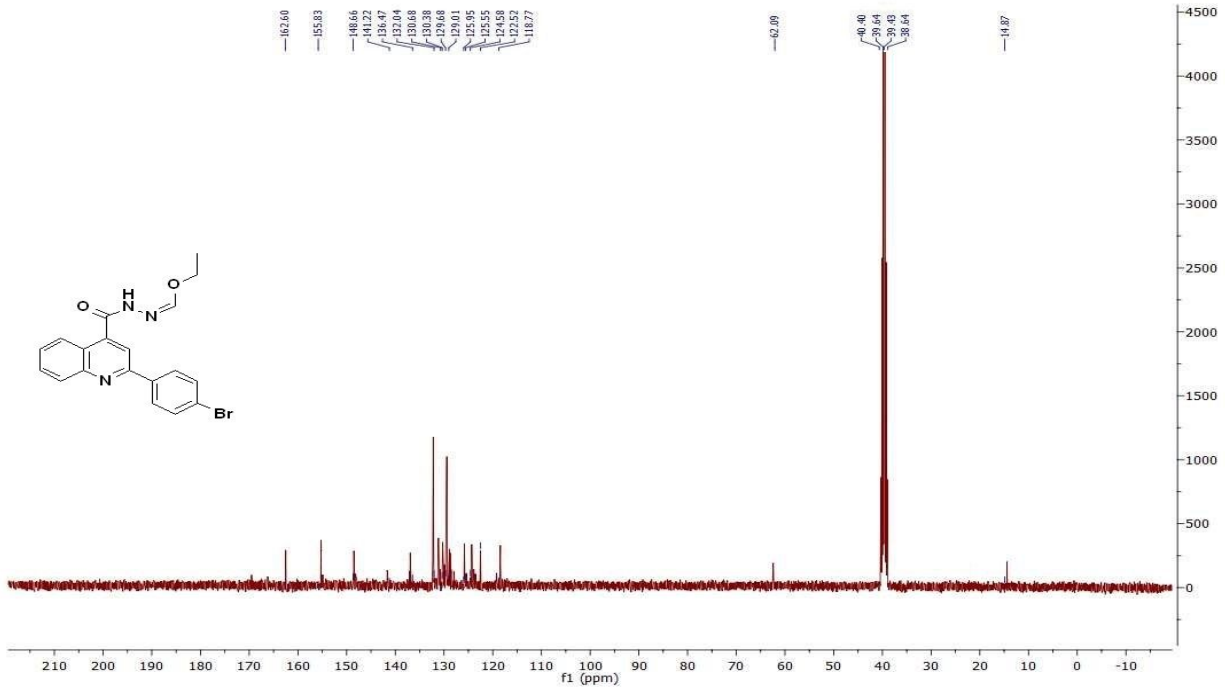


Figure S5: ^{13}C NMR spectrum of the compound **5**.

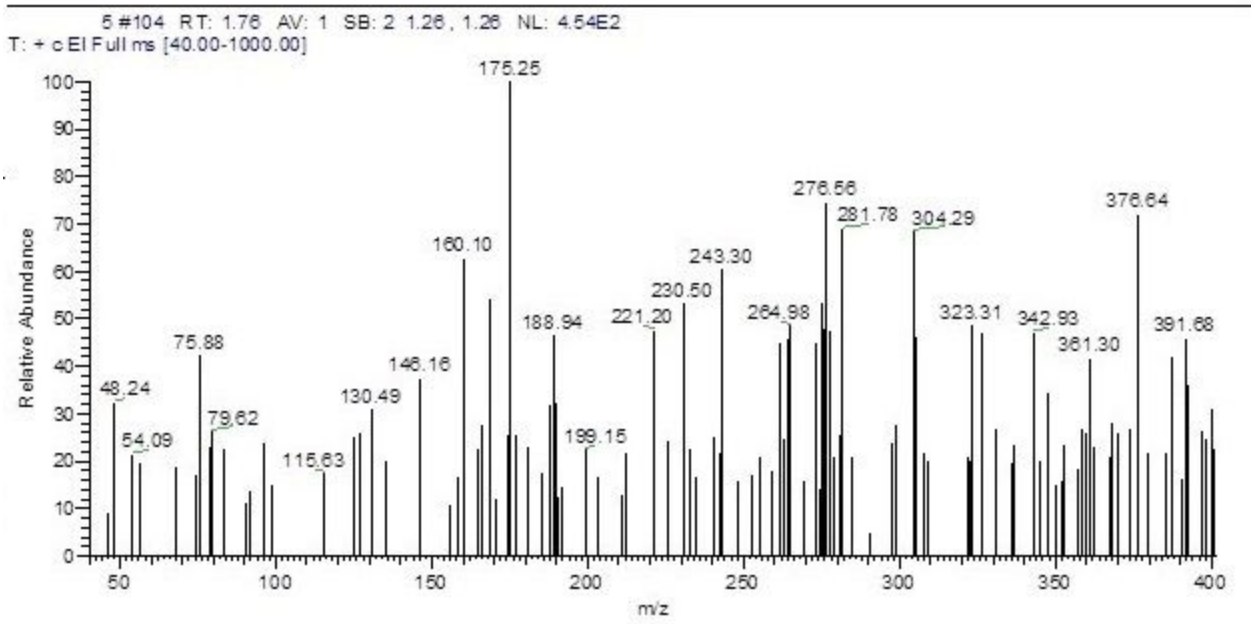


Figure S6: MS of the compound **5**.

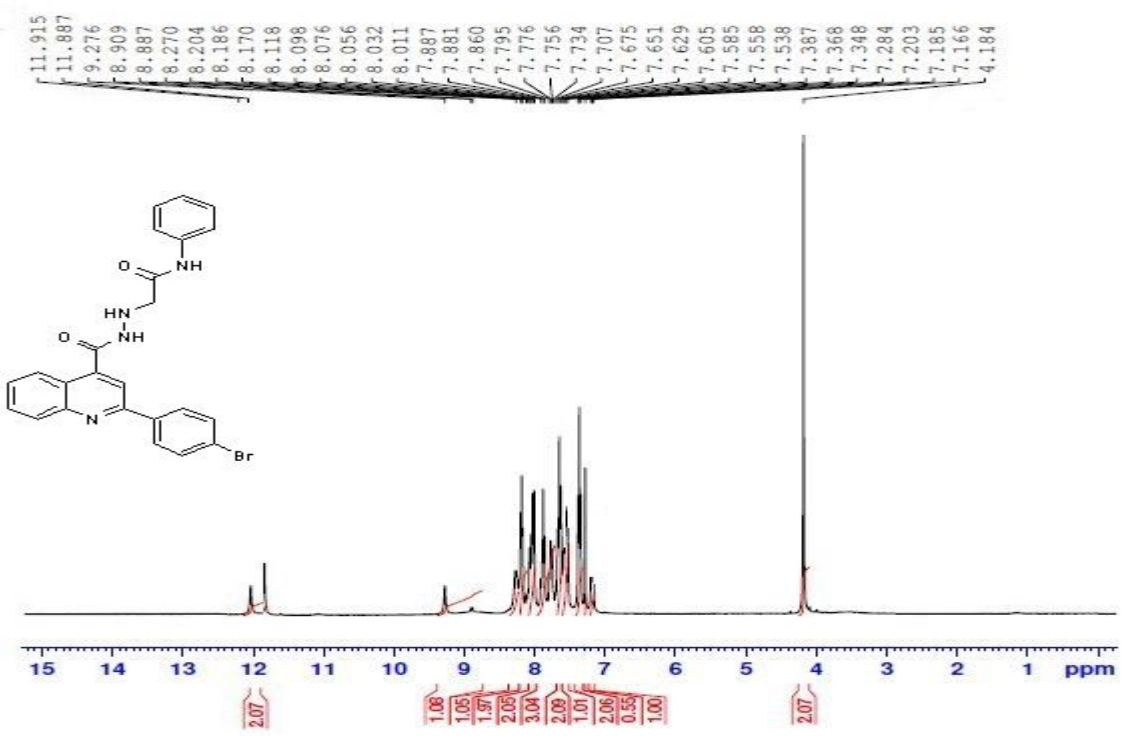


Figure S7: ^{1}H NMR spectrum of the compound **6a**.

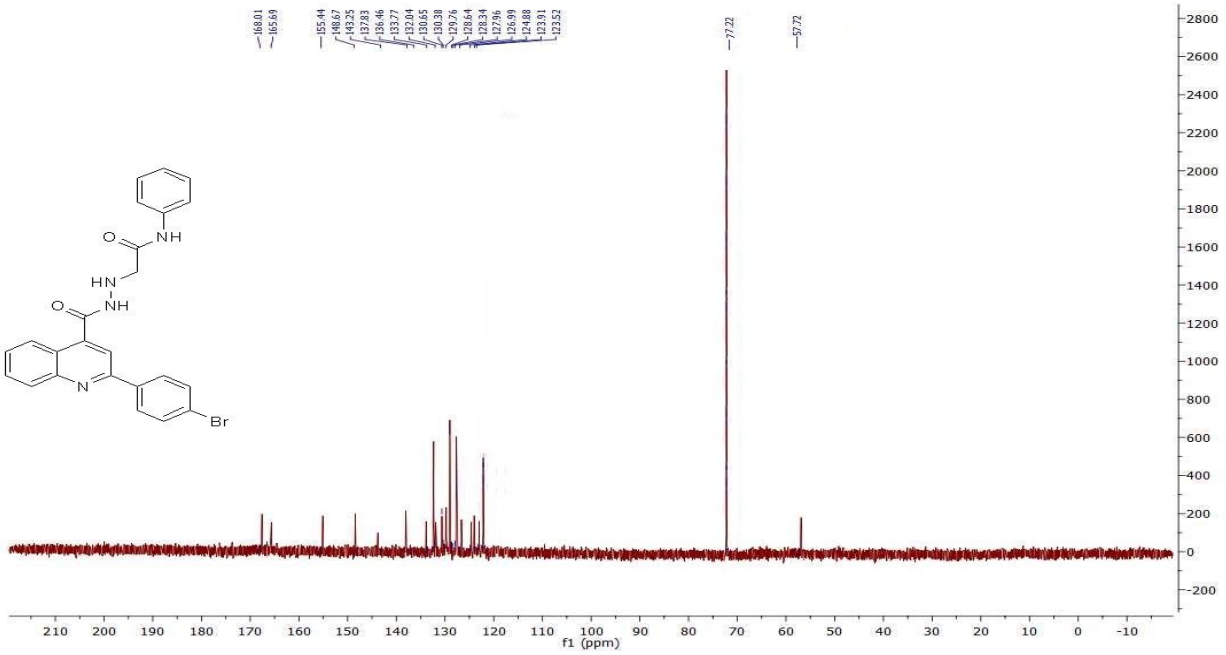


Figure S8: ^{13}C NMR spectrum of the compound **6a**.

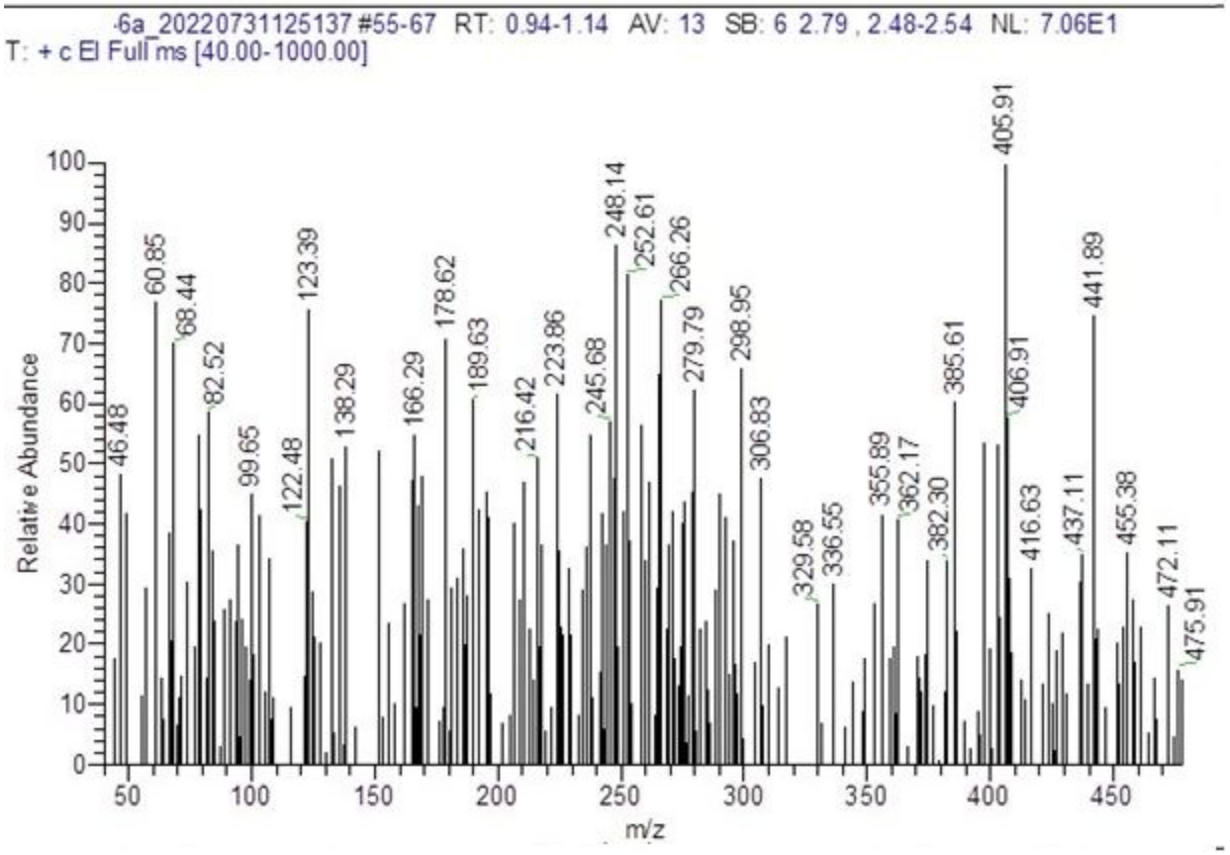


Figure S9: MS of the compound **6a**.

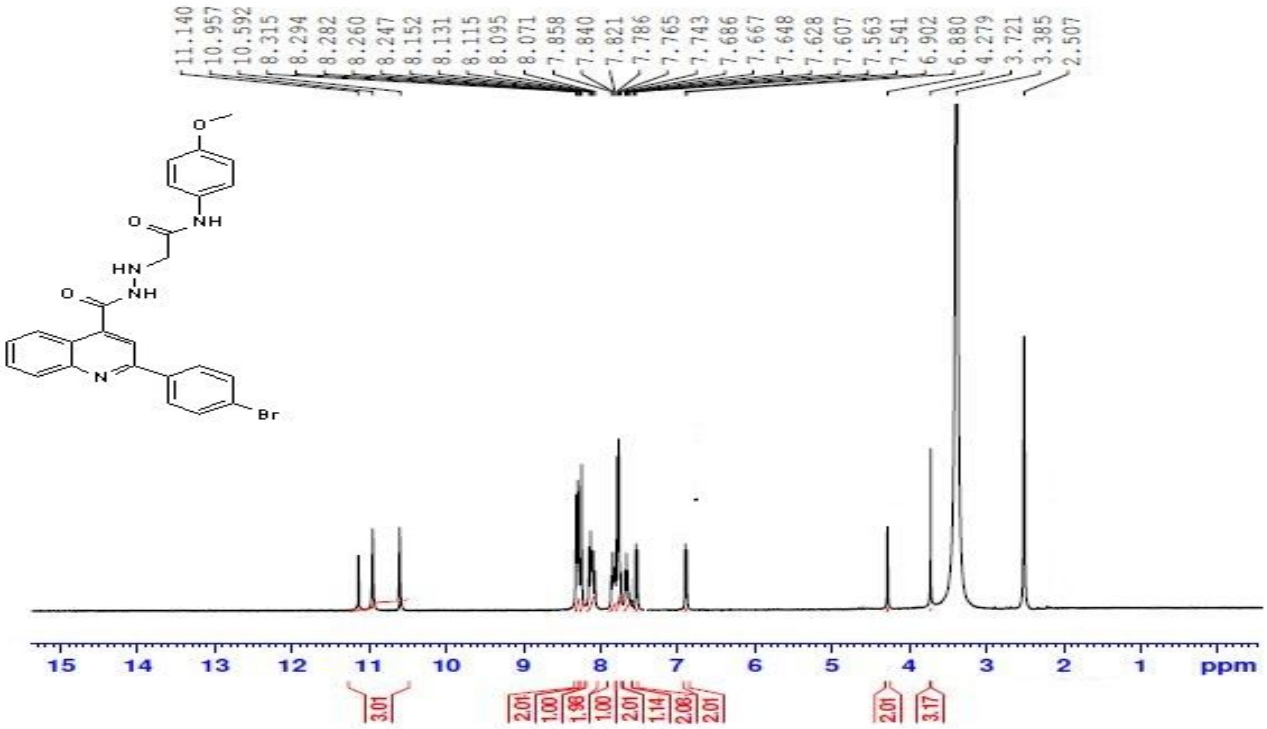


Figure S10: ^{1}H NMR spectrum of the compound **6b**.

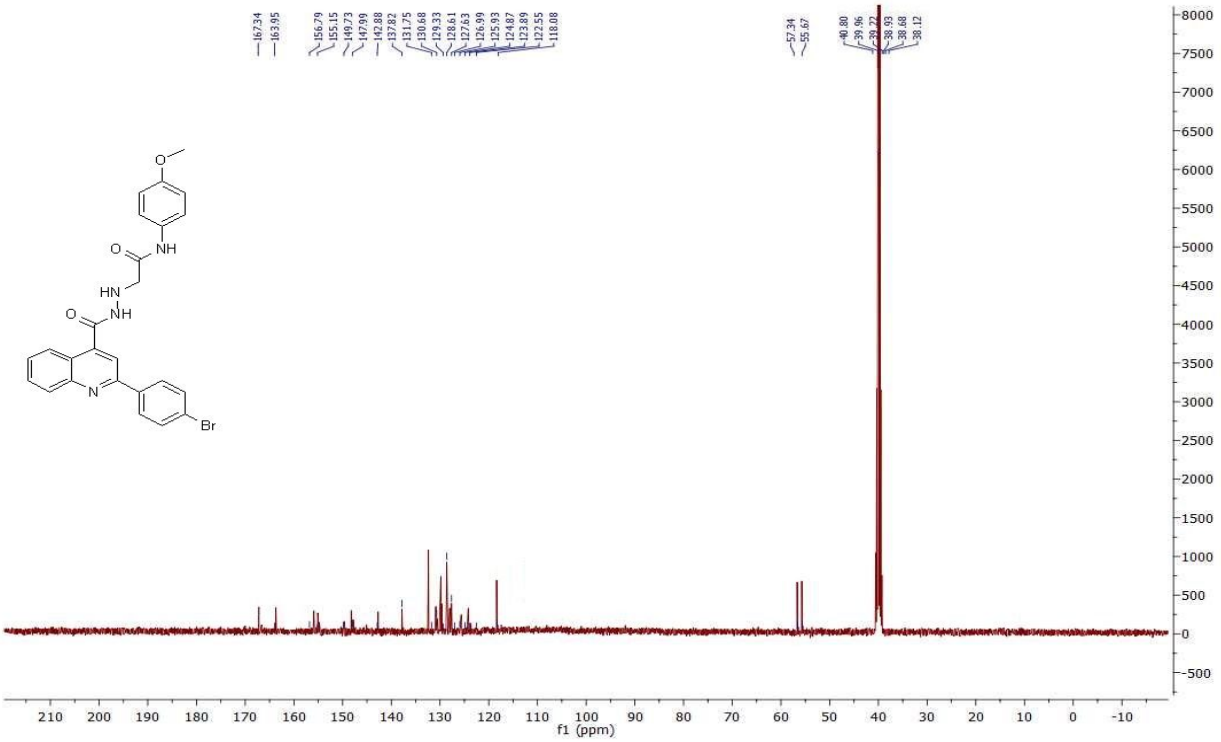


Figure S11: ^{13}C NMR spectrum of the compound **6b**.

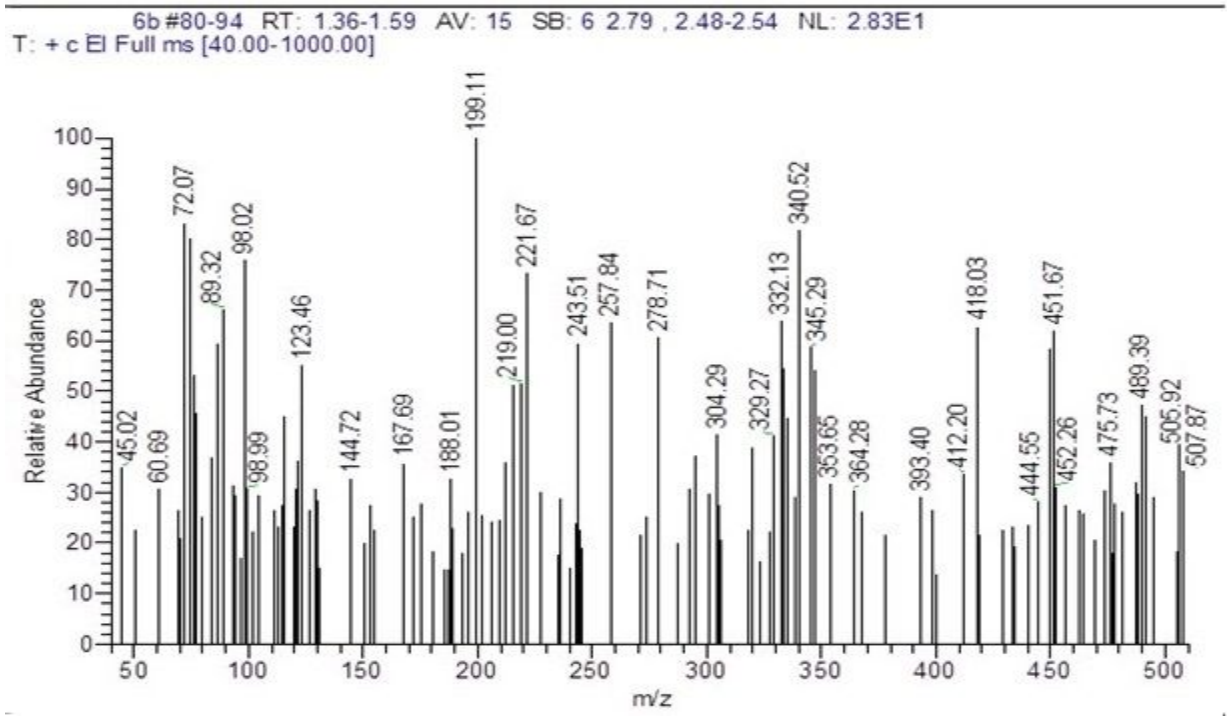


Figure S12: MS of the compound **6b**.

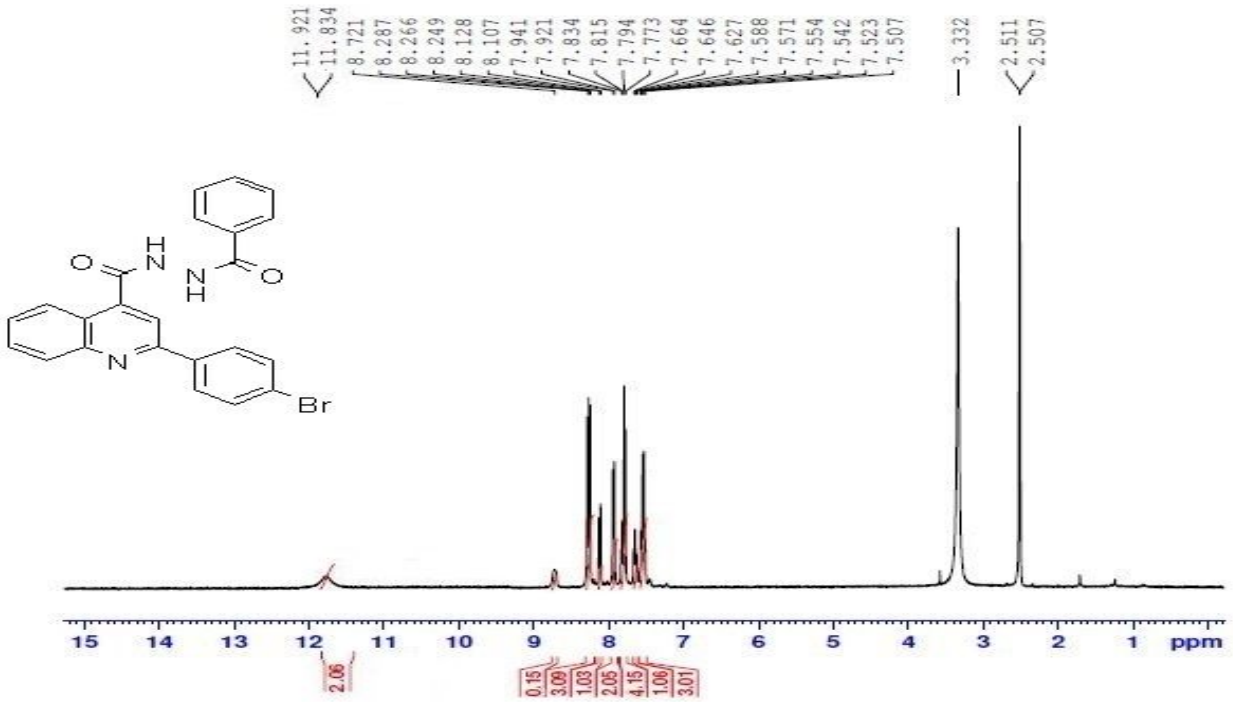


Figure S13: ^{1}H NMR spectrum of the compound **7a**.

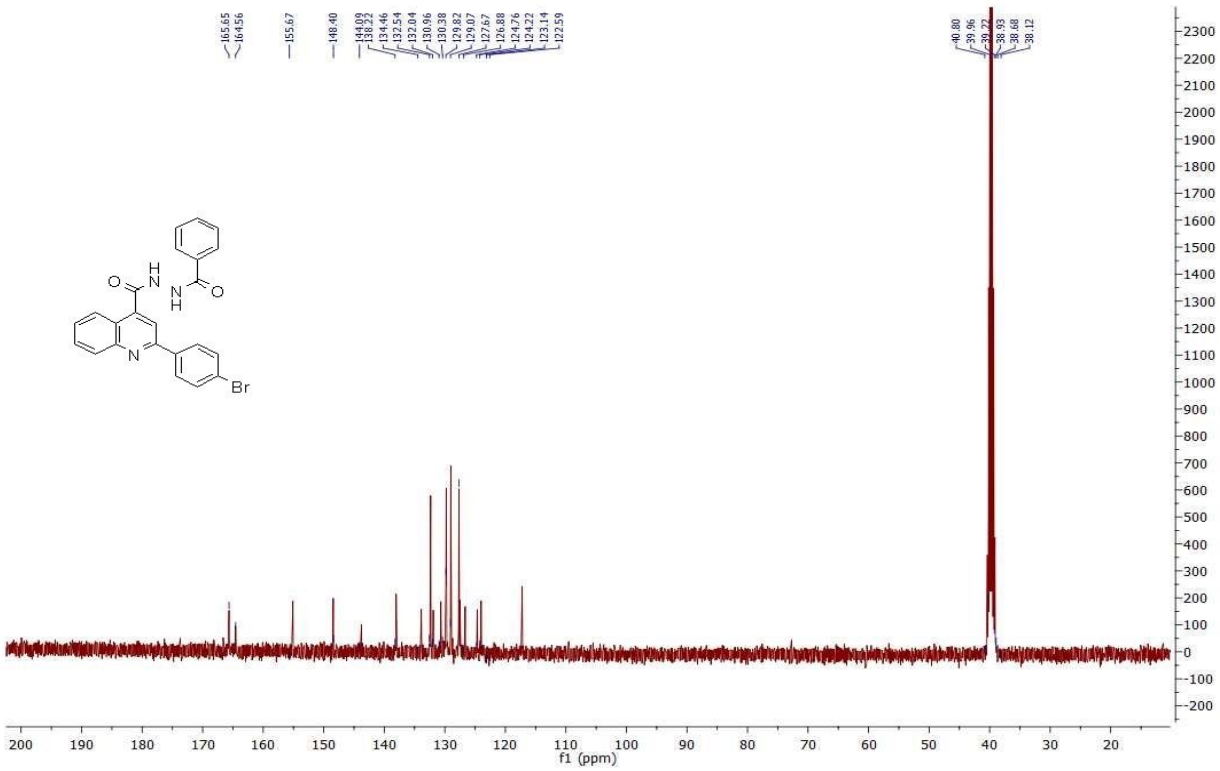


Figure S14: ^{13}C NMR spectrum of the compound **7a**.

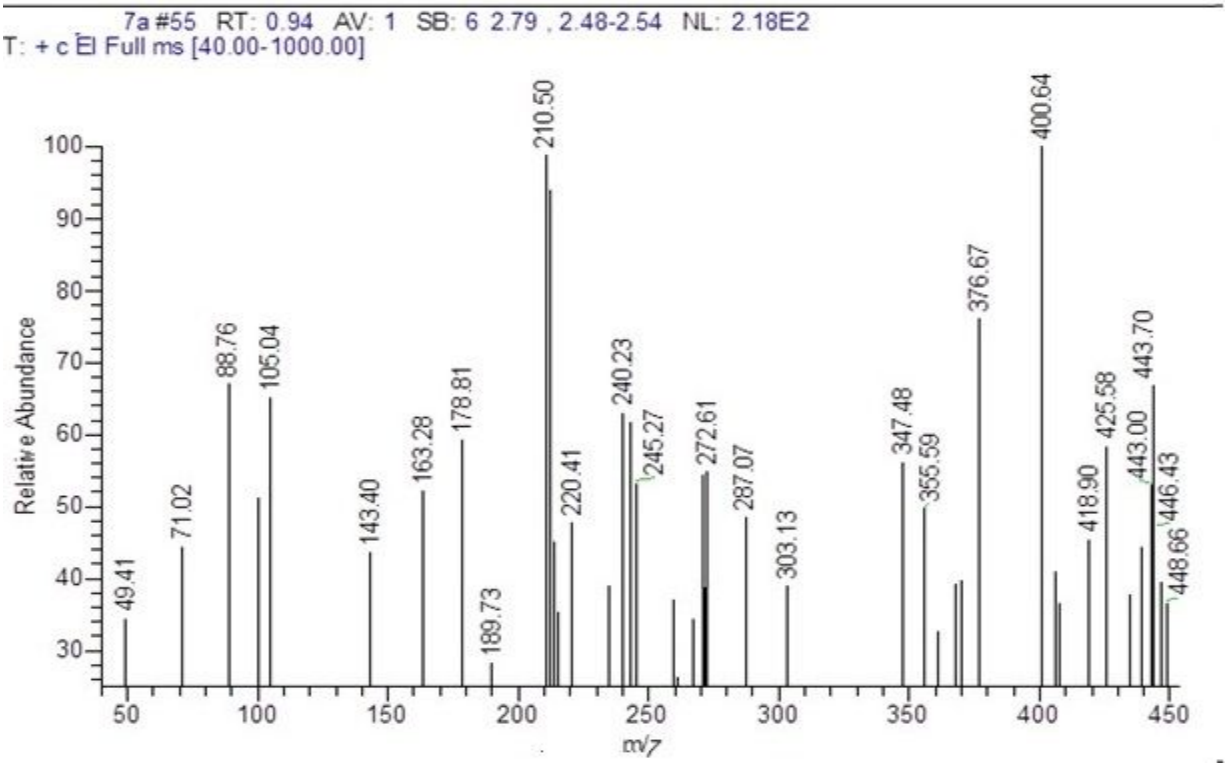


Figure S15: MS of the compound **7a**.

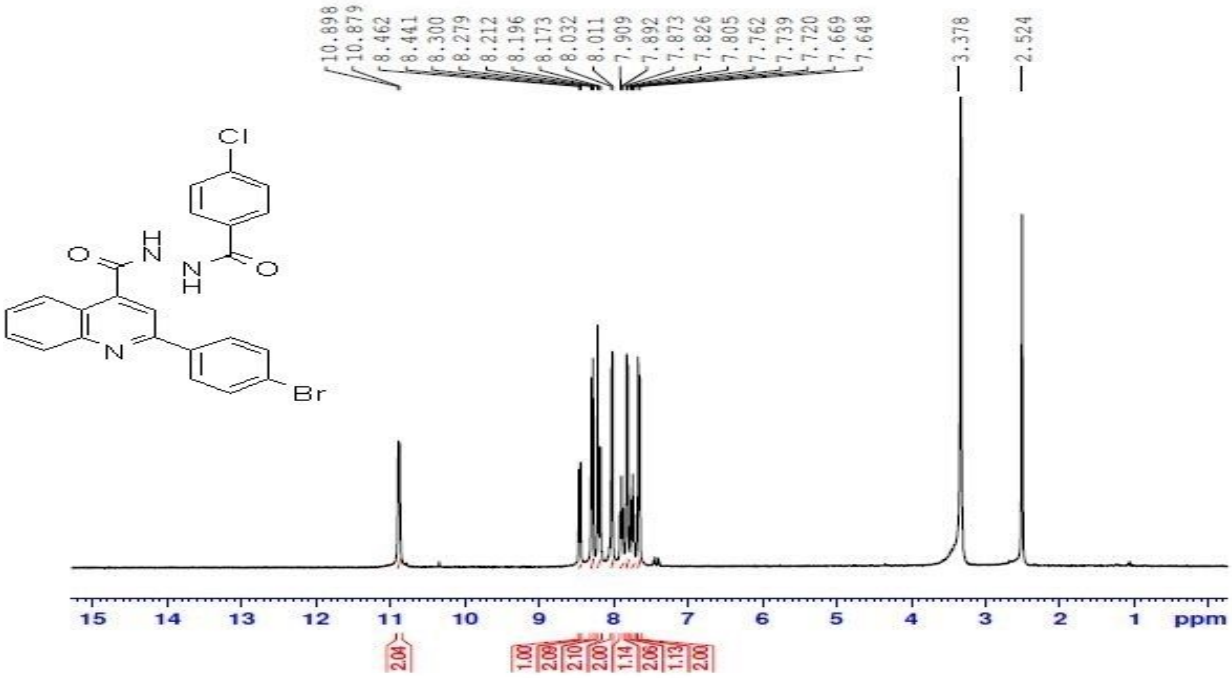


Figure S16: ^{1}H NMR spectrum of the compounds **7b**.

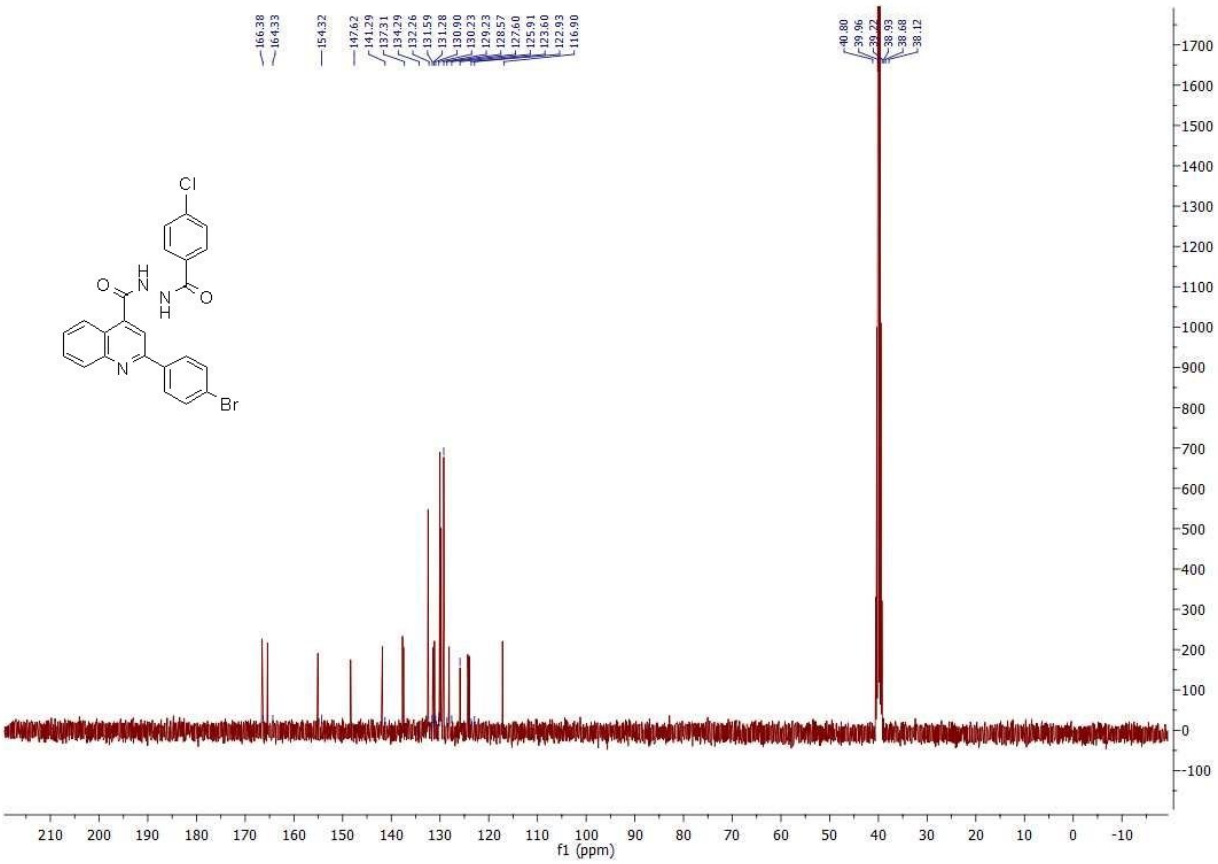


Figure S17: ^{13}C NMR spectrum of the compound **7b**.

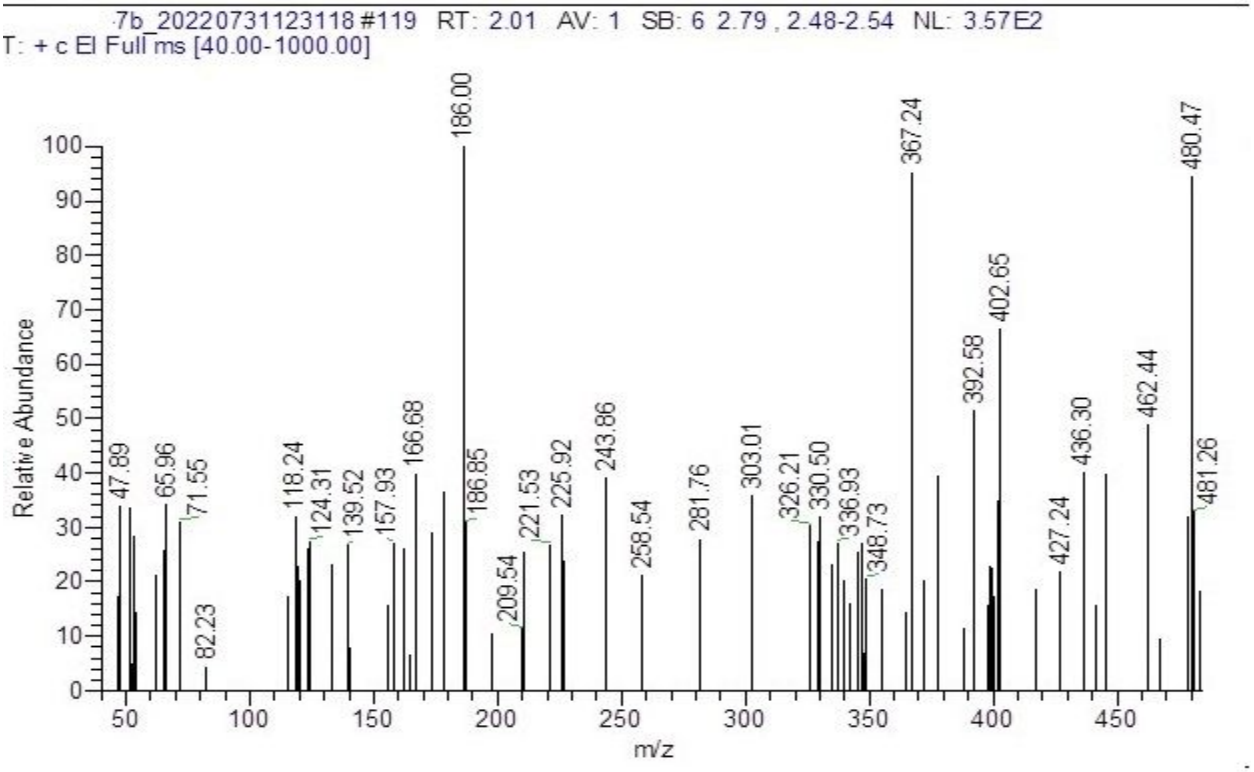


Figure S18: MS of the compound **7b**.

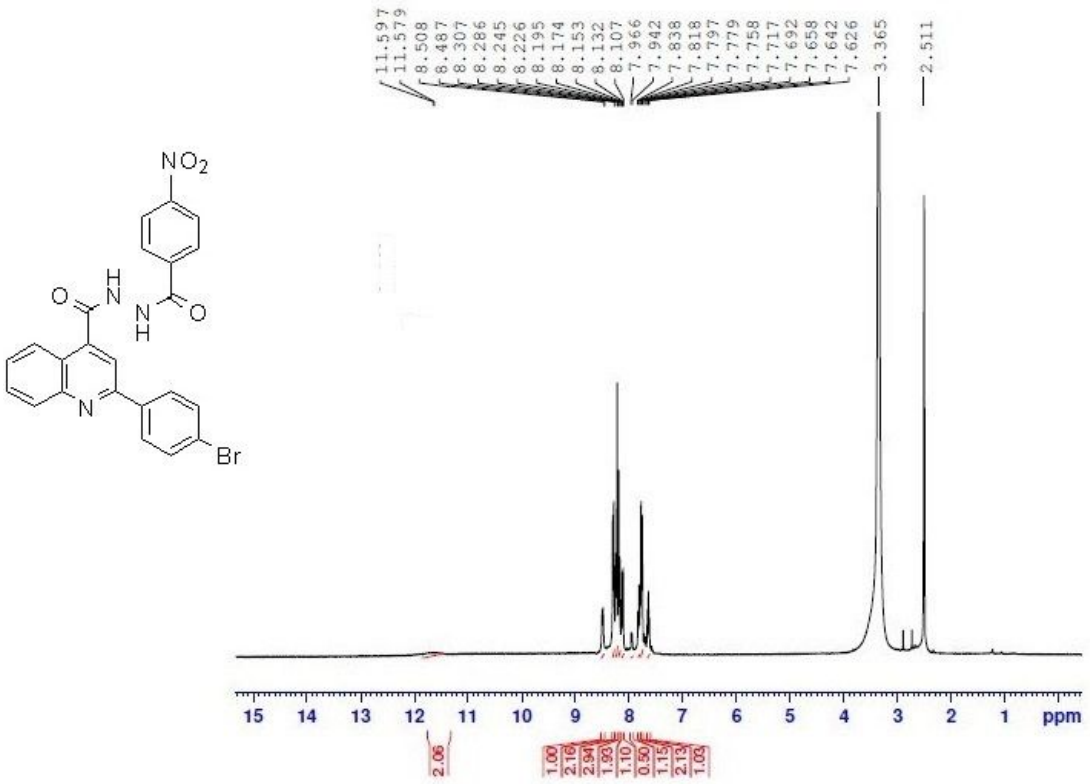


Figure S19: ^{1}H NMR spectrum of the compound **7c**.

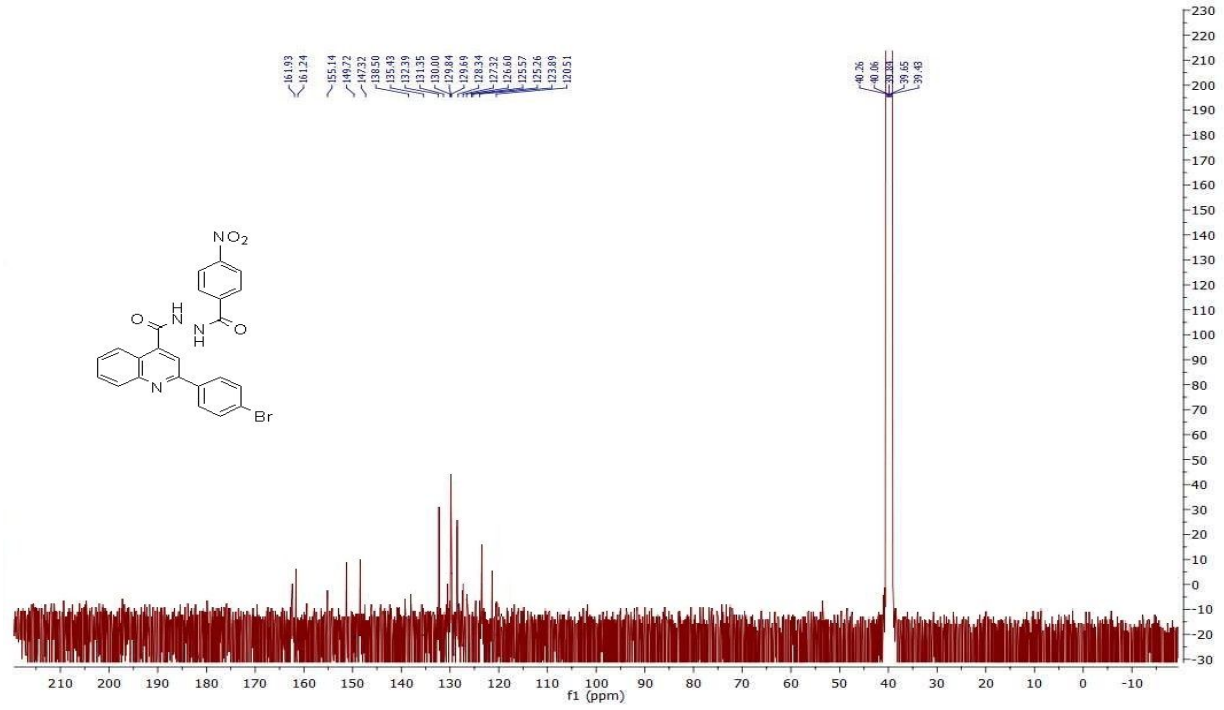


Figure S20: ^{13}C NMR spectrum of the compound **7c**.

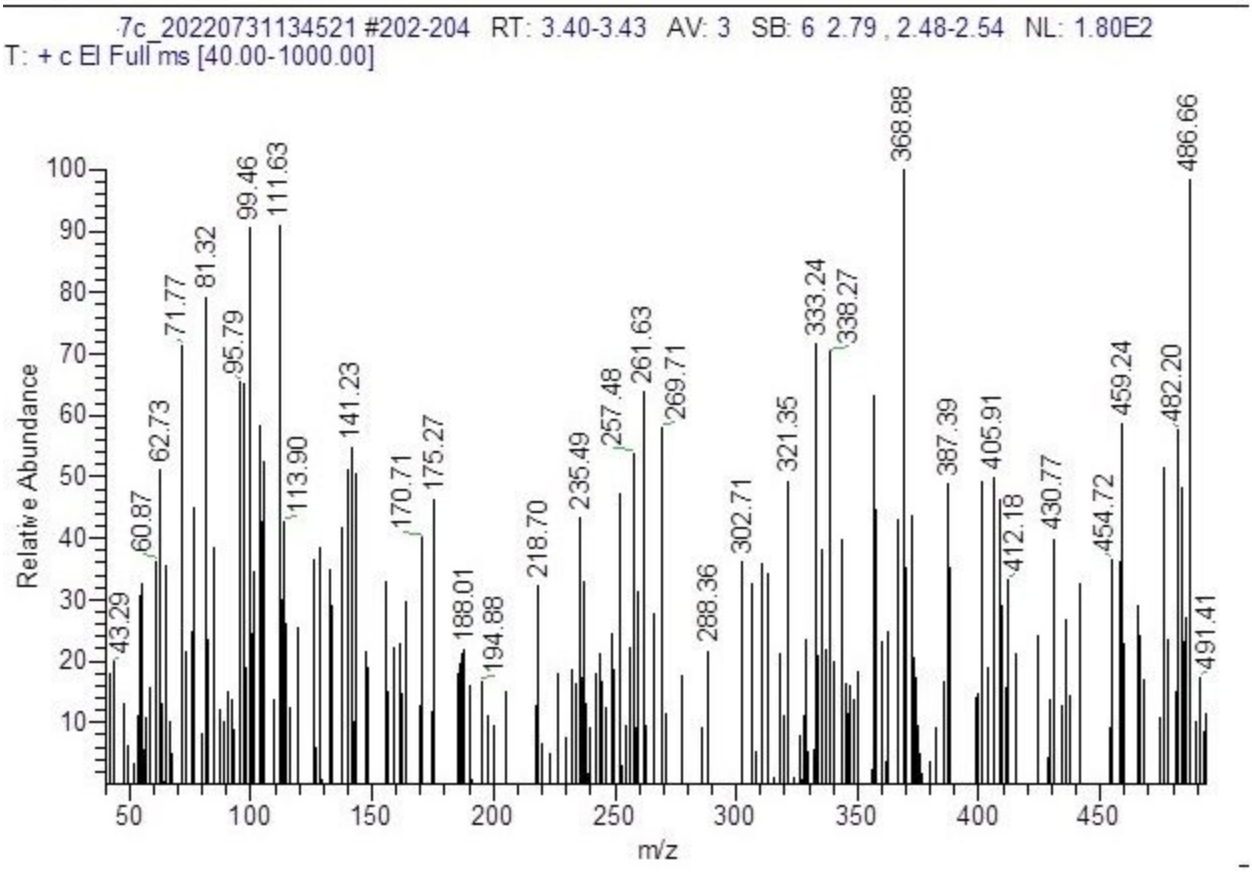


Figure S21: MS of the compound **7c**.

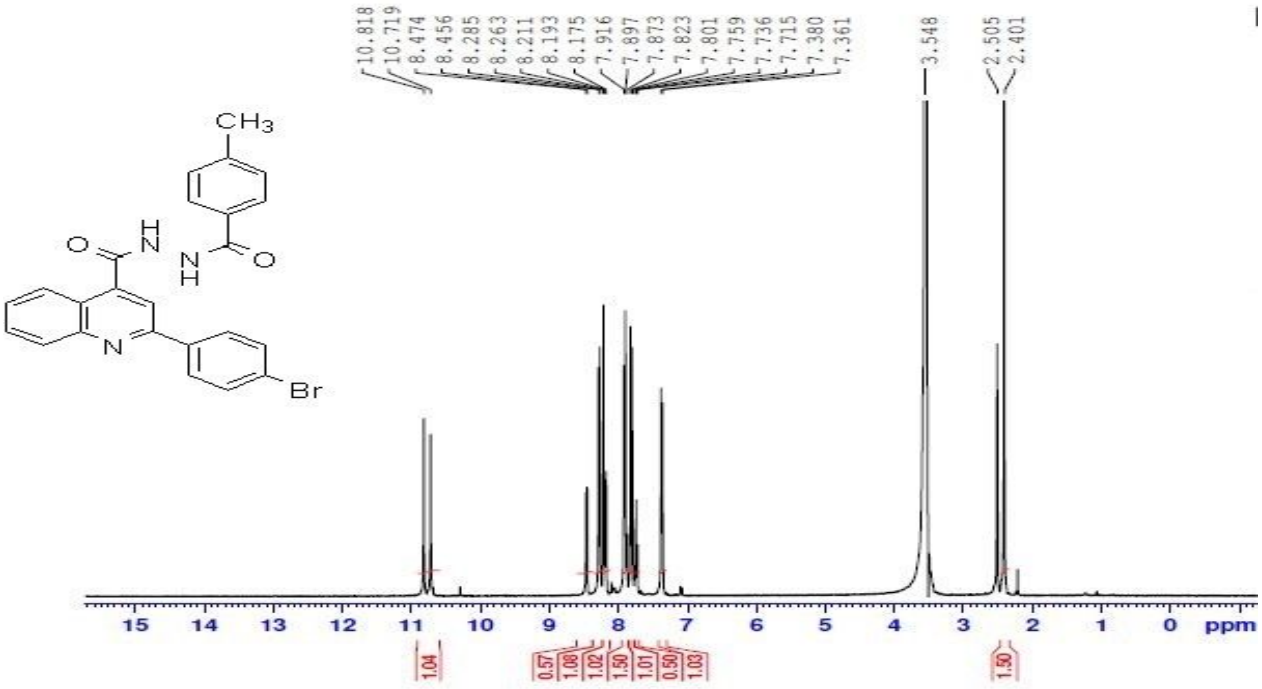


Figure S22: ^{1}H NMR spectrum of the compound **7d**.

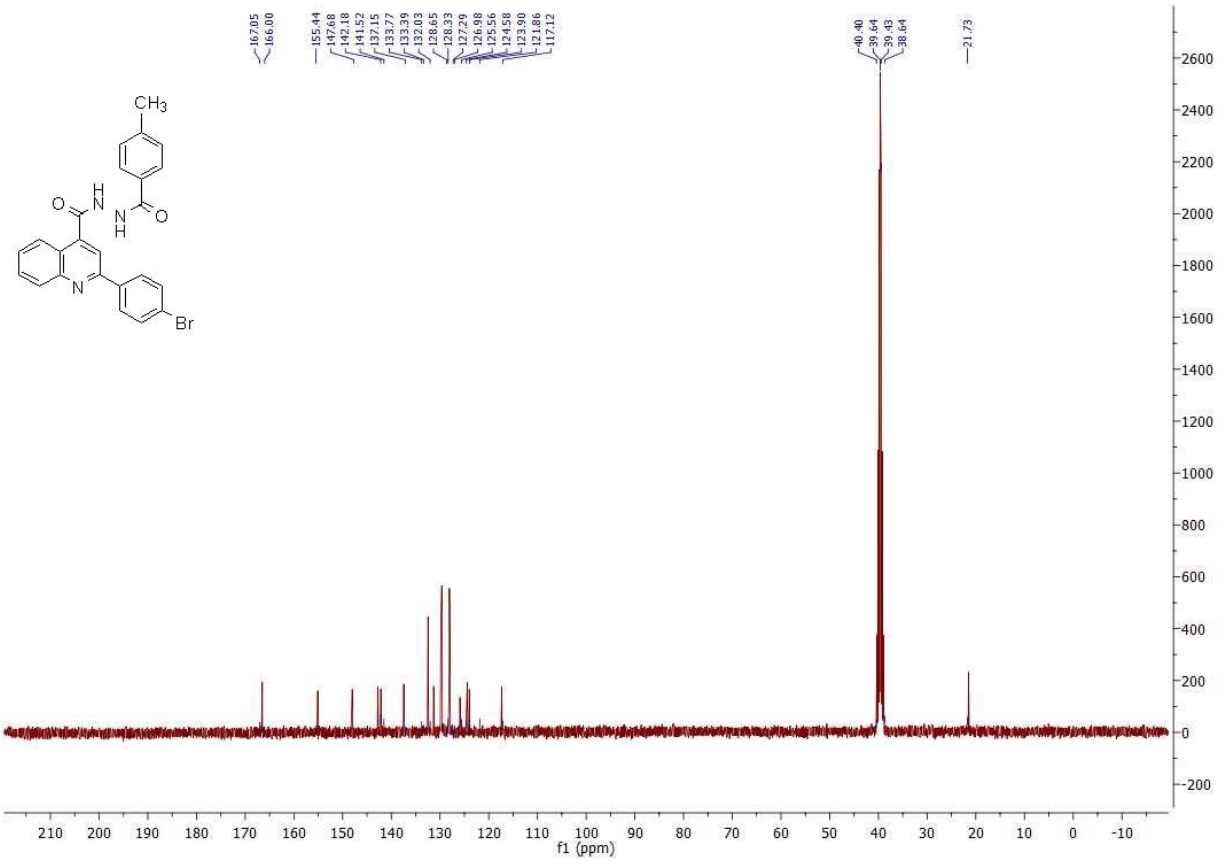


Figure S23: ^{13}C NMR spectrum of the compound **7d**.

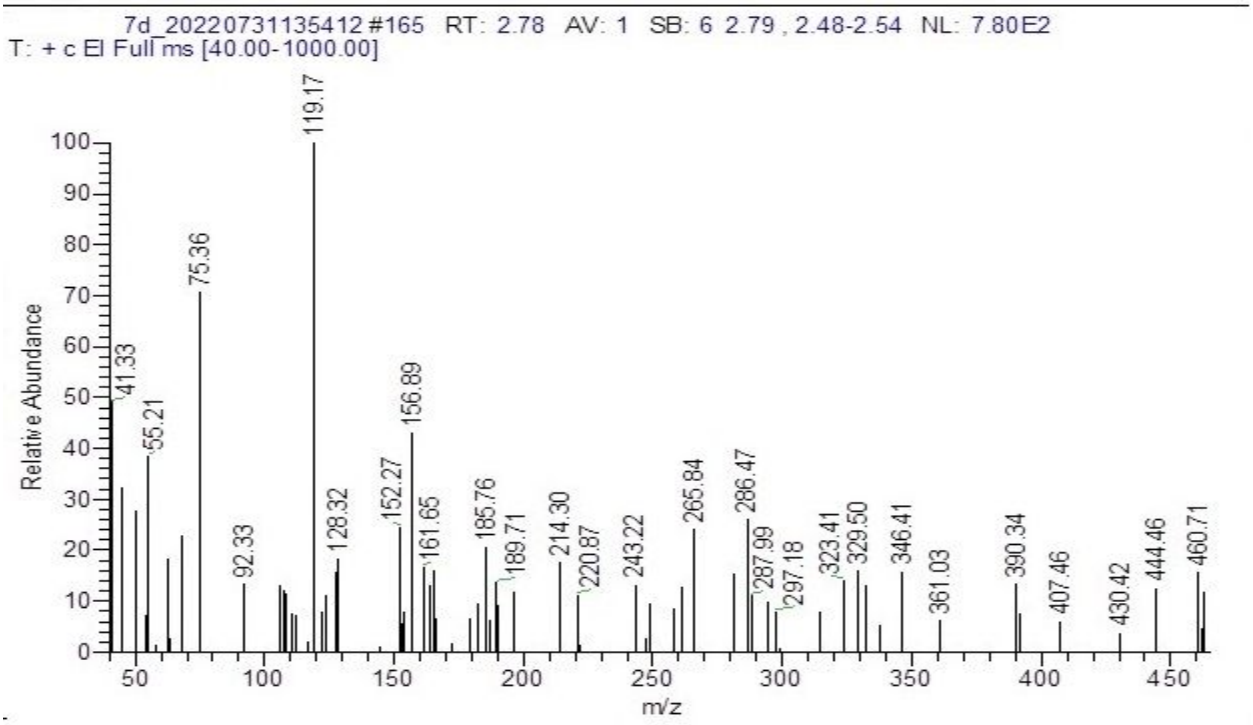


Figure S24: MS of the compound **7d.**

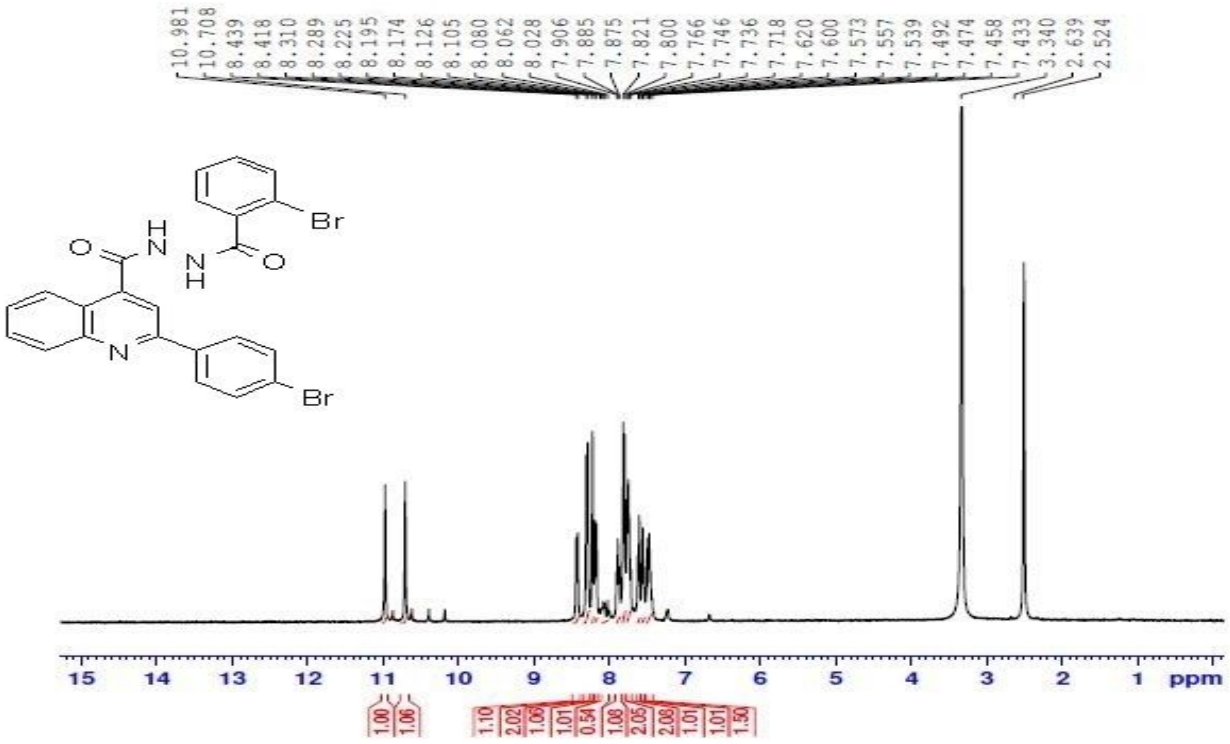


Figure S25: ^{1}H NMR spectrum of the compound **7e**.

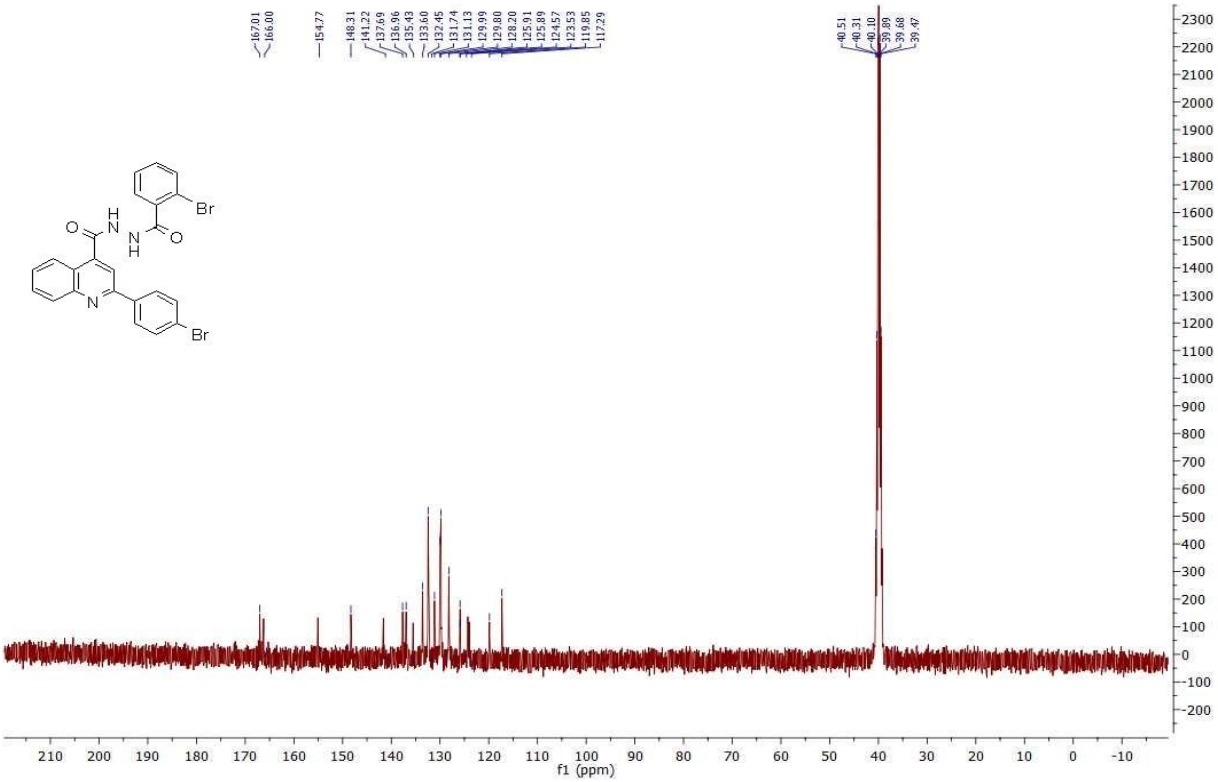


Figure S26: ^{13}C NMR spectrum of the compound **7e**.

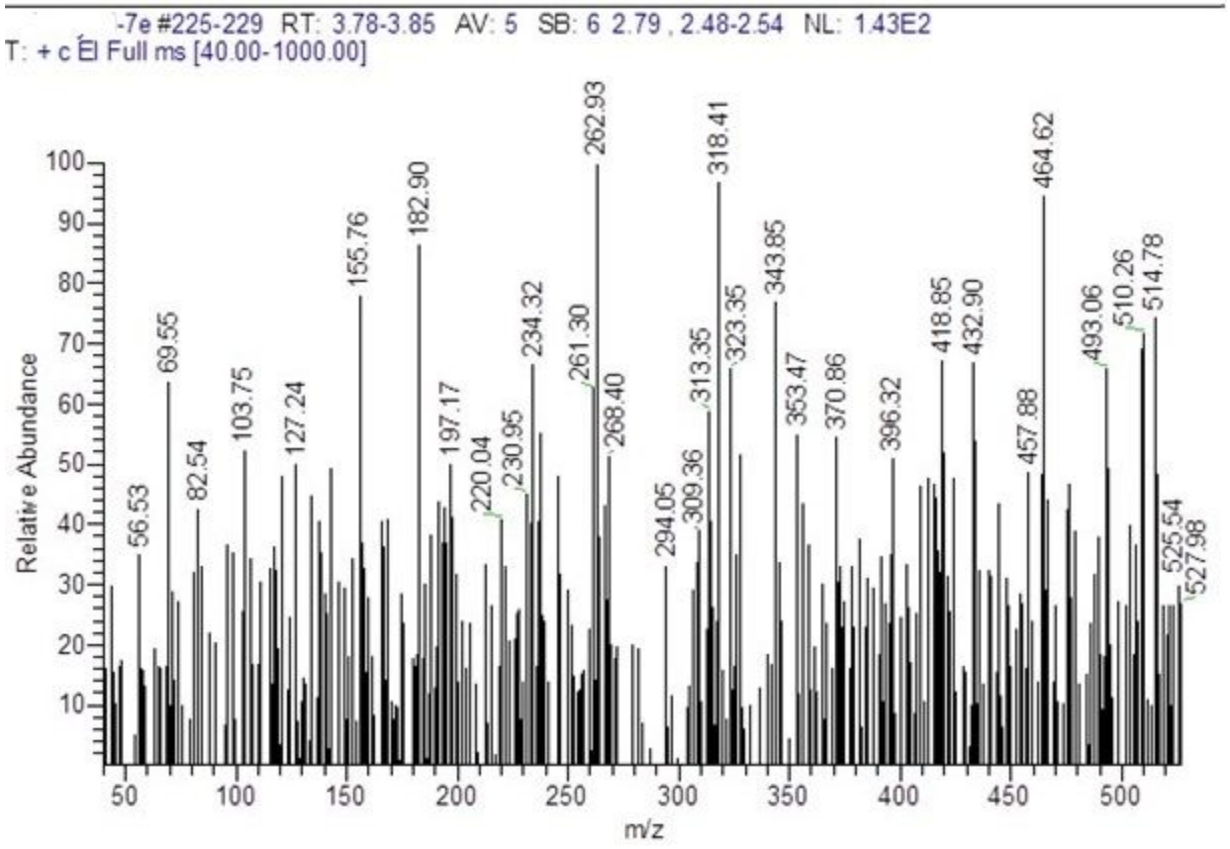


Figure S27: MS of the compound **7e**.

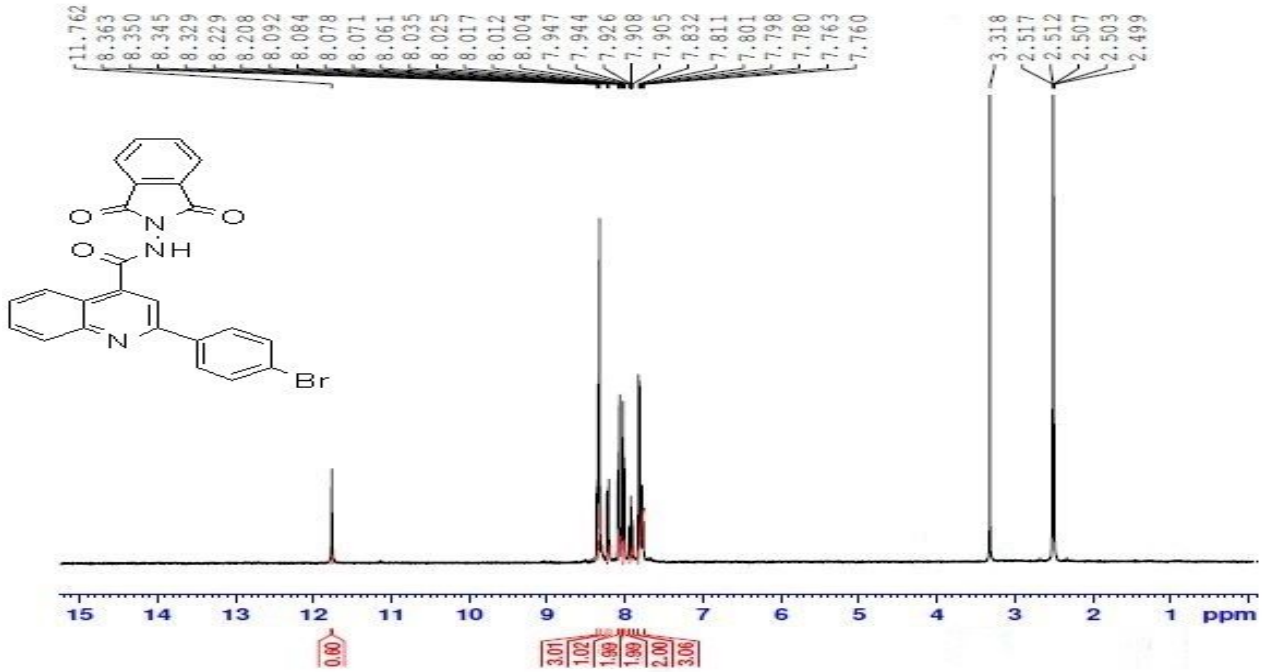


Figure S28: ^{1}H NMR spectrum of the compound **8**.

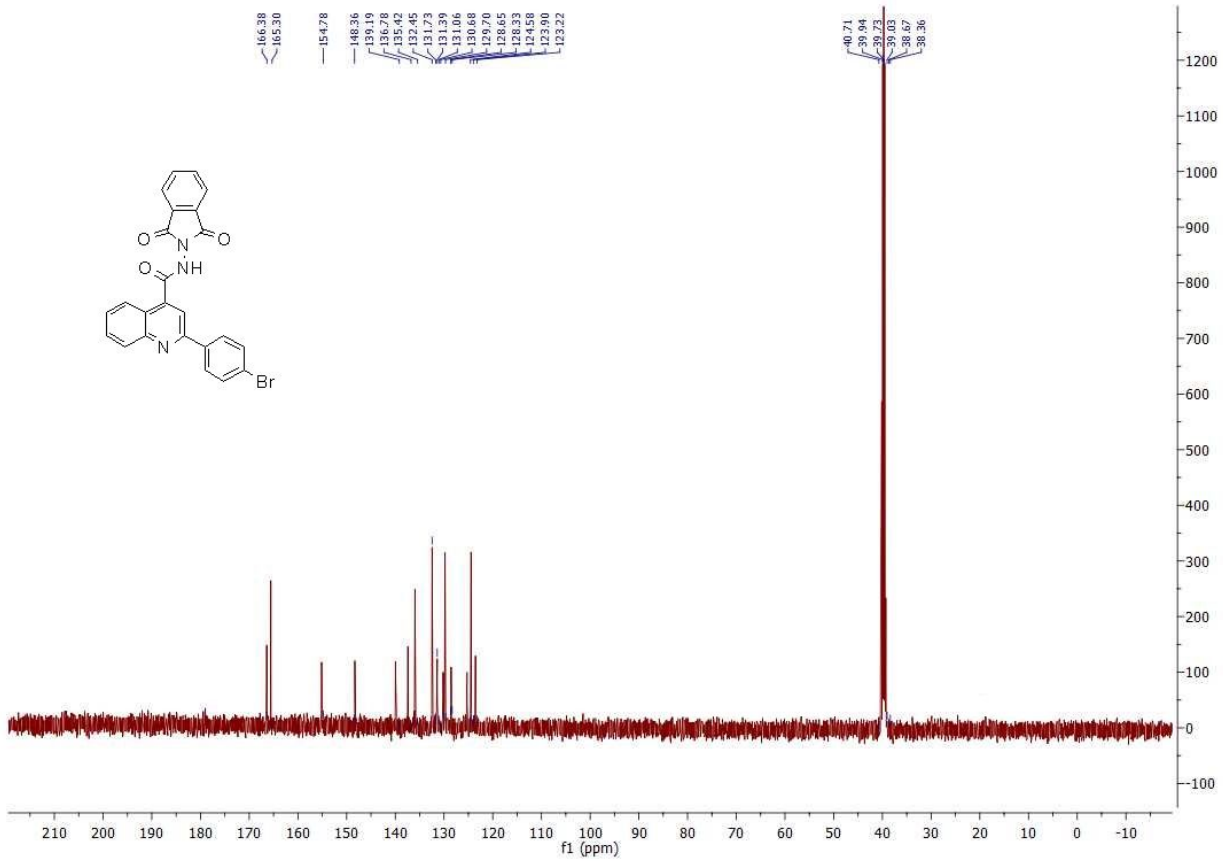


Figure S29: ^{13}C NMR spectrum of the compound **8**.

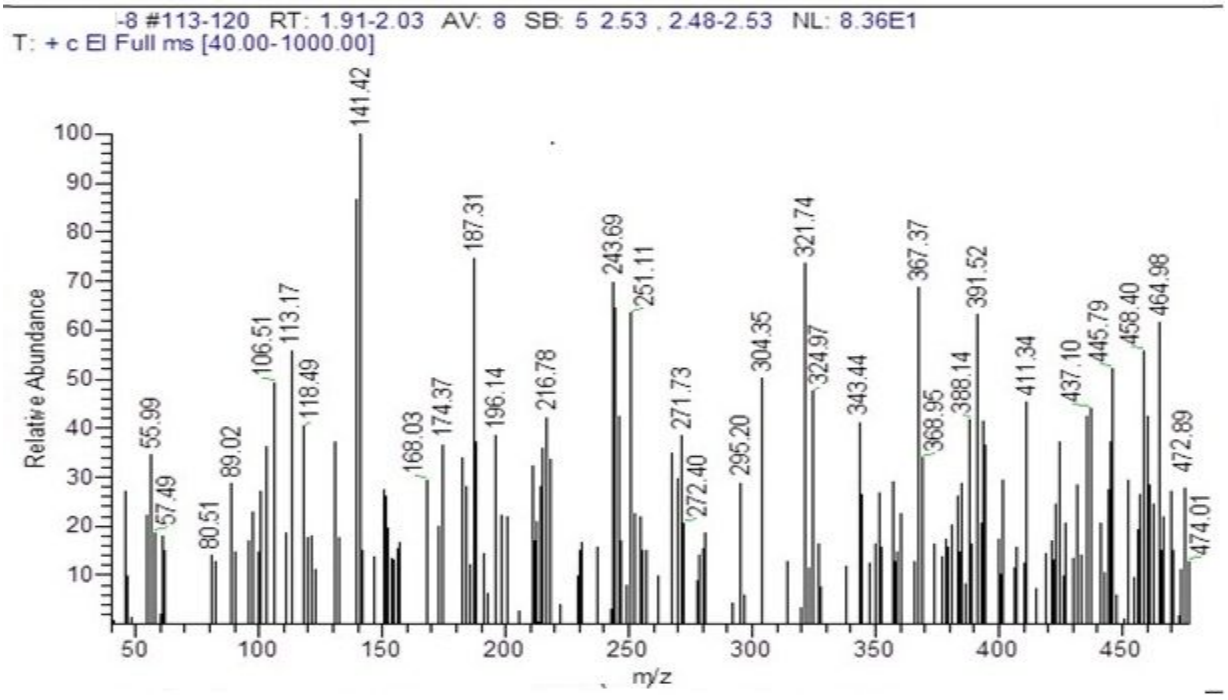


Figure S30: MS of the compound **8**.

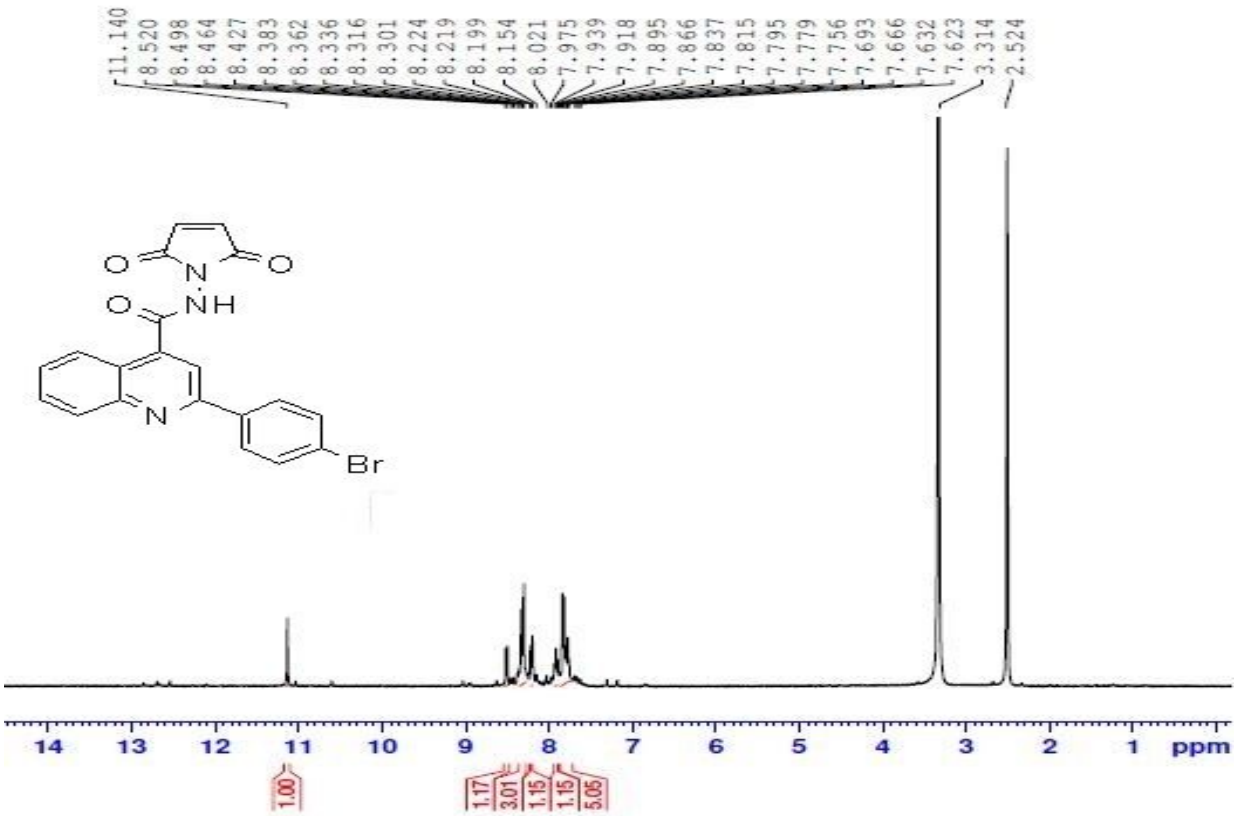


Figure S31: ^{1}H NMR spectrum of the compound **9**.

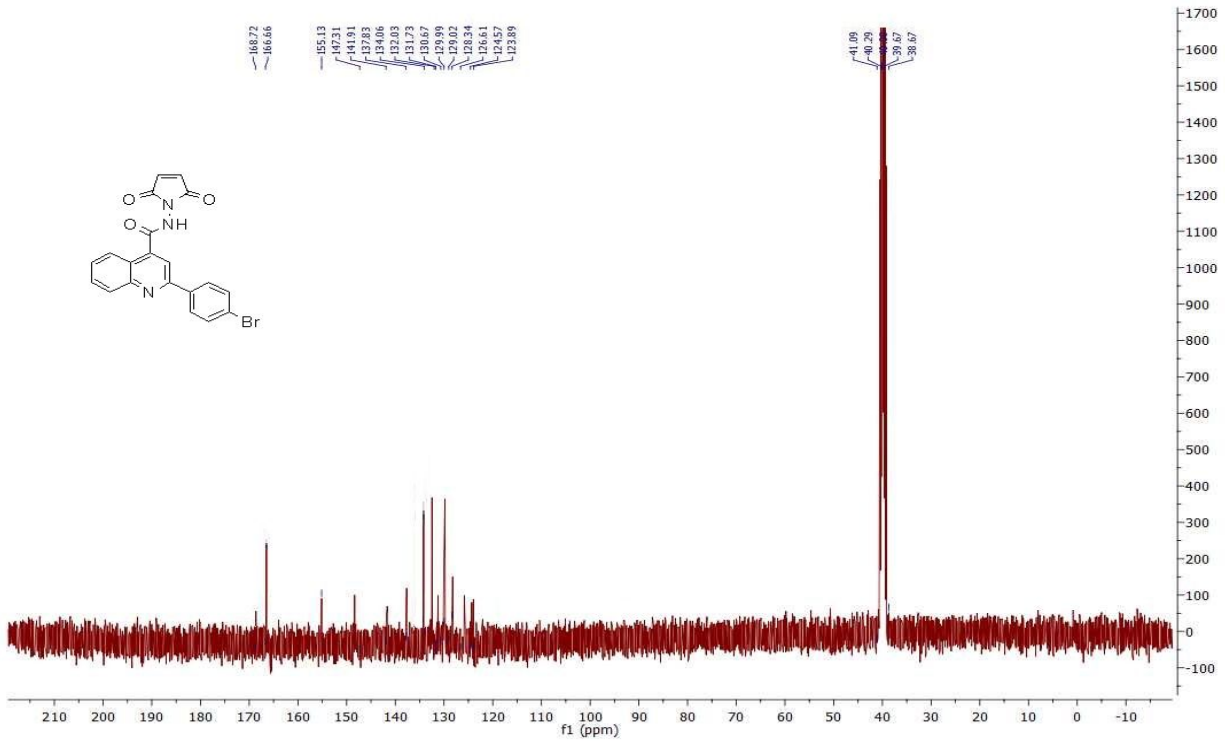


Figure S32: ^{13}C NMR spectrum of the compound **9**.

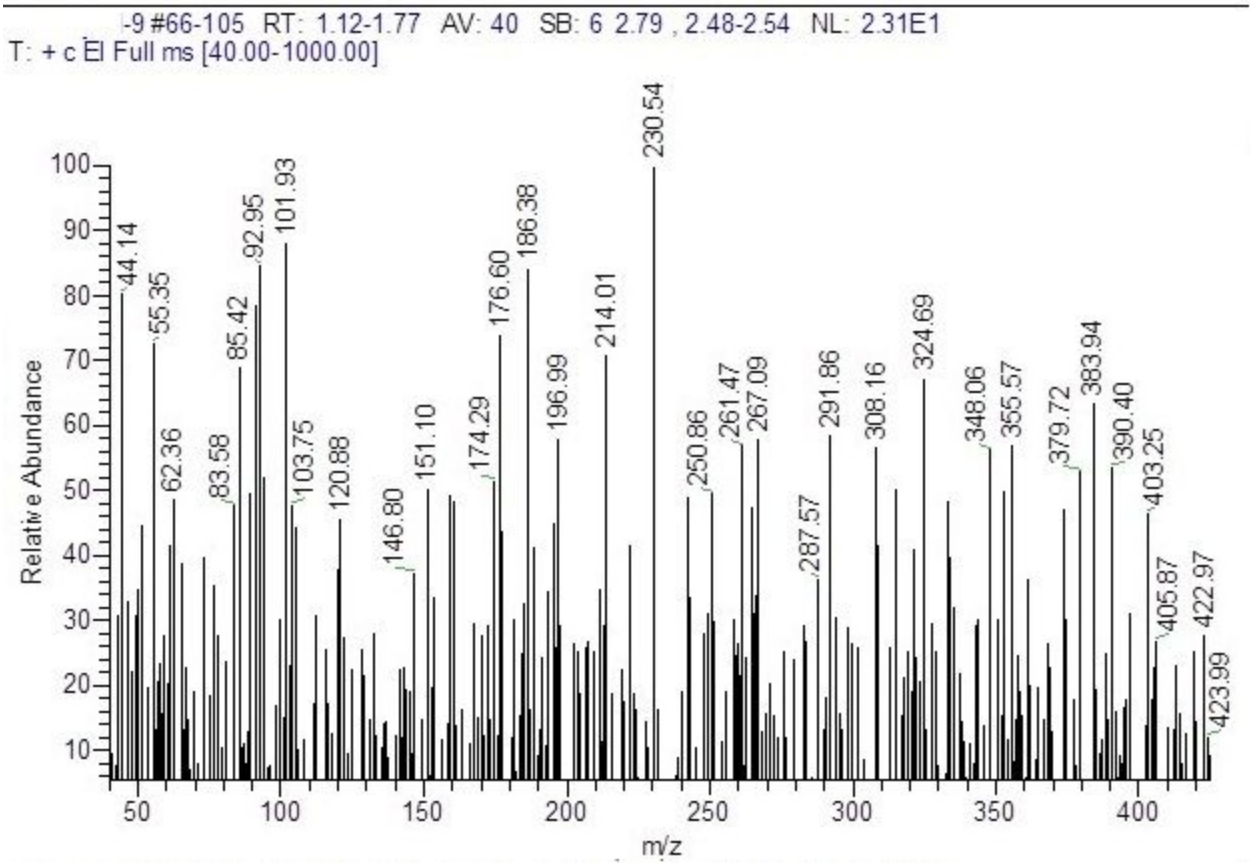


Figure S33: MS of the compound **9**.

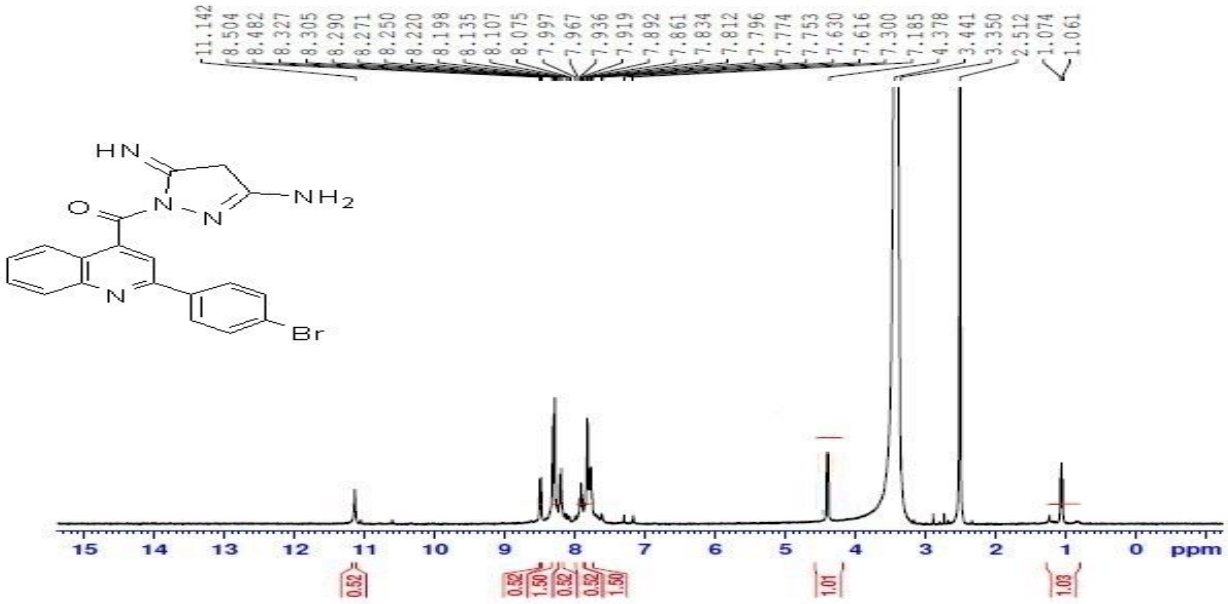


Figure S34: ^{1}H NMR spectrum of the compound **10**.

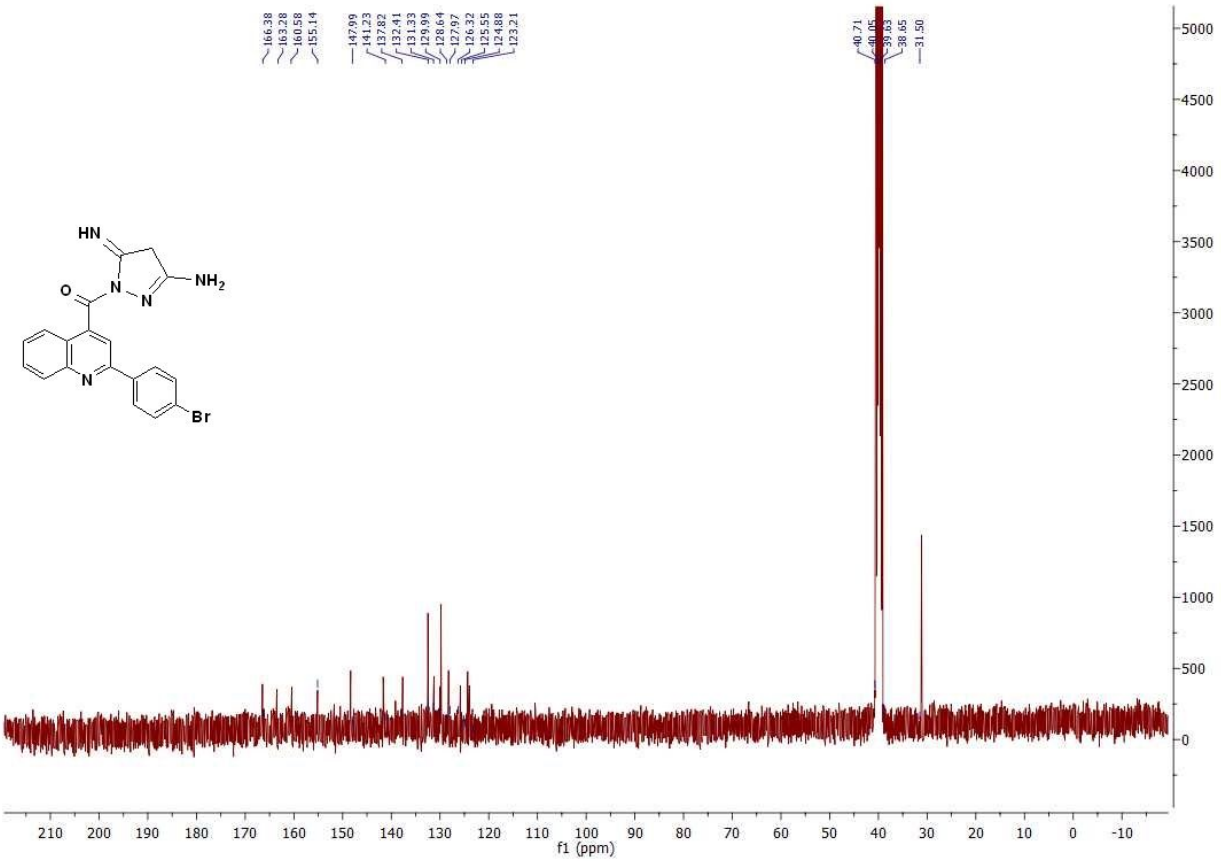


Figure S35: ^{13}C NMR spectrum of the compound **10**.

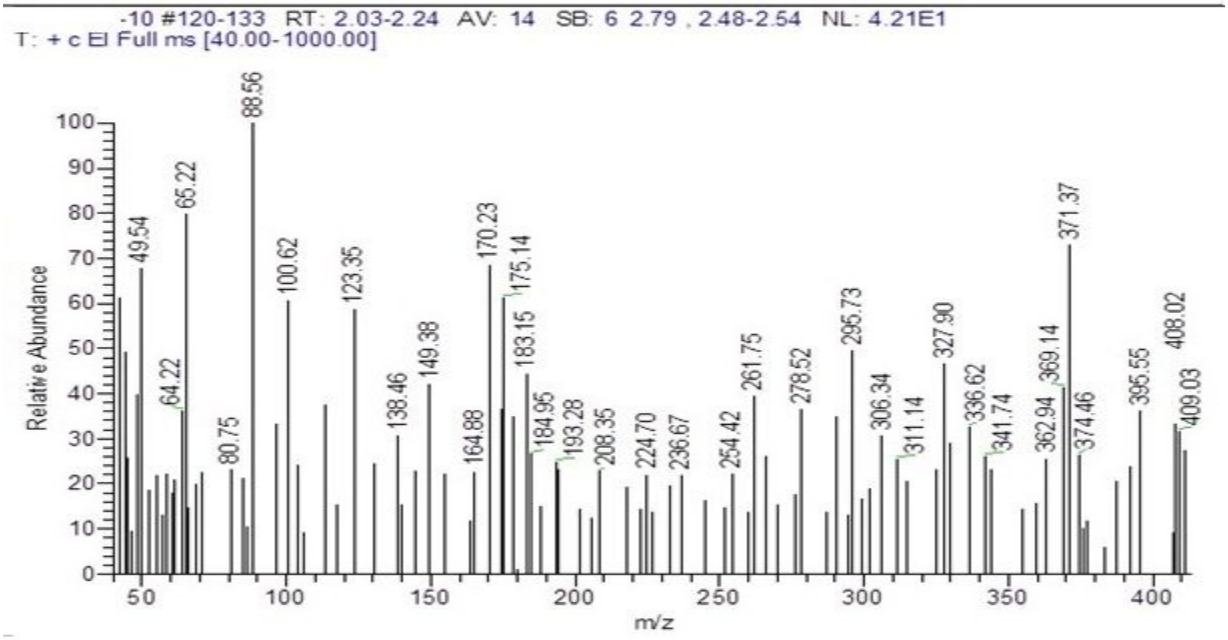


Figure S36: MS of the compound **10**.

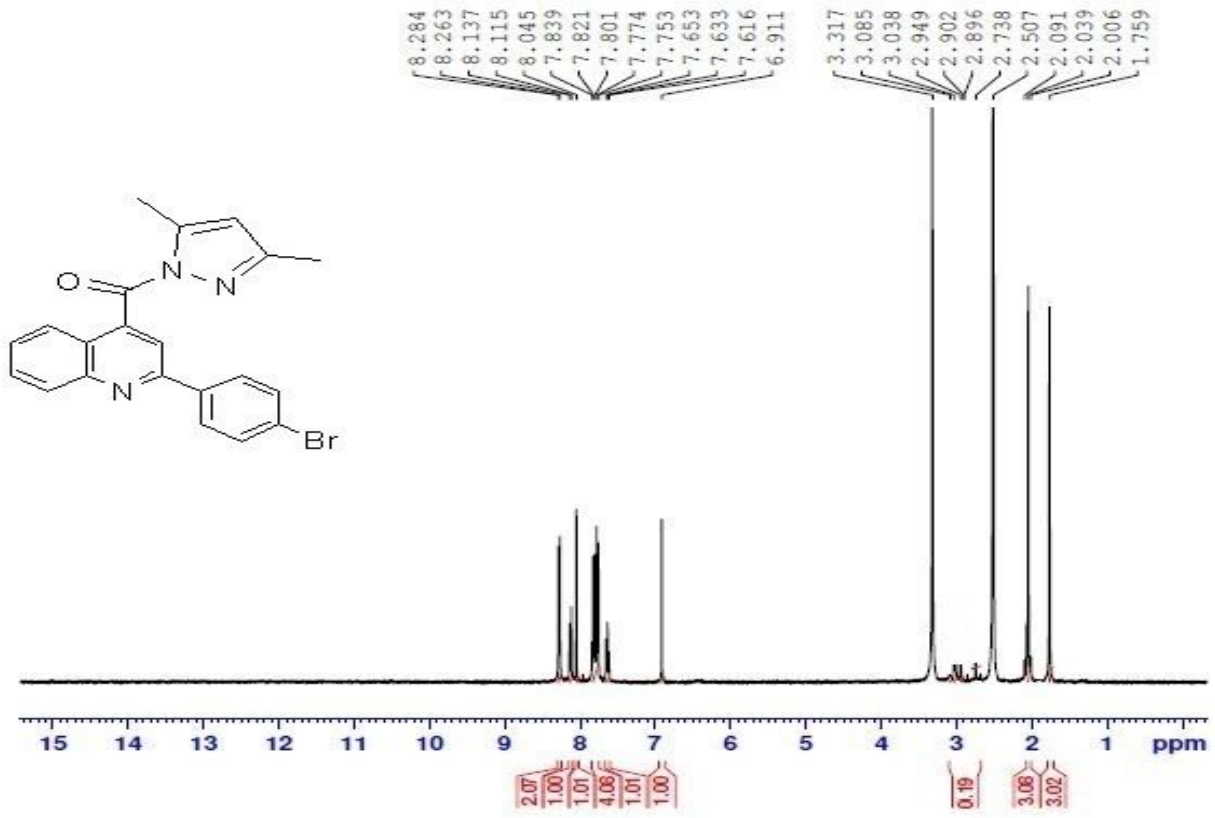


Figure S37: ^{1}H NMR spectrum of the compound **11**.

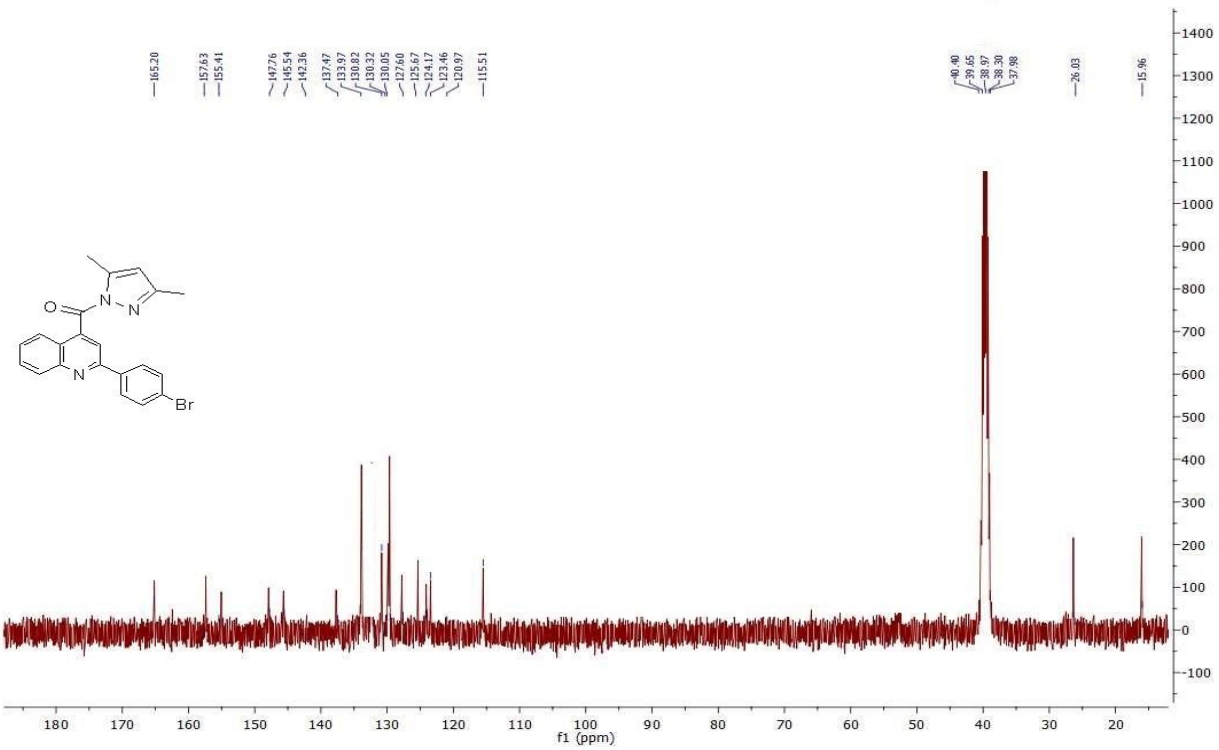


Figure S38: ^{13}C NMR spectrum of the compound **11**.

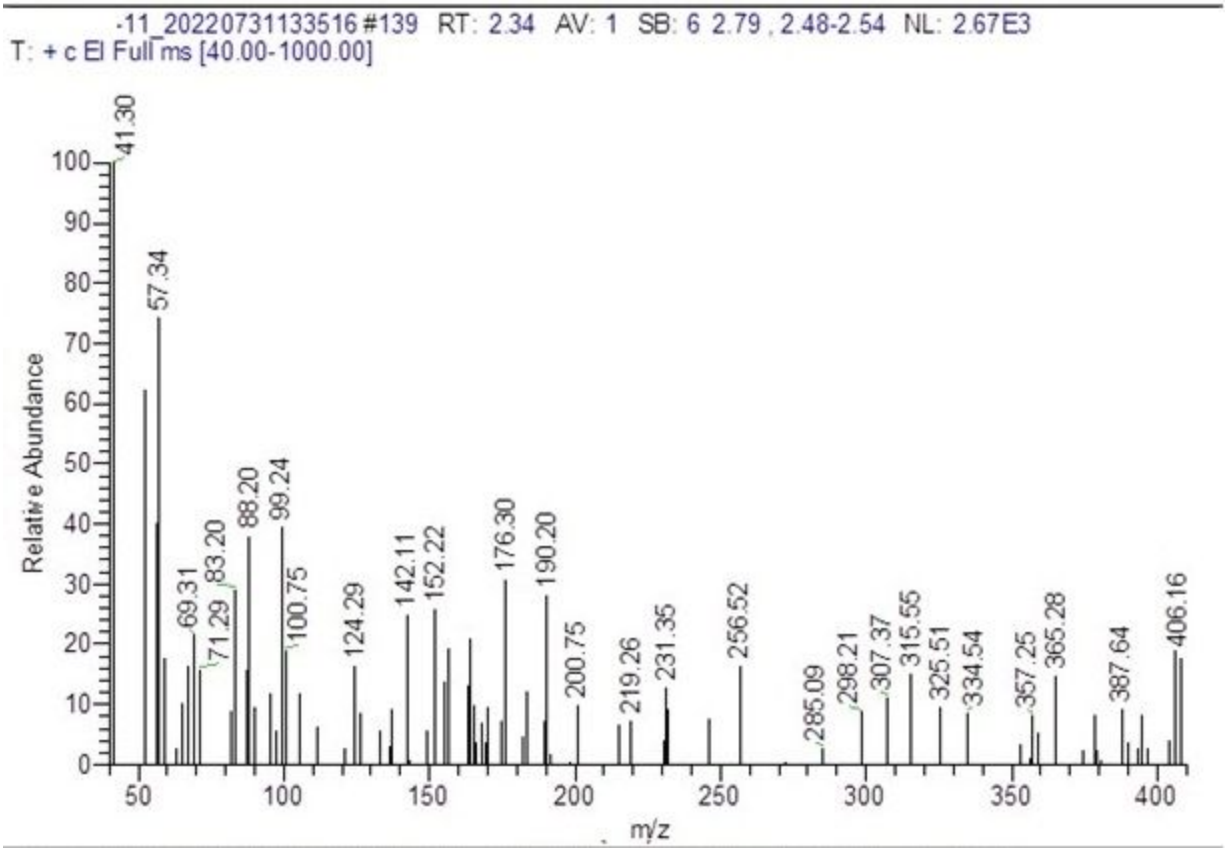


Figure S39: MS of the compound **11**.

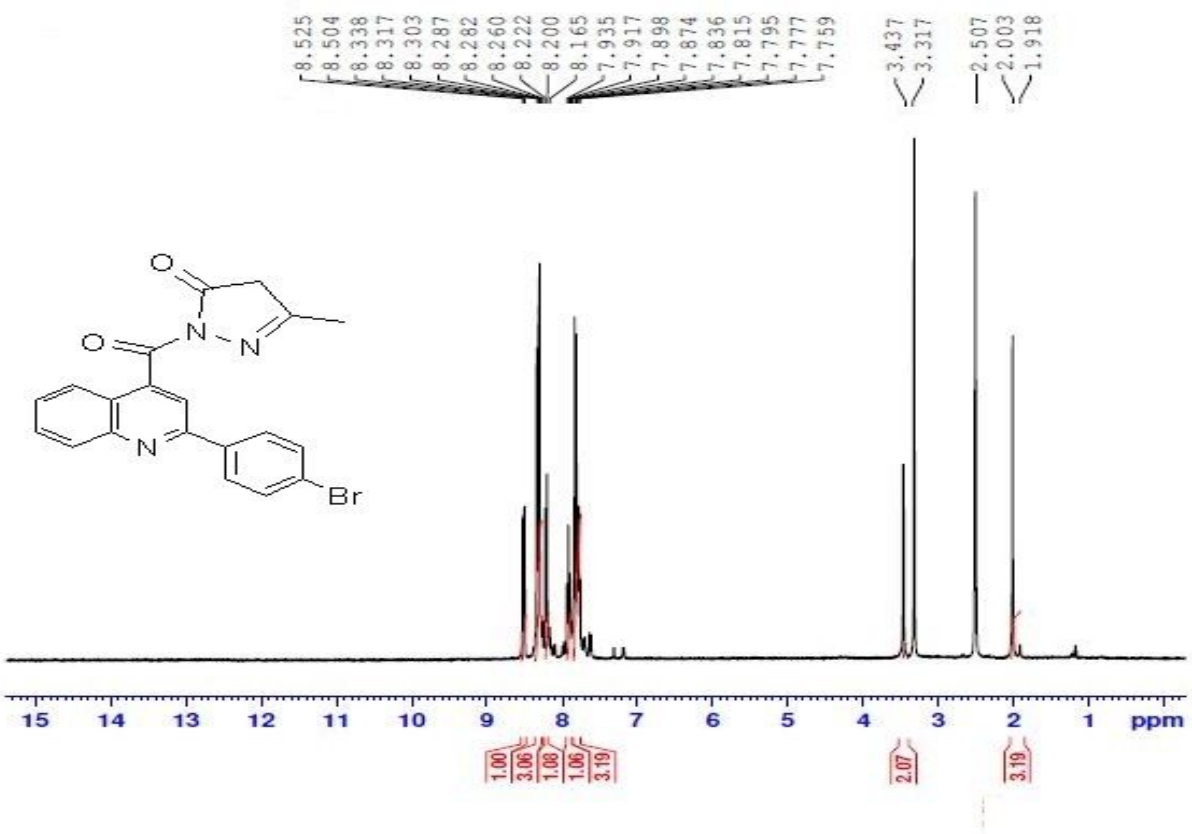


Figure S40: ^{1}H NMR spectrum of the compound **12**.

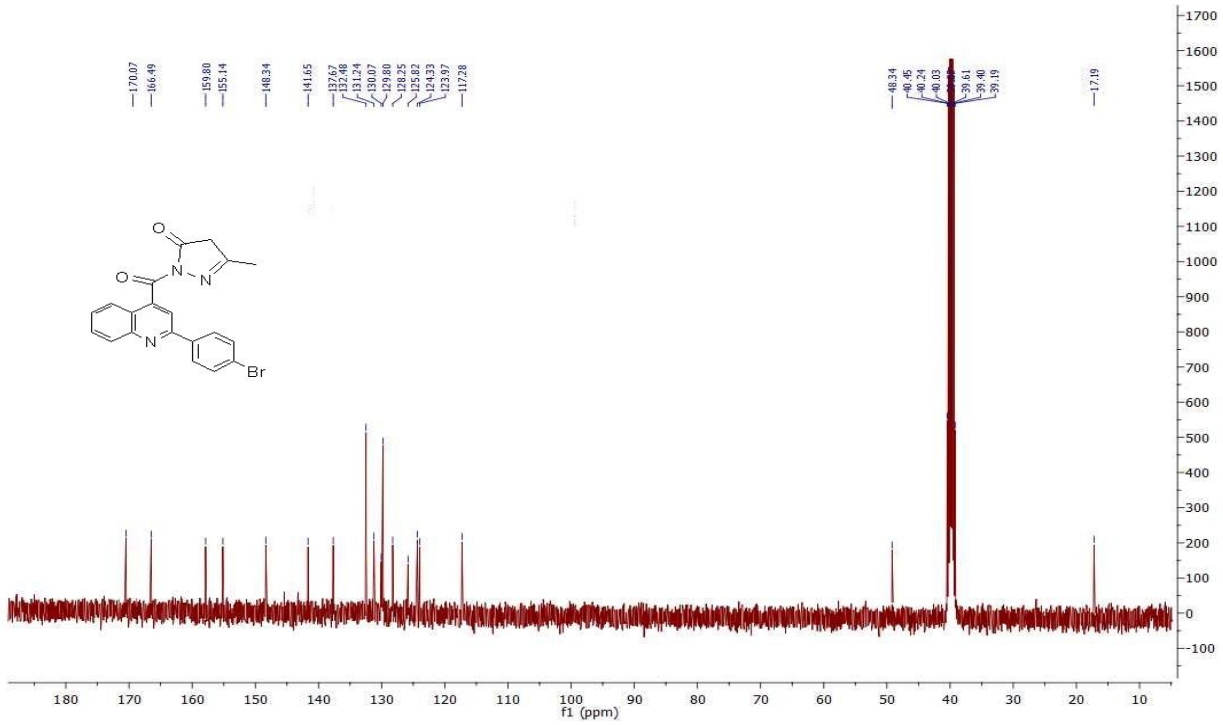


Figure S41: ^{13}C NMR spectrum of the compound **12**.

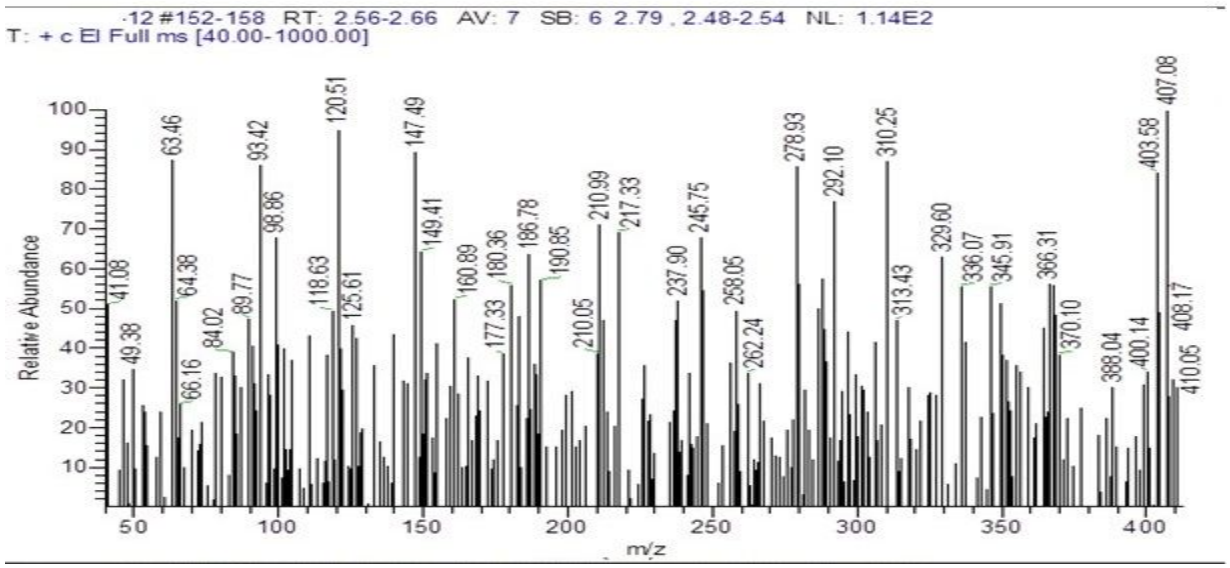


Figure S42: MS of the compound **12**.

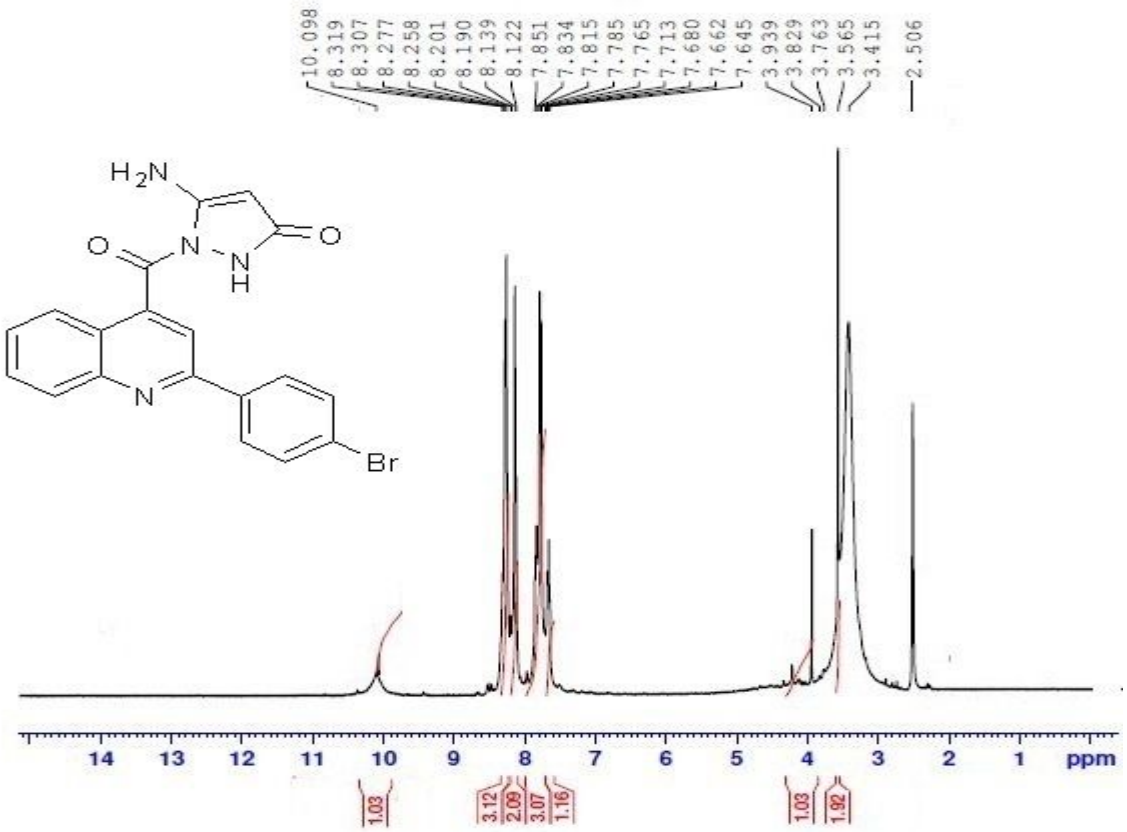


Figure S43: [1]H NMR spectrum of the compound **13**.

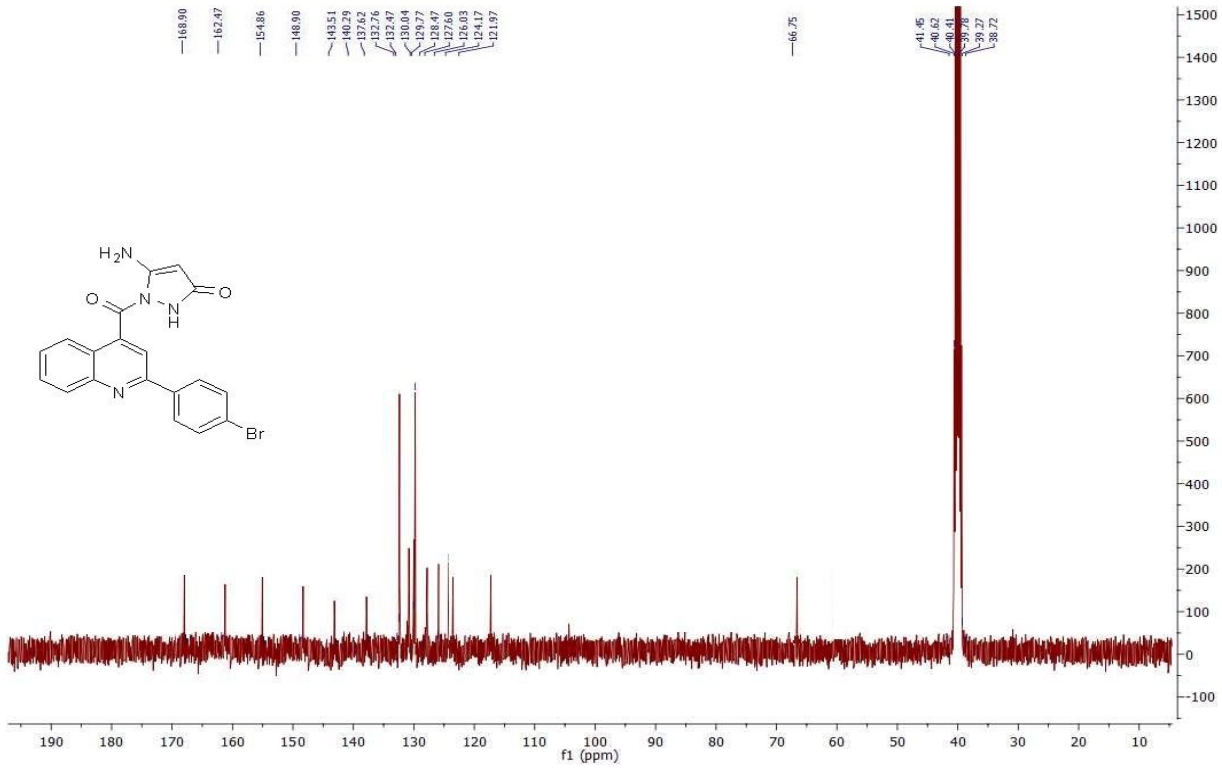


Figure S44: ^{13}C NMR spectrum of the compound **13**.

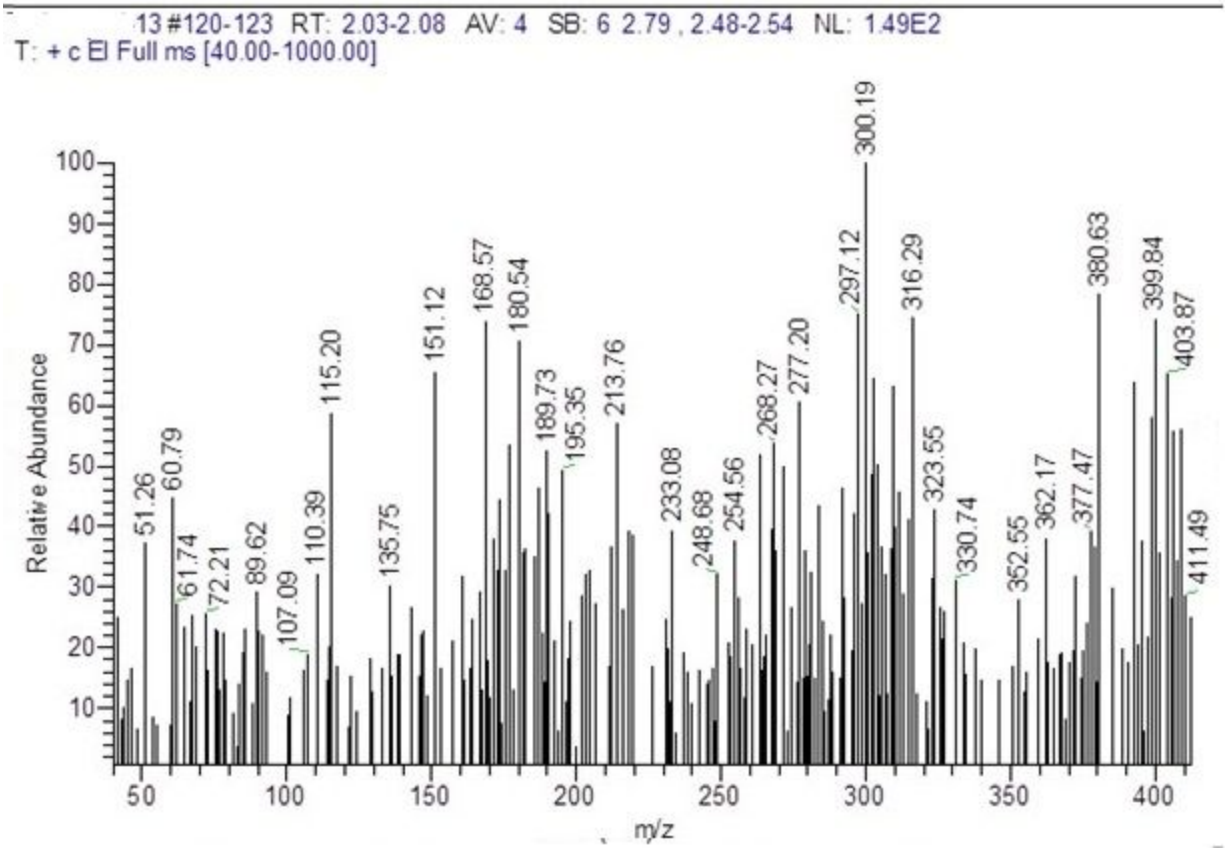


Figure S45: MS of the compound **13**.

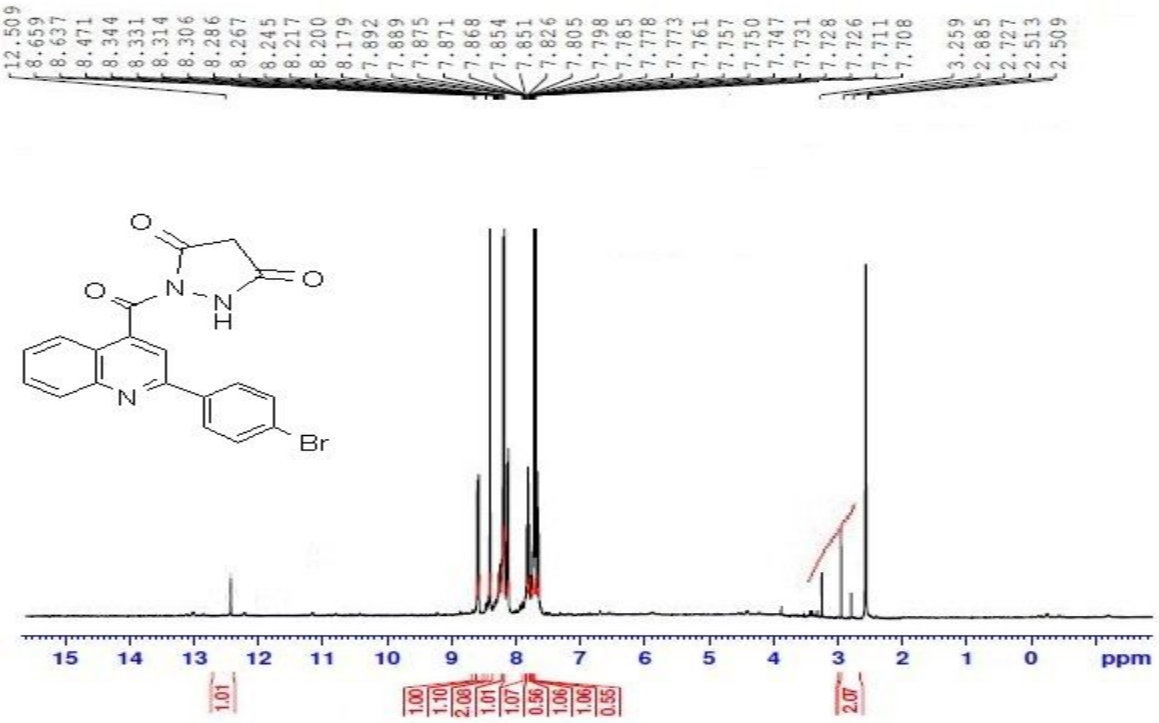


Figure S46: ^{1}H NMR spectrum of the compound **14**.

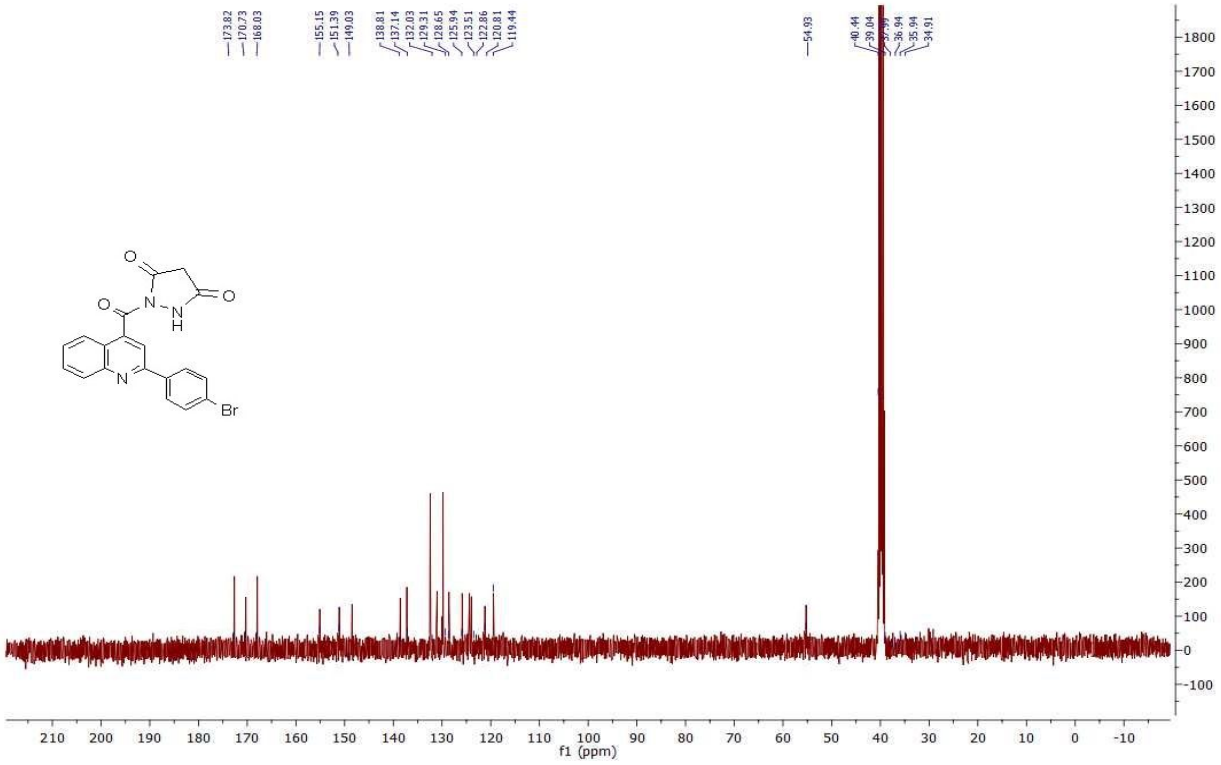


Figure S47: ^{13}C NMR spectrum of the compound **14**.

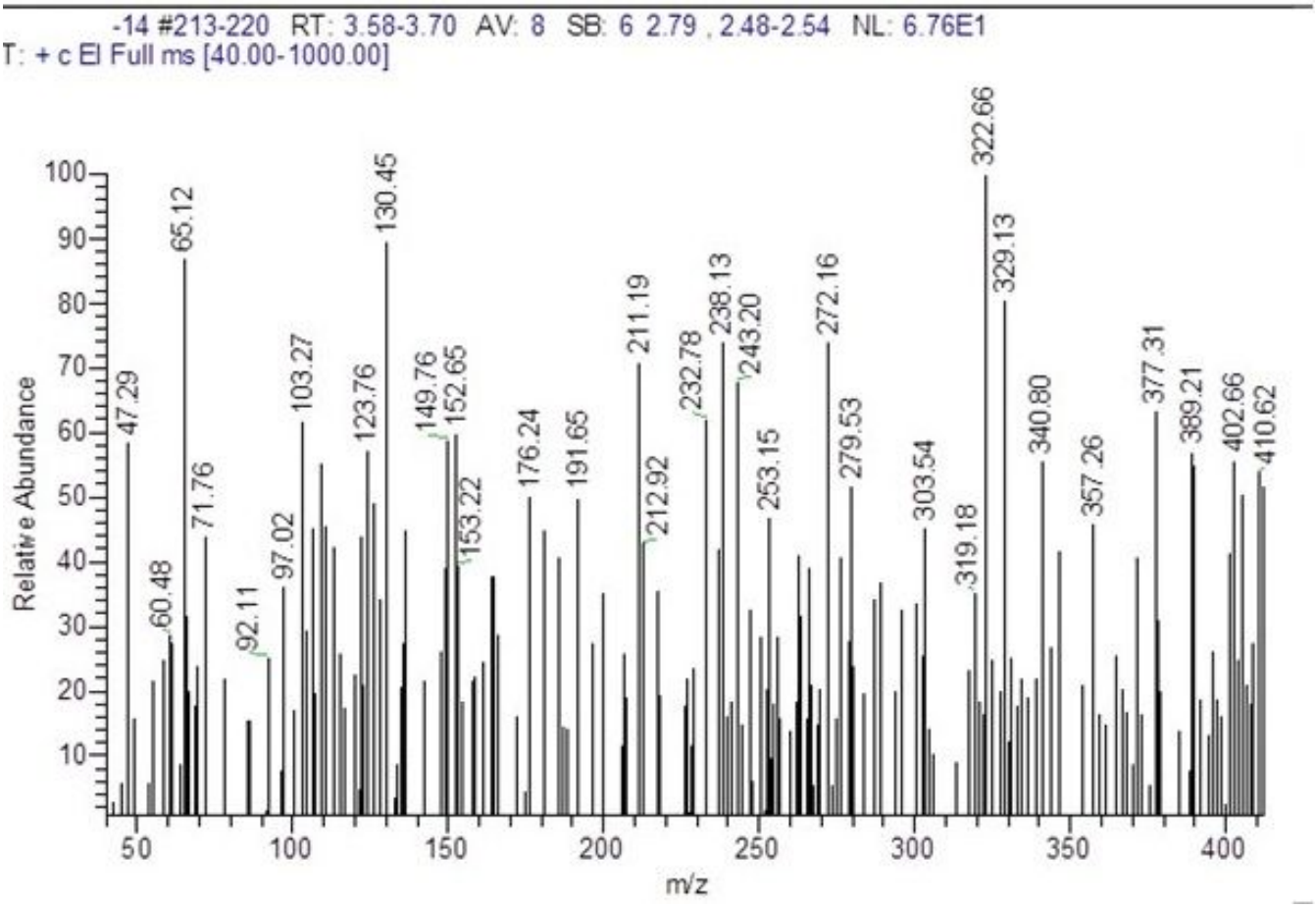


Figure S48: MS of the compound **14**.

Part 3: Biological studies

Appendix A

S4.2. Biology

S4.2.2. Agar well diffusion method

10 mg from each compound was dissolved in 2ml of DMSO and 100µl from each compound was tested with a final dose of 500µg for each treatment. Nutrient agar plates were heavily inoculated regularly with 0.1ml of 10^5-10^6 cells/ml in case of bacteria and yeast. Czapek-Dox agar plates seeded by 0.1ml (10^6 cells/ml) the fungal inoculum was used to evaluate the antifungal activities. Three holes were initiated in each inoculated plate. 100microleter from each sample were dispensed in each cup. Then plates were kept at low temperature (4°C) for 2-4 hours to allow maximum diffusion. The plates were then incubated at 37°C for 24 hours for bacteria and at 30°C for 48 hours in upright position to allow maximum growth of the organisms. The antimicrobial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter (mm). The experiment was carried out more than once and mean of reading was recorded.

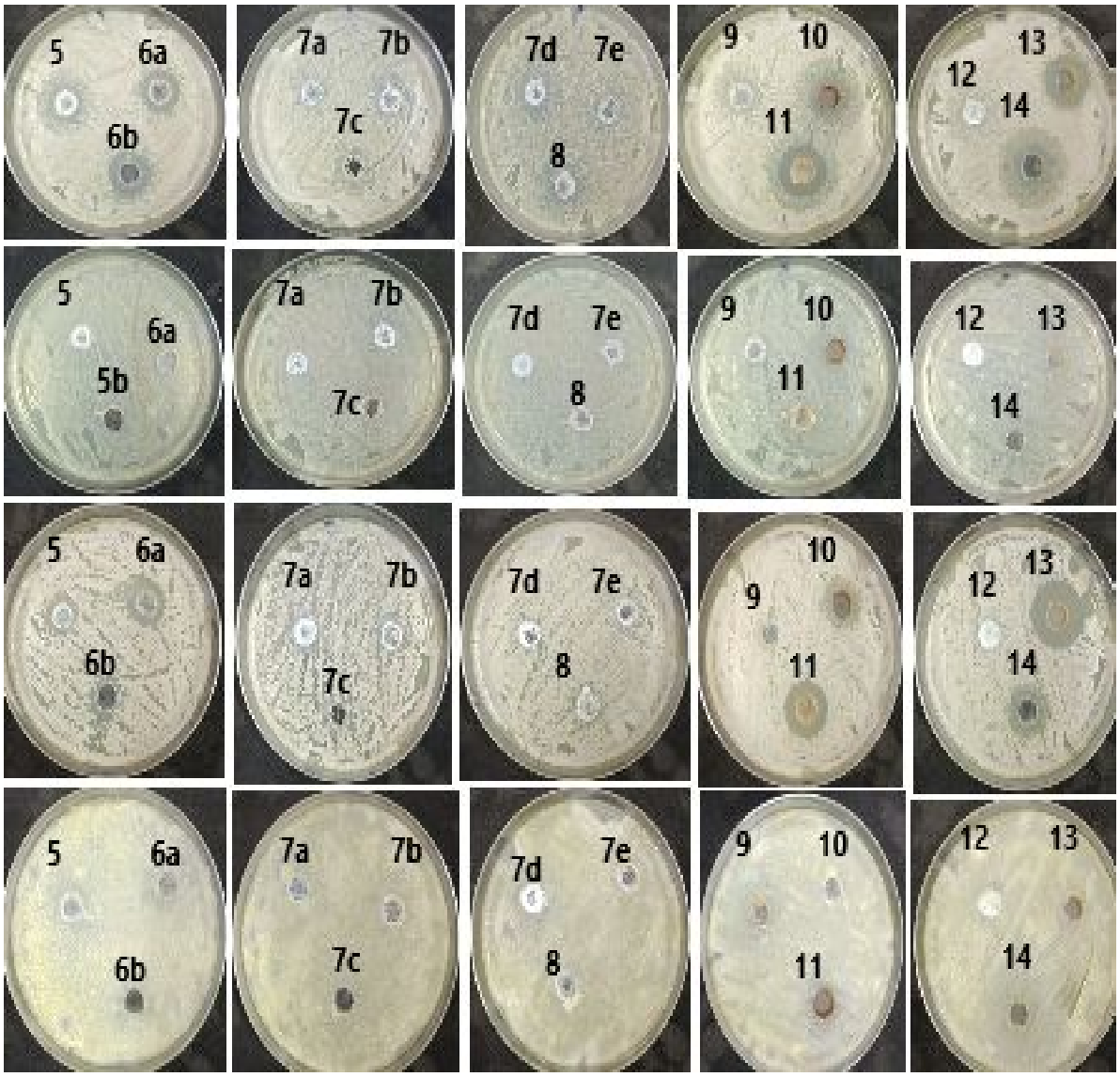


Figure S49: Agar well diffusion method

S4.2.3. MIC and MBC methodology

S4.2.3.1. Preparation of bacterial culture

Bacterial cultures were prepared under sterile conditions by incubating 100 ml bottle with each test microbe, capped and incubated at 35 ^{o}C for 24 h. Clean bacterial cells were prepared by centrifuging the growth culture, under sterile condition, in cooling centrifuge at 400 rpm for 15 min. The bacterial cells were re-suspended using 20 mL of sterile normal saline and centrifuged again at 4000 rpm for 5 min. This step was repeated until the supernatant was clear. The pellet was then suspended in 20 mL of sterile normal saline. The optical density of the bacterial suspension was recorded at 500 nm, and serial dilutions were carried out with appropriate aseptic techniques until the optical density was in the range of 0.5–1.0. The actual number of colony-forming units was carried out to obtain a concentration of $5x10^6$ cfu/mL.

S4.2.3.2. Preparation of resazurin solution

The resazurin solution was prepared by dissolving a 675 mg in 100 mL of sterile distilled water and shake well with vortex mixer and sterilized by filtration through membrane filter (pore size of (0.22- 0.45 μm).

S4.2.3.3. Preparation of the plates

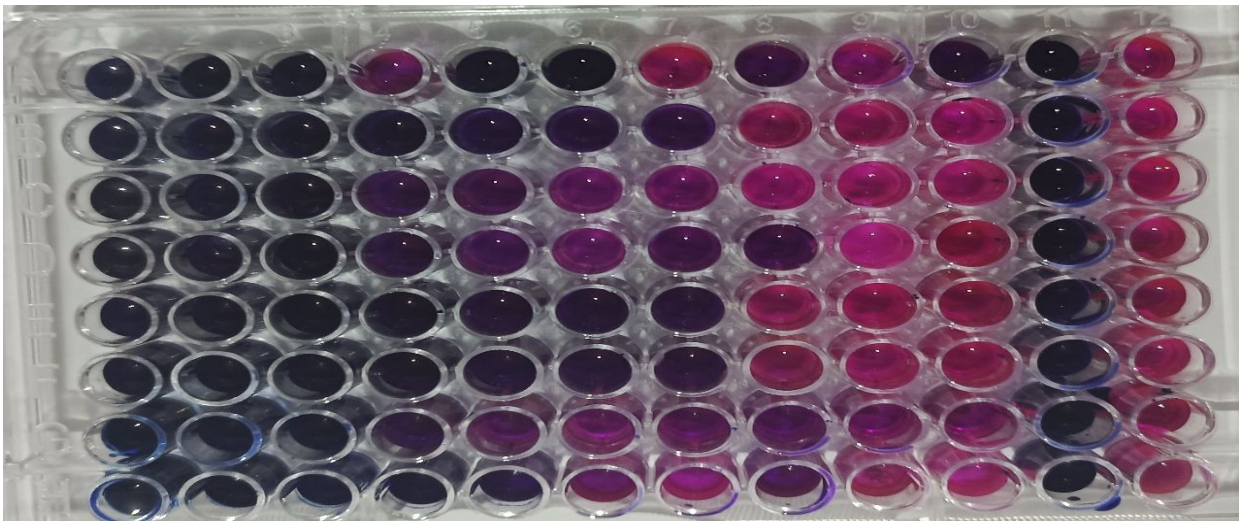
Microplates, 96 well were prepared and labelled under aseptic conditions. A volume of 500 μL of test material in DMSO (a stock concentration of 5mg/mL for purified compounds) was pipetted into the first row of the plate. To all other wells 50 μL of broth medium was added. Serial dilutions were performed. To each well 10μL of resazurin indicator solution was added, 10μL of bacterial suspension (5 $x10^6$cfu/mL) was added to each well. Each plate was wrapped loosely with parafilm to ensure that bacteria did not become dehydrated. The plates were prepared in duplicate and placed in an incubator set at 37°C for 18–24 h. The colour change was then assessed visually. Any colour changes from purple to pink or colourless were recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value.

S4.2.3.4. Determination of minimum bactericidal concentrations (MBC's) of the effective plants extract

Streaks were taken from the two lowest concentrations of the plant extract plates exhibiting invisible growth (from inhibition zone of MIC plates) and subcultures onto sterile nutrient agar plates. The plates were incubated at 35^{o}C for 24 h. then examined for bacterial growth in corresponding to plant extract concentration. MBC was taken as the concentration of plant extract that did not exhibiting any bacterial growth on the freshly inoculated agar plates.[1]

S4.2.3.5. Minimum biofilm inhibitory concentration (MBIC) assay

Inhibin of biofilm formation has been evaluated using 96-well microplates assay method.[2] In each well 200 µl of LB (Lauria broth) has been added. To the first well, 100 µl of the tested compound was added and two-fold dilution has been performed leaving the last two wells as control. To each well, 10 µl of the previously prepared microbial culture (0.5 McFarland standard, 5x10^{5} CFU/ml) were added. After incubating the plates at 37 ^{o}C for 24 h, the culture was gently decanted and then washed by phosphate buffered saline (PBS) buffer. The plates were left to dry for 30 min and 200 µl of crystal violet 0.1% was added to each well and left for 30 min. the excess crystal violet was decanted and washed-out three times with distilled water and left to dry for 30 min. Finally, 200 µl of 95% ethanol was added to each well and the absorbance was measured at 492 nm using ELISA reader.



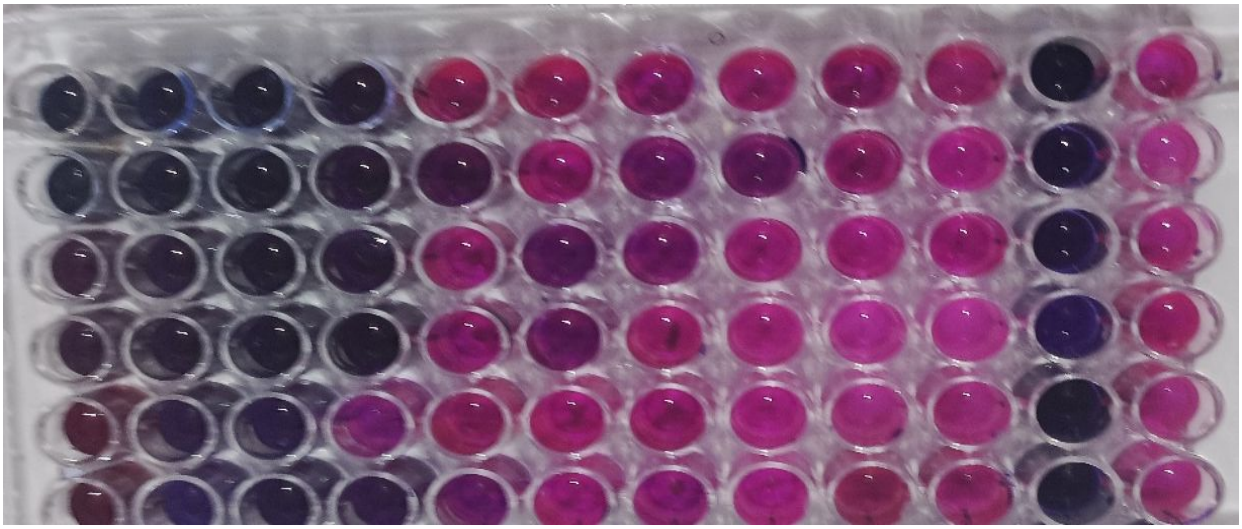


Figure S50: The MIC and MBC assay of compounds **5, 6a, 6b, 10, 11, 13 and 14** against *S. aureus*

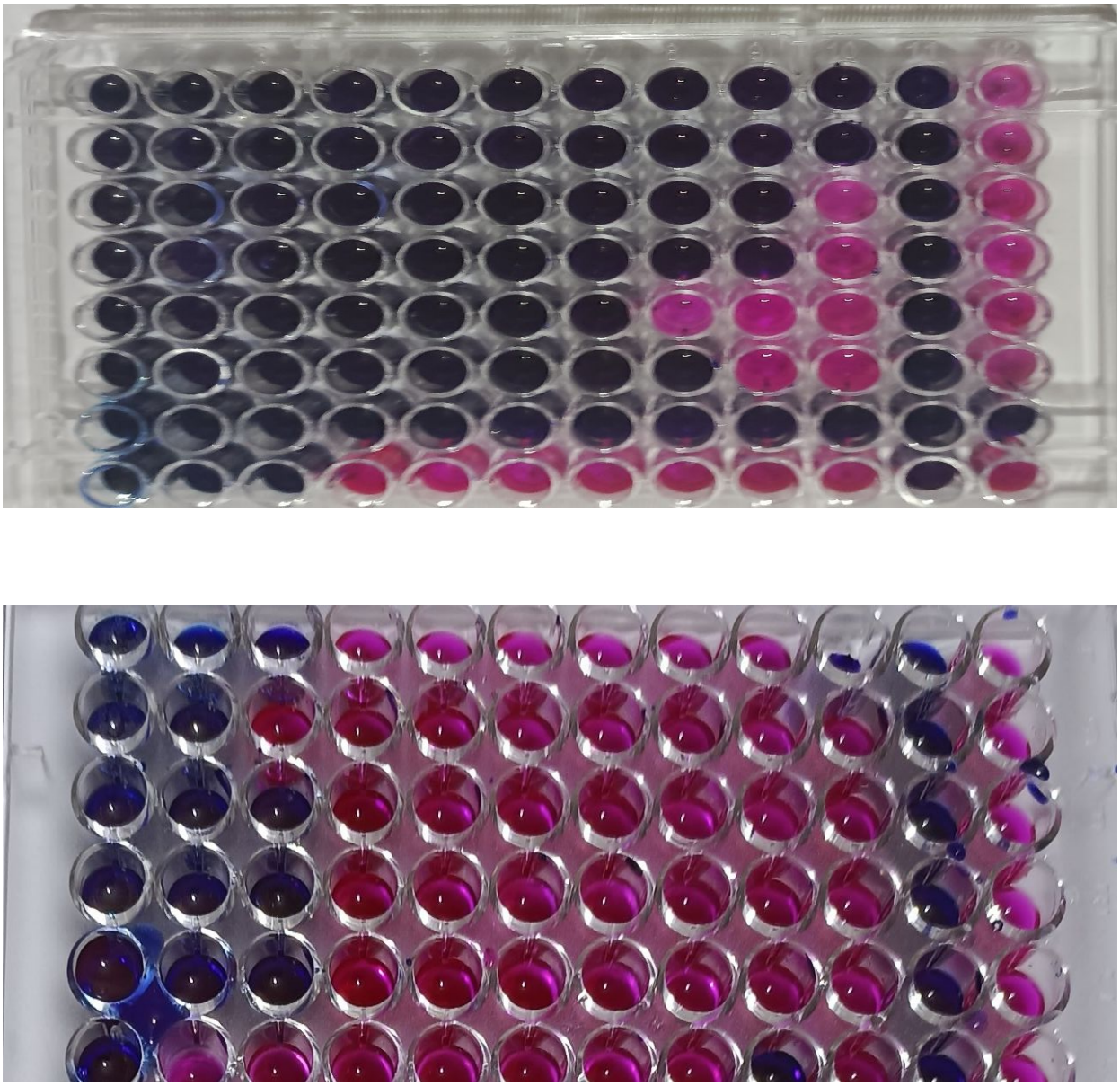


Figure S51: The MIC and MBC assay of compounds of **5, 6a, 6b, 10, 11, 13 and 14** against *C. albicans*

References

1. Sarker, S. D.; Nahar, L.; Kumarasamy, Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. *Methods* **2007**, *42*, pp. 321-324.

2. Ceri, H.; Olson, M. E.; Morck, D. W.; Storey, D. G. *Minimal biofilm eradication concentration (MBEC) assay: susceptibility testing for biofilms*. in *Biofilms, infection, and antimicrobial therapy*. CRC Press, 2005; pp. 275-288.